ASSESSMENT OF ARGINASE IN THE MESOCARP OF RIPE AND UNRIPE TOMATOES (*Solanum lycopersicum* L.)

BY

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(PSB/14/2354)

A FINAL YEAR PROJECT SUBMITTED TO THE DEPARTMENT OF PLANT SCIENCE AND BIOTECHNOLOGY, FACULTY OF SCIENCE IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN PLANT SCIENCE AND BIOTECHNOLOGY, FEDERAL UNIVERSITY OYE-EKITI, EKITI STATE, NIGERIA.

MARCH, 2019.

CERTIFICATION

This is to certify that this final year project was carried out under my supervision by AJAYI OLUWASEUN IREWOLE with the matriculation number PSB/14/2354, in the Department of Plant Science and Biotechnology, Faculty of Science, Federal University Oye-Ekiti, Ekiti State, Nigeria.

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DEDICATION

This project is dedicated to the Almighty God who made it possible for me to complete this research work.

ACKNOWLEDGEMENTS

I appreciate the Almighty God who has preserved my life throughout this entire Bachelors' programme. To Him be all glory, honour and adoration for seeing me through till the completion of this project work. I am grateful to my supervisor and Head of the Department of Plant Science and Biotechnology, Dr. A. A Ajiboye for his encouragement, constant support and guidance which was of greatest help for me during this project work.

I extend my gratitude to all my Lecturers, all non- Academic staff and the laboratory Technicians in the Department of Plant Science and Biotechnology. I appreciate Dr. Okonji Raphael for his technical support while using his laboratory at Obafemi Awolowo University (OAU). Ile Ife, Osun State, Nigeria.

My special thanks goes to my wonderful parents, Mr And Mrs Ajayi for their tutelage, counsel and advise as well as spiritual and financial support. Finally, I appreciate the contributions and support of my friends, course mates and Tobiloba Biochemistry student of Obafemi Awolowo University (OAU) Ile Ife, Osun State, Nigeria.

TABLE OF CONTENTS

CHAPTER THREE

CHAPTER FOUR

LIST OF FIGURES

Page No

LIST OF TABLES

Page No

LIST OF PLATES

Page No

ABSTRACT

Arginase a manganese-dependent and metal activated enzyme which result in ornithine and urea production, it is important for nitrogen metabolism in all organisms as well as for defence responses. This research was carried out to evaluate the quantities of arginase present in the mesocarp of ripe and unripe mesocarp of Tomato fruit (*Solanum lycopersicum*). Ripe Tomato fruits used in this study were collected from Oye market, Oye town in Ekiti State, Nigeria and unripe tomato fruits were gotten from a vegetable garden at Akure, Ondo State, Nigeria. The fruits were identified in the Herbarium section of Plant Science and Biotechnology laboratory, Federal University Oye-Ekiti. Samples of tomato were homogenized with 25ml of tris buffer and centrifuged, Arginase assay was performed and its concentration was determined at wavelength of 450nm. The unripe tomato fruit was discovered to have higher absorbance of arginase (0.202) in comparison to ripe tomato (0.[illegible]) and highest specific activity of (1.50×10^{-3}) in comparison to the ripe tomato (1.46 [illegible]). The Michealis (Km) constant for unripe and unripe tomato was 6mM and 80mM respectively and the arginase preferred arginine as substrate. The optimum pH for unripe and ripe tomato was 4.0 and 5.0 respectively. The urea and mercaptoethanol strongly inhibited arginase activity while EDTA enhanced arginase activity in ripe tomato in unripe tomato EDTA and urea while mercapethanol strongly inhibited enhanced arginase activity. The enzyme was markedly enhanced by 1.0Mn and 10Na for unripe tomato and 0.1Na and 0.1Fe markedly enhanced arginase activity in ripe tomato.The result of this study provides information on the presence of arginase in ripe and unripe tomato mesocarp, it will also provide useful information on some inhibitors and activator of the enzyme activity.

CHAPTER ONE

1.0 INTRODUCTION

Solanum lycopersicum (tomato) is one of the most important vegetable crops grown all over Nigeria and in the tropical and subtropical belts of the world. It is the world's largest vegetable crop after potato and sweet potato but it tops the list of canned vegetables. It is an important condiment in most diet and a very cheap source of vitamins, calcium and niacin all of which are of great importance in the metabolic activities of man. Tomato is a good source of vitamins A, C and E and minerals that are very good for the body and protect the body against diseases (Olaniyi *et al.*, 2010).

Tomatoes have also been found to be important for human life in many ways. Tomato is ranked at the top of all fruits and vegetables as a source of vitamins and minerals in the world (Eurostat statistics, 2016). Tomatoes are widely known for their outstanding antioxidant content; they also have anti-cancer potential which comes from being a non-starchy vegetable as well as a source of vitamin C and carotenoids (American institute for Cancer Research, 2017).

Tomatoes contain proteinaceous compounds and these include enzymes such as arginase,β-1,3-glucanases and chitinases, a-amylases and also other proteins and peptides which are responsible for their antimicrobial activity and have helped them to develop protective mechanisms that enable them to successfully resist different unfavourable conditions (*Rao and Marcos, 2013*; *Candido et al., 2011*).

Tomato plays a major role in human nutrition. It is an excellent source of phosphorus, iron and vitamin A, B and C; (Cobley and Steele, 1976, Varela *et al.*, 2003 and Naika*et al.*, 2005). As vegetable, it constitutes an important component in man's diet, especially in developing countries. There is no doubt that vegetables are very important in improving the quality of life and people's economic status.

Arginase, amanganase-dependent enzyme is widely distributed in all creatures (Jenkinson*et al.*, 1996). Arginase is the final enzyme of the urea cycle that catalyses the hydrolysis of L-arginine to generate L-ornithine and urea and it plays an important role in the excretion of ammonium in the body (Ash 2004; Knox and Greengard, 1965), it is present in all forms of life and plays a crucial role in nitrogen metabolism (Ash, 2004; Muszynska*et al.*, 1972; Ikemoto*et al* .. 1990). It is ubiquitous in all forms of life.

Some of the best sources for enzymes are fresh fruits, vegetables, and sprouted grains. Just as the body needs enzymes to function, so do plants need enzymes for growth, reproduction, and health. Foods are such rich sources of enzymes that some enzyme supplements are actually derived from food sources. All fresh fruits, vegetables, or grains are potential enzyme sources, but only if the enzymes have not been destroyed by heat, radiation, or any of the other processes. Arginase has also been reported in some plants like tomatoes, apple, water, melon, oranges etc, in varying distribution (Okonji R.E. and Agboola O.S. 2014).

This study therefore aimed at making available information on the presence of Arginase in tomato (*Solanum lycopersicum)* and to ascribe possible roles for the enzymes.

1.1: STATEMENT OF RESEARCH PROBLEMS

Arginase plays various physiological and metabolic roles in plant tissues, that is nitrogen mobilization during development of fruits, bulbs and tuber and seed germination. Many Farmers face challenges in breeding tomatoes as there might be poor uptake of nutrient, susceptibility to pests and pathogens, poor yield in the field and so on. Research has shown that exogenous application of this enzyme promotes plant growth and productivity and mediates plant environmental stress tolerance. Therefore, it becomes imperative to assess the levels of arginase in Tomato fruit.

1.2: OBJECTIVES OF THE RESEARCH

The objectives of the research are:

General Objective

1. To provide information on the presence of Arginase in tomatoes and to ascribe possible roles for the enzyme.

Specific Objectives

1 To assess the protein concentration levels in ripe and unripe tomatoes

2 To determine the level of arginase in ripe and unripe tomatoes

3 To compare the level of arginase in ripe and unripe tomatoes

1.3: CONTRIBUTION TO KNOWLEDGE

The results of this study will provide information on the presence of arginase in the mesocarp of ripe and unripe tomato fruits, its properties and the possible importance of this enzyme to plant breeders.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 THE TOMATO PLANT

Tomato (*Solanum lycopersicum),* is a tender warm season crop. It is characterized by glandular hairs (trichomes) that emit strong aroma when broken. Tomato plants are typically viny, prostrate, and are either determinate, semi determinate or indeterminate based on whether the apical stem terminates in an inflorescence. Most shoots form in the axils of leaves. It has got a deep tap root which may extend to three meters with extensive secondary roots (Peirce, 1987, Purseglove, 1988). The fruits are mostly red but there are some other colours such as yellow. There is a lot of variation among cultivars in the size and shape of the fruits, in the thickness of the fleshy mesocarp and in the development of the placenta (Prashant, 2003;Veershetty, 2004).

