TITLE

EFFECTS OF DIFFERENT RIPENING REGIMES OF *MUSA ACUMINATA* (BANANA) ON ALBINO RATS USED AS RISK ASSESSMENT MODEL

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF MASTER OF SCIENCE (M.Sc) IN ENVIRONMENTAL BIOCHEMISTRY, UNIVERSITY OF NIGERIA, NSUKKA.

BY

NWANKWO, JOY UCHECHI
PG/M.Sc/17/00156

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF NIGERIA, NSUKKA

SUPERVISOR: DR. UBANI, CHIBUIKE SAMUEL

FEBRUARY, 2020.

CERTIFICATION

Nwankwo, Joy Uchechi, a postgraduate student with Registration Number PG/MSc/17/00156 in the Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, has satisfactorily completed the requirements for the course work and research for the award of Masters of Science (M.Sc.) in Biochemistry (Environmental Biochemistry). The work embodied in this dissertation is original and has not been submitted in part or full for any other diploma or degree of this or any other university

DR. C. S. UBANI
(Supervisor)

PROF. H. A. ONWUBIKO
(Head of Department)

External Examiner

DEDICATION

This work is dedicated to the Almighty God.

ACKNOWLEDGEMENTS

I thank the Almighty God, the source of all knowledge for his guidance and protection throughout my studies in the University of Nigeria, Nsukka. This work wouldn't have been a success story without him.

My profound gratitude goes to my amiable supervisor, Dr. C. S. Ubani for his support, fatherly advice, patience and guidance. All that I have done was only due to his supervision and assistance, despite his busy schedule.

I wish to thank my Head of Department, Prof. H. A. Onwubiko; my lecturers, Prof. F. C. Chilaka Prof. O. U. Njoku, Prof. L. U. S. Ezeanyika, Prof. B. C. Nwanguma, Prof. S. O. O. Eze, Dr. V. N. Ogugua, Dr. (Mrs.) C. A. Anosike, Dr. V. E. O. Ozougwu, Dr. O. C. Enechi, Dr. (Mrs). U. O. Njoku, Dr. E. C. Ossai and Dr. A. L. Ezugwu. I also wish to extend my heartfelt gratitude in a special way to Dr. A. C. Osuji, Mr. I. Okagu, Mr. J. Nwachukwu and all my friends. I also appreciate all the staff and postgraduate students of Department of Biochemistry, University of Nigeria, Nsukka for their numerous assistance to me during the course of this work.

I also wish to acknowledge the countless efforts of the Postgraduate coordinator Dr. P. E. Joshua, whose tireless efforts ensured that this programme was a huge success.

To my parents, Mr. & Mrs. G. M Nwankwo, my siblings Mrs. N. Ofobike, Mazi B. Nwankwo, Mr. C. Nwankwo, Mrs. E. Ukoh and Mr. S. Nwankwo, thank you for your financial support, advice and encouragement, I remain indebted to you all. In a special way, I wish to thank Prof & Mrs C. O. Okeke for their immeasurable support towards the success of this work and my academic pursuit.

ABSTRACT

Fruit ripening is a natural process in which a fruit goes through various physical and chemical changes and gradually becomes sweet, colored, soft and palatable. The use of artificial ripening agents to enhance fruit ripening has become prevalent mostly due to the commercial purposes. People consume fruits ripened with chemicals such as calcium carbide, these can pose great health risk to the consumers. Therefore, the study was aimed to investigate the effect of different ripening regimes of *Musa acuminata* on albino rats used as risk assessment model. Two ripening agents namely calcium carbide and African bush mango were used and compared with a control with no ripening agent. Results showed that banana ripened with 30g calcium carbide (RB4) was the first to ripen in 3days followed by banana ripened with 7g calcium carbide (RB3) and banana ripened with African bush mango (RB2) with naturally ripened banana (RB1) ripening on the 10^{th} day. Proximate composition result of the fruit showed an increased moisture from 77.77% in RB1 to 85.15% in RB4, carbohydrate content reduced from 18.16% to 12.94%, Fibre, protein, fat and Ash content were found to decrease with the use of ripening agents in the following range 1.10-0.50, 1.24-0.35, 0.73-0.30 and 1.05-0.77. A total of twenty-five (25) adult female albino rats were divided into five (5) groups (1, 2, 3, 4 and 5), with five (5) animals in each group. Group 1 received distilled water only, group 2 received 20g of naturally ripened banana, group 3 received 20g of banana ripened with African bush mango while groups 4 and 5 received 20g each of banana ripened with 7g and 30g calcium carbide respectively for 30days. Heavy metals result showed a significant increase ($p < 0.05$) in the levels of Lead (Pb) and Arsenic (As) for groups 2, 3, 4 and 5 when compared with group 1. However, group 4 and 5 for Chromium were significantly higher when compared with group 1 while group 2 and 3 showed no significant difference ($p < 0.05$). Results from human health risk assessment model for Estimated Daily Intake showed an increase above the tolerable daily intake for heavy metals such as Pb (for group 2, 3, 4 and 5) and As (for group 5). There was also a fall in the Tolerable daily intake for Cr (all groups were below the TDI) and As (for group 1, 2, 3 and 4). Toxic Hazard Quotient showed increased values above the standard THQ ($0<x<1$) for an acceptable human such as Pb (for group 2, 3, 4 and 5) and As (for group 5), whereas values for Cr and As (for group 1, 2, 3 and 4) were below the standard THQ. Carcinogenic Risk (CR) values showed that all values estimated were within the value of United States Environmental Protection Agency (USEPA) incremental lifetime carcinogenic risk (ILCR > 10^{-3}) while Arsenic for group 5 was otherwise. Antioxidant enzyme activities showed a significant increase ($p < 0.05$) in catalase activity for group 2 when compared with group 1, while groups 3, 4 and 5 recorded no significant change. Glutathione Peroxidase and Glutathione Reductase activities for all the groups were not significant ($p < 0.05$). Likewise, Superoxide Dismutase activity for groups 4 and 5 were significant ($p < 0.05$) when compared with group 1 but recorded no significance for group 2 and 3. Liver Function Test (LFT) result for Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) activities showed no significant ($p < 0.05$) difference. Alanine amino (ALT) transferase activity was significantly increased across the groups when compared with the control group (Group 1). The observed results showed that calcium carbide contains traces of potential toxic elements (heavy metals) and that its use in fruit ripening will pose a potential health risk on the consumers.

TABLE OF CONTENTS

		Page
Title		i
Certification		ii
Dedication		iii
Acknowledgements		iv
Abstract		v
Table of Contents		vi
List of Figures		x
List of Tables		xi
List of Abbreviations		xii
CHAPTER ONE: INTRODUCTION		**1**
1.1	Banana	2
1.1.1	Nutritional properties and health benefits of Banana	3
1.1.2	Free radical scavenging property of Banana fruit	4
1.1.3	Bioactive compounds in Banana	4
1.1.3.1	Phenolics	5
1.1.3.2	Carotenoids	6
1.2	Fruit Ripening	6
1.2.1	Mechanism of fruit ripening	7
1.3	Characterization of ripening agents	8
1.3.1	Natural ripening agents	8
1.3.1.1	African bush mango	9
1.3.1.2	Apple	9
1.3.2	Artificial ripening agents	9
1.3.2.1	Ethylene	10
1.3.2.2	Calcium carbide	10

1.3.2.3	Ethephon	11
1.4	Effect of artificial ripening agents	11
1.4.1	Arsenic	13
1.4.2	Lead	13
1.4.3	Chromium	14
1.5	Permissible limits of heavy metals in fruits	15
1.6	Liver markers	15
1.6.1	Aspartate aminotransferase	15
1.6.2	Alanine aminotransferase	16
1.6.3	Alkaline phosphatase	16
1.7	Antioxidant compounds	16
1.7.1	Enzymatic antioxidants	17
1.7.1.1	Superoxide Dismutase	17
1.7.1.2	Catalase	18
1.7.1.3	Glutathione Peroxidase	18
1.7.1.4	Glutathione Reductase	18
1.8	Health Risk Assessment	19
1.8.1	Steps to Human Health Risk Assessment Process	21
1.8.1.1	Hazard Identification	21
1.8.1.2	Dose Response Assessment	22
1.8.1.3	Exposure Assessment	22
1.8.1.4	Risk Characterization	22
1.9	Justification of the Study	22
1.10	Aim and Objectives of the Study	23
1.10.1	Aim of the Study	23
1.10.2	Objectives	23
CHAPTER TWO: MATERIALS AND METHODS		**24**
2.1	Materials	24
2.1.1	Plant Material	24
2.1.2	Animals	24

2.1.3	Reagents and Chemicals	24
2.1.4	Instruments and Equipment	24
2.2	Methods	24
2.2.1	Sample Preparation	24
2.2.2	Proximate Composition of Musa acuminate	25
2.2.2.1	Determination of Moisture Content	25
2.2.2.2	Determination of Ash Content	25
2.2.2.3	Determination of Fat Content	25
2.2.2.4	Determination of Crude Fibre Content	26
2.2.2.5	Determination of Protein Content	26
2.2.2.6	Determination of Carbohydrate Content	27
2.2.3	Treatment	27
2.2.3.1	Collection of blood samples from experimental animals	27
2.2.4	Heavy metals	28
2.2.5	Antioxidant Studies	28
2.2.5.1	Assay for Superoxide Dismutase (SOD) Activity	28
2.2.5.2	Assay for Catalase Activity	28
2.2.5.3	Assay for Glutathione Reductase Activity	29
2.2.5.4	Assay for Glutathione Peroxidase Activity	29
2.2.6	Liver Function Biomarkers	30
2.2.6.1	Assay for Aspartate Aminotransferase	30
2.2.6.2	Assay for Alanine Aminotransferase	30
2.2.6.3	Assay for Alkaline Phosphatase Activity	31
2.2.7	Human Health Risk Assessment	31
2.2.7.1	Estimated Daily Intake	31
2.2.7.2	Toxic Hazard Quotient	31
2.2.7.3	Carcinogenic Risk	31

2.2.8 Statistical Analysis 31

CHAPTER THREE: RESULTS 33

3.1 Duration of Ripening 33

3.2 Proximate Composition of Musa acuminate 33

3.3 Heavy Metal Levels of Albino Rats 33

3.4 Human Health Risk Assessment 37

3.4.1 Estimated Daily Intake 37

3.4.2 Toxic Hazard Quotient 37

3.4.3 Increment Lifetime Carcinogenic Risk (ILCR) 37

3.5 Antioxidant Enzyme Activities 44

3.6 Liver Enzyme Activities 44

CHAPTER FOUR: DISCUSSION 47

4.1 Discussion 47

4.2 Conclusion 52

4.3 Suggestions for Further Studies 52

REFERENCES 54

LIST OF FIGURES

Figure 1: Banana fruit 3

Figure 2: Ethylene biosynthesis pathway 7

Figure 3: Steps to human health risk assessment 21

LIST OF TABLES

Table 1: Tolerable daily intake of selected heavy metals

Table 2: Ingestion Reference Dose (IRD)

Table 3: Duration of ripening

Table 4: Proximate composition of *Musa acuminata* treated with different ripening agents

Table 5: Heavy metal content of albino rats fed *Musa acuminata* treated with different ripening agents.

Table 6a: Estimated daily intake (EDI) of heavy metal through the consumption of banana treated with different ripening agents as compared to the tolerable daily intake (TDI) (Children)

Table 6b: Estimated daily intake (EDI) of heavy metal through the consumption of banana treated with different ripening agents as compared to the tolerable daily intake (TDI) (Adult).

Table 7a: Toxic hazard quotient (THQ) of heavy metals through the consumption of bananas treated with different ripening agents (Children).

Table 7b: Toxic hazard quotient (THQ) of heavy metals through the consumption of banana treated with different ripening agents (Adults).

Table 8a: Carcinogenic risk of heavy metal through the consumption of bananas treated with different ripening agents (Children).

Table 8b: Carcinogenic risk of heavy metal through the consumption of bananas treated with different ripening agents (Adults).

Table 9: Antioxidant enzyme activities of albino rats fed *Musa acuminata* treated with different ripening agents.

Table 10: Liver enzyme activities of albino rats fed *Musa acuminata* treated with different ripening agents

LIST OF ABBREVIATIONS

ACC: 1-aminocyclo-propane-1-carboxylic Synthase

AOAC: Association of Official Analytical Chemists

AsH_3: Arsine

C_2H_4: Ethylene

Ca_3As_2: Calcium Arsenide

Ca_3P_2: Calcium Phosphide

Cr (iii): Trivalent Chromium

Cr (vi): Hexavalent Chromium

CR: Carcinogenic Risk

CSF: Carcinogenic Slope Factor

DMRT: Duncan Multiple Range Test

DNA: Deoxyribonucleic acid

EDI: Estimated Daily Intake

EDTA: Ethylene diamine tetra acetic acid

EFSA: European Food Safety Authority

FAO: Food and Agricultural organization

GR: Glutathione reductase

GSH: Reduced Glutathione

GSSG: Glutathione disulphide

GST: Glutathione-S-Transferase

HCN: Hydrogen Cyanide

HNO3: Nitric acid

IARC: International Agency for Research on Cancer

IRD: Ingestion Reference Dose

KMB: 2-keto-4-methylthiobutyrate

MACC: Malonyl-1-aminocyclopropane-1-carboxylic acid

$MgCl_2$: Magnesium Chloride

MTA: 5′-methylthioadenosine

MTR: 5-methylthioribose

NADPH: Nicotinamide adenine dinucleotide phosphate

Ph_3: Phosphine

RFD: Reference Oral Dose

RNA: Ribonucleic Acid

RON: Reactive Nitrogen Species

ROS: Reactive oxygen species

SAM: S-Adenosyl Methionine

SD: Standard Deviation

SGOT: Serum Glutamate Oxalate Transaminase

TCR: Target Cancer Risk

TDI: Tolerable Daily Intake

THQ: Toxic Hazard Quotient

CHAPTER ONE

INTRODUCTION

In recent years, the use of artificial fruit ripening agents has become prevalent mostly due to the commercial purposes (Nazibul *et al*., 2018). Different ripening agents are reported to be used to initiate the ripening process in fruits such as banana. Fruit ripening is a natural process in which the fruit goes through various physical and chemical changes and gradually becomes sweet, colored, soft and palatable (Kendrick, 2012). The ripening of fruits is as a result of the changes in the carbohydrate composition of the fruit, which is converted from starch to sugar giving it a sweet taste. Ethylene is the natural plant growth regulator, it is a gaseous hormone naturally produced in fruit during the ripening stage. Ethylene being a natural hormone does not pose any health risk for consumers of the fruits. It is a de-greening agent, which can turn the peel from green to perfect yellow (in the case of bananas) and maintain the sweetness and aroma of the fruit, thus value addition in the fruit is possible as it looks more appealing (Mursalat *et al*., 2013). Science and technology today, have introduced various methods of ripening to meet consumers demand. Ripening agents can be categorized into natural and artificial groups (Singal *et al*., 2012). The natural ripening agent includes African bush mango, apple and tomatoes. They are a group of fruits capable of emitting ethylene gas (Siddiqui and Dhua, 2010). Artificial methods of fruit ripening involve the use of synthetic chemicals such as ethylene, ethephone, ethylene glycols, kerosene and calcium carbide. Generally, majority of the fruits are ripened artificially using some of these chemical agents in order to meet consumers demand as they tend to give a faster ripening time (Dhembare, 2013a; 2013b). Out of the entire ripening agents, the most commonly used chemical for artificial ripening is calcium carbide because it is inexpensive, effective and easily purchased in local markets (Asif, 2012). Hence, the use of calcium carbide for the study.

However, most of the artificial ripening agents are hazardous, carcinogenic and their consumption can cause serious health problems, such as heart disease, skin disease, lung failure and kidney failure (Hakim *et al*., 2012). It has been reported that regular consumption of artificial-ripened fruits may cause dizziness, weakness, skin ulcer and heart related diseases (Fattah and Ali, 2010, Asif, 2012). In addition, these ripening agents may contain potential toxic

elements (PTEs) as impurities. These toxic elements such as arsenic (As), lead (Pb), cadmium (Cd) and chromium (Cr) are nonessential elements that are not required for biological function and may be deleterious to human health (Basapor and Ngabaza, 2015). Humans are at risk of short term and long term health effects simply by eating bananas that are induced to ripen (Asif, 2012). PTEs bio-accumulate in the system of most organism and with time bio-magnify (Orisakwe *et al*., 2012), leading to a disruption of various biological processes in the body.

The ability of the human defense mechanism such as antioxidants to combat these exposures of toxic chemicals may diminish with frequent exposure. These enzymes such as catalase, superoxide dismutase, glutathione peroxide and glutathione reductase may be activated or inhibited under such conditions. According to Hamlet *et al*., 2012, bioaccumulation of PTEs may affect the antioxidant enzyme properties leading to an abnormality in their function. Also the liver which is the main organ for metabolism and detoxification of toxicants are exposed to significant concentrations of these chemicals, therefore liver functions can be adversely affected by acute or chronic exposure (Salminen *et al*., 2012). Therefore, consuming artificially ripened bananas are capable of eliciting different health issues (Giacomini, 2012).

