EFFECT OF ASH AS A PRESERVATIVE ON THE NUTRITIONAL QUALITY OF ORANGE (Citrus *sinensis)*

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AN UNDERGRADUATE PROJECT SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCE, UNIVERSITY OF BENIN, BENIN CITY EDO STATE, NIGERIA; IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN ENVIRONMENTAL MANEGEMENT AND TOXICOLOGY

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CERTIFICATION

This is to certify that this research titled "Effect of ash as a preservative on the nutritional quality of orange was carried out by "**SANDRA NKOYEN EHIEDU**" and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences. University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

DECLARATION

I**,** Nkoyen Sandra EHIEDU, hereby declare that this project, “Effects of ash as a preservative on the nutritional quality of orange” is a collection of my original research work and it has not been presented for any other qualification elsewhere. Information from any scholars (published or unpublished) and their contributions hereby have been duly acknowledged.

DEDICATION

This research project is dedicated to Almighty God the creator of heaven and the earth the only true God who know the end right from the beginning the architect of my life all Glory belong to him and to my family. May God bless you all and you will all eat the fruit of your labour.

ACKNOWLEDGEMENT

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ABSTRACT

The natural ripening process of fruit involves a series of physical and chemical transformations, leading to sweetness, coloration, softness, and enhanced taste. This research aimed to explore the impacts of two ripening methods on the nutritional value, namely; calcium carbide and wood ash, on orange. Freshly plucked green matured oranges were used for the determination of proximate analysis and mineral content (potassium (k), phosphorus (p), calcium (Ca), sodium (Na). Calcium carbide had the highest Ca, Mg, P, Na (111.10 mg/100g, 108.25mg/100g, 50.05mg/100g and 50.30mg/100g respectively and also shows significant differences between the two ripening methods based on the contents respectively. The results of the study showed that using calcium carbide as a ripening agent significantly reduced the nutrients in the fruit. Because of its possible effects on nutritional quality, ash has been used as a preservative in food preservation. The study evaluates changes in important nutritional characteristics such vitamins, minerals, and antioxidants using analytical techniques, such as nutritional analysis with untreated oranges. The results shed light on the effectiveness of ash as a preservative and how it affects oranges' nutritional value.

CHAPTER ONE

1.1 INTRODUCTION

In the world, the most widely grown fruit tree is the sweet orange (Citrus *sinesis (L)*). Mandarins, limes, lemons, grapefruits, sour and sweet oranges, and grapefruits are all members of the Rutaceae family, (Karoui and Marzouk, 2013). At the top of the fruit production hierarchy, citrus fruits are extremely valuable economically. The sweet fruit of orange trees is commonly grown in tropical and subtropical regions for eating whole or processing to obtain orange juice (Pandharipande and Makode, 2009). The fruit is peeled or chopped to avoid the bitter rind. Oikeh & Associates (2013). Large amounts of vitamin C, a powerful water-soluble vitamin that is vital for good health, are known to be present in citrus fruits. Additionally, it is known that they include other bioactive substances including carotenoids and a wide array of phenolic compound.

Ash has historically been used as a preventive measure for a variety of objectives,; due to its alkaline nature which helps inhibit the growth of bacteria and fungi from agriculture to food preservation, in a variety of cultures. For example, because of its alkaline qualities, which make circumstances unfavourable for pests, ash has been sprayed on crops to prevent pests and diseases (Ndakidemi *et al.,* 2006). This approach demonstrates the inventiveness of traditional knowledge and has been reported in areas where access to conventional pesticides may be restricted (Kubo *et al.,* 2002).

Ash has also been used to prolong the shelf life of perishable foods by preventing microbiological development. According to (Montagville and Matthews 2008), ash alkalinity elevates the pH of food, resulting in a less favourable atmosphere for microbial growth. This age-old practice of preserving food has been handed down through the centuries in societies that

may not have had access to refrigeration or other contemporary preservation methods. Ripening, which is the final step of fruit development, is an irreversible process that involves numerous physiological, biochemical, and organoleptic changes (Maduwanthi and Marapana, 2019). A number of intricate, independently connected events or changes result in the ripening of fruit. Seed maturation, colour, abscission, respiration rate, ethylene production rate, and tissue permeability are a few of these. Similar changes are seen in the content of proteins, organic acid, softening, development of wax on skin, generation of taste volatilities, and changes in the composition of carbohydrates (Maduwanthi and Marapana, 2019; Abbaset, 2021). Ripe fruits spoil quickly and are readily lost in transit (Mursalat *et al.,* 2013). Consequently, fruits are usually picked before they are fully ripe, transported, and then artificially ripened at their destination. Furthermore, fruit dealers gather immature fruits and employ artificial ripening agents to hasten the ripening process in order to meet high demand and increase their profits. While artificial fruit ripening is a quick process, it degrades the fruit's safety, sensory appeal, and nutritional value (Hossain *et al.,* 2015).

Artificial ripening has been shown to have detrimental impacts on the nutritive content of fruits, which can lower the fruits' nutritional quality and lead to important nutrient deficits in consumers, which can typically have a negative impact on their health (Rahman *et al.,* 2008). National and international emphasis has been focused on the impact of these artificial ripening agents on human health and food nutritional value. The nutritional content of artificially ripened fruits and the ensuing impact on human health, however, are the subject of little scientific research. Examining the impact of ash and calcium carbide as a ripening agent on the nutritional makeup (proximate, mineral, vitamin, and sucrose) and content of orangutans was the main goal of this study.