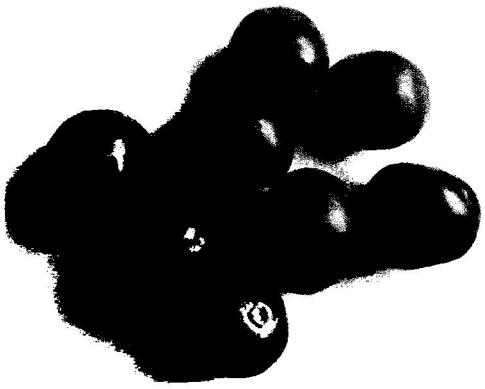

Plate1: Matured tomato fruits.

Nutritional Value of *Solanum lycopersicum* L.

Table2.1: Nutrient Composition of Tomato

Nutrient	Value	%DV(daily value)
Biotin	----	24%

Vitamin A	833IU	8%
Vitamin B6	0.1 mg	8%
Vitamin C	18.9 mg	32%
Vitamin E	0.54 mg	6%
Vitamin K	7.9 µg	16%
Potassium	237 mg	12%
Calcium	14.9 mg	1%
Copper	0.1 mg	12%
Dietary Fiber	1.8 g	7%
Manganese	0.15 mg	11%
Molybdenum	-----	20%
Folate	15 µg	7%
Phosphorus	24 mg	6%
Magnesium	11 mg	5%
Iron	0.3 mg	1.5%
Zinc	0.17 mg	1.5%
Choline	12.1 mg	2.2%
Pantothenic acid	0.2 mg	4%
Alpha-lipoic acid	0.56 g	---
Folic acid	68 mcg	7%
Beta-carotene	1.9 mg	2%
Lutein and Zeaxanthin	40 mcg	1.8%
Niacin	0.9 mg	4%
Riboflavin	0.0 mg	2%

Thiamin	0.1 mg	4%
Total Carbohydrate	5.8 g	2%

Source: Ravi Teja, 2017

Table 2.2: Protein & Amino Acids composition of *Solanum lycopersicum*

Protein and amino acids	Amounts Per Selected Serving
Protein	23g
Tryptophan	17.9mg
Threonine	58.7mg
Isoleucine	56.1mg
Leucine	89.3mg
Lysine	89.3mg
Methionine	20.4mg
Cysteine	30.6mg
Phenylalanine	63.7mg
Tyrosine	40.8mg
Valine	63.7mg
Arginine	56.1mg
Histidine	35.7mg
Alanine	68.9mg
Aspartic acid	334mg
Glutamic acid	887mg
Glycine	58.7mg
Proline	43.4mg

Serine	66.3mg
Hydroxyproline	~

Source:CondéNast, 2014

2.2 Plant Height

Tomato is an annual plant which can reach a height of over two meters (Naika *et al.*, 2005). Joshi *et al.*, (2004), have reported tomato mean plant heights of 121.36 cm. Height is among characters with high heritability (Veershetty, 2004, Mohanty, 2003, Singh *et al.*, 2000). Apart from fruit characteristics, the plant habit of tomato separates them into two distinct groups, those that are determinate and indeterminate cultivars. Determinate cultivars reach a height of 1.0 to 1.2 meters, at which stage the lead growth develops into a flower truss and similar things happen to all lateral branches. Indeterminate plants produce one or two stems, which grow on and on (as do laterals that are not removed) until they are stopped by removing the growing point. Indeterminate types usually have smaller fruits and reach maturity later. They bear fruits over a long period and are ideally suited to staking and pruning, both in open ground and in tunnels. They have much smaller pedicel scar than larger fruited sorts (Naika *et al.*, 2005). They allow continuous production of high quality fruits (Van der vooren *et al.*, 1986). The Determinate types have a relatively concentrated fruit set which lasts only two or three weeks and the fruit ripen much faster than those from indeterminate types (Naika *et al.*, 2005).

3.3 Tomato flower and maturity period

Tomato flowers had been reported to grow up to 2 cm in diameter. Flowers are borne in inflorescences of between four to twelve flowers. Its six petals are yellow and up to 1cm in length (Rice *et al.*, 1990). Marimbe, (1995), reported that maturity period of tomatoes

varieties differ. The period varies from 83 to 89 days. The crop reaches 50 percent maturity at 66 to 71 days (Nyirongo, 1995, Marimbe, 1995). Maturity period of Expresso (semideterminate) and Sixpack (determinate) had been reported to be 80 days after transplanting (Bok *et al.* 2006). Naika *et al.* (2005), also reported that the first harvest is possible 45 to 55 days after flowering, or 90 to 120 days after sowing.

2.4 Taxonomy of Tomato

In 1753, Linnaeus placed the tomato in the genus *Solanum* (alongside the potato) as *Solanum lycopersicum*. In 1768, Philip Miller moved it to its own genus, naming it Lycopersicon esculentum. This name came into wide use, but was in breach of the plant naming rules. Although the name *Lycopersicon lycopersicum* may be found, it is not used because it violates the International Code of Nomenclature barring the use of tautonyms in botanical nomenclature. Also, *Lycopersicum esculentus* is now an outdated name.

Genetic evidence has now shown that Linnaeus was correct to put the tomato in the genus *Solanum*, making *Solanum lycopersicum* the correct name.

Scientific classification

Kingdom: *Plantae*

Subkingdom: *Tracheobionta*

Division: *Angiosperms/ magnoliophyta*

Class: *Eudicots/ magnoliopsida*

Subclass: *Asteridae*

Order: *Solanales*

Family: *Solanaceae*

Genus: *Solanum*

Species: *Solanum lycopersicum*

2.5 Methods of Planting and Care of Tomato

Tomato need strong, direct light and should be exposed to area of intense light and they should be kept warm: day time temperatures between 65 and 85°F, and night time temperatures of 60 to 65°F. The seedlings should be transplanted from nursery when they about 20-25days old when the soil is warm. Two weeks before transplanting seedlings to the field, the soil should be tilled to about 1 foot and mixed with aged manure, compost, or fertilizer.

Tomato stakes or cages should be made at the time of planting. Staking keeps developing tomato fruit off the ground, while caging allows the plant hold itself upright. Though sprawling can also yield fine crops if there is space, and if the weather cooperates. Tomatoes need a lot of water to get the best fruit and should therefore be watered consistently throughout the growing season. Plants should be mulched five weeks after transplanting to retain moisture.

To help tomatoes through periods of drought, some flat rocks should be placed one next to each plant to pull water up from under the ground and keep it from evaporating into the atmosphere. The tomato plants should be fertilized with feed containing nitrogen, phosphorus and more of potassium if using stakes, plants should be pruned by pinching off suckers so that only a couple stems are growing per stake. Crop rotation should be practiced from year to year to prevent diseases that may have over wintered (Sue Sanderson, 2017).

2.6 Soil Requirements.

Tomatoes grow best in light, free draining, and fertile loam soil with pH of 5 – 7. However tomatoes can be grown in a variety of soils (Purseglove, 1988, Naika *et al.*, 2005). Regarding fertilizer requirements, tomatoes require an abundance of the three major elements

namely, nitrogen, phosphorus, and potassium. Adequate soil nitrogen application is important to enhance foliage growth which has a major bearing on crop maturity and protects the fruits from sunscald. Phosphorus influences fruit quality by stimulating vigorous root growth that enables more nutrients to enter the plant thereby promoting sturdy stem growth and healthy leaf formation.

Tomatoes do very well on most mineral soils but they prefer deep, well drained sandy loams. Upper layer of soil should be porous with little sand and good clay in the subsoil. Soil depth 15-20cm proves to be good for healthy crop. Deep tillage can allow for adequate root penetration in heavy clay type soils, which allows for production in these soil types. Tomato is a moderately tolerant crop to a wide pH range. A pH of 5.5-6.8 is preferred. The soils with proper water holding capacity, aeration, free from salts are selected for cultivation.