1.1 BANANA (*Musa acuminata)*

Banana is a climacteric fruit belonging to the family *Musaceae*, kingdom *plantae* and genus *Musa* (Bhaskar *et al*., 2012). Banana is a leading tropical fruit and one of the most popular fruit throughout the globe. It is estimated that 100 million people depend on banana and plantain as their main energy source (Taposhe *et al*., 2017). It is one of the fruits within the buying capacity of poor people and it is usually eaten as supplementary food or as a whole meal (Adekalu *et al*., 2011, Vaidya *et al*., 2016). The banana tree is a perennial herb that grows 5-9m in height, with a tuberous rhizome and a hard, long pseudo-stem. The ripe fruits are sweet, juicy and the peel is thick (Imam and Akter 2011; Onasanwo *et al*., 2013). Banana is the most popular fruit in industrialized countries, it is cultivated in over 130 countries as the second-most produced fruit after citrus (FAO, 2013; D'Hont *et al*., 2012). It contributes approximately to 17% of the world's total fruit production (FAO, 2013). It is one of a good number of consumed fruits in tropical and subtropical regions of the world (Alkarkhi *et al*., 2010).

Figure 1: Banana fruit

Source: (Rajesh, 2017)

1.1.1 Nutritional Properties and Health Benefits of Banana

Fruits are important in human diet because they supply vitamins and minerals to the body, provide variety to food and make food appetizing (Nura *et al.*, 2018). Banana is a tropical fruit with high calorie, it provides exceptional nutrition in different forms. Banana pulp is firm when the fruit is not ripe but softens during maturation. The pulp comprises of 75% water, 20 g of carbohydrates per 100 g of fresh pulp out of which fibre (2 g per 100 g fresh weight) is useful for regulation of intestinal transit. In general, bananas are a good source of Potassium , Iron, Magnesium, Copper and Manganese but do not supply much vitamin C or vitamin A relative to other fruits (Rajesh, 2017). The Pulp of bananas contains free phenolic compounds, mainly catechin and gallic acid, Phenolic compounds are present in greater quantity in the skin than in the pulp. Also, dopamine a powerful antioxidant is found at higher concentration in the pulp of colored bananas (*Musa acuminata* and *Musa sapientum*).

Consumption of fruits is essential for a diversified and nutritious diet. Sufficient consumption of fruit and vegetables significantly reduce the incidence of chronic diseases, such as cancer, cardiovascular diseases and other aging-related pathologies (Prakash *et al.*, 2012). Fruits offer protection against free radicals that damage lipids, proteins, and nucleic acids. Polyphenols, carotenoids (pro-vitamin A), vitamins C and E present in banana have antioxidant and free radical scavenging activities and play a significant role in the prevention of many diseases

(Prakash *et al.*, 2012). A number of trace elements also protect the cell from oxidative cell damage as these minerals are the cofactor of antioxidant enzymes.

1.1.2 Free Radical Scavenging Property of Banana Fruit

The reactive oxygen species (ROS) and reactive nitrogen species (RON) are two types of free radicals in the body. These compounds are produced during normal metabolism of cells and may take part in the pathological process named oxidative stress, which is promoted when the balance between free radicals and antioxidants tends to favor the former (Márcio *et al.*, 2018). As a result, the excess of free radicals within the human body leads to oxidative stress, resulting in the development of many types of diseases such as, chronic disorders, cardiovascular problems, diabetes, cancer, and rheumatoid arthritis (Mirończuk-Chodakowska and Zujko, 2018). Kandaswamy and Aradhya, (2014) has shown that the banana rhizome is a rich source of many polyphenolic compounds having antioxidant activities. They identified various anthocyanins such as cyanidin-3-rutinoside and 3-rutinoside derivatives of delphinidin, pelargonidin, peonidin, and malvidin.

1.1.3 Bioactive Compounds in Banana

Fruits and vegetables are rich sources of various health beneficial phytochemicals, such as vitamins, minerals, carbohydrates, flavonoids and phenols. Extensive researches on these phytochemicals by various groups have been revealed that they have immense role in the reduction of certain degenerative and malnutrition related diseases (Sampath *et al.*, 2012). Banana fruits are rich source of nutrients and their biochemical composition varies with growth stage and maturity, it also depends on the cultivar, abiotic and environmental factors, the nutrient status and the nature of the soil (Merlene *et al.*, 2012). The banana pulp, pseudo stem and banana fruit peel are good source of antioxidants. Higher content of polyphenols, flavonoids, total dietary fiber, insoluble dietary fiber, lignin, hemicellulose, cellulose, antioxidant capacity, and free-radical scavenging capacity are found in the native banana pseudo-stem. Mineral compositions of banana include being rich in calcium, phosphorus and iron. In addition to this they are also rich sources of antioxidant potential phytochemicals such as polyphenols, carotenoids, flavonoids, vitamin C and lesser in the quantity of anti-nutritional factors such as phytates and oxalates (Kookal and Thimmaiah, 2018). The phytochemistry and pharmacology of

banana (*Musa acuminata*) by Mathew and Negi (2017) have suggested the use of banana pulp and peel for the development of drugs and its use in functional foods.

1.1.3.1 Phenolics

These compounds are of particular interest due to their occurrence and the properties they possess, the main sources of phenolic compounds are fruits and vegetables. Phenolic compounds or polyphenols are one of the most frequent and widespread groups of substances in the world of plants, with more than 8,000 identified phenolic structures (Tsao, 2010). These compounds can be found in almost all organs of a plant. According to their structure, they have different functions ranging from skeletal constituents of different tissues to pigmentation of several plant organs (Ignat *et al.*, 2011). Polyphenols are secondary metabolites essential for the growth and development of plants and their reproduction. Similarly, they help to control growth in diameter, pigmentation and defense against various pathogens (Popa, 2015) or act as signaling molecules to distinguish symbionts. These compounds, as natural antioxidants, have important properties that involve the inhibition of lipid peroxidation, inhibition of carcinogenesis, antimicrobial activity, direct constrictive action on capillaries, naturally occurring phytohormones and stabilization of ascorbic acid (Tanase *et al.*, 2014). Phenolic present in banana fruits are the major bioactive compounds having antioxidant properties and are known for providing health benefits. Various phenolics present in banana have been identified as follows: gallic acid, catechin, epicatechin, tannins, and anthocyanins (Kandasamy and Aradhya, 2014). Banana peel is also a rich source of phenolic compounds. Tsamo *et al.* (2015) analysed banana pulp and peel from two dessert banana cultivars, according to their results hydroxycinnamic derivatives such as ferulic acid-hexoside were the major ones in banana pulp. They observed large variations in the phenolic contents among the cultivars tested. In the peel from plantain cultivars, rutin was the most abundant flavonol glycoside. Thus, both the banana peel and pulp are good sources of health-promoting phenolic compounds. Among the flavonoids possessed by banana are quercetin, myricetin, kaempferol, and cyanidin which provide health benefits mainly because they act as free radicals, reactive oxygen species and reactive nitrogen species scavengers (Jiwan and Tasleem, 2018). Most of these phenolic are known to also exhibit antibacterial, antiviral, anti-inflammatory, antiallergenic, antithrombotic and vasodilatory activities. With few exceptions, the peel extracts have higher total phenolic and total antioxidant activities than the

pulp. Among minerals, potassium was the major element found in both the peel and pulp followed by phosphorus and magnesium (Sulaiman *et al*., 2011).

1.1.3.2 Caroteniods

Carotenoids are precursors for vitamin A, and are also known to have strong antioxidant capacity to scavenge reactive oxygen species (ROS). Among the carotenoids present in banana fruit, α-carotene, β-carotene, and β-cryptoxanthin have provitamin A activity, but others like lycopene and lutein have a strong antioxidant capacity (Jiwan and Tasleem, 2018). Lycopene is known to provide protection against prostate cancer among men and lutein offers human health benefits to serve as an inhibitor of age-related macular degeneration. Certain banana cultivars rich in provitamin A carotenoid can be grown and consumed by the poor population of the world that is having serious vitamin A deficiency, the consumption of such banana fruit would alleviate vitamin A deficiency (Fungo and Pillay, 2013).

1.2 Fruit Ripening

Fruit ripening is an irreversible phenomenon involving a series of physiological, biochemical and organoleptic changes that leads to development of soft and edible fruit with a desirable quality (Bouzayen *et al*., 2010). Ripening is the process by which fruits attain their desirable flavor, quality, color, palatable nature and other textural properties. Ripening is associated with change in composition *i.e*. conversion of starch to sugar. It also involves co-ordination of different metabolism with activation and deactivation of various genes leading to a change in the tissues (Singal *et al*., 2012). These genes encode enzymes that participate directly in biochemical and physiological changes. They also encode regulatory proteins that participate in the signaling pathways and in the transcriptional machinery that regulate gene expression setting in motion the ripening process. On the basis of ripening behavior, fruits are classified as climacteric and non-climacteric fruits. Climacteric fruits are fruits that enter climacteric phase after harvest *i.e*. they continue to ripen. During the ripening process the fruits emit ethylene along with increased rate of respiration. Ripe fruits are soft and delicate and generally cannot withstand rigors of transport and repeated handling. These fruits are harvested hard and green, but fully mature and are ripened near consumption areas. Small dose of ethylene is used to induce ripening process under controlled conditions of temperature and humidity. Climacteric fruits include Mango, banana, papaya, guava, apple, passion fruit, apricot and plum. Non-climacteric fruits once harvested do

not ripen further (Singal *et al*., 2012). Non climacteric fruits produce very small amount of ethylene and do not respond to ethylene treatment. There is no characteristic increased rate of respiration or production of carbon dioxide. Non-climacteric fruits include Orange, Grapefruit, Grapes, Pomegranate, Watermelon, Cherry**,** Blackberry, Strawberry and Cashew**.** In order to improve external skin color and market acceptance, citrus like orange, lemon can be treated with ethylene as a de-greening agent. Ethylene treatment breaks down the green chlorophyll pigment in the exterior part of the peel and allows the yellow or orange carotenoid to be expressed.

1.2.1 Mechanism of fruit ripening

Fruit ripening is a natural process, in which the fruit goes through various chemical changes and gradually becomes sweet, colored, gets soft and becomes palatable (Bouzayen *et al*., 2010). Unripe fruits often contain various types of organic acids, namely citric acid, malic acid, ascorbic acid, formic acid and tartaric acid. These acids are held responsible for the sour taste of fruits. After certain chemical changes, these acids are transformed into sugars and the fruits turn sweet (Kendrick, 2012). In fruit ripening process, Chlorophyll is produced and at the same time decomposed. Starch is acted on by amylase to produces sugar. Pectin converts into pectinase and decomposition of pectin, in this case unglues the fruit cells. The cells being able to slip past one another makes the fruit further soft, (Koning, 2012). With the advancement of science and technology, various artificial methods of fruit ripening have been observed mostly to meet consumer's demand and other economic factors. However, in the recent years, artificial fruit ripening has been considered a matter of concern and the effect of artificial ripening has become questionable because of various health related issues. In climacteric fruit varieties, ripening is characterized by automatic catalytic ethylene responses (Sushmitha *et al*., 2018). Ethylene, a phytohormone regulates the ripening process in fruits. It is usually present in gaseous form in the physiological conditions and it is also involved in the senescence process. The biosynthesis of ethylene begins with the activation of 1-aminocyclo-propane-1-carboxlyic acid (ACC) synthase genes which causes an increased production of ACC synthase. The first step is the conversion of methionine to S-AdenosylMethionine (SAM) and the second step is the conversion of s-adenosylmethionine to ACC in the presence of ACC synthase. The ACC synthase is a pyridoxal-5'-dependent enzyme and it is the main regulating enzyme of the ethylene biosynthesis. ACC is then converted by oxidation to ethylene by ACC oxidase enzyme, leading to the process of

ripening (Sushmitha *et al.*, 2018). *S*-adenosyl-methionine (*S*-AdoMet) is synthesized from methionine by the enzyme *S*-adenosyl-methionine synthetase (SAM synthetase) with one ATP molecule expensed per *S*-AdoMet synthesized. *S*-AdoMet is then converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase with 5′-methylthioadenosine (MTA) being the by-product. MTA is recycled to methionine by successive enzymatic reactions involving various intermediates (MTR, 5-methylthioribose; KMB, 2-keto-4-methylthiobutyrate), which constitute the methionine (Yang) cycle. *S*-AdoMet is also the precursor of the spermidine/spermine biosynthesis pathway (Arc *et al.*, 2013). Ethylene production is catalyzed by the ACC oxidase using ACC as substrate, and generates carbon dioxide and hydrogen cyanide. Malonylation of ACC to malonyl-ACC (MACC) reduces ACC content and consequently ethylene production.

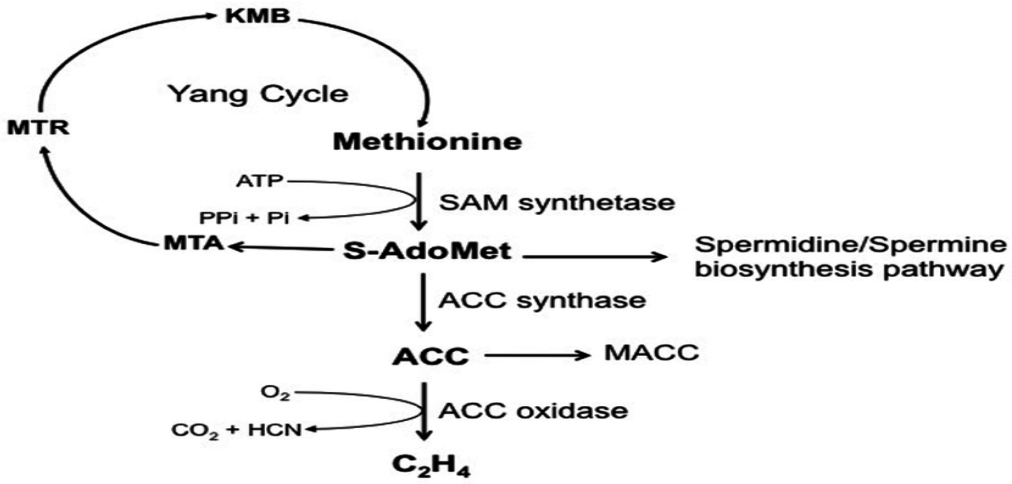

Figure 2: Ethylene biosynthesis pathway

Source: (Arc *et al.*, 2013)

1.3 Characterization of Ripening Agents

1.3.1 Natural Ripening Agents

Natural ripening agents are made up of climacteric fruits, capable of speeding up the process of ripening in other fruits. Natural ripening agents have increased rate of respiration and also emits ethylene (Singal *et al.*, 2012). They include fruits such as African bush mango, apple, banana, tomatoes etc.

1.3.1.1 African Bush mango

African Bush mango fruit (*irvingia gabonesis*), also known as wild mango, is commonly called 'Ogbono' in Igbo and 'Apon' in Yoruba (Katnap *et al*., 2017). The fruit is greenish yellow with a fleshy fibrous pulp surrounding a large hard stone. The fruit pulp is eaten and the kernel is also used for medicinal purposes and as a source of oil for making soap (Edouard *et al*., 2017). It produces edible fruits and seeds. The dried seed of *Irvingia gabonesis* is widely consumed by humans in Nigeria as a good thickener for soup. *Irvingia gabonesis* is a climacteric fruit capable of emitting ethylene gas, the fruit is now being used as a ripening agent to accelerate ripening period by local farmers (Adewale and Duruji, 2010)

1.3.1.2 Apple

Apple is one of the most commercially grown fruit crop in the world after banana, orange and grapes. Apple can be eaten raw or used as an important ingredient in many desserts like apple pie, maple crumble and apple (Gourab, 2015). It has excellent health benefits, hence it is recommended for daily consumption and it is also a climacteric fruit that takes part in the initiation of ripening.

1.3.2 Artificial Ripening Agents

Artificial ripening is done to achieve faster and uniform ripening. It is the process by which ripening is controlled and products may be achieved as per requirement by controlling different parameters. Artificially ripened fruits may develop uniform and attractive surface color but the tissue inside remains hard and the fruit generally have shorter shelf life (Hossain *et al*., 2015). Artificial ripening accelerates the ripening process but affects the nutritional quality, sensory and safety of the fruits (Hossain *et al*., 2015). Dhembare (2013) reported that substances used as artificial ripeners have several effects on humans which include memory loss, cerebral edema, prostate and lung cancer, quick-buck syndrome, changes in DNA and RNA as well as hematological changes. Fruits ripened artificially are over soft, inferior in taste and flavor. Precautionary measures observed to avoid toxic effects of the substances were through washing of fruits before eating and avoidance of eating the skin of the fruits (Fattah and Ali, 2010). Chemicals commonly used to induce ripening include ethylene gas, ethephon, ethylene glycol, and calcium carbide (Hossain *et al.* 2015).

1.3.2.1 Ethylene

Ethylene is the simplest of the organic compounds known as alkenes, which contain carbon-carbon double bonds. It is a colorless, flammable gas having a sweet taste and odor. Ethylene is an important industrial organic chemical produced by heating either natural gas especially its ethane and propane components (Maduwanthi and Marapana, 2019). Ethylene is an invisible gas which has no known hazardous effect on humans at the concentrations encountered within the fruit supply chain. Ethylene is associated with plants under stress and fruit maturation in horticulture. The most popular method practiced in developed countries is application of ethylene gas in ripening rooms. Ethylene gas filled in pressurized cans promote fruit ripening in 24-48 hour.