1.2 Aim and objectives of the study

The aim of this work was to examine the effects of ash as a preservative and other preservative method and its implications for the nutritional contents of oranges.

OBJECTIVE OF THIS STUDY

I. To determine the proximate analysis of the citrus fruit (orange)

II. to examine the impact of ash and calcium carbide on the nutritional elemental and content of orange

III. Its effects on sensory quality and nutritional value

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF ASH PRESERVATIVE

A food preservative is a chemical that, as a result of microorganism activity, inhibits, delays, or eliminates the growth of microbes or any other deteriorating molecule (Delores *et al.,* 2004). In order to ensure food security and sustainable farming practices, it is imperative that perishable agricultural produce be preserved. Of all the techniques used, the application of ash as a preservative has drawn interest due to its historical relevance and possible advantages in preserving the organic nature of fruits (Ryder, 2017). Ash application is an interesting option in fruit preservation, especially with regard to oranges. Oranges are known for their high nutritious content and delicate flavour, but because of enzymatic reactions and microbial activity, they can deteriorate and perish quickly. Given its historical use in food preservation and its possible modern significance in reducing spoiling and prolonging the shelf life of this prized fruit, the investigation of ash as a preservative for oranges piques intrigue. Ash has been used historically to preserve food since ancient cultures found it to be a simple yet efficient way to prolong the shelf life of a variety of perishable goods (Smith, 2015). Since ash is alkaline, it has long been known to prevent microbial growth and enzyme activity, which helps to preserve foods like grains, fruits, and vegetables. Ash has long been used as a preservative in a variety of cultures, from ancient civilizations to indigenous tribes, demonstrating its effectiveness in preserving food quality and edibility (Jones *et al.,* 2019). The objective of this literature review is to conduct a thorough analysis of how oranges' natural quality is affected by ash used as a preservative. This review tries to clarify the biochemical mechanisms behind ash's potential as a preservative, clarify its effect on oranges' sensory qualities and nutritional value, and investigate its usefulness

in the context of fruit preservation in contemporary agricultural and food systems by examining the body of research that has already been done. The study of ash as an orange preservative is a complex project motivated by a number of variables. First of all, because oranges are perishable, new preservation methods must be investigated in order to extend the fruit's shelf life without sacrificing its nutritional value or flavour profile. Second, because ash has long been used to preserve food and has natural antibacterial qualities, researchers are looking into whether ash may be applied to contemporary farming methods. The goal of this literature study is to provide a thorough analysis of how oranges' natural quality is affected by ash used as a preservative. This review looks at the body of research to clarify the molecular mechanisms behind ash's potential as a preservative, clarify its effect on oranges' sensory qualities and nutritional value, and investigate its usefulness in the setting of fruit preservation in contemporary food and agricultural systems. Furthermore, the use of ash as a natural preservative presents a promising way to reduce post-harvest losses while reducing the need for artificial preservatives and chemicals, in line with the ideals of environmentally friendly and sustainable food systems, as the world struggles with issues of food waste and sustainable agricultural practices (Garcia *et al.,* 2021).

2.2 HISTORY OF CITRUS FRUIT

Oranges were likely domesticated in south-east Asia by 2500 BC (Nicolosi *et al.,* 2008), when they were called "Chinese" apples (Ehler, 2011). It is currently the most extensively planted fruit tree in the world, having originated in southern China where it was long produced for commercial purposes. Today, it is grown worldwide in tropical, semi-tropical, and some warm temperate countries (Nicolosi *et al.,* 2000).

Nigeria and many other tropical and subtropical countries grow a lot of citrus (Piccinelli *et al,* 2008). Citrus ranks second globally in terms of production volume, behind bananas, with about 108 million tonnes produced (FAO Statistics 2006). A member of this family, the orange (Citrus sinensis) is a rich source of vitamins, particularly C, as well as adequate amounts of folacin, calcium, potassium, thiamine, niacin, and magnesium (Angew, 2007). Oranges are a significant fruit crop from an economic standpoint; as of 2005, production of 60 million metric tonnes of oranges was valued at $9 billion globally. Half of this total originated in the United States and Brazil (Goudeau *et al.,* 2008; Bernardi *et al.,* 2010). According to FAO estimates from 2009, there were nine million hectares of citrus planted worldwide, and the crop produced 122.3 million tonnes, making oranges the most popular fruit crop worldwide (Xu *et al,* 2013). Because of its many nutritional benefits, high vitamin content, and other applications, it is now produced practically everywhere in the world for human use.

2.3 NUTRIENT CONTENT AND FUNCTION OF CITRUS FRUIT

Most people consider citrus to be an excellent source of vitamin C. Citrus fruits, like most whole foods, also contain an impressive array of other essential nutrients, such as potassium, folate, calcium, thiamin, niacin, vitamin B6, phosphorus, magnesium, copper, riboflavin, pantothenic acid, and both glycaemic and non-glycaemic carbohydrate (sugars and fibre). Citrus also has no cholesterol because it is a plant food and has no fat, sodium, or fat. Fresh citrus also has a low average energy value which is significant for customers who are worried about gaining too much weight. For instance, a medium orange has between 60 and 80 calories, (Whitney and Rolfes, 1999).