Soils extremely high in organic matter are not recommended due to the high moisture content of these medium and nutrient deficiencies. But, as always, the addition of organic matter to mineral soils will increase yield. Adequate soil nitrogen application is important to enhance foliage growth which has a major bearing on crop maturity and protects the fruits from sunscald. Phosphorus influences fruit quality by stimulating vigorous root growth that enables more nutrients to enter the plant thereby promoting sturdy stem growth and healthy leaf formation. Tomatoes use large amount of potassium. This element is important in stimulating early plant growth and regulating normal carbohydrate and protein metabolism.

2.7 Climatic Requirements

Tomato is more successful where there are long sunny periods. The optimum growing temperatures are 21°c to24°c. At these temperatures good quality seeds will take about seven days to emerge. Temperature affects flowering and pollination. The hot and dry weather leads to drying of the flowers and stops pollination. If temperatures are below 15°c or above 29°c,

pollen release is restricted resulting in incomplete fertilization of ovules. This causes collapsed fruit walls and formation of deep indentation in the fruit, a phenomenon called catface (Peirce, 1987, Bok *et al.* 2006).

2.8 Importance of Tomatoes in global Agriculture

Tomato plays major role in human nutrition as a vegetable; it constitutes an important component in human diet, especially in developing countries (Stevens, 1974). It is the second most consumed vegetable in the world behind potato. Tomatoes are eaten fresh in salads or processed and can be stewed, fried, baked and used to produce soup, or used as juice. In addition to this versatility, tomatoes are also an important source of vitamins and minerals. They are an excellent source of phosphorus, iron and vitamin A, B and C. They also contain small amounts of the B complex vitamins; thiamine, niacin and riboflavin (Cobley and Steele, 1976, Peirce, 1987, Purseglove, 1988, Varela *et al.*, 2003 and Naika *et al.*, 2005).

Tomato is called a functional food, that is, it does more than providing basic nutrition. There are lots of advantages in consuming fruits and vegetables including tomatoes. Studies have shown that tomato constituent like lycopene has prevented prostate cancer (American Institute for Cancer Research, 2016) and reduced cholesterol. They are rich in vitamin A that improves vision and low level of the vitamin can cause blindness. Also, lycopene, vitamin C and copper are eye-beneficial, lycopene counters free radical damage (Ravi Teja, 2017); vitamin C fights age-related cataracts and copper produces melanin, an essential black pigment in the eyes. It regulates blood pressure because it is rich in potassium, a mineral that lowers blood pressure by reducing the effect of sodium (American Heart Association, 2016). Vitamin C in tomatoes help protect both mother and baby.

Tomato deals with ulcerative colitis, a major issue pregnant woman face in their first trimester (Centres for Disease Control and Prevention, 2011). Tomato is rich in beta-

carotene, folate and flavonoids which promote cardiovascular health (The American Journal of Clinical Nutrition, 2015). They also help decrease platelet aggregation and homocysteine; two phenomena that affect the heart. Tomato is rich in iron, vitamins C and E and all these relieve diabetic symptoms (American Diabetes Association). Consumption of tomatoes help to prevent urinary tract infections as they contain vitamin C that inhibits E.coli bacteria growth thereby making urine less acidic and prevents infection (Megan Ware RDN LD; Helen Webberley 2016).

Consumption of leaves of tomato plant should be avoided as they contain large concentrations of alkaloids. If tomatoes are consumed in reasonable amounts, there should be no serious adverse effects. However, consumption of more than 30mg of lycopene daily could cause indigestion, bloating, nausea or diarrhea (Jessie Szalay, 2016).

2.9 Pest and Diseases.

Table1.3: Pests affecting Tomato and their control measures.

S/N	Pests	Control measures
1.	Leaf eating caterpillar	Spraying of cypernethrin at the rate of 3-4ml or for phosphamidon (85 SL) 5ml per 10 litre of water at the interval of 8-10 days.
2.	Tomato fruit borer	Spraying of monocrotophos (36 SL) 5ml/ 10 litre of water at the interval of 8-10 days. Trichograma and campoletic chloride as a predator and heliocil as biological control.

3.	Aphids	Spraying dimethoate (30 E.C.) 10ml/10 litre of water.
4.	Whiteflies	Soil application, treating the nursery beds with granular insecticides

Source: www.indiaagronet.com

Table1.4: Some diseases affecting Tomato, symptoms, control and method of application.

Diseases	Symptoms	Control	Method of application
Bacterial fruit spot	On leaves small, water soaked, brown spots appear on the older leaflets. The affected leaves turn yellow and blighting of the foliage may take place.	Streptocycline (100PPm) or copper fungicides.	Spraying
Bacterial wilt	Yellowing and wilting of leaves until the entire plant wilts and dies prematurely. Often the stem tissue is discoloured throughout the plant.	Streptocycline @ 1g/40 lit, Benoymyl 0.1% carbendazim	Seed treatment for 30min, soil application
Early blight	On established plants, dark brown spots with concentric rings develop first on leaves. Spotted leaves die prematurely leading to early defoliation	Mancozeb @ 0.2%	Foliar spray
Powdery mildew	White talcum-like coverings on the lower surface of leaves while the corresponding upper surface turns yellow. Premature dropping of infected leaves are common.	Dinocap at 0.1% or wet sulphur at 0.2%	Spraying

Septoria leaf spot	Appearance of numerous, small, grey coloured circular leaf spots with dark margin is the characteristic symptom of the disease	Mancozeb @ 2 g/kg. 0.2% Dithane Z-78	Seed treatment foliar spraying

Source: www.indiaagronet.com

2.10 Harvesting and Storage of Tomato

The stages of maturity when the fruit is picked and the intervals between picks, is determined by several factors. For distant markets or where there are large time delays between picking and marketing, the fruit needs to be harvested at a less mature stage. This also applies under high temperature conditions, which hasten fruit ripening and deterioration. The market preference also plays a role: processors and certain communities prefer fully coloured fruits whereas most retail outlets favour less mature fruits. Tomatoes are picked at the following stages of maturity (Amy Grant, 2016).

2.11: Tomato Ripening Stages

The tomato fruit stops growing and starts ripening from mature green stage when the development of the fruit is complete and final size is attained. Tomato ripening process sequentially passes through six stage, based on the percentage of the external colour: Mature green (no external red coloration), Breaker (<10% red colour at blossom end), Turning (10% to 30% of fruit surface having red colour), Pink (30% to 60% of fruit surface having red shade), Light red or Orange (60% to 90% of fruit surface having red colour), and Red (at least 90%-95% of fruit surface having red colour). In developing countries usually fruit growers pick the fruits before breaker stage and apply exogenous ethylene to the fruits to induce ripening after reaching the destination (Margaret Roach, 2012).

2.12: Storage of Tomato

Tomatoes are quite sensitive to cold which can stop ripening process and destroy their flavor. They should be stored at room temperature preventing direct sunlight and consume as soon as possible. Tomatoes that are overripe and soft should be avoided. Slightly unripe tomatoes should be wrapped in paper bag with bananas and apples and left overnight to ripen by emitted ethylene gas from the fruits (Isaac *et al.*, 2016). They should then be placed in the refrigerator after they are fully ripe where they will remain fresh for 2 to 3 more days. For use, tomatoes should be brought out 30 minutes before to enhance flavor and juiciness. Canned tomatoes can be consumed within 6 months while opened tomatoes should be consumed within a week (Ravi Teja, 2017)

2.13: ARGINASE

Arginase a manganase-dependent enzyme is widely distributed in all creatures (Jenkinson et al., 1996). Arginase is one of the important enzymes in urea cycle and is important in ammonia detoxification (Mohamed SA et al., 2005; Mavri-Damelin et al., 2007: Peters SJ et al., 2008). The reaction products may be further metabolized to other amino acids and polyamines, including proline, putrescine, spermidine, and spermine, all of which play crucial roles in wide developmental processes and in biotic and abiotic stress responses (Flores et al., 2008; Brauc et al., 2012; Shi et al., 2014).