1.3.2.2 Calcium Carbide

Calcium carbide is a whitish grey powder which is readily available in markets. Apart from its industrial use, it is frequently used in domestic settings for unclogging the drain as well as ripening fruits (Jindal *et al.*, 2013). Calcium carbide is an alkali, it releases acetylene gas when it comes in contact with moisture. Acetylene gas is a chemical analogue of ethylene gas which is a natural fruit ripening agent. Calcium carbide absorbs moisture and produces acetylene, which is a weak analog of ethylene, responsible for triggering ripening process (Asif, 2012). Calcium carbide is indiscriminately used in preference to other recommended practices of inducing ripening because it is readily available and also very much affordable (Singal *et al.*, 2012). However, use of calcium carbide poses lots of potential health hazards to human health. The acute (short-term) health effects may include the irritation of the skin causing rashes, redness, and burning feeling on contact. Irritation of the mouth, nose, throat and lungs causing cough or shortness of breathe. Higher exposures may cause a build-up of fluid in the lungs (pulmonary edema), a medical emergency with severe shortness of breath. Other effects on acute exposure include headache, vertigo, dizziness, delirium, seizures and even coma. Immediately after consumption, there may be abdominal pain, vomiting and diarrhea. Prolonged and repeated exposure may cause dry, cracked skin and eye lid irritation. Consumption of carbide ripened fruits is extremely hazardous for health, mainly for the nervous system. This is because acetylene, generated from carbide reduces oxygen supply to the brain. In the long term, it may produce mood disturbance and loss of memory. Commercial grade calcium carbide is impure,

they contain impurities such as arsenic and phosphorus in the form of Calcium phosphide (Ca_3P_2) and calcium arsenide (Ca_3As_2), which may reacts with water to form phosphene (PH_3) and arsine (AsH_3) respectively. These hydrides are fat soluble and may dissolve in the wax layer of fruits, diffuse from peel to flesh of fruits thereby causing health hazards when consumed (Igbinaduwa and Aikpitanyi-Iduitua 2016). Arsenic is one of the most toxic metals derived from the natural environment, in chronic arsenic ingestion, arsenic accumulates in the liver, kidneys, heart, and lungs and smaller amounts in the muscles, nervous system, gastrointestinal tract and spleen.

1.3.2.3 Ethephon

Ethephon (2-chloroethylphosphonic acid) is an ethylene releasing compound, categorized as non-carcinogenic to humans by International Agency for Research on Cancer (IARC). It penetrates into the fruit and decomposes to ethylene (Singal *et al.*,2012), it has been shown to hasten ripening of several fruits including bananas, apples, tomatoes, mango, peaches, citrus fruits, and guava (Gill *et al.*, 2014). Apart from being used to initiate ripening, ethephon has been recorded as plant growth regulator which can be used to increase fruit size, induce flowering, enhance colour, and induce flower abscission. Ethephon is used throughout the world as an insecticide. It is an organophosphorus compound and in experimental animals it has been reported to get rapidly absorbed in the gut (Maduwanthi and Marapana, 2019). Toxic effects of ethephon reported in human adults till date are salivation, lacrimation, diarrhea, urgency of bowel movement, stomach cramps and frequency of urination with decreased appetite (Raouf and Girgis, 2011).

1.4 Effects of Artificial Ripening Agents

Most of the ripening agents used by the fruit-sellers are of industrial grade, they are collected from unauthorized sources and may contain a high percentage of toxic impurities that are deleterious to human health (Rahimzadeh and Rastegar, 2017). Contamination of fruits with heavy metals can cause serious health problems. Industrial grade calcium carbide generally contains impurities of arsenic, phosphorus as well as lead and chromium that pose a number of health problems (Nura *et al.*, 2018). This is the reason why it is banned in most of the countries. But because of their cheap prices and easy availability, it is still in use in many parts of the world (Gupta, 2017).

In most developing countries like Nigeria, the quest for rapid economic growth through industrialization and modern agriculture have resulted in a concomitant inflow of several contaminants (such as heavy metals) into the environment (Otitoloju, 2016). Heavy metals are harmful compounds even when present at low concentration. They have non-biodegradable nature and long half-lives. Since the body does not have enough mechanisms for heavy metal elimination, serious damage to human and animal tissues may happen upon contamination (Sardar *et al*., 2013). Accumulation of heavy metals in body may cause a disruption in the function of vital organs such as heart, brain, kidneys, prostrate, ovary, bone, and liver; on the other hand, they displace the vital nutritional minerals from their original place (Singh *et al*., 2011). Despite the benefits of fruit consumption on human health, heavy metal contents in fruits can be toxic when they exceed the recommended levels or when they bio-accumulate in the body over a long period (Orisakwe *et al*., 2012). Direct consumption of acetylene from calcium carbide has been found to be detrimental as it reduces oxygen supply to the brain and can further cause prolonged hypoxia (Fattah and Ali, 2010). Carbide is known to be a carcinogenic (cancer inducing) substance and its consumption for a long periods of time predispose one to a high risk of developing cancer and some other abnormalities (Hakim *et al*., 2012). Toxic (non-essential) metals have been shown to induce conditions of oxidative stress either through their ability to undergo redox-cycling and generate ROS such as superoxide or as a consequence of enhanced production of ROS by damaged mitochondria. For example, lead has the ability to generate ROS, also enhanced formation of ROS from mitochondria occurs in cells exposed to arsenic, probably as a result of the ability of the metal ion to bind to protein thiol groups and induce mitochondrial damage through opening of the mitochondrial permeability transition pore. A unique feature of toxic metals is the ability of the complexes formed between the metal ion and the nucleophilic sites of the cellular proteins to mimic endogenous substrates or conformations. This property is responsible for the selective transport of metal ions into or across cells and their interference with the functioning of target enzymes. When damage to the cell becomes excessive as a result of damage to mitochondria, cell death pathways are activated and these typically take the form of apoptosis, autophagic cell death or necrosis.

1.4.1 Arsenic

Arsenic is one of the most notorious poisons since the ancient times, it is a highly toxic element, and its presence in food composites is a matter of concern to the world scientists. Humans are exposed to arsenic predominantly through drinking water, air, food, occupation and other environmental sources. Individuals could be exposed to arsenic through several sources, with food consumption and drinking water as the two most important ones. Although dermal and inhalation exposure is possible, food and drinking water are the principal routes of exposure to arsenic (WHO 2011a; IARC 2012; EFSA 2014). Long-term or chronic exposure to arsenic is related to severe adverse health effects including dermatitis, cardiovascular diseases, diabetes mellitus, chronic bronchitis, immune disorders, peripheral neuropathy, liver damage, renal failure, adverse reproductive outcomes, hematological effects and other ailments (Ali *et al*., 2010; Argos *et al*., 2010). In fact, arsenic affects almost all vital organs of human body causing the damage or dysfunction. Arsenic usually causes disease conditions even at low concentration. The type of disease condition caused depends on the oxide and sources. In organic sources (sources through which it is mainly contacted), arsenic can cause nerve injury and stomach aches. According to Izah and Srivastav (2015) and Tchounwou *et al*., (2012), high arsenic exposure could lead to cardiovascular, hematological, neurological, respiratory, gastrointestinal and developmental disorders, dermatitis and cancer, diabetes and hearing loss. High concentrations of arsenic could affect kidney, liver, bladder and skin. Inorganic and semi-metal arsenic compounds are carcinogenic. Specifically, long-term, low-dose exposure to inorganic arsenic compounds could lead to mutagenesis (Pflaum *et al*., 2016).

1.4.2 Lead

Lead is identified as a cumulative toxicant that is distributed in the brain, liver, kidneys, and bones. It can cause several unwanted effects such as disruption in biosynthesis of hemoglobin resulting in anemia, nervous system damage, increased blood pressure, kidney dysfunction and miscarriages (Iyer *et al*., 2015; Ogunkun *et al*., 2014). This metal is particularly harmful to infants and young children because of ongoing development of central and peripheral nervous system. A decreased intelligence quotient and behavioral deficits are the most common side effects of lead poisoning in children (Zhong *et al*., 2016; Chen *et al*., 2015). Lead is known to cause toxicity and poisoning in individuals exposed to them. Lead poisoning can lead to various

disease symptoms, including anemia, convulsion, central-nervous system disorders (Garba *et al*., 2015), anorexia, headache, malaise, diarrhea, lead-palsy, encephalopathy, insomnia, high blood pressure, weakness in fingers, wrists and ankles, hair loss, lung fibrosis, skin allergies, outbreak of eczema and variable degrees of kidney operation, loss of appetite, vomiting, irritability and behavioral changes (Iweala *et al*., 2014). Some notable damage caused by high exposure to lead includes kidney, liver and brain (cerebral edema), reproductive system, cardiovascular and nervous system, gastrointestinal and neuromuscular disorders (Izah and Srivastav, 2015).

1.4.3 Chromium

Chromium is one of the toxic essential heavy metals. It is highly detrimental to humans when its concentration exceeds tolerable limits by humans, it exists in the environment at different oxidation states ranging from -2 to +6, the most stable forms of chromium are the trivalent (Cr (III)) and hexavalent (Cr(VI)) chromium (Kirti *et al*., 2015). Trivalent chromium has poor absorption inside the cell as compared to hexavalent chromium. It aids in the biosynthesis of glucose tolerance factor (Iwegbue *et al*., 2013), utilization of sugar, protein and fats (Orisakwe and Ajaezi, 2014) catabolism of fat, carbohydrates and the maintenance of blood glucose especially in diabetic patients (Salako *et al*., 2016). Exposure routes of chromium to humans are ingestion, dermal contact and inhalation. The primary health hazards caused by chromium are bronchial asthma, lung and nasal ulcers, cancers, skin allergies, reproductive and developmental problems (Thompson *et al*., 2011). When taken in excess it may cause death also. Chromium is carcinogenic in nature, human health is adversely affected due to the exposure of chromium and these health effects are categorized into two types, carcinogenic and non-carcinogenic with three types of exposure duration (Fathima *et al*., 2010), Acute (14 days or less), Intermediate (75-364 days) and Chronic (365 days or more). Acute and chronic toxicity of chromium occurs depending on the level of exposure and the occurring form of the element. Typically, chronic forms of chromium are characterized by eczematous dermatitis and acute toxicity which could lead to death. Other organs significantly affected by chromium toxicity includes kidney, skin, neurons and liver (Muhammad *et al*., 2014). Some of the symptoms associated with chromium toxicity include breathing difficulty, nose problems, ulcers, asthma and cough. Chromium can also cause a reduction in glucose tolerance factor and an increase in cardiovascular diseases (Adegbola *et al*., 2015).

1.5 Permissible Limits of Heavy metals in Fruits

The effects of heavy metal contamination of fruits cannot be underestimated as these foodstuffs are important components of human diet. However, the intake of heavy metal-contaminated fruits may pose a risk to human health, hence heavy metal contamination of food is one of the most important aspects of food quality assurance (Rubaljot *et al.*, 2014). Heavy metals in general are not biodegradable, they have long biological half-life and its accumulation in different body organs leads to unwanted side effects (Elbagermi *et al.*, 2012). It is important to know the permissible limits of these heavy metals in fruits, as it gives an idea of the tolerable levels of metals in fruits still considered to be safe for human consumption and would elicit no reason for public health concern.

1.6 Liver Biomarkers

The liver is the largest internal organ in the human body and it is the main organ for the metabolism and detoxification of drugs and environmental toxicants. Due to blood flow from the stomach and intestine, the liver is the first internal organ to encounter a number of insults including ingested metals, drugs and environmental toxicants (Salminen *et al.*, 2012). As a result, liver cells are exposed to significant concentrations of these chemicals and liver functions can be adversely affected by acute or chronic exposure. These enzymes used in determining the state of the liver includes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) (Malakouti *et al.*, 2017).

1.6.1 Aspartate Aminotransferase (AST)

Aspartate Aminotransferase is a transaminase enzyme that catalyzes the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate. The enzyme is located in the cytosol but higher concentrations are found in the mitochondria. The AST enzyme was formerly known as serum glutamate oxalate transaminase (SGOT) and is present in all tissues except bone, with highest levels in liver and skeletal muscle (Malakouti *et al.*, 2017). Injury to hepatocytes causes leakage of AST into the extracellular compartment with subsequent elevation in serum AST activity. The AST activity may also be elevated in times of skeletal muscle injury in preclinical species. Aspartate aminotransferase is considered a less specific marker for liver injury than ALT, due to expression from other tissues, such as brain, myocardial cells and skeletal muscle cells (Wenqian *et al.*, 2019).

1.6. 2 Alanine Aminotransferase (ALT)

The serum ALT activity (hereafter termed ALT) has been regarded as a reliable and sensitive marker of liver disease, it is primarily located in liver and is considered a more specific test for liver damage (Odiba *et al*., 2014). Alanine aminotransferase (ALT) is an enzyme that catalyzes the transfer of amino groups to form the hepatic metabolite oxaloacetate.

1.6.3 Alkaline phosphatase

As an enzyme it is located on the outer layer of the cell membrane, it catalyzes the hydrolysis of organic phosphate esters present in the extracellular space (Wenqian *et al*., 2019). Zinc and magnesium are important co-factors of this enzyme. Alkaline phosphatase is made mostly in the liver and bone, with some produced in the intestines and kidneys. It is also made by the placentas of pregnant women (Heinrich *et al*., 2018). Conditions that cause rapid bone growth (puberty), bone disease (osteomalacia), hyperparathyroidism, or liver cell damage can lead to increases in alkaline phosphatase levels.

1.7 Antioxidant Compounds

Antioxidants are compounds that inhibit oxidation reactions (Kumar *et al*., 2016). Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that can damage the cells of organisms. Antioxidants are very useful in ameliorating these chain reactions. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants such as glutathione and enzymes (e.g. catalase and superoxide dismutase) produced internally or the dietary antioxidants vitamins such as vitamin C and E (Pawar *et al*., 2016). Production and accumulation of free radicals is a common denominator in many disorders and environmental issues, it can cause serious cell damage leading to physiological dysfunction and cell death in almost all aerobes (Maes *et al*., 2018). Enzymatic and non-enzymatic antioxidant systems in the body including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), lipid-soluble vitamin E, carotenes and water-soluble vitamin C regulates the balance between ROS and antioxidants. Oxidative stress is a cellular phenomenon or condition which occurs as a result of physiological imbalance between the levels of antioxidants and oxidants (free radicals or reactive species) in favor of oxidants. On the other hand, dietary antioxidants mostly obtained from fruit and vegetable consumption, has also been associated with a great balance between free radicals and antioxidant status which

helps to minimize oxidative stress and reduce the risk of cancer, cardiovascular diseases and aging (Kumar *et al.*, 2016). The cells in our body are equipped with a range of powerful defense and repair mechanisms, toxicity is only observed once this protective barrier has been overwhelmed (Sylvia, 2018). The key defense mechanisms comprise among others, small antioxidant molecules such as ascorbic acid and a-tocopherol, the tripeptide glutathione (GSH) and a range of antioxidant enzymes such as superoxide dismutases, catalase, GSH transferases and GSH peroxidases. Our cells are therefore well equipped to deal with toxic compounds that induce conditions of oxidative stress. Indeed, the majority of toxic drugs, environmental compounds and the effects caused by ionizing and non-ionizing radiation, through the direct generation of reactive oxygen species or reactive nitrogen specie, or through the depletion of cellular thiols via oxidation or conjugation, lead to conditions of oxidative stress (Rout and Sikdar, 2017). These result in direct or indirect damage to cellular proteins, phospholipids, and nucleic acids and in turn to a spectrum of cellular effects ranging from cancer to cell death.

1.7.1 Enzymatic Antioxidants

1.7.1.1 Superioxide dismutase (SOD) (EC 1.15.1.1)

Superoxide dismutase (SOD) is a necessary antioxidant enzyme that protects cells from reactive oxygen species (ROS). Decreased levels of SOD or mutations that affect their catalytic activity have serious phenotypic consequences. Through their activity, SOD enzymes control the levels of a variety of reactive oxygen species (ROS) and reactive nitrogen species by limiting the potential toxicity of these molecules and controlling broad aspects of cellular life that are regulated by their signaling functions (Wang *et al.*, 2018). Superoxide dismutase (SOD) is the first detoxification enzyme and most powerful antioxidant in the cell. It is an important endogenous antioxidant enzyme that acts as a component of first line defense system against reactive oxygen species (ROS). It catalyzes the dismutation of two molecules of superoxide anion (O_2-) to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), consequently rendering the potentially harmful superoxide anion less hazardous. SOD is a metalloenzyme and hence requires a metal cofactor for its activity (Hayyan *et al.*, 2016). The metal ions which are normally bound by SOD are iron (Fe), zinc (Zn) copper (Cu) and manganese (Mn).

1.7.1.2 Catalase (EC 1.11.1.1)

Catalase is a common antioxidant enzyme present almost in all living tissues that utilize oxygen. The enzyme uses either iron or manganese as a cofactor and catalyzes the degradation or reduction of hydrogen peroxide (H_2O_2) to water and molecular oxygen, consequently completing the detoxification process initiated by SOD (Asaduzzaman *et al.*, 2010). Catalase has one of the highest turnover rates for all enzymes. One molecule of the enzyme can convert approximately 6 million molecules of hydrogen peroxide to water and oxygen each minute (Aoyama and Nakaki, 2015). Highest enzyme activity of catalase occurs in the liver and erythrocytes where it continuously scouts for hydrogen peroxide molecules within cells, catalase is located in the peroxisomes (a strategic location because of the presence of many H_2O_2-producing enzymes) and mitochondria as both soluble and membrane-bound forms (Cobanoglu *et al.*, 2010). It is highly efficient and can break down millions of hydrogen peroxide molecules in one second. A deficiency or mutation of the enzyme has been linked with various disease conditions and abnormalities.