2.4 FACTORS AFFECTING CITRUS FRUITS QUALITY

Fruit quality (FQ) is determined by a number of factors, some of which are irrigation, hormones, fruit thinning, harvest time, cultivar or rootstock, and mineral nutrition.

2.4.1 Climate

Climate Basically, from blossoming to harvest, the effects of climate on citrus fruit quality are the result of rainfall and heat accumulation According to (Volpe *et al.,* 2002), Furthermore, in assessing the fruit quality (FQ) of "Shogun" tangerines grown in Yala and Pattani, India, (Chelong & Sdoodee 2013) noted that lower soil moisture, lower precipitation, and higher mean temperatures had a detrimental impact on fruit development.

2.4.2 Cultivar

Specific fruit features within each citrus group can manifest differently based on other factors influencing quality. It was discovered that tangerine had the highest SS and carotenoid values, oranges had the highest total phenol content, and lemons had the highest TA and the lowest pH. (Roussos *et al.* 2013) characterised 22 cultivars of orange tree (Citrus sinensis), tangerine (Citrus reticulata Blanco), and lemon (Citrus limon Burm. f.). These scientists also mentioned that there was FQ variety among the cultivars of each citrus category. Researchers (Domíngues *et al.* 2003), (Cavalcante *et al.* 2006), and (Tazima *et al.* 2009) discovered similar outcomes while assessing the fruits of various orange tree cultivars.

2.4.3 Rootstock

To demonstrate how rootstock affects CTF quality, various rootstocks and cultivars have been tested in studies. That is to say, other from controlling the tree cycle and its phenology, rootstock effect on FQ is predicated on its impact on precocity and yield, according to Castle (1995).

2.4.4 Fruit Thinning and Hormone

Administration and fruit thinning are examples of horticultural methods that impact CTF quality. Thinning produces better fruit in terms of size, shape, colour, and SS content, as noted by (Ouma 2012). One can increase sink capacity or the amount of carbohydrates available from the source to increase fruit size. The availability of carbohydrates is enhanced at the source when auxins are employed during the physiological decline. Auxin application increases sink capacity during the fruit's linear growth stage. Applying ethylene can speed up ripening, whereas applying gibberellic acid can slow it down (Agusti *et al*., 2002). Citrus fruit output can be increased by using growth regulators (auxins, gibberellins, and cytokinins) to promote fruit set, according to (Galván-Luna *et al*., 2009).

2.4.5 Irrigation

Of all the horticultural disciplines, irrigation is one of the most important for CTF quality. In this sense, appropriate and uniform soil moisture can guarantee good FQ, according to (Vélez *et al.,* 2012). The potential evapotranspiration of "Tahiti" lime, which is produced in Piracicaba, São Paulo, Brazil, however, did not show any effects of irrigation on quality when (Alves Júnior *et al*., 2011) took varying water supply levels into factor. Furthermore, neither the traditional application method nor fertirrigation of fertiliser was seen to have an effect on the growth of Valencia orange FQ by (Duenhas *et al.,* 2005). On the other side, inadequate irrigation may be linked to a lack of water, which would be detrimental to FQ.

2.5 ORANGE; Human Health and Nutrition

Important micronutrients, such as vitamins C and E, carotenoids, and flavonoids, are found in the human diet and are necessary for maintaining health. These chemicals can be found in nearly every plant material and have multiple dietary sources (Di Majo *et al.,* 2005). These functional

food components, together with antioxidant nutraceuticals or phytochemicals, are what give foods their nutritional significance. Edible fruits and vegetables include phytochemicals that, when consumed, may favourably alter human metabolism and fend off degenerative and chronic illnesses (Tripoli *et al*., 2007). Consuming more fruits and vegetables helps protect against degenerative diseases including cancer and therosclerosis (Keys, 1995). Epidemiological studies have demonstrated a negative correlation between citrus-based dietary flavonoid intake and cardiovascular disorders (Hertog *et al.,* 1993; Di Majo *et al.,* 2005). Citrus fruits have long been prized for their healthy, nutrient-dense, and antioxidant qualities. They are the primary source of significant phytochemical nutrients. Oranges' high vitamin and mineral content has been scientifically shown to provide numerous health advantages. Furthermore, it is now recognised that other physiologically active, non-nutrient substances present in citrus fruits, such as soluble and insoluble dietary fibres and phytochemical antioxidants, may help lower the risk of cancer and a number of chronic illnesses, including obesity, coronary heart disease, and arthritis (Crowell, 1999).