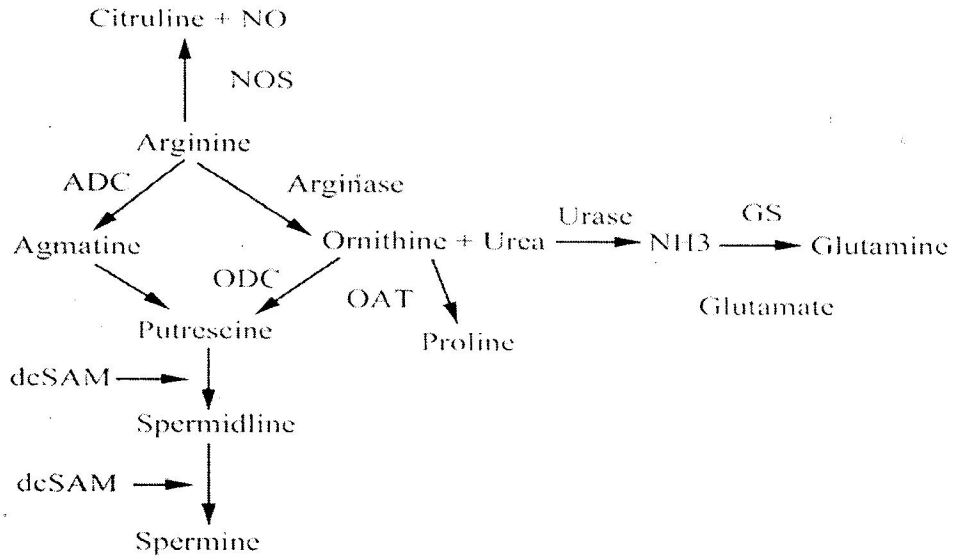

Figure 1 The pathway of Arginine Metabolism in plant

Source: www.wiki.com

L-ornithine is the precursor of polyamine synthesis; urea, a precursor of nitrite, nitrate and NO synthesis is involved in nitrogen metabolic cycle (Brownfield *et al.*, 2008). Polyamine produced as a result of breakdown of ornithine produced from arginase activities serves as a scavenger of reactive oxygen species (ROS) and a stress signal in plants regulating plant growth as well as biotic and abiotic stress (Wang *et al.*, 2011; Wimalasekera *et al.*, 2011).

It has been proved that majority of environmental stress such as dehydration, high salinity, chilling and other stresses will result in excessive accumulation of ROS quickly in plants which leads to oxidative stress and may lead to death (Mittler2006; Miller *et al.*, 2010; Zhu *et al.*, 2002, Zhu *et al.*, 2016). Plant development and a range of biotic and abiotic stress responses can be regulated by NO and polyamines in plants (Ji-Hong Liu *et al.*, 2015; Lozano-Juste *et al.*, 2011; Wang *et al.*, 2011).

In plants tissues this enzyme play various physiological and metabolic role, that is nitrogen mobilization during development of fruifts, bulbs and tubers, seed germination,

biosynthesis of glutamine and polyamine and biodegradation of canavanine to canaline and urea. Arginase may also participate in plants responses to herbivorous insects since it catalyses transformation of essential protein amino acid arginine to non-protein ornithine. Arginase activity increases sharply during germination in several species including shade plants, ginseng (Hwang et al., 2001), Arabidopsis and loblolly pines (Todd et al., 2001).

It has been shown in many studies that arGrow (arginine fertilizer) treated plants or plants with high arginine level form better improved root systems with optimal nitrogen level which results in faster establishment and growth of plants and higher average survival rate (SweTree Technologies, 2014).

2.14: History of Arginase

Arginase has been reported to be a metal activated enzyme (that is a metallo-enzyme) and was first reported by Hellerman and Perkins in 1935. They showed that arginaseis activated by divalent metal ion including cobalt, nickel, manganese and iron (Hellerman and Perkins, 1935). Robbins and Shields in 1956, further improved the "partial" purification of arginase. In their report they demonstrated that the activity of arginase was dependent upon manganese and found the optimal pH to be 9.4 (Robbins and Shields, 1956; Xie et al., 2004).

On inhibition, several workers in the late1970s and early 1980s investigated the effect of inhibitors on the activity of arginase (Rosenfeld et al., 1977; Bedino, 1977; Pace and Landers, 1981). Bedino in 1977, investigated the effect of the product inhibitor, ornithine, and thereafter proposed an allosteric model for regulation of the enzyme's activity.

2.15: Sources of Arginase

Arginase apart from being ubiquitous present in mammalian tissues has also been characterized from various insects, worms, molluses, fishes, bacteria, fungi, yeast actinomycetes, algae and plants.

Among bacteria producing arginase, the prominent ones include *Bacilli*. Mycobacteria (Zellar et al., 1954), *Proteus spp.* (Prozesky et al., 1973). Arginase has been found in the cotyledon of the broad leaves Victafaba and needles of scouts pine and its function in the germination of several species including soybeans (Matsubara and Sazuki, 1984; Kang and Cho, 1990. Arabidopsis and loblolly pines (Zomia et al., 1995; King and Gifford, 1997).

2.16: Arginine

Arginine is one of the most functionally diverse amino acid in living cells and a major storage and transport for organic nitrogen in plants. In addition to serving as constituent of proteins and essential metabolites for many cellular and developmental processes, arginine is a precursor for biosynthesis of proline and nitric oxide (winter*et al.*, 2015). Numerous researchers have showed that exogenous application of arginine significantly promoted plant growth and productivity (Nasser*et al.*, 2003) and mediates plant environmental stress tolerance (Zeid 2009, Nasibi*et al.*, (2011).

A research showed the role of arginine in alleviating the effect of salinity stress on mung beans (*Vigna radiate* L.Wilczek).Salinity stress reduced most yield components and nutritional value of produced seeds. However, spraying mung bean plants with arginine alleviated the harmful effect of salinity on plant height and plant dry weight. (Abd El-Monem, 2007).

Exogenous application of polyamine (a product of arginine), formed by indirect effect of arginase, to several plant species have been shown to promote cell division, cell differentiation and growth. (Xuet al. 2001; Nassar *et al.*, 2003). Study showed that putrescine, a product of arginine was exogenously supplied on the salt stressed plant, the grain yield of wheat increased (El-Bassiouny and Bekheta 2001)

In a research work, the application of arginine significantly promoted the growth and increased the fresh and dry weights, certain endogenous plant growth regulators, chlorophylls a and b and carotenoids in bean (*Vignasp*) (Nassar *et al.*, 2003); in wheat (Abd El-Monem *et al.*, 2007; El-Bassiouny*et al.*, 2008). It was observed induced disease resistance in tomato fruits via its effects on nitric oxide biosynthesis and defensive enzyme activity (Zheng*et al.*, 2011).

The alkalinization of the cytosol by induction of arginase and urease activity due to rapid hydrolysis is an active component of pathogen defense mechanisms (Polacco *et al.*, 2013).Overproduction of arginase in plants provides enhanced resistance to herbivores by acting as an anti-nutritive defence against phytophagous insect (Gregg A. Howe and Hui Chen, 2011). It has been proved that arginasealong with threonine deaminase (TD) induced by Jasmonic acid inducible protein (JIP) in *S.lycopersicum* plants attacks arginine in the mid-gut causing intestinal issues in *Maducasexta*larvae which infest the tomato plant hence conferring plant protection against herbivores (Chen *et al.*, 2004).

Treatment of *S. lycopersicum* fruit with hot air at 38°C enhanced the transcript levels of LeARG1 and LeARG2, the two genes encoding arginase, and thus arginase activity. Results showed that arginase induction may be partly involved in HA-induced chilling tolerance in tomato fruit, possibly by a mechanism involving activation of antioxidant enzymes and an increase in proline levels (Chen *et al.*, 2004).Treatment of *Solanum lycopersicum*with methyl jasmonate enhanced transcription levels of arginase genes. Results

showed that arginase is involved in methyl jasmonate -induced chilling tolerance of tomato fruit, possibly by ameliorating the antioxidant enzyme system of fruit and increasing proline levels (Chen *et al.*, 2004). The physiological role of arginase in physiologically and metabolically has been well described in several species of plants.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of samples

Unripe Tomato fruits was harvested on a vegetable garden in Akure, Ondo State, Nigeria while the ripe one was purchased at Oye market in OyeEkiti, Ekiti State, Nigeria. Thereafter the tomatoes were washed thoroughly in distilled water in the laboratory.