1.7.1.3 Glutathione peroxidase (EC 1.11.1.9)

It is an important intracellular enzyme that breakdown hydrogen peroxides (H_2O_2) to water and lipid peroxides to their corresponding alcohols mainly in the mitochondria and sometimes in the cytosol (Noblanc *et al.*, 2011). The reaction is very important in removing low levels of hydrogen peroxide that might damage the cell. Such levels are not efficiently decomposed by catalase. Most times, its activity depends on a micronutrient cofactor known as selenium. There are two forms of glutathione peroxidase; selenium dependent and selenium-independent otherwise known as Glutathione-S-transferase (GST). The enzyme plays a crucial role in inhibiting lipid peroxidation process, thereby protecting cells from oxidative stress (Burk *et al.*, 2011).

1.7.1.4 Glutathione reductase (EC 1.6.4.2)

Glutathione Reductase is a flavo-protein oxidoreductase which catalyses the reduction of glutathione disulphide (GSSG) to the sulphydryl form glutathione (GSH), it employs NADPH as a reductant (Esma *et al.*, 2019). The enzyme is predominantly found in chloroplast. However, a small amount of the enzyme isoforms is also found in mitochondria, cytosol and peroxisomes.

The activity of glutathione reductase is used as an indicator for oxidative stress, a high GSH/GSSG ratio is essential for protection against oxidative stress.

1.8 Health Risk Assessment

The health impact of chemicals is determined by a process of assessment which aims to provide a consensus scientific description of the risks of chemical exposures (WHO, 2011). Health risk assessment study seeks to evaluate the consequences of human activities and weighs the adverse effects to public health against the contributions to economic development. The role of risk assessment is to undertake the analysis, estimate the risk and anticipate how it will change under various courses of action and provide guidance in the way of precedents, benchmarks, comparisons and lateral solutions. These can then be further developed and communicated with stakeholders and interested parties. It is one of the fastest methods currently used to evaluate the impact of the hazards on human Health (Yujun *et al*., 2011).

Estimated Daily Intake

The Estimated Daily Intake (EDI) for a chemical contaminant represents the total exposure from all known or suspected exposure pathways for an average person. It is the maximum amount of a chemical that can be ingested daily over a lifetime with no appreciable health risk, it is based on the highest intake that does not give rise to observable adverse effects (Bamuwamye *et al*., 2015). In calculating the estimated daily intake, the value obtained is compared with the tolerable daily intake (TDI) and inference can be drawn. It is important to note that estimated daily intake varies for both adults and children (Reham *et al*., 2013).

Table 1: Tolerable daily intake of selected heavy metals (USEPA, 2017)

Heavy metals	Tolerable daily intake (mg/kg)
Arsenic (As)	<0.00210
Chromium (Cr)	<0.15000
Lead (Pb)	<0.00357
Nickel (Ni)	<0.00400

Source: (Bamuwamye *et al*., 2015).

Toxic Hazard Quotient

Toxic hazard quotient (THQ) is the ratio between exposure to the toxic element and the reference oral dose (RfD) which is the highest level at which no adverse health effects are expected (Johann *et al.*, 2017). The reference oral dose is usually specific for a particular trace element being assessed. The THQ describes the non-carcinogenic health risk posed by exposure to the respective toxic element. If the THQ is less than one (<1), non-carcinogenic health effects are not expected (no adverse health effect is expected). If the THQ is greater than one (>1), there is a possibility that adverse health effects could be experienced (adverse health effect could be possible). Sometimes, a THQ exceeding 1 is not a statistical probability that adverse non-carcinogenic health effects will occur (Onuoha *et al.*, 2016).

Carcinogenic Risk/Target Cancer Risk

The target cancer risk (TCR) is used to determine the potential risk associated with the exposure of an individual to carcinogenic agents throughout the lifetime exposure period (Liang *et al.*, 2017). Instead of oral reference dose which is used in the determination of THQ, carcinogenic slope factor (CSF) is used when assessing cancer risk. The carcinogenic slope factor is used to estimate the probability of cancer risk over the lifetime of the exposed individual. It is a toxicity value that quantitatively defines the relationship between dose and response. The cancer slope factor is a plausible upper-bound estimate of the probability that an individual will develop cancer if exposed to a chemical for a lifetime of 70 years. Ingestion cancer slope factor are usually expressed in mg/kg/day.

Table 2: Ingestion Reference Dose (IRD)

Heavy metals	Ingestion Reference Dose (mg/kg/day)	Carcinogenic slope factor (mg/kg/day)
Arsenic (As)	0.0050	1.5000
Chromium (Cr)	1.5000	0.5000
Lead (Pb)	0.0035	0.0085
Nickel (Ni)	0.0200	1.7000

Source: (Bamuwamye *et al.*, 2015; Onuoha *et al.*, 2016)

1.8.1 Steps to human health risk assessment process

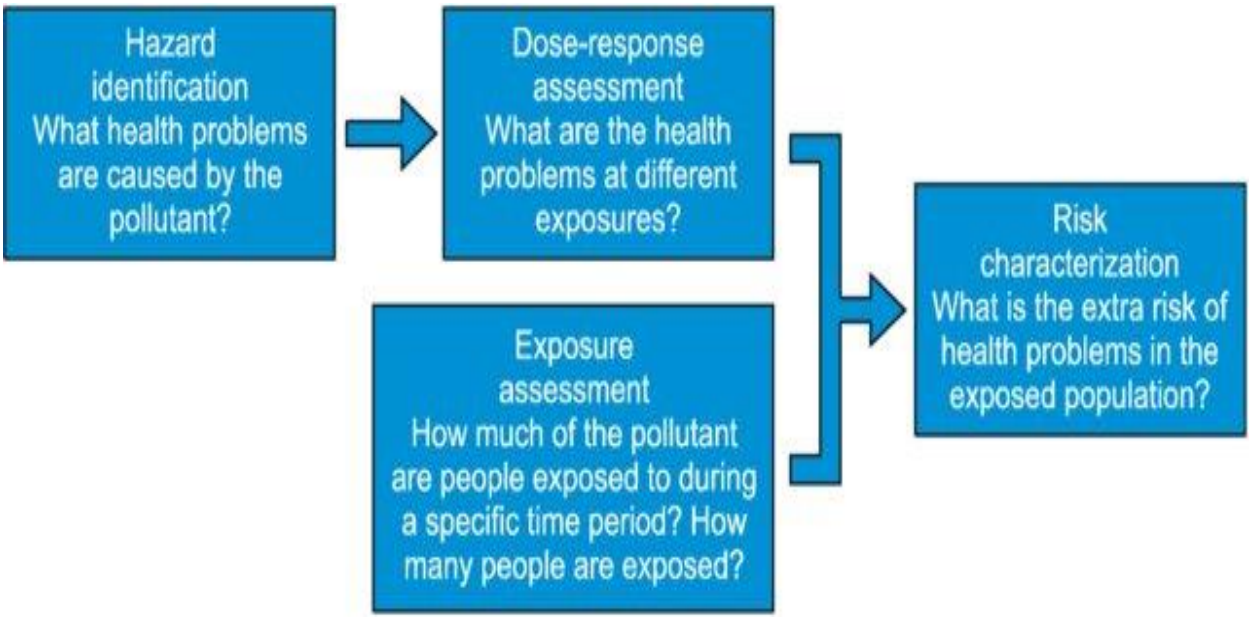

Figure 3: Steps to human health risk assessment
Source: Sylvia, 2018.

1.8.1.1 Hazard identification

Hazard means a source or a situation with a potential for harm in terms of human injury or ill health, damage to property, damage to the environment or a combination of these (Yaneira *et al.*, 2014). The identification of biological, chemical and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods is referred to as hazard identification. The main purpose of hazard identification applied to metals is to evaluate the weight of evidence for adverse health effects, based on an assessment of all the available data regarding toxicity and mode of action of that particular metal. In practice, a review of studies regarding the mode of action, the toxico-kinetics of the metal (absorption, distribution, metabolism and excretion), the nature of any toxicity or adverse health effect occurring and the affected target cells/organs/tissues site is performed (Jean-Lou *et al.*, 2011). Toxicological studies in animals (mainly mouse, rat, rabbit and dog) play a critical role in hazard identification and ideally use international guidelines and good laboratory practices.

1.8.1.2 Dose response assessment

A dose-response assessment describes how the likelihood and severity of adverse health effects (the responses) are related to the amount and condition of exposure to an agent (Rout and Sikdar, 2017). As the dose increases, the measured response also increases. At low doses there may be no response. At some level of dose, the responses begin to occur in a small fraction of the study population or at a low probability rate.

1.8.1.3 Exposure assessment

Exposure assessment is the qualitative and/or quantitative evaluation of the likely intake of biological, chemical and physical agents via food as well as exposure from other sources if relevant (Yaneira *et al.*, 2014). For chemical contaminants in the food and the feed chain, exposure assessment integrates the occurrence and the concentrations of the compound in the human diet measured using validated analytical techniques and the human consumption patterns for the different food categories available (Rout and Sikdar, 2017). Additionally, a range of intake/exposure scenarios are taken into account so that special subgroups of the population that may be at either high dietary exposure or high consumers are taken into account.

1.8.1.4 Risk characterization

Risk characterization is the final step and represents the qualitative and quantitative estimation including attendant uncertainties of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment (Jean-Lou *et al.*, 2011). In practice, risk characterization integrates hazard identification and characterization, leading to a health-based guidance value and the human exposure estimated from either a deterministic or probabilistic method to conclude on the likelihood of adverse effects for public health.

1.9 Justification of the Study

The use of different ripening agents to enhance banana ripening helps fruit sellers meet up with the market demands. However, some of these ripening agents are toxic and contain some potential toxic elements (PTEs). Bioaccumulation of PTEs in the body affects different biological processes giving rise to several health problems. There is a need to assay for the

quantity, effect of these trace metals and also estimate the risk involved in the consumption of artificially ripened banana.

1.10 Aim and Objectives of the Study

1.10.1 Aim of the study

The aim of this study is to determine the effects of different ripening regimes of *Musa acuminata* on albino rats used as risk assessment models.

1.10.2 Specific Objectives of the Study

The purpose of the study was to:

1. To determine the proximate composition of *Musa acuminata* affected by different ripening regimes.
2. To determine the heavy metal content of albino rats fed *Musa acuminata* treated with different ripening regimes.
3. To determine the estimated daily intake (EDI), toxic hazard quotient (THQ) and carcinogenic risk (CR) using albino rats as tool for human health risk assessment.
4. To determine the effect of different ripening regimes of *Musa acuminata* on antioxidant enzymes of albino rats.
5. To determine the effect of different ripening regimes of *Musa acuminata* on liver markers of albino rats.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant material

Freshly harvested Unripe but matured banana fruits were purchased from Obollo Afor market in Nsukka, Enugu state, Southeastern Nigeria. The ripening agent (*Irvingia gabonesis*) used for the study was also bought from the same market.

2.1.2 Animals

Twenty-five (25) albino rats (female) with weight ranging from 120-150 grams were used for the study. The animals used were obtained from the animal house of the Department of Veterinary Medicine, University of Nigeria Nsukka. They were separated in groups of 5 using an aluminum cage. The experimental animals were left to acclimatize for two (2) weeks in a conducive environment, they were fed standard growers mash with clean water before administration of the banana flour.

2.1.3 Reagents and Chemicals

All chemicals used in this study were of analytical grade. The reagents used for the biochemical assays were commercial kits and products of Randox (USA). The industrial grade calcium carbide used was bought from Obollo Afor market in Nsukka, Enugu state, Southeastern Nigeria,

2.1.4 Instruments and Equipment

The instrument used includes UV Spectrophotometry, Atomic Absorption Spectrophotometer (model AA-7000 Shimadzu, Japan ROM version 1.01, S/N A30664700709), weighing balance and animal cage.

2.2 METHODS

2.2.1 Sample Preparation

The banana fruits were carefully separated from the bunch, washed with clean water to remove dirts and subjected to different ripening methods. They were cut and separated into 4 groups made up of fourteen (14) banana fingers of approximately the same size each. Each of the four

methods of ripening was carried out in five replicate. The banana samples were kept in clean polyethylene bags and treated with different ripening agent to induce the ripening process. Batch A was allowed to ripen naturally, batch B was ripened using African bush mango and batch C & D were ripened using 7 g and 30 g of calcium carbide respectively. Change in skin color and texture of the fruit was considered as the stage for ripening of the fruit. After ripening was achieved, the fruits were peeled, sliced and dried in a hot-air oven. The dried fruits were pulverized using a mechanical grinder and stored in plastic rubbers. The dried banana samples was mixed with the animal feed and administered to the animals.

2.2.2 Proximate Composition of *Musa acuminata*

2.2.2.1 Determination of Moisture Content

This was determined by the method of Association of Official Analytical Chemists (AOAC, 1990). The method used involved measurement of weight loss due to evaporation of water. This method gives accurate result when considered on a comparative basis. Crucibles were washed and dried in an oven at 110 ^{0}C for 10 min and cooled in a desiccator. About 20 g of ripened banana sample for each of the groups were placed in the crucibles, kept in a hot oven and dried to a constant weight at 105 ^{0}C for 2 hr.

2.2.2.2 Determination of Ash Content

Ash content was determined by the method of Association of Official Analytical Chemists (AOAC, 1990). Exactly 2.0 g of the test sample was weighed into a previously dried and weighed porcelain crucible. The crucible with its content was placed in a furnace preheated to 600°C for 2 hr. The sample was allowed to cool in the furnace to 250 °C. The crucible and the ash were then transferred into an oven at 100 °C for 30 min cooling. After this period, the crucible with its content was cooled in a desiccator and weighed. The weight of the ash was expressed as a percentage of the initial weight of the sample.

2.2.2.3 Determination of Fat Content

Crude fat was determined based on Sohxlet extraction method described by AOAC (1990). A 250 ml round bottom flask was washed and dried in an oven at 103 °C for 25 min and allowed to cool to room temperature before it was weighed. About 2g of the test sample was weighed into an extraction thimble (double thickness 22 x 88 mm) and was inserted into the extraction column with

the condenser connected. Two hundred milliliters (200 ml) of the extracting solvent (petroleum ether, boiling point 40 to 60°C) was poured into the round bottom flask and fitted into the extraction unit. The flask was then heated with the aid of electro thermal heater at 60 ˚C for 2 hr. Losses of solvent due to heating were checked with the aid of the condenser so that it cooled and refluxed the evaporated solvent. After extraction, the thimble was removed and the solvent salvaged by distillation. The flask containing the fat and residual solvent was placed on a water bath to evaporate the solvent, followed by a further drying in an oven at 103 °C for 30 minutes to completely evaporate the solvent. It was then cooled in a desiccator and weighed. The fat obtained was expressed as a percentage of the initial weight of the sample.

2.2.2.4 Determination of Crude Fibre Content

This was determined by the method of Association of Official Analytical Chemists (AOAC, 1990). In determining the fibre content of the sample, the protein, starch, fat and other digestible carbohydrate was hydrolyzed and separated out of the sample. The residue was then weighed and ashed to determine the organic component. The removal of ash content from the original residue gives the fibre. About 2.0 g of the sample was weighed into a beaker and 180 ml preheated, 0.13 M H_2SO_4 was added and boiled for 30 min using a water pressure filter system. The mixture was filtered and residue washed 3 times with hot water. The residue was then collected and 150 ml preheated 0.22M KOH was added and boiled for another 30 minutes. Finally, the mixture was filtered and residue washed on the water pressure system 3 times with acetone. The residue was collected in a crucible, dried at 130 ^{0}C for 1hr and weighed.

2.2.2.5 Determination of Protein Content

Crude protein was determined by the method of the Association of Official Analytical Chemists (AOAC, 1990). Exactly 2.0 g of the sample was weighed into a digestion flask and 0.5 g of selenium catalyst was added followed by 25 ml of concentrated H_2SO4, the flask was agitated to mix the contents. The flask was then placed on a digestion burner for 8 hr and heated until the solution turned green and clear. The sample solution was then transferred into a 100 ml volumetric flask and made up to the mark with distilled water. About 25 ml of 2 % boric acid was pipetted into a 250 ml conical flask and two drops of mixed indicator (20 ml of bromocresol green and 4 ml of methyl red) solution was added; and into the decomposition chamber of the distillation apparatus was added 15 ml of 40 % NaOH solution. From the digested sample, 10 ml was

introduced into the Kjedahl flask. The condenser tip of the distillation apparatus was then dipped into the boric acid contained in the conical flask. The ammonia in the sample solution was then distilled into the boric acid until it changed completely into bluish-green. The distillate was then titrated with 0.1 N HCl solutions until it became colorless. The percent total nitrogen and crude protein were calculated using a conversion factor of 6.25.