2.5.1 Antioxidants

An orange that is mature and evenly spread with good colour intensity is considered to be of high grade. These oranges need to be solid, devoid of decay, flaws, and other imperfections, and have a fairly smooth texture and shape that are typical of the variety. Citrus flavonoids have been shown to have biological activity and positive health eThese plant-derived pigments, together with anthocyanin, contribute to the colour of flowers and fruits. Additionally, they can be found in dietary fruits and vegetables (Macheix *et al.,* 1990). They can also exert their antioxidant activity through a variety of mechanisms, such as metal chelation (Bombardelli and Morazzoni, 1993). Based on research, flavonoids effectively scavenge hydroxyl radicals by inhibiting them

and donating hydrogen atoms (Cillard and Cillard, 1988; Darmon *et al*., 1990). (Tripoli *et al.* 2007; Di Majo *et al.,* 2005). In addition to being a great source of vitamin C, oranges also include dietary fibre, folate, carotenoids, and flavonoids, which are bioactive substances that guard against degenerative illnesses and cancer (Ejaz *et al.,* 2006). Eating meals high in vitamin C strengthens the body's defences against infections and helps the blood remove dangerous, pro-inflammatory free radicals. Hesperetin and narigenin are just two of the many phytochemicals found in sweet oranges. As an antioxidant, free radical scavenger, anti-inflammatory, and immune system modulator, naringenin has a bioactive impact on human health.

2.5.2 Anti-inflammation

Citrus flavonoids have anti-inflammatory properties because they contain regulatory enzymes (phosphodiesterase, phospholipase, lipoxygenase, and cyclooxygenase) that regulate the production of biological mediators, which activate specialised cells and endothelial cells involved in inflammation. These enzymes may be inhibited by flavonoids, which can also reduce the immunological and inflammatory responses (Tripoli *et al*., 2007). Sure enough, citrus Flavonoids have the ability to block the kinases and phosphodiesterases that are necessary for the activation and transmission of cellular signals. Additionally, they have an impact on the activation of T and B lymphocytes, two types of cells involved in the immunological response (Manthey *et al.,* 2001). Citrus flavonoids also stop atheroma development, which in turn prevents atherosclerosis (Hertog *et al*., 1993). Hesperidin derived from citrus cultures may be useful as a mild anti-inflammatory agent in therapeutic settings, according to (Tripoli *et al.,* 2007). It may also be a good precursor to novel flavonoids that possess this property (Da Silva *et al*., 1994). Hesperidin also inhibits the overexpression of cyclooxygenase-2, inducible nitric oxide synthase (iNOS), prostaglandin E2 overproduction, and nitric oxide synthase (iNOS)

induced by lipopolysaccharide (LPS), according to studies conducted using mouse macrophage cells. prostaglandin E2 overproduction, nitric oxide (NO), and oxide synthase (iNOS) (Sakata *et al*., 2003).

2.5.3 Anti-Cancer and Anti-Arteriosclerosis

According to (Elangovan *et al*., 1994 and Hirano *et al.,*1994), citrus flavonoids can prevent cancer via selectively cytotoxic, antiproliferative, and apoptotic effects. Because flavonoids are antimutagenic and can absorb UV light, they shield DNA from oxidative damage (Stapleton and Walbot, 1994). They counteract free radicals, which when produced close to DNA, encourage mutations. This has been demonstrated in mice with c-ray-irradiated bodies (Shimoi *et al.,* 1994). By directly interacting with the tumor-causing chemicals, such as bleomycin-induced chromosomal abnormalities, flavonoids can also preserve DNA (Heo *et al.,* 1994). Citrus flavonoids in the cardiac and hepatic tissue of syngenetic rats have been shown to prevent the growth of rat malignant cells and their ability to form tumours (Bracke *et al*., 1989). According to (Bracke *et al.* 1989, 1991), citrus flavonoids' capacity to act as such is predicated on their ability to hinder cell motility. Aside from these nutrients, oranges are also high in calcium, folic acid, iron, chlorine, manganese, zinc, salt, phosphorus, iodine, pectin, beta-carotene, and fibre and amino acids. A solitary orange is purported to possess over 170 phytonutrients and more than 60 flavonoids possessing anti-tumor, anti-inflammatory, antioxidant and blood clot-inhibiting qualities, all of which support general health (Cha *et al*. 2001).

2.5.4 Anti-obesity

Sweet oranges are low in calories, have no cholesterol or saturated fats, and are high in dietary fibre and pectin, which is particularly beneficial for obese people. By attaching to compounds in

the colon that cause cancer, pectin, a bulk laxative, shields the mucous membrane from exposure to harmful substances. It has also been demonstrated that pectin lowers blood cholesterol levels by binding to bile acids in the colon and preventing the colon from reabsorbing cholesterol (Walton *et al.,* 1945). The alkaloid synephrine, found in orange peels, lowers the liver's production of cholesterol. Oranges include antioxidants that fight against oxidative stress, which oxidises blood LDL (low-density lipoprotein).

2.5.5 Wholesome Health

Oranges are also a good source of vitamin A and other flavonoid antioxidants with antioxidant qualities, including lutein, beta-cryptoxanthin, beta-carotenes, and zeaxanthin. For the skin, mucous membranes, and eyes to remain healthy, vitamin A is required. It is also an excellent source of B-complex vitamins, including folate, pyridoxine, and thiamine. In the sense that the body needs to obtain certain vitamins from outside sources, they are vital. Minerals like calcium and potassium are also abundant in orange fruit. Blood pressure and heart rate can be regulated with the help of potassium, an essential component of cell and body fluids.