3.2 PREPERATION OF REAGENTS AND BUFFERS

3.2.1 Preparation of 0.33m 0f Arginine

About 57.49g of Arginine was dissolved in 100ml of distilled water

3.2.2 Enrlich Reagents

About 2g of p-dimethyl benzaldehyde was dissolved in 20ml of HCL and then diluted to 100ml by distilled water using a measuring cylinder

3.2.3 Preparation of 1mM $Mncl_2$

About 0.2g of $Mncl_2$ was dissolved in 100ml of distilled water

3.2.4 Tris buffer of pH 7.2

About 1.99g of tris base was dissolved in 1.3L of distilled water after which concentrated HCL was added to the solution to reduce the pH and NaOH was also added to bring the pH to 7.2.

3.2.5 Bradford Reagent

About coomassive brilliant blue was dissolved in 50ml of 95% ethanol and then added 100ml of 85% phosphoric and the solution was made up to 1L and then filtered.

3.3 Preparation of Crude Extract

The Tomato fruit was cut open and the seeds were separated from the mesocarps. 150g of the mesocarp was weighed and homogenized with 25ml of tris buffer in a blender and the homogenate was centrifuge at 4000rpm for 30minutes and the supernatant was collected.

3.4: ASSAYS FOR PROTEIN AND ARGINASE ACTIVITY.

3.4.1: Arginase Assay

Arginase activity was determined by the measurement of urea produced by the reaction with Ehrlich reagent according to the modified method of kaysen and strecker (1973). The reaction mixture, which contained 0.33mM arginine solution, 0.2 ml tris buffer (pH 7.2) containing 1mM manganese chloride and 0.05ml of the enzyme preparation was added. The mixture was incubated for 10minutes at 30^0c. The reaction was terminated by the addition of 1.25ml of Ehrlich reagent (containing 2.0g of p-dimethyl-aminobenzaldehydein 20ml 0f concentrated hydrochloric acid and made-up to 100ml by adding distilled water). The optical density reading was taken after 10minutes at wavelength of 450nm. The urea produced was estimated from the urea curve prepared by varying the concentration of urea between 0.1 and 1.0 micromole and 2.5ml of erhlich reagent and water were also added and a graph of optical density against urea concentration was plotted for extrapolation of the arginase activity. The unit of arginase activity is defined as the amount of enzyme that will produce 1micromole of urea per minute at 37^0c.

3.4.2 Protein Concentration

Protein concentration was determined using Bradford's reagent. The reaction mixture consist 200ml of the enzyme solution and 1000ml of Bradford's reagent (Bradford *et el*; 1976). The absorbance was read at 460nm.

Table 3.1: Summary of Arginase Assay

Reagent	Blank(ml)	Test(ml)
2mMTris Buffer	0.20	0.20
0.33M Arginine	0.15	0.15
1mM $MnCl_2$	0.05	0.05
Distilled Water	0.10	-
Crude Enzyme	-	0.10
Erhlich Reagent	1.25	1.25

Table 3.2: Summary of Protein Concentration

Reagent	Blank(ml)	Test(ml)
Distilled Water	0.2	-
Enzyme	-	0.2
Brandford Reagent	1.0	1.0

3.5 Kinetic Parameter

The kinetic parameters (K_m and V_{max}) of the enzyme were determined according to Kaysen and Strecker (1973). The K_m of arginine was determined by varying the concentration of L-arginine between 30 and 300mM. Both K_m and V_{max} were determined by using double reciprocal plot of Lineweaver and Burk (1934)

3.6 Effect of pH on Enzyme Activity

The effect of pH on the enzyme activity was determined according to the methods of Agboola and Okonji (2014). The enzyme was assayed using different buffer with different pH. 0.1ml of citrate buffer (pH 3-5), phosphate buffer (pH 6-7), tris base pH8 and borate buffer (pH 9.4-10) were used.

3.7 Effect of Metal ion on the Enzyme Activity

Arginase is a metallo-enzyme in which manganese acts as a co-factor as well as activator (Jenkinson *et al*, 1996; Dabir *et al*, 2005). The effect of divalent cations on the enzyme activity were determined following the assay method as described earlier. The divalent cation includes Mn^{2+}, Fe^{3+} and Na^{+}.

3.8 Inhibiting Effect of Some Compounds on Enzyme Activity

The effect of some inhibiting compounds on enzyme activity was performed as described by Agboola and Okonji (2014) which was used to determine their effect on the enzyme activity. The compounds include EDTA, UREA AND MERCAPTOETHANOL.

CHAPTER FOUR

4.0: RESULT

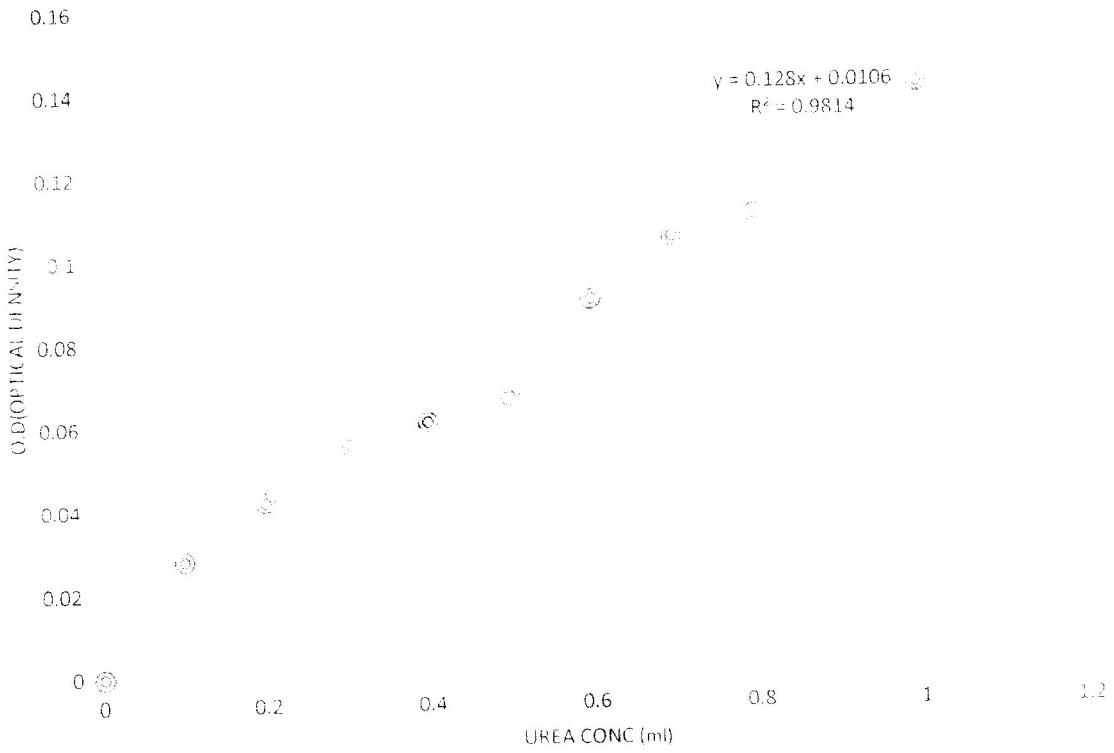

Figure 4.1: Standard Urea Curve

Note: Urea standard curve is a standard curve where the enzyme activity can be extrapolated or interpolated. The unit of arginase activity is defined as the amount of enzyme that will produce 1micromole of urea.