2.2.2.6 Determination of Carbohydrate Content

This was determined by the method described by Onyeike *et al*. (1995). This involves finding the difference after adding the % crude protein, moisture, ash, crude fibre and fat content from 100 %. Percentage carbohydrate = 100 - (% moisture + % ash + % crude protein + % crude fat + % crude fiber)

2.2.3 Treatment

Twenty-five (25) albino rats were used. The animals were randomly divided into five (5) main experimental groups, each containing five (5) animals. After two weeks of acclimatization, 20g of the pulverized banana was mixed with the rats feed and served to them for thirty days.

Groups	**Treatments**
Group 1	Received normal feed and water
Group 2	Received 20g of naturally ripened banana
Group 3	Received 20g of banana ripened with African bush mango
Group 4	Received 20g of banana ripened with 7g calcium carbide
Group 5	Received 20g of banana ripened with 30g calcium carbide

2.2.3.1 Collection of Blood Sample from Experimental Animals

After 30 days of administration, the animals were sacrificed after being anaesthetized using chloroform. Blood samples were collected through occular puncture using anticoagulant bottles for heavy metal analysis. Liver organ was also harvested and used for antioxidant and liver function test.

2.2.4 Heavy metal studies

Each of the banana samples (1 g) were weighed into the digestion flask and 30 cm^3 of aqua regia (a mixture of HNO_3 and HCl in the ratio of 1:3) was added and digested in the fume cupboard, for the evaporation of HCl until a clear solution was obtained, it was cooled, filtered and then made up to 50ml mark in a standard volumetric flask with de-ionized water. The digested samples were analyzed for Arsenic (As), chromium (Cr), Lead (Pb), using atomic absorption spectrophotometer (AAS) at respective wavelengths. A blank sample was prepared to zero the instrument before running other series of samples. Standards (2 ppm, 4 ppm and 6 ppm) were prepared from 1000 ppm stock solution of the metals and used to plot the calibration curve. The curve was plotted automatically by the instrument. Atomic Absorption Spectrometer (model AA-7000 Shimadzu, Japan ROM version 1.01, S/N A30664700709) was used for the analysis (Gray, 1980).

2.2.5 Antioxidant Studies

2.2.5.1 Assay for Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activity was determined by the method of Xin *et al*, 1991.

Principle: Superoxide dismutase (SOD) reduces superoxide to hydrogen peroxide. The theory of this method is based on the competition between SOD activity and iodonitrazolium violet in reacting with superoxide, which is generated by xanthine oxidase (XOD) reaction.

Procedure: About 0.1 ml of the sample was mixed with 0.9ml of distilled water. From the mixture, 0.1 ml was mixed with 0.9 ml of carbonate buffer and 75 ul of xanthine oxidase was added. The absorbance was read at 500 nm for 3 minutes at 20 seconds interval. Change in the rate of absorbance was used to determine the superoxide activity.

2.2.5.2 Assay for Catalase Activity

The activity was determined using the method of Aebi, 1983.

Principle: The ultra violet absorption of hydrogen peroxide can be easily measured at 240 nm. On the decomposition of hydrogen peroxide (H_2O_2) by catalase the absorption decreases with time and this decrease is a measure of catalase activity.

Procedure: Hydrogen peroxide buffer (2 ml) and 2.5 ml of phosphate were added to a beaker. The mixture was mixed with 0.5 ml of the sample. About 1 ml portion of the reaction mixture was

added to 2 ml of dichromate acetic acid reagent. The absorbance was read at 240 nm (at a minute interval in triplicates). Catalase activity was calculated using the following equation:

$$\text{Catalytic concentration}\left(\frac{\text{unit}}{\text{L}}\right) = \frac{\log(\text{Abs 1}/\text{Abs 2}) \times 0.23}{0.00693}$$

Where Abs 1= Absorbance at t=0
Abs 2= Absorbance at t=15 seconds

2.2.5.3 Assay for Glutathione Reductase Activity

Glutathione reductase was estimated by the method described by Goldberg (1983). This method involves the catalytic reduction of glutathione (GSSG) by the glutathione reductase in the presence of NADPH, which is oxidized to $NADP^+$. The decrease in absorbance at 340 nm was measured.

Principle: Glutathione reductase catalyses the NADPH – dependent reduction of glutathione disulphide (GSSG) to glutathione (GSH). The oxidation of $NADP^+$ is accompanied by a decrease in absorbance at 340 nm. The reaction is thus measured by the decrease in absorbance at 340 nm.

Procedure: To 40 ml of serum sample, 1 ml of working solution (dilute potassium phosphate) was added and mixed. Also 200 ml of NADPH was added and mixed. The change in absorbance was recorded at 340 nm for 5 minutes at a minute interval.

2.2.5.4 Assay for Glutathione Peroxidase Activity

Glutathione Peroxidase (GPx) enzymes belong to a family of seleno proteins whose function is to catalyze the reduction of various peroxides. Four types of GPx (GPx-1 to 4) have been identified in mammalian species. GPx-1 is the classic intracellular form while GPx-2 to 4 is gastrointestinal, plasma and phospholipid hydro peroxide metabolizing forms respectively.

Principle

This assay was done using the method of Paglia and Valentine (1967). Glutathione peroxidase (GPX) catalyzes the reduction of an organic peroxide (ROOH), oxidizing reduced glutathione (GSH) to form disulfide glutathione (GSSG). The oxidized glutathione is then reduced by glutathione reductase (GR) with b-nicotinamide adenine dinucleotide phosphate (NADPH) forming $NADP^+$ (resulting in decreased absorbance at 340 nm) and recycling the GSH. Because glutathione peroxidase (GPx) is limiting, the decrease in absorbance at 340 nm is directly proportional to the glutathione (GPx) concentration.

$$R\text{-}O\text{-}O\text{-}H + 2\,GSH \xrightarrow{GPx} R\text{-}O\text{-}H + GSSG + H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2GSH + NADP^+$$

Procedure

The sample tissue homogenate was added to a solution containing glutathione, glutathione reductase, and NADPH. The enzyme reaction was initiated by adding the substrate, hydrogen peroxide and the absorbance at 340nm was recorded. The rate of decrease in the absorbance at 340nm is directly proportional to the GPx activity in the sample.

2.2.6 Liver function biomarkers

2.2.6.1 Assay for Aspartate Aminotransferase

Aspartate aminotransferase (AST) catalyses the transfer of amino group from aspartate to a keto-acid to form a new amino acid and oxaloacetate.

Principle: AST activity is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Procedure: The standard method described by Reitman and Frankel (1957) was used. 0.1 ml of the sample was mixed in a test tube with 0.5 ml Sodium azide buffer, 0.5ml distilled water and incubated for 30 minutes at 37 ^{0}C. Subsequently, 0.5 ml of 2, 4-dinitrophenylhydrazine was added, mixed and allowed to stand for 20 minutes at 20-25 ^{0}C. About 5 ml of Sodium hydroxide was added and the absorbance of the sample was read against the blank at 546 nm.

2.2.6.2 Assay for Alanine Aminotransferase (ALT)

Principle: ALT activity is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Procedure: The standard method described by Reitman and Frankel (1957) was used. From the sample 0.1 was mixed in a test tube with 0.5 ml Sodium azide buffer and 0.1ml distilled water, the mixture was incubated for 30 minutes at 37 ^{0}C. Subsequently, 0.5 ml of 2, 4-dinitrophenylhydrazine was added, mixed and allowed to stand for 20 minutes at 20-25 ^{0}C.

Sodium hydroxide (5 ml) was added and the absorbance of the sample was read against the blank at 546 nm.

2.2.6.3 Assay for Alkaline Phosphatase

Principle: Alkaline Phosphatase in serum catalyse the hydrolysis of p. nitrophenyl phosphate to p-nitrophenol and phosphate. The rate of formation of p-Nitrophenol is measured as an increase in the absorbance which is proportional to the ALP activity in the sample.

Procedure: Alkaline phosphatase (ALP) was estimated by the method of Rec (1972). From the sample 0.01 ml was mixed with 0.5ml buffer solution (Diethanolamine buffer and $MgCl_2$) and 0.5ml P-nitrophenylphosphate at 37^0C. Absorbance was read at 405nm at time interval of 1, 2 and 3minutes.

2.2.7 Human Health Risk Assessment

2.2.7.1 Estimated Daily Intake (EDI)

Estimated Daily Intake of metals for adults and children was determined by the equation

$$EDI = \frac{\text{Concentration of Metals X Daily Banana Intake}}{\text{Average Body Weight}}$$

2.2.7.2 Toxic Hazard Quotient (THQ)

Toxic Hazard Quotient was calculated using the equation below

$$THQ = \frac{\text{Concentration of Metals X Daily Banana Intake}}{\text{RfD x Average Body Weight}}$$

Where RfD is the Oral Reference Dose (Onuoha, 2016).

2.2.7.3 Carcinogenic Risk (CR)

The lifetime probability of cancer or carcinogenic risk was estimated according to (USEPA, 2017) by,

CR = Estimated Daily Intake (mg/kg/day) x Ingestion Carcinogenic Slope Factor (mg/kg/day)-1.

2.2.8 Statistical Analysis

All experiments were carried out in replicate. The data obtained were analysed using IBM Statistical Product and Service Solution (SPSS) version 20.0 and microsoft excel 2010. The results

were expressed as mean ± standard deviation (SD). One way analysis of variance (ANOVA) was carried out as $p < 0.05$ was considered statistically significant. Duncan's Multiple Range Test (DMRT) was used to compare mean values.

CHAPTER THREE

RESULTS

3.1 Duration of Ripening

The duration of ripening was seen to increase from 3days in RB4 to 10days in RB1.

3.2 Proximate Composition of *Musa acuminata.*

The result of the proximate compositions of *Musa acuminata* revealed an increase in moisture content and a decrease in carbohydrate, fat, ash, crude fiber and crude protein contents in the order of RB1, RB2, RB3, RB4 and RB5. Also, the values were statistically significant at $p < 0.05$ except for crude fiber which showed no significant difference.

3.3 Heavy Metal Levels of albino Rats

Results from Table 2 for albino rats fed banana treated with different ripening agents showed a significant increase ($p < 0.05$) in the level of Lead (Pb) and Arsenic (As) for groups 2, 3, 4 and 5 when compared with group 1. However, groups 4 and 5 for Chromium were significantly higher when compared with group 1 while groups 2 and 3 showed no significant difference ($p < 0.05$).

Table 3: Duration of ripening

Group	Duration of ripening
RB1	10days
RB2	5days
RB3	5days
RB4	3days

RB1 = Naturally ripened banana, RB2 = banana ripened with African bush mango, RB3 = banana ripened with 7g calcium carbide, RB4 = banana ripened with 30g calcium carbide.

Table 4: Proximate composition of *Musa acuminata* treated with different ripening agents.

	PROPERTY (%)					
TREATMENT	Moisture (%)	Ash content (%)	Crude fiber (%)	Fat content (%)	Crude protein (%)	Carbohydrate (%)
RB1	77.77±0.32^{a}	1.05±0.04^{d}	1.10 ± 0.00	0.73±0.04^{d}	1.24±0.01^{d}	18.16±0.29^{d}
RB2	79.07±0.05^{b}	0.90±0.01^{c}	1.00 ± 0.00	0.60±0.00^{c}	0.99 ±0.00^{c}	17.45±0.06^{c}
RB3	80.67±0.11^{c}	0.84±0.00^{b}	0.80 ± 0.00	0.45±0.00^{b}	0.75± 0.00^{b}	16.50±0.08^{b}
RB4	85.15±0.16^{d}	0.77±0.01^{a}	0.50 ± 0.00	0.30±0.00^{a}	0.35 ±0.00^{a}	12.94±0.15^{a}

Values are expressed as Mean ± SD, n=2. Values in the same column having different superscripts differ significantly ($P < 0.05$). RB1 = banana ripened without any ripening agent, RB2 = banana ripened African bush mango fruit, RB3 = banana ripened with 7g calcium carbide, RB4 = banana ripened with 30g calcium carbide.

Table 5: Heavy metal content of albino rats fed *Musa acuminata* treated with different ripening agents.

Treatments	Pb (mg/kg)	Cr (mg/kg)	As (mg/kg)
Group1 = Feed and water	0.25 ± 0.00^{a}	0.05 ± 0.01^{a}	0.06 ± 0.01^{a}
Group 2 = Naturally ripened	0.50 ± 0.00^{b}	0.06 ± 0.00^{a}	0.09 ± 0.00^{bc}
Group 3 = African Bush mango	0.50 ± 0.00^{b}	0.06 ± 0.00^{a}	0.08 ± 0.01^{ab}
Group 4 = 7g calcium carbide	0.75 ± 0.00^{c}	0.12 ± 0.00^{b}	0.11 ± 0.01^{c}
Group 5 = 30g calcium carbide	1.08 ± 0.08^{d}	0.16 ± 0.01^{c}	1.23 ± 0.14^{d}

Results expressed in means ± SD, n = 3. Values in the same column having different superscripts differ significantly ($P < 0.05$). Pb = Lead, Cr = Chromium, As = Arsenic.

Group 1 = Received normal feed and water

Group 2 = Received 20g of naturally ripened banana

Group 3 = Received 20g of banana ripened with African bush mango

Group 4 = Received 20g of banana ripened with 7g calcium carbide

Group 5 = Received 20g of banana ripened with 30g calcium carbide

3.4 Human Health Risk Assessment

3.4.1 Estimated Daily Intake (EDI)

Results from human health risk assessment model for Estimated Daily Intake showed an increase above the tolerable daily intake for heavy metals such as Pb (for groups 2, 3, 4 and 5) and As (for group 5). There was also a fall in the Tolerable daily intake for Cr (all groups were below the TDI) and As (for groups 1, 2, 3 and 4).

3.4.2 Toxic Hazard Quotient

Results from human health risk assessment model for Toxic Hazard Quotient showed increased values above the standard THQ ($0<x<1$) for an acceptable human such as Pb (for groups 2, 3, 4 and 5) and As (for group 5), whereas values for Cr and As (for groups 1, 2, 3 and 4) were below the standard THQ, thus being within the acceptable range. The results also showed that children were more at risk than adults

3.4.3 Incremental Lifetime Carcinogenic Risk (ILCR)

Carcinogenic Risk (CR) values from the human health risk assessment model showed that all values estimated were within the value of USEPA incremental lifetime carcinogenic risk (ILCR $> 10^{-3}$) having more than 1 cancer case per 10,000 persons. However, CR values fell within an acceptable range ($10^{-3} < \text{ILCR} < 10^{-6}$) while Arsenic for group 5 was otherwise.

Table 6a: Estimated daily intake (EDI) of heavy metal through the consumption of banana treated with different ripening agents as compared with the tolerable daily intake (TDI) (Children)

Treatments	Pb (mg/kg/day)	Cr (mg/kg/day)	As (mg/kg/day)
Group1 = Feed and water	0.0025	0.0005	0.0006
Group 2 = Naturally ripened	0.0050	0.0006	0.0009
Group 3 = African Bush mango	0.0050	0.0006	0.0008
Group 4 = 7g calcium carbide	0.0070	0.0012	0.0011
Group 5 = 30g calcium carbide	0.0108	0.0016	0.0123
TDI	0.0036	0.1500	0.0021

TDI = Tolerable daily intake

Pb = Lead, Cr = Chromium, As = Arsenic.

Group 1 = Received normal feed and water

Group 2 = Received 20g of naturally ripened banana

Group 3 = Received 20g of banana ripened with African bush mango

Group 4 = Received 20g of banana ripened with 7g calcium carbide

Group 5 = Received 20g of banana ripened with 30g calcium carbide

Table 6b: Estimated daily intake (EDI) of heavy metal through the consumption of banana treated with different ripening agents as compared to the tolerable daily intake (TDI) (Adult).

Treatments	Lead (mg/kg/day)	Chromium (mg/kg/day)	Arsenic (mg/kg/day)
Group1 = Feed and water	0.0017	0.0003	0.0004
Group 2 = Naturally ripened	0.0033	0.0004	0.0006
Group 3 = African Bush mango	0.0033	0.0004	0.0005
Group 4 = 7g calcium carbide	0.0050	0.0008	0.0007
Group 5 = 30g calcium carbide	0.0072	0.0011	0.0082
TDI	0.0036	0.1500	0.0021

TDI = Tolerable daily intake

Group 1 = Received normal feed and water

Group 2 = Received 20g of naturally ripened banana

Group 3 = Received 20g of banana ripened with African bush mango

Group 4 = Received 20g of banana ripened with 7g calcium carbide

Group 5 = Received 20g of banana ripened with 30g calcium carbide

Table 7a: Toxic hazard quotient (THQ) of heavy metals through the consumption of bananas treated with different ripening agents (Children).

Treatments	Lead	Chromium	Arsenic
Group1 = Feed and water	0.7143	0.0003	0.1200
Group 2 = Naturally ripened	1.4286	0.0004	0.1800
Group 3 = African Bush mango	1.4286	0.0004	0.1600
Group 4 = 7g calcium carbide	2.1429	0.0008	0.2200
Group 5 = 30g calcium carbide	3.0857	0.0012	2.4600

Group 1 = Received normal feed and water

Group 2 = Received 20g of naturally ripened banana

Group 3 = Received 20g of banana ripened with African bush mango

Group 4 = Received 20g of banana ripened with 7g calcium carbide

Group 5 = Received 20g of banana ripened with 30g calcium carbide

Table 7b: Toxic hazard quotient (THQ) of heavy metals through the consumption of banana treated with different ripening agents (Adults).