In addition to being necessary for healthy skin and mucous membranes, vitamin A is also crucial for eyesight. Eating fruits that are naturally high in flavonoids protects the body against cervical and lung cancer. Additionally, orange fruit has excellent mineral content, including calcium and potassium. Potassium regulates blood pressure and heart rate and is a crucial component of body and cell fluids. The orange's alkaline qualities encourage

the digestive fluids, relieving constipation in the process. Frequent use of orange juice lowers the risk of kidney stone formation from calcium oxalate. Oranges include polyphenols that shield the body against viral illnesses. Oranges keep your skin looking young and fresh by shielding it from

the damage produced by free radicals (Tsuda *et al.,* 2004). Oranges can be juiced and then further processed into concentrate, which can be consumed straight away or used in a variety of soda and cocktail drinks, punches, orangeades, and liqueurs (though many of these are produced using sour oranges instead of sweet ones, or a combination of the two). Orange peels and fruits are used in a variety of sweets, including candies, biscuits, cakes, and jams and marmalades. Orange seed oil is also used in cooking and as a component in plastics. Orange peel oil is used as an essential oil in perfumes. It is also extracted from blossoms, leaves, and twigs.

2.6 CALCIUM CARBIDE ($Cacl_2$)

Another inexpensive chemical that was once widely used for orange ripening in developing nations was calcium carbide, which costs about Rs 25–30 per kilogramme and can ripen 200 kg of orange. A trader only needs to wrap a tiny amount of CaC_2 in a paper package and store it next to a box or pile of fruits (Hossain *et al.,* 2015). Fruit ripening is accelerated when CaC_2 combines with moisture to produce acetylene gas, an analogue of ethylene. An immature fruit may occasionally acquire ripening hue when calcium carbide is used. CaC_2 was employed carelessly, preferring to be used above other suggested methods of ripening, like exposing fruits to ethylene gas. The industrial grade of calcium carbide has up to 95 parts per million of arsine and 3 parts per million of phosphine gas released, respectively, and contains traces of arsenic and phosphorus hydride, all of which are harmful to human health if consumed (Rahman *et al.,* 2008) which cause cancer. A considerable proportion of expectant mothers ingested fruit ripened with carbide, resulting in malformations in the offspring (Rahim, 2012). Alkaline in nature, calcium carbide irritates the stomach mucosal tissue (Fattah and Ali, 2011). It was clear that eating carbide-ripened orange caused gastrointestinal upsets, as (Siddiqui and Dhua 2010) reported. CaC_2 ripened fruits are also excessively soft, less flavorful, and have a shorter shelf life. A fruit

that has been artificially ripened would have a yellow exterior peel, but its internal tissue would either not be ripe or would still be raw and green (Rahman *et al.,* 2008). According to (Rahman *et al.,* 2008), applying CaC_2 to unripe fruit necessitates adding more of the chemical to ripen it, making the fruit even more bland and potentially hazardous. While it was discovered that these artificially ripened fruits had improved cosmetic quality, their organoleptic quality suffered, particularly when collected fruits were treated without taking into account their maturity status (Medlicott *et al.,* 1988; Rahman *et al.,* 2008).

2.7 HEALTH EFFECTS OF ARTIFICIAL RIPENING

Ripening agents often include calcium carbide. This is a result of its low cost and ease of acquisition. However, consuming fruits that have been ripened with calcium carbide presents a serious risk to consumers' health (Rahim, 2012); traces of phosphorous and arsenic have been discovered in them. Neurological issues can also be brought on by the carcinogen calcium carbide. It can result in peripheral neuropathy and tingling in the hands and feet (Rahim, 2012). Calcium carbide-ripened fruits are extremely detrimental to one's health. particularly the nervous system. Acetylene, which is derived from carbide, restricts the quantity of oxygen that the brain can use. In its early stages, it can produce delirium, headaches, vertigo, disorientation, seizures, and even coma. In the long term, it may lead to mood fluctuations and memory loss. There have been reports of vomiting, diarrhoea, and abdominal pain following the consumption of fruits ripened using calcium carbide. Other side effects include skin burns, allergies, and jaundice (Fattah and Ali, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 samples collection and preparation

The unripe fruit (orange) were plucked from university of Benin (Hall 1), Edo state, Nigeria. Unripe oranges with uniform color and size were selected. A powdery residue of wood ash was collected from a fireplace, Calcium carbide was procured from the local chemical vendor in the local market in Uselu, Egor local Government, Edo state Nigeria. The unripe fruit were separated into two equal batches A and B. Batch A was rubbed by ash and allowed to ripen, Batch B was exposed to a different type of ripening agent (calcium carbide). Each were placed in separate plastic bags. The ripening of treated and untreated oranges were carried out in a closed laboratory.