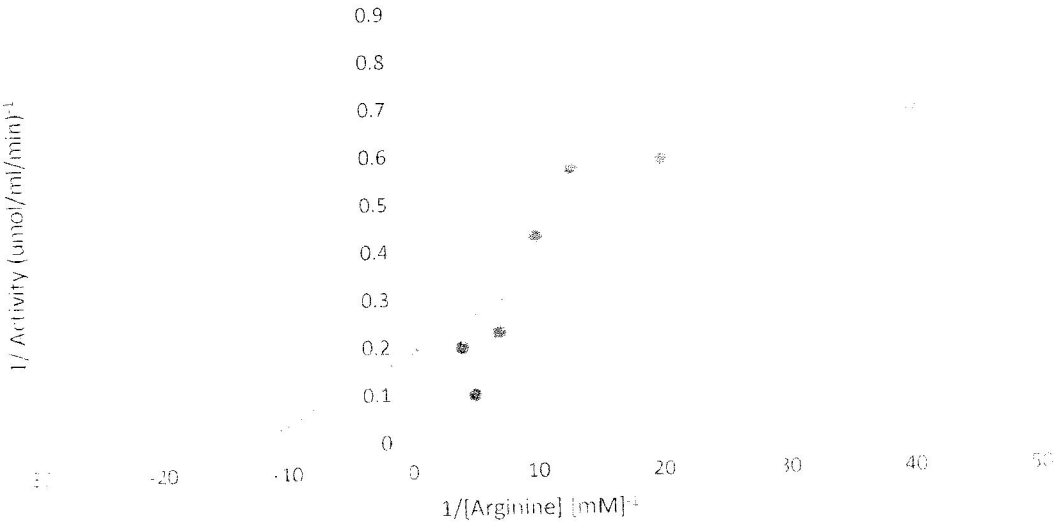

Figure 4.2: Lineweaver-Bork Plot for Varying Concentration of Arginine in Mesocarp of ripe tomato.

Lineweaver-Bork plot of 1/V at varying concentration of Arginine between 25mM and 225mM. The kinetic parameter is used for determining the Km and Vmax which helps us to determine the enzyme specificity and the velocity at which the reaction progresses.

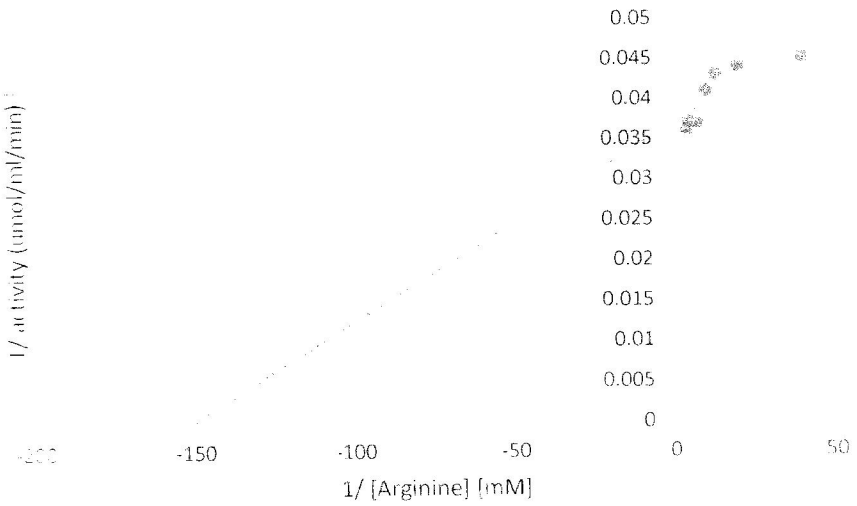

Figure 4.2.1: Lineweaver-Bork Plot for Varying Concerntration of Arginine in Mesocarp of Unripe Tomato.

Lineweaver-Bork plot if 1/V at varying concentration of Arginine between 25mM and 225mM.The kinetic parameter is used for determining the Km and Vmax which helps us to determine the enzyme specificity and the velocity at which the reaction progresses.

Table 4.2: Summary of kinetic parameters from the mesocarp of ripe Tomato

Substrate	K_m(mM)	V_{max}
Arginine	80	5

Table 4.3: Summary of kinetic parameters from the mesocarp of unripe Tomato.

Substrates	K_m(mM)	V_{max}
Arginine	6	27

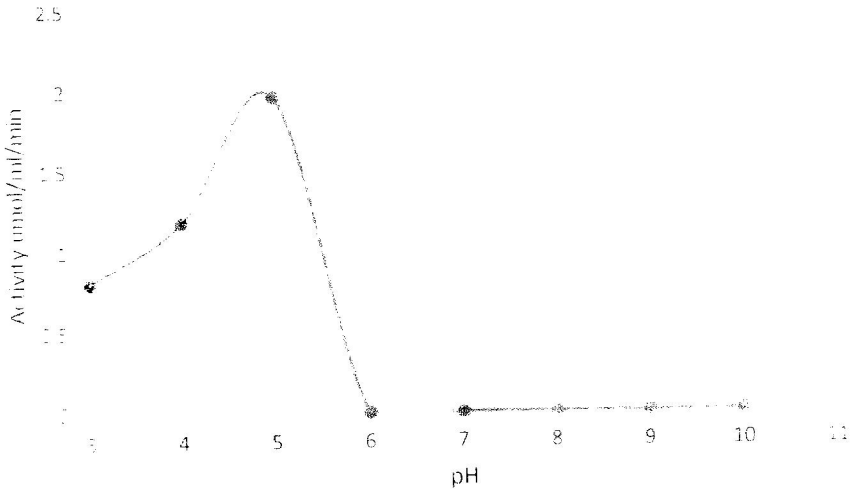

Figure 4.3: Effect of pH on the Arginase Activity in the Mesocarp of Ripe Tomato.

The optimum pH was determined using 50mM citrate buffer (pH 3.0-5.0); 50mM phosphate buffer (pH 6.0-7.0); 50mM borate (9.0-10.0). The optimum pH was 5.0 in the presence of $MnCl_2$ as shown in the pH activity curve.

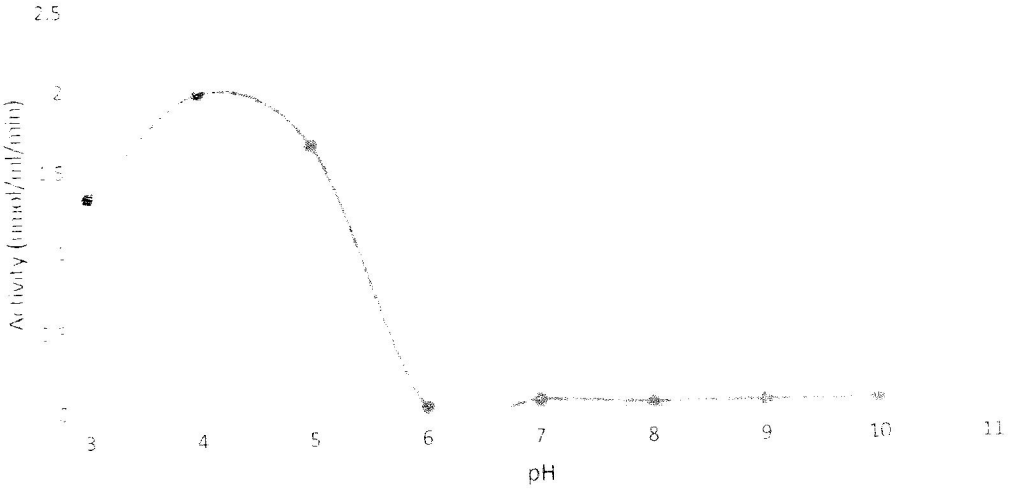

Figure 4.3.1: Effect of pH on Arginase Activity in the Mesocarp of Unripe Tomato.

The optimum pH was 4.0 in the presence of $MnCl_2$ as shown in the pH activity curve.

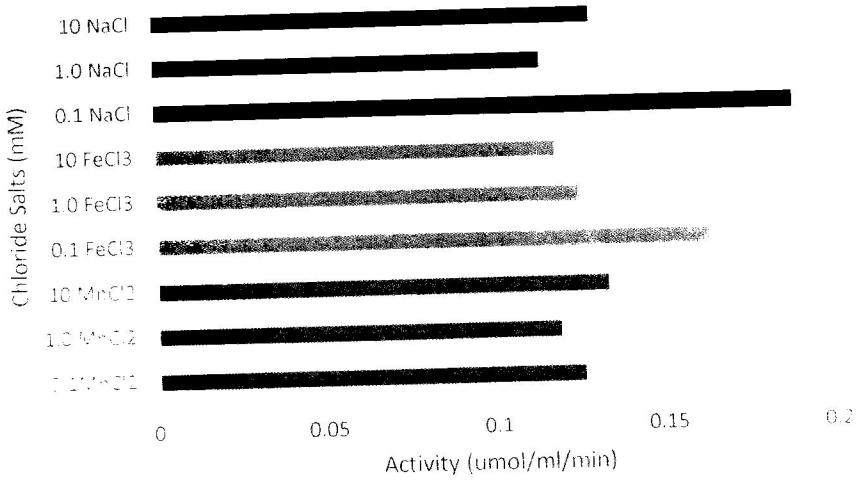

Figure 4.4: Effect of Metal ion on Arginase Activity in the Mesocarp of Ripe Tomato.