Treatments	Lead	Chromium	Arsenic
Group1 = Feed and water	0.4857	0.0002	0.0800
Group 2 = Naturally ripened	0.9429	0.0003	0.1200
Group 3 = African Bush mango	0.9429	0.0003	0.1100
Group 4 = 7g calcium carbide	1.4286	0.0005	0.1500
Group 5 = 30g calcium carbide	2.0571	0.0007	1.6400

Group 1 = Received normal feed and water

Group 2 = Received 20g of naturally ripened banana

Group 3 = Received 20g of banana ripened with African bush mango

Group 4 = Received 20g of banana ripened with 7g calcium carbide

Group 5 = Received 20g of banana ripened with 30g calcium carbide

Table 8a: Carcinogenic risk of heavy metal through the consumption of bananas treated with different ripening agents (Children).

Treatments	Lead	Chromium	Arsenic
Group1 = Feed and water	2.1E-5	2.5E-4	9E-4
Group 2 = Naturally ripened	4.3E-5	3E-4	1.4E-3
Group 3 = African Bush mango	4.3E-5	3E-4	1.2E-3
Group 4 = 7g calcium carbide	6.4E-5	6E-4	1.7E-3
Group 5 = 30g calcium carbide	9.2E-5	8E-4	1.8E-2

Group 1 = Received normal feed and water

Group 2 = Received 20g of naturally ripened banana

Group 3 = Received 20g of banana ripened with African bush mango

Group 4 = Received 20g of banana ripened with 7g calcium carbide

Group 5 = Received 20g of banana ripened with 30g calcium carbide

Table 8b: Carcinogenic risk of heavy metal through the consumption of bananas treated with different ripening agents (Adults).

Treatments	Lead	Chromium	Arsenic
Group1 = Feed and water	1.4E-5	1.5E-4	6E-4
Group 2 = Naturally ripened	2.8E-5	2E-4	9E-4
Group 3 = African Bush mango	2.8E-5	2E-4	8E-4
Group 4 = 7g calcium carbide	4.3E-5	4E-4	1.1E-3
Group 5 = 30g calcium carbide	6.1E-5	5.4E-4	1.2E-2

Group 1 = Received normal feed and water

Group 2 = Received 20g of naturally ripened banana

Group 3 = Received 20g of banana ripened with African bush mango

Group 4 = Received 20g of banana ripened with 7g calcium carbide

Group 5 = Received 20g of banana ripened with 30g calcium carbide

3.5 Antioxidant Enzyme Activities

Results from the antioxidant enzyme activities through the consumption of banana treated with different ripening agents indicates a significant increase ($p < 0.05$) in catalase activity for group 2 when compared with group 1, while groups 3, 4 and 5 recorded no significant change. Glutathione Peroxidase and Glutathione Reductase activities for all the groups were not significant ($p < 0.05$). However, Superoxide Dismutase activities for groups 4 and 5 were significant ($p < 0.05$) when compared with group 1 but recorded no significance for groups 2 and 3.

3.6 Liver Enzyme Activities

Liver Function Test (LFT) result through the consumption of banana treated with different ripening agents for Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) activities showed no significant **($p < 0.05$)** difference. Alanine amino transferase activity was significantly increased across the groups when compared with the control group (Group 1).

Table 9: Antioxidant enzyme activities of albino rats fed *Musa acuminata* treated with different ripening agents.

Treatments	SOD (mg/ml)	CAT (U/L)	Gpx (mg/ml)	Gpr (mg/ml)
Group1 = Feed and water	10.23 ± 0.76^{a}	6.68 ± 0.27^{a}	30.60 ± 3.27^{a}	22.79 ± 2.67^{a}
Group 2 = Naturally ripened	10.32 ± 0.57^{a}	10.79 ± 0.32^{b}	29.65 ± 2.29^{a}	20.30 ± 1.15^{a}
Group 3 = African Bush mango	10.80 ± 0.59^{ab}	6.94 ± 0.38^{a}	31.67 ± 6.06^{a}	20.94 ± 1.61^{a}
Group 4 = 7g calcium carbide	11.52 ± 0.40^{b}	6.73 ± 0.38^{a}	30.39 ± 2.06^{a}	20.34 ± 1.33^{a}
Group 5 = 30g calcium carbide	11.32 ± 0.15^{b}	7.17 ± 1.01^{a}	25.92 ± 0.71^{a}	20.68 ± 1.25^{a}

Results expressed in means ± SD, n = 3. SOD = Superoxide Dismutase, CAT = Catalase, Gpx = Glutathione Peroxidase, Gpr = Glutathione Reductase. Values in the same column having different superscripts differ significantly at $p<0.05$ while values in the same column having the same superscript does not have any significant difference.

Group 1 = Received normal feed and water

Group 2 = Received 20g of naturally ripened banana

Group 3 = Received 20g of banana ripened with African bush mango

Group 4 = Received 20g of banana ripened with 7g calcium carbide

Group 5 = Received 20g of banana ripened with 30g calcium carbide

Table 10: Liver enzyme activities of albino rats fed *Musa acuminata* treated with different ripening agents.

Treatments	AST (U/L)	ALT (U/L)	ALP (U/L)
Group1 = Feed and water	38.67 ± 1.53^{ab}	85.00 ± 5.20^{a}	56.00 ±3.61^{a}
Group 2 = Naturally ripened	33.33 ± 5.77^{a}	97.00 ± 9.54^{ab}	59.33 ± 4.73^{a}
Group 3 = African Bush mango	30.67 ± 2.52^{a}	90.67 ± 4.93^{ab}	51.67 ± 5.51^{a}
Group 4 = 7g calcium carbide	37.67 ± 4.51^{ab}	98.33 ± 5.51^{b}	57.00 ± 3.61^{a}
Group 5 = 30g calcium carbide	44.00 ± 8.72^{b}	95. 67 ± 4.93^{ab}	59. 67 ± 2.08^{a}

Results expressed in means ± SD, n = 3. AST = Aspartate aminotransferase ALT = Alanine aminotransferase, ALP = Alkaline phosphatase. Values in the same column having different superscripts differ significantly at $p<0.05$ while values in the same column having the same superscript does not have any significant difference.

Group 1 = Received normal feed and water

Group 2 = Received 20g of naturally ripened banana

Group 3 = Received 20g of banana ripened with African bush mango

Group 4 = Received 20g of banana ripened with 7g calcium carbide

Group 5 = Received 20g of banana ripened with 30g calcium carbide

CHAPTER FOUR

DISCUSSION

The duration of ripening as shown in table 3:1 was seen to be 3days for RB4, RB2 and RB3 ripened on the 5th day while RB1 took 10days to ripen. This result also agreed with previous research works that ripening agents accelerates ripening faster than when done naturally (Hakim *et al*., 2012). Also, Calcium carbide was able to induce ripening faster and the fact that it is cheaper makes it a popular ripening agent among banana sellers especially in developing countries (Sogo-Temi *et al*., 2014).

Table 3:2 presents the proximate composition of *Musa acuminata* treated with different ripening agents. The moisture content of the ripe bananas ranged between 77.77% - 85.15%. It increased from 77.77% in RB1 to 85.15% in RB4. Moisture content values were found to increase with increase in calcium carbide concentrations. This agreed with the findings of Sogo-Ttemi *et al*. (2014) and Nura *et al*. (2018) who reported increase in moisture content in bananas ripened with calcium carbide. Also, during banana ripening, moisture content in banana pulp is observed to increase because of respiratory breakdown of starch to sugar, migration of water from peel to pulp and excess moisture formation (Sen *et al*, 2012; Ayo-Omogie, 2010; Hakim *et al*, 2012). Moisture rich bananas are easily susceptible to the microbial attack, short storage life and high postharvest loss. Thus the shelf life of the food material is determined by the moisture content in the food, low moisture foods usually slow down growth of microorganisms.

Fibre and Ash content were found to decrease with the use of ripening agents in the following order RB1> RB2 > RB3 > RB4. Highest fibre and ash content values were found in RB1 while RB4 has the least fibre and ash content. The result was in agreement with Adewole and Duruji, (2010) who observed a reduction in ash content on exposure to calcium carbide and African bush mango fruit. However, it does not agree with Sogo-Temi *et al*, (2014) and Nura *et al* (2018) who observed an increased ash content in calcium carbide ripened bananas.

The protein content of the bananas reduced during ripening in artificially ripened banana. RB4 reduced to about 50% of RB1, this is in agreement with Adewole and Duruji (2010), Nura *et al* (2018) and Sogo-Temi *et al* (2014) who observed a reduction in the protein content during

ripening, which may be due to a reduction in nitrogen content during ripening. However, it was not in agreement with Sen *et al* (2012) who stated that protein contents increased during ripening. Therefore, the use of ripening agents might have contributed to the loss of Nitrogen.
There was a reduction in carbohydrate content of the bananas during ripening from 18.16% in RB1 to 12.94% in RB4, however there was no significant difference in the reduction except for RB4. According to Sen *et al* (2011) one of the biochemical changes that occur during ripening is a decrease in carbohydrate content. Starch is degraded by starch degrading enzymes α and β amylases which convert starch to simple sugars (Ayo-Omogie *et al*., 2010).

Table 3:3 presents the heavy metal results of rats fed with banana treated with different ripening agents. Heavy metals such as Lead, Arsenic and Chromium are considered as toxic contaminants making their importance in the body insignificant. Rats which served as control had the lowest concentration of Pb, while carbide ripened bananas had the highest values. The values for rats fed the naturally ripened bananas with no ripening agent and the biological agents(African bush mango) were almost the same but differed significantly when compared to the control. Though the Pb values increased when carbide ripened bananas were fed to the rats, Pb values for both the naturally and artificially ripened were higher than the permissible limits of 0.003ppm for fruits (Orisakwe, 2012). The Presence of Pb in the fruit may be from the soil as high levels of Pb have been reported in different farmlands in Eastern Nigeria (Orisakwe, 2012) and in fruits like banana, apple and pineapple (Sobukola *et al*., 2010). Also the presence of Pb detected in the ripened bananas could be due to the type of ripening agent, storage/packaging material used during ripening (Sajib *et al*., 2014). Banadda *et al* (2011) reported the migration of Pb from black polythene bag into banana. They reported that Pb which is a residue from the polymerization process of polythene can migrate at high temperature. Since chemical ripening agents like carbide are used to generate heat, the high temperature needed for the heavy metals to migrate is provided (Sogo-Temi *et al*., 2014).

Chromium was also detected in all the groups with highest amount occurring in the group fed carbide ripened banana. Chromium toxicity is related to Chromium (VI), due to its high absorption, easy penetration of the cell membranes and its genotoxicity and oxidizing properties.

Groups 1, 2, 3 and 4 fell within the recommended intake of chromium except for group 5 which had a higher Chromium value.

Arsenic values for groups 4 and 5 were increased when compared to the groups 1, 2 and 3. Highest level of As was seen in group 5, this may be as a result of high level of calcium carbide used in ripening the fruit in order to achieve quick ripening, this is in agreement with Igbinaduwa *et al*. (2018). Arsenic is one of the most toxic metals in the natural environment, chronic arsenic ingestion leads to accumulation of arsenic in the liver, kidneys, heart and lungs, smaller amounts in the muscles, nervous system, gastrointestinal tract and spleen (Hakim *et al.*, 2012).

Bio-accumulation of heavy metals in the body poses a serious health risk to the consumers of artificially ripened fruits especially those with high consumption rates. The use of Estimated Daily Intake (EDI) helps in the identification of the tolerable level of heavy metal ingestion. Therefore, estimated daily intake or 'tolerable intake' is widely used to describe 'safe' levels of intake of heavy metals. From the results shown in table 3:4, the EDI values of Lead (Pb) for both children and adults were above the tolerable daily intake except for the control group (Group 1) which fell within the acceptable limit. Chromium (Cr) EDI was found to be below the TDI of 0.15mg/kgbwt/day for both children and adult for all the groups. Also, Arsenic for all groups fell within the established limits except for group five which was above the TDI value. From the EDI values of all the heavy metals, it is evident that the EDI for both adult and children were within the same value, children who consume banana exposed calcium carbide stands a chance of having a higher quantities of heavy metals and given that their rate of metabolism is much less than adults, they stand a higher risk of being affected by the adverse effect being caused by heavy metals, as such children should be prevented from regular consumption of carbide ripened bananas.

Toxic Hazard Quotient (THQ) studies through the consumption of banana treated with different ripening agents is a measure of the chemical contaminants in the fruit, it is a dimensionless index of risk associated with long term exposure to chemicals. THQ is not a measure of risk but indicates a level of concern. THQ values can either be <1 or >1. When THQ >1 indicates reason for public Health concern. From the results established, the THQ of Pb for group 1 (children)

and groups 1, 2 and 3 (adults) were less than one (<1) indicating no reason for public health concern, while groups 1, 2, 3 and 4 (children) and groups 4 and 5 (adults) were significantly greater than one (>1), giving reasons for public health concern. The THQ of Cr (as shown in table 3:5) for both adult and children were significantly less than 1, this indicates that people who consume artificially ripened banana at that particular dosage are not exposed to the health risk of chromium. Also, THQ value for As for groups 1, 2, 3 and 4 (children and adults) were < 1 indicating that consumers are not exposed to the health risk of arsenic, while group 5 (children and adults) was > 1 indicating a reason for public health concern. From the THQ values obtained, it is evident that Pb especially for children have incredible high values, these makes it a reason for serious public health concern because the hazard potential that could be caused by this high toxicity may be deleterious to human health. Also, the difference in THQ values for adults and children can be attributed to difference in body weight (Samuel *et al*., 2018). According to Onuoha *et al.* (2016) and Patrick-Iwuanyanwu and Chioma. (2017), significantly higher THQ value has a relatively higher potential health risk to human beings who are the consumers.

Carcinogenic Risk (CR) is expressed as the probability of contracting cancer over a lifetime of 70 years as a result of continuous consumption of carbide ripened banana over one's entire lifetime (Onuoha *et al.* 2016). Pb, Cr, and As are classified by the IARC as being carcinogenic agents (Mahfuza *et al*., 2017). Chronic exposure to low doses of Pb, Cr and As could therefore result into many types of cancers. In general, USEPA considers excess cancer risks that are below about 1 chance in 1,000,000 (1×10^{-6} or 1E-06) to be so small as to be negligible, and risks above 1 in 10,000 (1×10^{-4} or 1E-04) to be sufficiently large that some sort of remediation is required. The benchmark for gathering additional information is an incremental lifetime carcinogenic risk (ILCR) greater than one in ten thousand ($> 10^{-4}$) whereas 1 in 1000 or greater (ILCR $> 10^{-3}$) is moderate increased risk and should be given high priority as a public health concern (Bamuwamye *et al*., 2015).

According to the results of table 3:6, carcinogenic risk values for Pb in groups 1, 2, 3, 4 and 5 are 2.1E-5, 4.3E-5, 4.3E-5, 6.4E-5 and 9.2E-5 respectively, showing probable cancer cases of 21 in 100,000 people, 43 in 100,000 people, 43 in 100,000, 64 in 100,000 and 92 in 100,000 people respectively. This indicates that the values obtained for Pb probable cases is below the USEPA standard (ILCR < 1E-6) and as such is as small as to somewhat negligible. Carcinogenic risk

values of Cr for groups 1, 2, 3, 4, and 5 were 2.5E-4, 3E-4, 3E-4, 6E-4, 8E-4., indicating cancer cases of 25 in 10,000 people, 3 in 10,000 people, 3 in 10,000 people, 6 in 10,000 people, and 8 in 10,000 people respectively. The values were also below the USEPA standard (ILCR < 1E-6) and as such is not indicative of high chances of cancer. The result for As for groups 1, 2, 3, 4, and 5 were 9E-4, 1.4E-3, 1.2E-3, 1.7E-3 and 1.8E-2. These values indicate that consumption of artificially ripened bananas will result in 14 cancer cases per 1000 people, 12 cancer cases per 1000 people, 17 cancer cases per 1000 people and 18 cancer cases per 100 people for groups 2, 3, 4 and 5 respectively as opposed to 9 cancer cases per 10,000 people for group 1, Arsenic values exceeds USEPA standards (ILCR>10-3), It is indicative of a highly increased chance of contracting cancer over one life's time and should be given high priority as a public health concern.