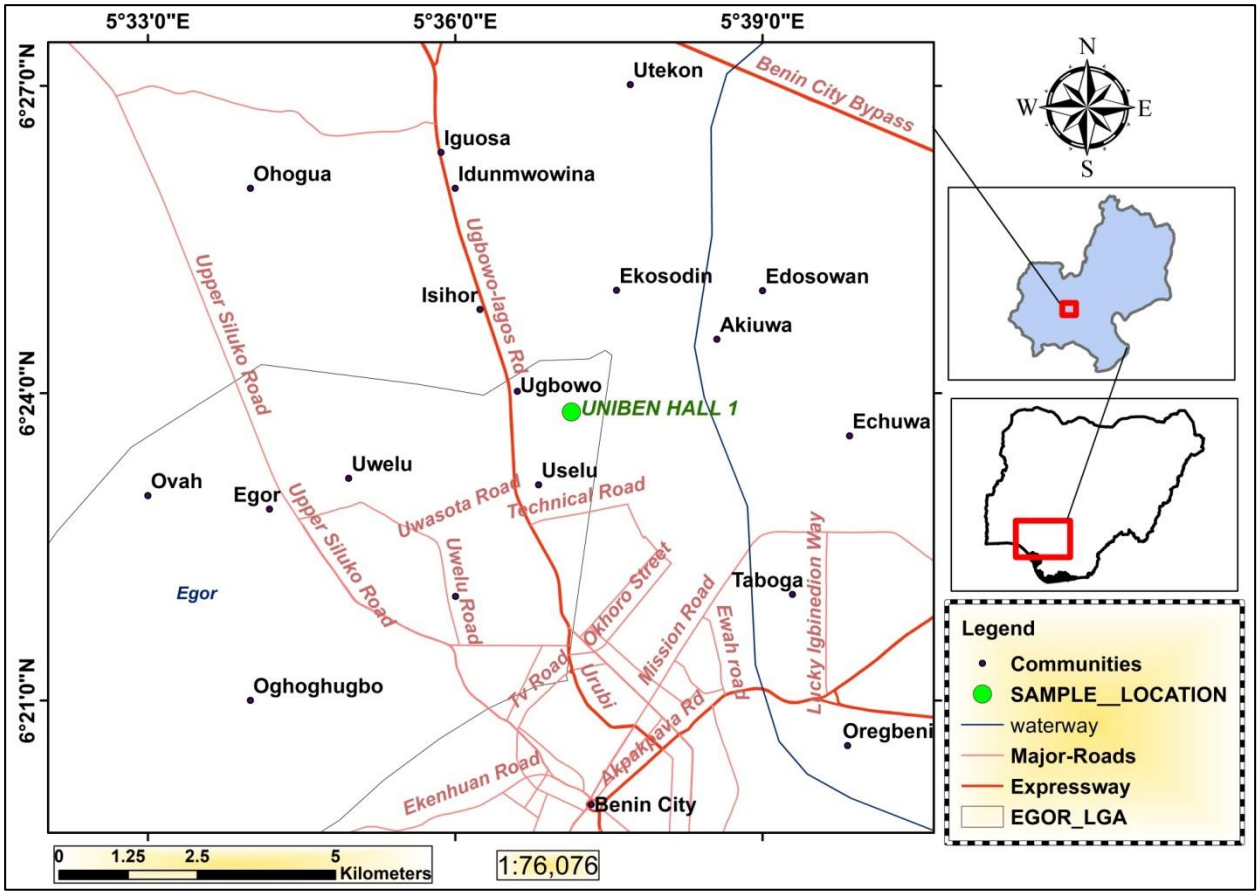

Figure 1: Map showing study area

3.2 Proximate Analysis of Sample

Moisture content, ash content, crude fat, crude fibre, and crude protein were all assessed by proximate analysis of the samples using the techniques outlined in AOAC, the method of difference was used to quantify the amount of carbohydrates present in the samples.

3.3 Determination of Moisture Content

2g of each processed samples were placed in a crucible and heated at 1050C until a constant weight was attained. According to (Rangma method 2018). The moisture content of the sample was calculated in percentage base on the equation

%Moisture Content = X 100

Where: W1= initial weight of empty crucible; W2= weight of crucible + sample before drying; W3 = weight of crucible + sample after drying

3.4 Determination of Ash Content

2g of each sample were placed in a crucible and was then placed in a muffle furnace at 500c and allowed to stay for 6 hours; after this process it was cooled in a desiccator , at room temperature. A.O.A.C. (2019) it was weighed to get the weight of the ash using the formula

%Ash Content = X 100

3.5 Determination of Crude Fiber

Crude fiber content was determined using the method of A.O.A.C. (2019) 5g of each of the sample and 200ml of 1.25% H_2SO_4 were heated for (30) minutes and filtered with a Büchner funnel. The residue was washed with distilled water until it was acid free. 200ml of 1.25% NaOH was used to boil the residue for (30) minutes. It was filtered and washed with distilled

water until it was alkaline free. It was then rinsed once with 10% of HCL and ethanol. Finally the residue was put in a crucible and dried at 105c in an oven. It was then cooled in a dessicator and then ignited in a muffle furnace at 500c for 60minutes to obtain the weight of the ash. The

%Crude Fiber = X 100

3.6 Determination of Crude Fat

This was carried out using the soxhlet extraction method. 10g of each of the sample were weighed and wrapped with a filter paper and placed in a thimble. The thimble was covered with a cotton wool and and placed in extraction column that was connected to a condenser. 200ml of age and was used to extract the lipid. The fat content was obtaine using