Arginase activity was strongly enhanced in the presence of 0.1mM of Na^{+} and 0.1mM Fe^{3+} in the mesocarp of ripe tomato as shown in bar above.

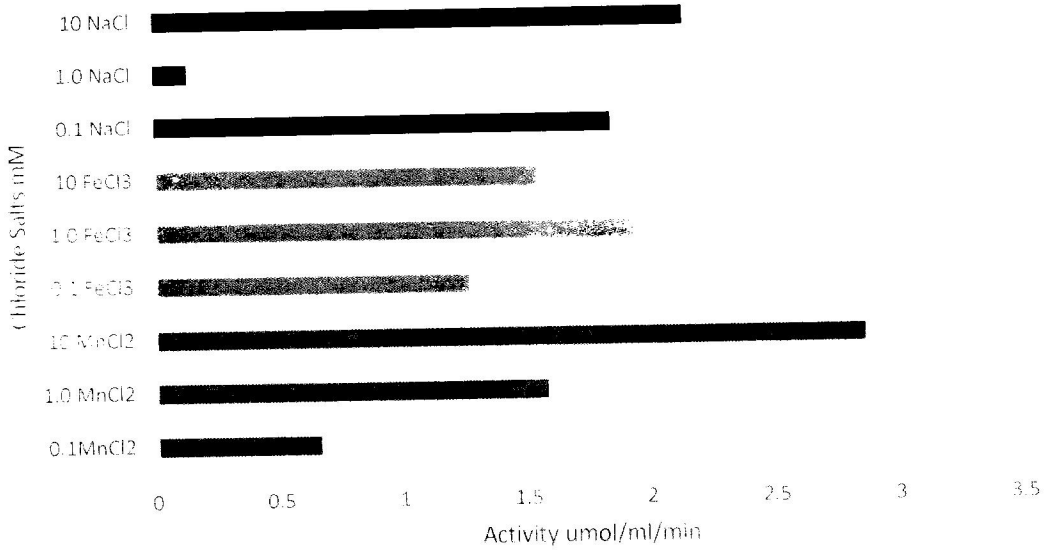

Figure 4.4.1: Effect of Metal ion on the Arginase Activity in the Mesocarp of Unripe Tomato.

Arginase activity was strongly enhanced in the presence of 10mM of $Mn^{2+}$and 10mM Na^{+} and was strongly inhibited in the presence of 1.0mMNa^{-} and 0.1mM Mn^{2+}in the mesocarp of unripe tomato as shown in the bar above.

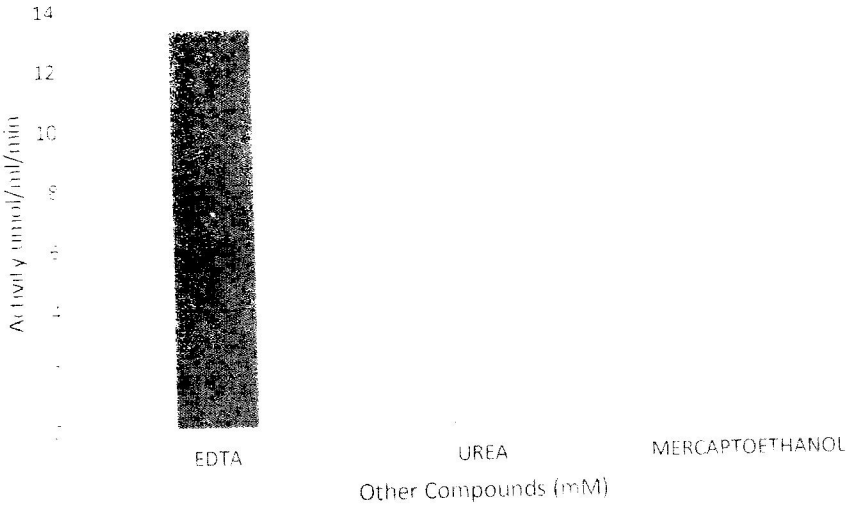

Figure 4.5: Inhibiting Effect of Some Compounds on Arginase Activity in the Mesocarp of Ripe Tomato.

Arginase activity was inhibited by urea and mercaptoethanol in the mecocarp of ripe tomato EDTA on the other enhance the activity of Arginase.

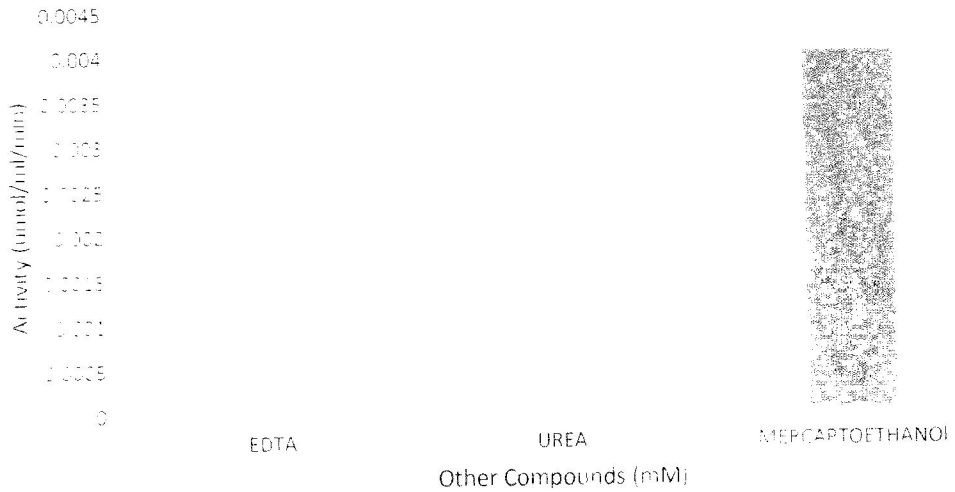

Figure 4.5.1: Inhibiting Effect of Some Compounds on the Arginase Activity in the Mesocarp of Unripe Tomato.

Arginase activity was inhibited by urea and EDTA in the mecocarp of unripe tomato, mercaptoethanol on the other enhanced the activity of Arginase.

DISCUSION

Arginase, a primordial enzyme, is found throughout the primary kingdoms of life (McGee *et al.*, 2004; Mohamed *et al.*, 2005). It plays a crucial role in the hepatic metabolism of most of the higher organism as a cardinal component of the urea cycle. Additionally, it occurs in numerous organism and tissue where there is no functioning urea cycle. The comparison of arginaseactivity in ripe and unripe tomato mesocarp reveals that unripe tomato have higher arginase activity when compared with the ripe tomato. Most of the studies on plant arginase have focused on its role in mobilizing arginine during early seedling germination. Arginase activity increases sharply during germination in several species including shade plant, ginseng (Hwang *et al.*, 2001), arabidopsis and loblolly pines (Todd *et al.*, 2001).

One of the most investigated factors influencing arginase activity is the effect of pH Many researchers have reported varied optimum pH for arginases from different sources. The effect of pH was carried out on ripe and unripe tomato mesocarp and an optimum pH of 5 was obtained for ripe tomato and an optimum pH of 4 was obtained for unripe tomato. The result was different from some reported pH for arginases from different sources. Okonji*et al.* (2013) reported an optimum pH of 8 for grasshopper argianse. A pH of 10.0 was reports for *Vigna catjang* cotyledon arginase (Dabir *et al.*, 2005). *F. gigantic* showed maximum activity at pH 9.5 (Mohamed*et al.*, 2005) while the pH of *H. niloticus* was 5.5. the purified arginase of Helicobacter pylori had an optimum acidic pH of 6.1 (McGee*et al.*, 2004).