The inhibition of free radical generation is important in providing protection against myocardial infarction (Khan *et al.*, 2012). The activities of the antioxidant enzymes in the liver of the rats are shown in Table 3:7. The study revealed a varied response in SOD activities, groups 4 and 5 were slightly increased when compared with groups 1 and 2. Superoxide dismutase is an enzyme that catalyses the dismutation of the superoxide (O_2-) radical into molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Superoxide is generated as a by-product of oxygen metabolism, cells may be destroyed if not regulated (Hayyan *et al.*, 2016). Therefore, increase in the activity of SOD especially in the groups fed with artificially ripened fruits is an indication that there was no alteration in the activity of the enzyme at that level of dosage and exposure to the calcium carbide, this was not in agreement with Eka *et al.* (2018) who attributed an increase in SOD activity in artificially ripened fruits to increased superoxide radical generation by calcium carbide. CAT activity was increased in group 2 when compared with the control group and the artificially ripened groups. However, this is in disagreement with Eka *et al.* (2018) who didn't observe any significant difference. The presence of non-enzymatic antioxidant such as vitamin C and the presence of cofactors Fe, Mg, and Zn in banana which are needed for the synthesis and proper functioning of CAT might have played some roles in the enhanced activity as observed in group 2, while the use of artificial ripening agent could have had a negative impact on CAT activities of group 4 and 5. The results of glutathione peroxidase and glutathione reductase activities for groups 3, 4 and 5 as shown in table 9 varied and showed no significant difference when compared with the control and naturally ripened groups. The varied results indicate the ability of the antioxidant

enzymes to stabilize or deactivate free radicals, thereby preventing them from attacking cellular components. This result is also in disagreement with Eka *et al*. (2018) who observed an increase in the Groups given artificially ripened fruits, with a significant reduction in the groups given naturally ripened fruits. Oxidative stress can be measured by the ratio of reduced glutathione to oxidized glutathione in the cell. In normal cells and tissues, above 90% of the total glutathione is found in the reduced form (GSH) and lower than 10% are in the disulphide form (GSSG). An elevated level ratio of GSSG to GSH is an indication of oxidative stress.

AST, ALP and ALT results showed a varied (though not statistically significant) response of the experimental animals to the treatments when compared with the control, this is in agreement with Igbinaduwa and Aikpitanyi-Iduitua. (2016). A slight rise in ALP levels in groups 4 and 5 maybe an indication that there is a disturbance in the activity of the liver. AST, ALT and ALP are liver enzymes, they were analyzed to investigate liver disease and myocardial infarction. Increase in the levels of these enzymes serves as reliable indices of assessment of damage to parenchymatus cells of the heart, liver and kidney. Thus the observed increases in the activities of these enzymes are pointers to calcium carbide induced lesions in the heart, liver and kidney. Since the various liver enzymes are slightly but not significantly increased, it is possible that the use of a higher dosage of the carbide and prolonged consumption of carbide ripened banana may result to findings that are statistically significant.

4.2 CONCLUSION

This study has shown that the use of artificial ripening agents accelerates fruit ripening but affects the nutritional quality of the fruits. Calcium carbide used in this study was seen to contain traces of potential toxic elements. Consumption of carbide ripened bananas is of great public health concern because accumulation of potential toxic elements in the body affects different biological process. Therefore, the use of calcium carbide as a ripening agent should be discouraged.

4.3 SUGGESTION FOR FURTHER STUDIES

Since the quantity of carbide used determines the duration of ripening, therefore

1. Higher concentrations of the calcium carbide should be used for ripening as the market sellers indiscriminately induce ripening to achieve a faster ripening rate.
2. The animals should be exposed for a longer duration to obtain a more significant response to the toxicant.

REFERENCES

Adegbola, R. A., Adekanmbi, A. I., Abiona, D. L. and Atere, A. A. (2015). Evaluation of some heavy metal contaminants in biscuits, fruit drinks, concentrates, candy, milk products and carbonated drinks sold in Ibadan, Nigeria. *International Journal of Biological and Chemical Sciences*, **9**:1691-1696.

Adekalu, J., Ojuawo, R. and Adekalu, O. (2011). Proximate and elemental analyses of Banana during ripening process. *Nigerian Food Journal*, **29**(1):29-30.

Adewole, M. B. and Duruji, R. W. (2010). Quality assessment of plantain (Musa paradisiaca L.) as affected by different ripening methods. *African Journal of Biotechnology*, **9**(38):6290-6293.

Aebi, H. E., (1983). Catalase. In: Bergmeyer, H.U., Ed., *Methods of Enzymatic Analysis*, Verlag Chemie, Weinhem, 273- 286.

Ali, N., Hoque, M. A., Haque. A., Salam, K. A., Karim, M.R., Rahman, A., Islam, K., Saud, Z. A., Khalek, M. A., Akhand, A. A., Hossain, M., Mandal, A. and Karim, M. R. (2010). Association between arsenic exposure and plasma cholinesterase activity: A population based study in Bangladesh. *Environmental Health*, **9**:36-45.

Alkarkhi, A. F. M., Ramli, S., Yeoh, S.Y. and Easa, A. M. (2010). Physiochemical properties of banana peel flour as influenced by variety and stage of ripeness: Multivariate statistical analysis. *Asian Journal of Food Agro-Industry*. **3**:349-362.

AOAC (1990). Official Methods of Analysis. (14th edition). *Association of Official Analytical Chemists*. Virginia U.S.A. Pp. 503-515.

Aoyama, K. and Nakaki, T. (2015). Glutathione in cellular redox homeostasis: Association with the Excitatory Amino Acid Carrier 1 (EAAC1). *Molecules*, **20**:8742-8758.

Arc, E., Sechet, J., Corbineau, F., Rajjou, L., & Marion-Poll, A. (2013). ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination. *Frontiers in Plant Science*, **4**:63-85.

Argos, M., Kalra, T., Rathouz, P. J., Chen, Y., Pierce, B., Parvez, F., Islam, T., Ahmed, A., Rakibuz-Zaman, M., Hasan, R., Sarwar, G., Slavkovich, V. and vanGeen, A. (2010). Arsenic exposure from drinking water, and all-cause and chronic-disease mortalities in Bangladesh (HEALS): a prospective cohort study. *Lancet*, **376**:252-258.

Asaduzzaman, M., Khan, M., Tania, D. Z. and Zhang, H. C. (2010). Antioxidant enzymes and Cancer. *Chinese Journal of Cancer Research*, **22**(2):87-92.

Asif, M. (2012). Physico-chemical properties and toxic effect of fruit-ripening agent calcium carbide. *Annals of Tropical Medicine and Public Health*, **5**:150-160.

Ayo-omogie, H. N., Adeyemi, I. A. and Otunola, E. T. (2010). Effect of ripening on some physiochemical properties of cooking banana (Musa ABB Cardaba) pulp and flour. *International journal of food science and technology*, **45**:2605-2611.

Bamuwamye, M., Ogwok, P. and Tumuhairwe, V. (2015). Cancer and non-cancer risks associated with heavy metal exposures from street foods. Evaluation of roasted meats in an urban setting. *Journal of Environmental Pollution and Human Health*. **3**(2):24-30.

Banadda, N., Namawejje, H., Ayaa, F., Kigozi, J. B. and Sendagi, S. (2011). Diffusive flux modeling of lead migration from black polythene bags into food: A case study of green bananas (Matooke). *African journal of Food science*, **5**(5):313-319.

Basapor N and Ngabaza T (2015). Toxicological effect of chlorpyrifis and lead on the acquatic snail Helisoma duryi. *Advances in Biology and Chemistry*. **5**:225-233.

Bhaskar, J. J. Mahadevamma, S., Nandini, D. C. and Paramahans, V. S. (2012). Banana (Musa sp. var elakki bale) Flower and Pseudostem: Dietary Fiber and Associated Antioxidant Capacity. *Journal of Agricultural and Food Chemistry*, **60**:427-432.

Bouzayen, M., Latché, A., Nath, P. and Pech, J. C. (2010), "Mechanism of fruit ripening", Plant Developmental Biology-Biotechnological Perspectives, *Springer Berlin Heidelberg*, **1**:319-339.

Burk, R. F., Olson, G. E., Winfrey, V. P., Hill, K. E. and Yin, D. (2011). Glutathioneperoxidase-3 produced by the kidney binds to a population of basement membranes in the gastrointestinal tract and in other tissues. *American Journal of Physiology*-Gastrointestinal and *Liver Physiology*, **301**:32-38.

Chen, J., Li, M., Lv, Q., Chen, G., Li, Y., Li, S., Mo, Y., Ou, S., Yuan, Z., Huang, M. and Jiang, Y. (2015). Blood lead level and its relationship to essential elements in preschool children from Nanning, China. *Journal of Trace Elements in Medicine and Biology*, **30**:137-141.

Cobanoglu, U., Demir, H., Duran, M., Şehitogullari, A., Mergan, D. and Demir, C. (2010). Erythrocyte catalase and carbonic anhydrase activities in lung cancer. *Asian Pacific* Journal of *Cancer Prevention*, **11**:1377-1382.

D'Hont, A., Denoeud, F. and Aury, J. M. (2012). The banana (Musa acuminata) genome and the evolution of monocotyledonous plants. *Nature*, **488**:213-217.

Dhembare, A. J. (2013). Eating artificially ripened fruits is harmful. *Current Science*, **99**(12):1664-1668.

Dhembare, A. J. (2013). The bitter truth about fruits with reference to artificial ripeners. *Archives of Applied Science Research,* **5**(5):45-54.

Edouard, K., Carl M. F., Mbofung, Z. T. and Felicite, M. T. (2017). Sensory Evaluation of Tropical Bush Mango (*Irvingia gabonensis*) Fruits. *Pakistan Journal of Nutrition,* **16**:562-570.

EFSA (European Food Safety Authority) (2014) Dietary exposure to inorganic arsenic in the European Population European Food Safety Authority (EFSA), Parma, Italy. *European Food Safety Authority Journal*, **12**(3):3597.

Eka, B. E., Onyegeme-Okerenta, B. M. and Onyema, J. O. (2018). Calcium Carbide as an Artificial Fruit-Ripening Agent and its Physiological Effects on Wistar Rats. *Clinical and Experimental Medical Sciences,* **6**(1):47-61.

Elbagermi, M. A., Edwards, H. G M. and Alajtal, A. I. (2012). "Monitoring of Heavy Metal Content in Fruits and Vegetables Collected from Production and Market Sites in the Misurata Area of Libya," ISRN *Analytical Chemistry*, Article ID 827645, 5 pages, 2012.

Esma, K., Oktay, T., Hüseyin, C., Murat, S., Claudiu, T. S. and Deniz, E. (2019). Determination of the inhibitory effects of N-methylpyrrole derivatives on glutathione reductase enzyme, *Journal of Enzyme Inhibition and Medicinal Chemistry*, **34**(1):51-54.

FAO (Food and Agriculture Organization). FAOSTAT; *Food and Agriculture Organization* Geneva, Switzerland, 2013.

Fathima, B. J., Suganthi, R., Rajvel, D., Padmini Pooja, M. and Mathithumilan, B. (2010): Bioremediation of chromium in tannery effluent by microbial Consortia. *African Journal of Biotechnology*, **9**(21):3140-3143.

Fattah, S. A. and Ali, M. Y. (2010). Carbide Ripened Fruits - A Recent Health Hazard. *Faridpur Medical College Journal*, **5**(2):37.

Fungo, R., Pillay, M. (2013). β-Carotene content of selected banana genotypes from Uganda. African Journal of Biotechnology, **10**:5423-5430.

Garba, Z. N., Ubam, S., Babando, A. A. and Galadima, A. (2015). Quantitative Assessment of Heavy Metals from Selected Tea Brands Marketed in Zaria, Nigeria. *Journal of Physical Science*, **26**:43-51.

Giacomini, D. G. (2012). Formal Recommendation by the National organic Standard Board (NOSB) to the National Organic Program (NOP). *U.S. Department of Agriculture, USA.*

Gill, P. S., Jawandha, S. K., Singh, N., Kaur, N. and Verma, A. (2014). "Influence of postharvest applications of ethephon on fruit ripening in mango," *International Journal of Advanced Biological Research*, **4**:438-441.

Goldberg, R., Catesson, A. M. and Czaninski, Y. (1983). Some properties of syringaldazine oxidase, a peroxidase specifically involved in the lignification processes. *Z Pflanzenphysiology*, **110**:267-279.

Gourab, B. (2015). An assessment of apple cultivation in kalpa, (Kinnaur) District, Himachal Pradesh. *Journal of Humanities and social science*, **20**(8):20-23.

Gray, D. C. (1980). Analytical chemistry, 3rd Edition, John Wiley and Sons Inc., New York. 46-68.

Gupta, R. (2017). Artificial ripening of fruits and effects on health. In: *proceedings of 6th International Conference on Recent Trend in Engineering, Science and Management* (ICRTESM-17), Punjab, India. 341-345.

Hakim, M.A., Obidul, A. K., Alam, M. A., Khatib, A., Saha, B. K., Haque, K. M. F. and Zaidul, I. S. M. (2012). Role of health hazardous ethephon in nutritive values of selected pineapple, banana and tomato. *Journal Food Agriculture and Environment*, **10**(2):247-251.

Hamlet, S. A., Bensoltane, S., Djekoun, M., Yassi, F. and Berrebbah, H. (2012). Histological changes and biochemical parameters in the hepatopancreas of terrestrial gastropod *Helix aspersa* as biomarkers of neonicotinoid insecticide exposure. *African Journal of Biotechnology*, **11**:16277-16283.

Hayyan, M., Hashim, M. A. and AlNashef, I. M. (2016). Superoxide Ion: Generation and Chemical Implications. *Chemical Reviews*. **116**:3029-3085.

Heinrich, D., Guise, T. A., Suzuki, H. and Sartor. O. (2018). Alkaline phosphatase in metastatic castration-resistant prostate cancer: reassessment of an older biomarker. *Future Oncology* 24:2543-2556.

Hettiaratchi, U. P. (2011) Chemical compositions and glycemic responses to banana varieties. *International Journal of Food Science and Nutrition*, **62**(4):307-309.

Hossain, M. F., Akhtar, S. and Anwar, M. (2015). Health hazards posed by the consumption of artificially ripened fruits in Bangladesh. *International Food Research Journal* ,**22**(5):1755-1760.

IARC (International Agency for Research on Cancer) (2012) Monographs on the evaluation of carcinogenic risks to humans. A review of human carcinogens: arsenic, metals, fibres, and dusts. *International Agency for Research on Cancer*, **100**:41-93.

Igbinaduwa, P. and Aikpitanyi-Iduitua, R. (2016). Calcium carbide induced alterations of some haematological and serum biochemical parameters of Wistar rats. *Asian Journal of Pharmaceutical and Health Sciences,* **6**(1):1396-1400.

Igbinaduwa, P. O., Omotoso, A. E., Aikpitanyi, R. and Uwaezuoke, C. E. (2018). Toxic levels of arsenic and phosphorous found in some commonly consumed fruits sold in the market in Benin city. E*uropean Journal of pure and applied chemistry.* **4**(1).

Ignat, I., Volf, I., Popa, V. I. (2011). A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chemistry*. **126**:1821-1835.

Imam, M. Z. and Akter, S. (2011). Musa paradisiaca L. and Musa sapientum L., A Phytochemical and Pharmacological Review. *Journal of Applied Pharmaceutical Science*, **1**:14–20.

Iweala, E. E. J., Olugbuyiro, J. A. O., Durodola, B. M., Fubara-Manuel, D. R. and Okoli, A. O. (2014). Metal contamination of foods and drinks consumed in Ota, Nigeria. *Research Journal of Environmental Toxicology*, **8**:92-97.

Iwegbue, C. M. A., Nwozo, S. O., Overah, C. L., Bassey, F. I. and Nwajei, G. E. (2013). Concentrations of Selected Metals IN Some Ready-to-Eat-Foods Consumed in Southern Nigeria: Estimation of Dietary Intakes and Target Hazard Quotients. *Turkish Journal of Agriculture-Food Science and Technology*, **1**:1–7.

Iyer, S., Sengupta, C. and Velumani, A. (2015). Lead toxicity: An overview of prevalence in Indians. *Clinica Chimica Acta*, **451**:161-164.

Izah, S. C. and Srivastav, A. L. (2015). Level of arsenic in potable water sources in Nigeria and their potential health impacts: A review. *Journal of Environmental Treatment Techniques*, **3**:15-24.

Jean-Lou, C. M. D., George, E. N. K., Luisa, R. B., Billy, A., Ulla, B., Anna, F. C., Claudia, H., Mari, E., Stefan, F., Pietro, F., Elena, S., Eugenia, D., Peter, F., Alan, R. B. and Philippe, V. (2011). Human Risk Assessment of Heavy Metals: Principles and Applications. *Metal Ions in Life Science*, **8**:27-60.

Jindal, T., Agrawal, N. and Sangwan, S. (2013) Accidental Poisoning with Calcium Carbide. *Journal of Clinical Toxicology*, **3**(2):159.

Jiwan, S. S. and Tasleem A., Z. (2018). Bioactive compounds in banana fruits and their health benefits, *Food Quality and Safety,* **2**(4):183-188.

Johann, M. R., AntoineLeslie, A. H. and FungCharles, N. G (2017). Assessment of the potential health risks associated with the aluminium,arsenic, cadmium and lead content in selected fruits and vegetables grown in Jamaica. *Toxicological reports,* **4**:181-187.

Kandasamy, S. and Aradhya, S. M. (2014). Polyphenolic profile and antioxidant properties of rhizome of commercial banana cultivars grown in India. *Food Bioscience*, **8**:22–32.

Katnap, R. S., Arome, A. F., Zakari, H., Juliet, O., Nanbol, K. K., Kumzhi, P. R. and Joseph, A. A. (2017). Occurrence of Aflatoxin Producing Mould in Irvingia gabonensis (Bush Mango) Seeds Sold within Jos Metropolis. *American Journal of Biomedical and Life Sciences,* **5**(5):92-95.