%Crude Fat = X 100

3.7 Determination of Protein Content

Two (2) grams of each sample was weighed along with 20cm of distilled water into a micro-Kjeldahl digestion flask. It was shaken and allowed to stand for some time. One tablet of selenium catalyst was added followed by the addition of 20cm concentrated hydrogen tetraoxosulphate. The flask was heated on the digestion block at 1000C for 2 hours, until the digest became clear. The flask was removed from the block and allowed to cool. The content was transferred into 50cm volumetric flask and diluted with water. An aliquot of the digest (10cm3) was transferred into another micro-Kjeldahl flask and placed in the distilling outlet of the micro-Kjeldahl distillation unit. A conical flask containing 5cm of boric acid indicator was placed under the condenser outlet. Sodium hydroxide solution (10cm3, 40%) was added to the content in the Kjeldahl flask by opening the funnel stopcock. The distillation starts and the heat supplied were regulated to avoid sucking back. When all the available distillate was collected in 5cm of

Boric acid, the distillation was stopped. The nitrogen in the distillate was determined by titrating with 0.01M of H2S04; the end point was obtained when the colour of the distillate changed from green to pink. The percentage nitrogen was calculated and multiplied by 6.25 to obtain the value of the crude protein.

% Nitrogen= X 100

Where: Vs = Titre value of the sample: Vb = Volume of acid required to titrate: Nacid = Normality of acic: W= weight of sample in grams

3.8 Determination of Carbohydrate Content

The carbohydrate content was determined by subtracting the summed-up percentage compositions of moisture, protein, lipid, fibre, and ash contents from 100. The percentage carbohydrate was obtained using

% carbohydrate =100 - (%protein + % moisture +% Ash + % Fiber)

3.9 Mineral Analysis

The method of A.O.A.C. was employed for the determination of mineral content. One (1) gram of the pulverized sample was placed in a crucible and ignited in a muffle furnace at 500C for 3 hours. The resulting ash was dissolved in10ml of 10% HNO3 and heated slowly for 20 minutes. After heating, it was filtered and the filtrate was used for the determination of mineral content. Atomic Absorption Spectrophotometer (AAS) was used to determine Ca while flame photometer was used for the determination of Na in the filtrate.

VITAMIN ANALYSIS

3.10 Determination of vitamin A

The beta carotene was determined by soaking 1g of the sample in 5ml of methanol for 2 hours at room temperature under dark condition in order to get a complete extraction. The beta carotene layer was separated using hexane through separating funnel. The volume was made up to 10ml with hexane and then this layer was again passed through Sodiumsulphonate through a funnel in order to remove any moisture from the layer.

Beta-carotene (100g) = Absorbance (436) x V x D x 100 x 100/ W x Y

Where: V = Total volume of extract: D= Dilution factor: W =Sample weight: Y = Percentage dry matter content of the sample

3.11 Determination of Vitamin C

10g of each sample was extracted with 50ml. Extracting solution for 1 hour and filtered through a Whatman filter paper into a 50ml volumetric flask and made up to the mark with the extracting solution. 20ml of the extract was pipette into a 250ml conical flask and 10ml of 30% KI was added and also 50ml of distilled water added. This was followed by 2ml of 1% starch indicator. This was titrated against 20% CuSO4 solution to a dark end point.

%Fat content = Vit . C ( ) = 0.88 X X X

Where: Vf = Volume of extract, T = Sample titre – blank titre

3.12 Statistical Analysis

Analysis of variance (ANOVA) was performed on the gathered data using SPSS version 21. We used the Duncan New Multiple Range Test (DMRT) to differentiate significant means ($p \leq 0.05$).

3.13 Sensory Attribute

The effect of wood ash and carbide treatment on the sensory attributes of orange (Citrus sinensis) in colour, appearance, firmness, odour and general acceptability and the difference was significant.

CHAPTER FOUR

RESULTS

4.1 Proximate Analysis

The proximate analysis of the orange (Table 1) showed that the moisture, fat, and protein levels in the sample (calcium carbide induced, ripened orange and ash ripened orange) and naturally ripened orange) differed significantly ($p<0.05$). Furthermore, there was no statistically significant variation ($p<0.05$) observed in the amounts of ash, fibre, and carbohydrates between the control and sample groups (ash and carbide ripened orange). The samples (ash and carbide ripened orange) contained the highest concentration of moisture, ash, and fibre, while the control had the highest concentration of fat, carbohydrate, and protein. In the same way, the sample (ash ripened), moisture, lipid, and protein content all differed significantly ($p<0.05$). Similarly, the levels of ash, fibre, and carbohydrates did not differ significantly ($p >0.05$).

Table1: Proximate composition of artificially (Ash and Carbide) and naturally ripened fruit

(%)	Sample A (Natural)	Sample B (Ash control)	Sample C (Carbide control)
Moisture	84.25±0.7	80.21±0.07	85.71± 0.1
Ash	0.18±0.00	0.45±0.01	0.25± 0.04
Fiber	0.49±0.01	0.07±0.01	0.52±0.01
Fat	0.15±0.00	0.12±0.01.	0.08±0.01
Carbohydrate	13.58±0.03.	12.75±0.01	13.61±0.01
Protein	0.88±0.04	0.15±0.06	0.29±0.04

Means of three determinations ±SD; no significant difference is observed between means within a row ($p>0.05$).