Several K_m values have been reported for arginases from varying sources. The K_m value for ripe tomato and unripe tomato arginase were found to be 83 and 60 respectively as shown in table and table and the arginase showed preference to arginine as substrate The K_m value of tomato is in the range of other reported K_m (40-83mM) of plant arginase (Matsubara et al., 1984; Butin JP 1982; Kollofel C et al., 1975). It is interesting to note that at a higher

concentration of substrate; double reciprocal plot is non-linear and seems to indicate excess substrate inhibition.

Arginase activity was completely inhibited in the presence of urea and mercaptoethanol and strongly activated in the presence of EDTA in ripe tomato. Enzyme activity was strongly inhibited in the presence of EDTA and urea and strongly activated in the presence of mercaptoethanol in unripe tomato. Reports have shown that these compounds have varying effects on arginases from different sources (O'Malley and Terwilliger, 1974; Jenkinson et al., 1996)

Arginase is a metalloenzyme in which manganese acts as a cofactor as well as activator in almost all reported arginase (Ash, 2004; Dabir*et al.*, 2005). Most arginases are activated by manganese (Jenkinson *et al.*, 1996; Ensunsa *et al.*, 2004; Mohamed *et al.*, 2005).The effect of metals on ripe tomato was similar to most inhibitory effects reported for arginases. 0.1 $Fe^{2+}$and 0.1 Na^{+} strongly activated enzyme activity where 1.0 Na+ and 1.0 Mn^{2+}showed slight inhibition on the enzyme. The enzyme was strongly activated in the presence of $10Mn^{2+}$ and strongly inhibited in the presence of 1.0 Na^{+} for unripe tomato. Dabir*et al.*, (2005) reported the replacement of Mn ion with other metal ion such as Mg^{2+}, Co^{2+}, restored more than 60% while Ni^{2+}, and Fe^{2+} restored about 50% activity where as Ca^{2+}, Zn^{2+}, and Cd^{2+} inhibited *Vigna catjang* cotyledon arginase. In *F. gigantic* arginase, enzyme was activated by Mn^{2+} but was inhibited by Fe^{2+}, Ca^{2+}, Fe^{2+} and Zn^{2+} (Dabir *et al.*2005). The amount of Mn^{2+} required for optimal activity of the soybean axes enzyme was reported as 1mM, 1-2mM for human liver (Carvajal N *et al.*, 1971), 8-10mM for human erythrocytes (Nishibi *et al.*, 1970).

CONCLUSION AND RECOMMENDATION

This study showed a wide spread distribution of Arginase in the mesocarp of ripe and unripe tomato fruits and gave a significant insight on some various activators and inhibitors of the enzyme. The presence of this enzyme in Tomato may be a determinant of some physiological functions which include nitrogen mobilization.

Based on the result of this research, It is therefore recommended that tomato breeders should be encouraged to employ the use of arginine to improve their plant yield through exogenous application.

PLATES

A B

Plate 2: A: mesocarp of unripe tomato fruit, B: mesocarp of ripe tomato

PLATES

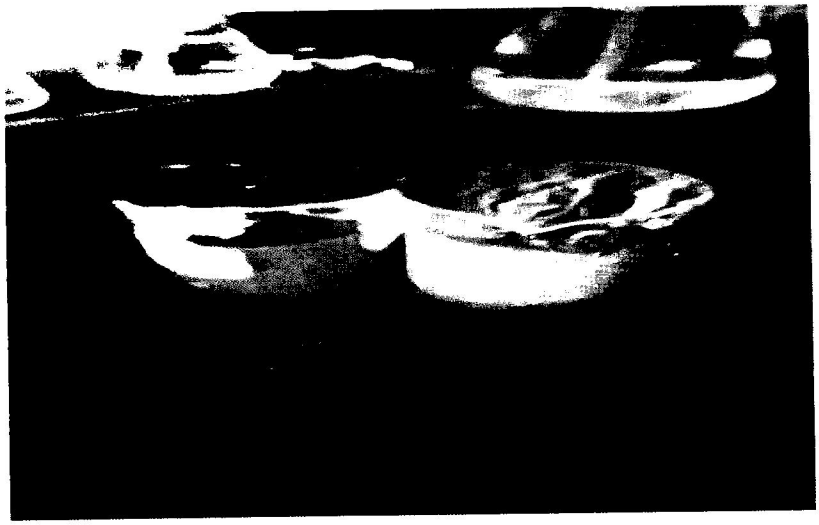

Plate 3: Enzyme source (homogenized tomato fruits).

PLATES

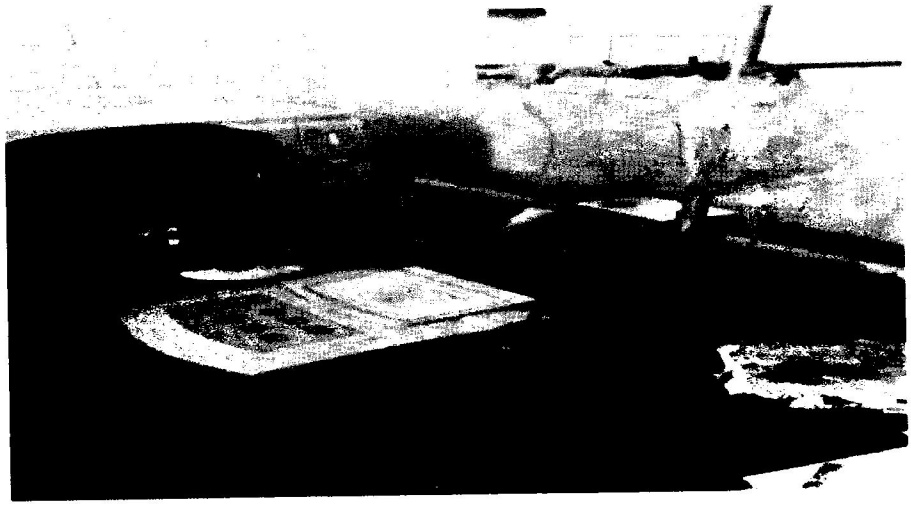

Plate 4: Preparation of tris- buffer (homogenization buffer).

PLATES

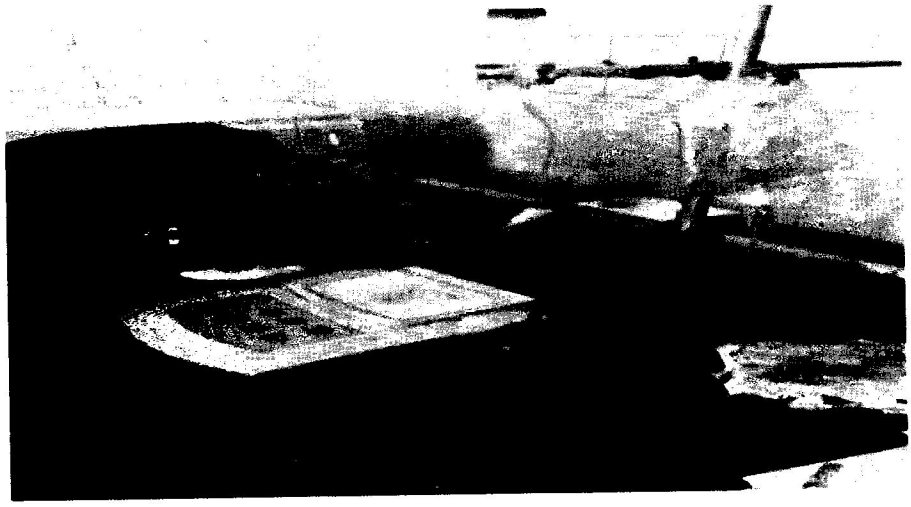

Plate 4: Preparation of tris- buffer (homogenization buffer).

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APPENDICES

EDTA	Ethylene Diamine Tetra-acetic Acid
NO	Nitric Oxide
OAT	Ornithine Aminotransferase
ODC	Ornithine Decarboxylase
ROS	Reactive Oxygen Specie
TD	Threonine Deaminase
JIP	Jasmonic Acid Inducible Protein