Kendrick, M. (2012). The Origin of Fruit Ripening, in Scientific AmericanTM. Nature America, Inc.: New York. *Homepage: www.scientificamerican.com; Retrived: 2 June 2012.*

Khan, M. R. and Ahmed, D. (2009). Protective effect of *Digera muricata* (L.) Mart. on testis against oxidative stress of CCl_4 in rats. *Food Chemistry and Toxicology*, **47**(6): 1393-1393.

Kirti, S., Sreemoyee, C. and Bhumika, J. (2015). Chromium Toxicity and its Health Hazards. *International Journal of Advanced Research*, **3**(7):167-172.

Koning, R. E. (2012). Fruit Ripening. Plant Physiology Information Website. Available from: *http://plantphys.info/plants_human/fruitgrowripe.shtml*.

Kookal, S.K. and Thimmaiah, A. (2018). Nutritional Composition of Staple Food Bananas of Three Cultivars in India. *American Journal of Plant Sciences*, **9**:2480-2493.

Kumar, P., Chand, S. and Maurya, P. K. (2016). Quercetin-modulated erythrocyte membrane sodium-hydrogen exchanger during human aging: Correlation with Atpase's. *Archives of Physiology and Biochemistry,* **122**:141-147.

Liang, Y., Yi, X., Dang, Z., Wang, Q., Luo, H. and Tang, J. (2017). Heavy Metal Contamination and Health Risk Assessment in the Vicinity of a Tailing Pond in Guangdong, China. *International Journal of Environmental Research and Public Health,* **14**(12):1557-1563.

Maduwanthi, S. D. T. and Marapana, R. A. U. J. (2019). Induced Ripening Agents and Their Effect on Fruit Quality of Banana. *International Journal of Food Science*, Article ID 2520179, 8 pages.

Maes, M., Bonifacio, K. L., Morelli, N. R., Vargas, H. O., Barbosa, D. S., Carvalho, A. F. and Nunes, S. O. V. (2018). Major differences in neurooxidative and neuronitrosative stress pathways between major depressive disorder and types i and ii bipolar disorder. *Molecular Neurobiology*, **55**:1-16.

Mahfuza, S. S., Rana, S., Yamazaki, S., Aono, T. and Yoshida, S. (2017). Health risk assessment for carcinogenic and noncarcinogenic heavy metal exposures from vegetables and fruits of Bangladesh. *Cogent Enviromental Science,* 3:1291107-1291124.

Malakouti, M., Kataria, A., Ali, S. K. and Schenker, S. (2017). Elevated Liver Enzymes in 11 Asymptomatic Patients - What Should I Do? *Journal of Clinical and Translational Hepatology*, **5**:394-403.

Márcio, C., Isabel C. F. R., Ferreira, P. M., and Marina, S. (2018). "Antioxidants and Prooxidants: Effects on Health and Aging." *Oxidative Medicine and Cellular Longevity*, vol. 2018, Article ID 1472708, 2 pages,

Mathew, N. S., Negi, P. S. (2017). Traditional uses, phytochemistry and pharmacology of wild banana (*Musa acuminata* Colla): A review. *Journal of Ethnopharmacology*, **196**: 124-140.

Merlene, A. B., Suriyakalu, M. A. and Gothandown, K. M. (2012). Varietal Impact on Phyto Chemical Contents and Antioxidant Properties of *Musa accuminata* (Banana). *Journal of Pharmaceutical Science and Research*, **4**:1950-1955.

Mirończuk-Chodakowska, A. M. W., and Zujko, M. E. (2018). "Endogenous non-enzymatic antioxidants in the human body," *Advances in Medical Sciences*, **63**(1):68-78.

Muhammad, I., Ashiru, S., Ibrahim, I. D., Salawu, K., Muhammad, D. T. and Muhammad, N. A. (2014). Determination of Some Heavy Metals in Wastewater and Sediment of Artisanal Gold Local Mining Site of Abare Area in Nigeria. *Journal of Environmental Treatment Techniques*, **1**:174-182.

Mursalat, M., Rony, A. H., Rahman, A. H. M. S., Islam, M. N., Khan, M. S. (2013). A critical analysis of artificial fruit ripening: Scientific, Legislative and Socio-Economic Aspects. *CHE THOUGHTS-Chemical Engineering and Science Magazine*, **4**(1): 6-12.

Nazibul, I., Mollik, Y. I., Sabrina, S. A., Farrhin, N., Swarit, A. S., Mohidus, S. K and Fatih, Y. (2018). Artificial ripening on banana (*Musa* Spp.) samples: Analyzing ripening agents and change in nutritional parameters. *Cogent Food & Agriculture*, **4**:1-7.

Noblanc,A., Kocer, A., Chabory, E., Vernet, P., Saez, F., Cadet, R., Conrad, M. and Drevet, J. R. (2011). Glutathione peroxidases at work on epididymal spermatozoa: an example of the dual effect of reactive oxygen species on mammalian male fertilizing ability. *Journal of Andrology*, **32**:641-650.

Nura, A., Dandag, M. A. and Wali, N. R. (2018). Effects of artificial ripening of banana (*Musa spp*) using calcium carbide on acceptability and nutritional quality. *Journal of Postharvest Technology*, **6**(2):14-20.

Odiba, A. S., Onosakponome. I., Iroha, O. K., Ukegbu, C. Y., Omeje, K. O. (2014). Transaminase [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] Activity of HIVFemale Patients on Drugs and Female Patients Not on Drugs. *Journal of Pharmacy and Biological Sciences*, **9**(2):60-65.

Ogunkun, A. T. J., Bello, O. S. and Ojofeitimi, O. S. (2014). Determination of heavy metal contamination of street-vended fruits and vegetables in Lagos state, Nigeria. *International Food Research Journal,* **21**:1725-1730.

Onasanwo, S. A., Emikpe, B. O., Ajah, A. A. and Elufioye, T. O. (2013). Anti-ulcer and ulcer healing potentials of Musa sapientum peel extract in the laboratory rodents. *Pharmacological Research*, **5**:173-178.

Onuoha, S. C., Anelo, P. C. and Nkpaa, K. W. (2016). Human health risk assessement of heavy metals in snail (Archachatina marginata) from four contaminated regions in rivers state, Nigeria. *American Chemical Science Journal,* **11**(2):1-8.

Onyeike, E. N., Olungwe, T. and Uwakwe, A. A. (1995). Effect of heat treatment and defatting on the proximate composition of some Nigerian local soup thickeners. *Food Chemistry*, **53**: 173-175.

Orisakwe, O. E., Nduka, J. K., Amadi, C. N., Dike, D. O. and Bede, O. (2012). Heavy metals health risk assessment for population via consumption of food crops and fruits in Owerri, South Eastern, Nigeria. *Chemical Central Journal*, **6**(1):77-85.

Orisakwe, O. F.; Ajaezi, G. C. (2014). Heavy metal hazards of functional beverages in Nigeria. Malays. *Journal of Nutrition,* **20**:121-131.

Otitoloju, A. A., 2016. Today's Apple: perspective of an Environmental Toxicologist. Twelfth (12^{th}) Inaugural Lecture, University of Lagos. University of Lagos Press.

Paglia, D. E. and Valentine, W. N. (1967) *Journal of Laboratory Clinicals in Medicine*, **70**:158-169.

Patrick-Iwuanyanwu, K. and Chioma, N. C. (2017). Evaluation of Heavy Metals Content and Human Health Risk Assessment via Consumption of Vegetables from Selected Markets in Bayelsa State, Nigeria. *Biochemistry and Analytical Biochemistry*, **6**:332-338.

Pawar, R. K., Bhagure, G. R., Chavan, R. P. (2016). Antioxidants and their role in nurture human life and industry: A review. *International Journal of Chemical Studies*, **4**:22-26.

Pereira, A., Maraschin, M. (2015). Banana (*musa* spp) from peel to pulp: ethnopharmacology, source of bioactive compounds and its relevance for human health. *Journal of Ethnopharmacology,* **160**:149-163.

Pflaum, T., Hausler, T., Baumung, C., Ackermann, S., Kuballa, T., Rehm, J. and Lachenmeier, D.W. (2016). Carcinogenic Compounds in Alcoholic Beverages: An update. *Archives of Toxicology*, **90**:2349-2367.

Popa, V. I. (2015) Wood bark as valuable raw material for compounds with biological activity. Celul. ¸Si, **64**:5-17.

Prakash, D., Upadhyay, G., Gupta, C., Pushpangadan, P. and Singh, K. K. (2012). Antioxidant and free radical scavenging activities of some promising wild edible fruits. *International Food Research Journal,* **19**(3):1109-1116.

Rahimzadeh, M. and Rastegar, S. (2017). Heavy metals residue in cultivated mango samples from Iran. *Journal of Food Quality and Hazards Control*, **4**:29-31.

Rajesh, N. (2017). Medicinal benefits of *Musa paradisiaca* (Banana). *International Journal of Biology Research*, **2**(2):51-54.

Raouf A. A. and Girgis, S. M. (2011). Mutagenic, teratogenic and biochemical effects of ethephon on pregnant mice and their fetuses. *Global Vetenary*, **6**:251-257.

Rec GSCC, (1972). Optimised standard colorimetric methods. Serum Alkaline phosphatase (DGKC). *Journal of Clinical Chemistry and Clinical Biochemistry*, **10**:182-182.

Reham, K. B., El-Gawad, M. A. and Hanan E. O. (2013). Health risks assessment of heavy metals and microbial contamination in water, soil and agricultural foodstuff from wastewater irrigation at Sahl El-Hessania area, Egypt. *Journal of Applied Sciences Research*, **9**(4):3091-3107.

Reitman, S. and Frankel, S. A. (1957). Colorimetric method for the determination of serum levels of glutamic oxaloacetic acid and pyruvic acid transaminases. *American Journal of Clinical Pathology*, **28**: 56-63.

Rout, B. K. and Sikdar, B. K. (2017). Hazard Identification, Risk Assessment and Control Measures as an Effective Tool of Occupational Health Assessment of Hazardous Process in an Iron Ore Pelletizing Industry. *Indian Journal of Occupational and Environmental Medicine,* **21**(2):56-76.

Rubaljot, k., Mahajan, B. V. C and Dhillon, W. S. (2014). Heavy Metal Contamination in Vegetables, Fruits, Soil and Water – A Critical Review. *International Journal of Agriculture, Environment and Biotechnology*, **7**(3):603-612.

Sajib, M. A. M., Hoque, M. M., Yeasmin, S. and Khatun, M. H. A. (2014). Minerals and heavy metals concentration in selected tropical fruits of Bangladesh. *International Food Research Journal* **21**(5):1731-1736.

Salako, S. G., Adekoyeni, O. O., Adegbite, A. A. and Hammed, T. B. (2016). Determination of Metals Content of Alcohol and Non-alcoholic Canned Drinks Consumed at Idiroko Border Town Ogun State Nigeria. *Journal of Applied Science and Technology*, **12**:1-8.

Salminen, W. F., Yang, X. and Shi, Q. (2012) Green tea extract can potentiate acetaminophen-induced hepatotoxicity in mice. *Food and Chemical Toxicology*, **50**:1439-1446.

Samuel, T. A., Samuel, J. C., Felix J. A., Abudu, B. D. and Zita, N. A (2018). Health risk assessment and heavy metal contamination levels in vegetables from Tamale Metropolis, Ghana. *International Journal of Food Contamination*, **5**:5-13.

Sampath, K., Bhowmik, D., Duraivel, S. and Umadevi, M. (2012) Traditional and Medicinal Uses of Banana. *Journal of Pharmacognosy and Phytochemistry*, **1**:51-63.

Sardar, K., Ali, S., Hameed, S., Afzal, S., Fatima, S., Shakoor, M. B., Bharwana, S. A. and Tauqeer H. M. (2013). Heavy metals contamination and what are the impacts on living organisms. *Greener Journal of Environmental Management and Public Safety*, **2**:172-179

Sen, C., Mishra, H. N. and Srivastav, P. P. (2012). Modified atmosphere packaging and active packaging of banana (Musa spp): A review of control of ripening and extension of shelf life. *Journal of stored products and postharvest research*, **3**(9):122-132.

Siddiqui, M. W. and Dhua, R. S. (2010). Eating Artificial Ripened Fruits is Harmful. *Current Science*, **99**(12):1664-1668.

Singal, S., Kumud, M. and Thakral, S. (2012). "Application of apple as ripening agent for banana" *Indian Journal of Natural Products and Resources*, **3**(1):61-64.

Singh, B., Singh, J. P., Kaur, A. and Singh, N. (2016). Bioactive compounds in banana and their associated health benefits – A review. *Food Chemistry*, **206**:1-11.

Singh, R., Gautam, N., Mishra, A. and Gupta, R. (2011). Heavy metals and living systems: an overview. *Indian Journal of Pharmacology*, **43**: 246-254.

Sobukola, O. P., Adeniran, O. M., Odedairo, A. A. and Kajihausa, O. E. (2010). Heavy metals levels of some fruits and leafy vegetables from selected markets in Lagos, Nigeria. *African Journal of Science* **4**(2):389-393.

Sogo-Temi, C. M., Idowu, O. A. and Idowu, E. (2014). Effect of biological and chemical ripening agents on the nutritional and metal composition of banana (Musa spp). *Journal of Applied Science and Environmental Management*, **18**(2):243-246.

Sulaiman, S. F., Yusoff, N. A. M., Eldeen, I. M., Seow, E. M., Sajak, A. A. B. and Ooi, K. L. (2011). Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (Musa sp.). *Journal of Food Composition and Analysis*, **24**:1-10.

Sushmitha, S., Sathya P. T. and Ganesan, J. (2018). Enhancement of Shelf Life of the Climacteric Fruits: A Review on Application of CRISPRi Technology. *Trends in Technological Science Research*, **1**(2):55 -557.

Sylvia, A. (2018). Introduction of Human Health associated with Risk Assessment. *Journal of Environmental Science and Public Health*, **2**:179 -187.

Tanase, C., Boz, I., Stingu, A., Volf, I .and Popa, V. I. (2014). Physiological and Biochemical responses induced by Spruce Bark Aqueous extract and deuterium depleted water with synergistic action in sunflower (Helianthus annuus l.) plants. *Industrial Crops and Products*, **60**:160-167.

Taposhe, R., Abdus, S., Zainul, A., Asaduzzaman, S., Imam, H. and Abu, Z. (2017). Study of Artificial Ripening Agent and Its Effects on Banana (*Musa spp*.) Collected fromTangail Area, Bangladesh. *Journal of Environmental Science, Toxicology and Food Technology*, **11**(9):14-19.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K. and Sutton, D. J. (2012). Heavy Metals Toxicity and the Environment. *National Institutes of Health*, **101**:133-164.

Thompson, C. M., Haws, L. C., Harris, M. A., Gatto, N. M. and Proctor, D. M. (2011): Application of the U.S. EPA mode of action Framework for purposes of guiding future research: a case study involving the oral carcinogenicity of hexavalent chromium. *Toxicological Sciences*, **119**:20-40.

Tsamo, P. C. V., Marie-France, H., Kodjo, T., Thomas, H. E., Joëlle, Q., Hervé, R., Yvan, L. and Christelle, A. (2015). Phenolic profiling in the pulp and peel of nine plantain cultivars (Musa spp.). *Food Chemistry*, **167**:197-204.

Tsao, R. (2010). Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients*, **2**:1231-1246.

United States Environmental Protection Agency (USEPA). (2017). Human Health Risk Assessment Model Ecological Risk Assessment; Retrieved from https://www.epa.gov/risk/human-health-risk-assessment on 26th March, 2017.

Vaidya, D., Sharma, A., Abrol, G. S., Sharma, S. and Vaidya, M. K. (2016). Evauation of technology for low cost drying of banana slices. *Indian Journal of Natural Products and Resources*, **7**(2):176-180.

Vu, H. T., Scarlett, C. J., Vuong, Q. V. (2018). Phenolic compounds within banana peel and their potential uses: a review. *Journal of Functional Foods*, **40**:238-248.

Wang, Y., Robyn, B., Alycia, N. and Siegfried, H. (2018). Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. *Journal of cell biology*, **217**(6):1915-1928.

Wenqian, Y., Junjun, L., Min, Q. and Minghui, Y. (2019) Detection of alkaline phosphatase activity and inhibition with fluorescent hydroxyapatite nanoparticle. *Analytical Methods***,** **11**:2272-2276.

WHO, (2011). Guidelines for drinking water quality, 4th ed. http:// whqlibdoc.who.int/publications/2011/9789241548151_eng.pdf

Xin, Z., Waterman, D. F., Henken, R. M. and Harmon, R. J. (1991). Effects of copper status on neutrophil function, superoxide dismutase and copper distribution in steers. *Journal of Diary science*, **74**:3078-3085.

Yaneira, E. S., Kumar, I. and Jeremy, G. (2014). Bow-Tie Diagrams in Downstream Hazard Identification and Risk Assessment. *Process Safety Progress*, **33**:26-35.

Yujun, Y., Zhifeng, Y. and Shanghong, Z. (2011). Ecological Risk Assessment of heavy metals in sediment and Basin. *Journal Environmental Pollution*, **159**:2575-2585.

Zhong, W. S, Ren, T. and Zhao, L. J. (2016). Determination of Pb (Lead), Cd (Cadmium), Cr (Chromium), Cu (Copper), and Ni (Nickel) in Chinese tea with highresolution continuum source graphite furnace atomic absorption spectrometry. *Journal of Food and Drug Analysis*, **24**:46-55.