4.2 Mineral Analysis

A statistically significant ($p<0.05$) variation was noted between the natural and artificially ripened orange mineral levels (calcium and sodium). With the greatest concentration was the artificially ripened group

Table 2: Mineral composition of artificially (Ash and Carbide) and naturally ripened fruit

(ppm)	Sample A (Natural)	Sample B (Ash control)	Sample C (Carbide control)
Calcium	80.35±0.07	97.70±0.28	111.10 ± 0.14
Magnesium	46.15±0.06	55.15±0.37	108.25±0.07
Potassium	30.0±0.00	20.05±0.02	50.05±0.07
Phosphorus	42.99±0.18	46.19±1.20	98.04±0.06
Sodium	58.55±0.2	36.10±0.00	50.30±0.28
Zinc	2.34±0.12	1.40±0.10	31.18±0.21

Means of three determinations ±SD; no significant difference is observed between means within a row ($p>0.05$).

4.3 Vitamin Analysis

The data in Table 3 below demonstrate that the levels of vitamins C and A in the samples (A, B and C) differed significantly ($p<0.05$). The concentration was highest across the artificially ripened group

Table3: Vitamins composition of artificially (Ash and Carbide) and naturally ripened fruit

	Sample A (Natural)	Sample B (Ash control)	Sample C (Carbide control)
Vitamin A (µg/100g)	565.33 ± 8.22	282.74 ± 0.23.	363.10± 8.22
Vitamin C (mg/100g)	102.07± 0.01	76.28 ± 1,33	73.92 ± 0.23

Means of three determinations ±SD; no significant difference is observed between means within a row ($p>0.05$).

CHAPTER FIVE

5.1 DISCUSSION

After ripening with Calcium carbide, the moisture, ash and fibre levels increased in orange. This finding is consistent with the findings of (Sogo-temi *et al*. 2014), who investigated the nutritional composition using biological and chemical ripening methods. This In addition, the fruits that were ripened naturally contained more protein than those that were ripened with calcium carbide. This is also consistent with the observations made by Sogo-temi *et al.* (2014), who reported a drop in protein concentration during calcium carbide ripening. This decline may be the consequence of a significant reduction in nitrogen during the fast ripening process.

Mineral analysis showed a drop in calcium levels following ripening with calcium carbide. Nonetheless, the results of (Oguntade and Fatumbi 2019 and Bawa *et al.,* 2020) refute this conclusion. These investigations show that after ripening with calcium carbide, calcium levels increased.

It has been noted that applying wood ash considerably raised the amount of nutrients in orange (Citrus simensis) This is in line with the (Akinmutimi *et al.*, 2013). Samples treated with wood ash fared better in all storage temperatures, but particularly at 18 oC. This could be explained by the fact that wood ash has higher concentrations of the minerals (sodium and calcium), which can increase the produce's shelf life. There hasn't been any work done on this.

The results from (Bawa *et al.,* 2020) regarding Sodium reduction in Calcium carbide-ripened fruits align with our current study's findings. Similarly, (Yeasmin *et al*., 2019) observed lower Sodium levels in carbide-ripened fruits compared to naturally - ripened ones. However, (Iroka *et al.*, 2016) reported an opposite trend, showing an increase in Sodium content for Calcium

carbide-ripened fruits, which contrasts with our observations. Additionally, the fruits ripened using Calcium carbide exhibited a decline in the levels of vitamin A and C.

The detection of arsenic and lead in fruits ripened with Calcium carbide aligns with findings from other studies (Bawa *et al.,* 2020; Abbas *et al.,* 2021) that have reported similar results. Industrial Calcium carbide is known to contain elevated levels of arsenic, lead, and phosphorus compounds, all of which pose health risks to humans and can contaminate artificially ripened produce, as reported in previous studies (Muanya, 2019). This elucidates the presence of arsenic and lead in the artificially ripened fruit examined in this study.

CONCLUSIONS

This research has investigated how different ripening methods impact the proximate and mineral composition of ripened Orange. The proximate composition of orange treated with calcium carbide was notably higher than what has been documented in previous studies. In contrast, orange ripened with wood ash showed similar levels of ash, moisture, fat, and carbohydrate content.

The application of Calcium carbide to induce artificial ripening leads to a notable reduction in the essential nutritional elements of fruits. Treatment with Calcium carbide decreases the levels of protein, fat, vitamin A and C, Calcium, Sodium, and sucrose in the fruits under investigation, while concurrently increasing moisture, ash, and fiber content. Although the carbohydrate content of bananas ripened with Calcium carbide increases, oranges subjected to the same treatment experience a decrease in carbohydrate content. Furthermore, the use of Calcium carbide results in the presence of traces of lead and arsenic in the samples. Overall, this study highlights that employing Calcium carbide as a ripening agent causes a significant depletion of fruit nutrients.

RECOMMENDATION

While Calcium carbide accelerates the ripening process, its usage should be discouraged due to the associated health risks. It's imperative for the Government, traders, and the public to collectively raise awareness about the dangers posed by Calcium carbide. Restrictions on the procurement and sale of this banned compound in the open market should be implemented.

Exploring the utilization of wood ash as an alternative approach for fruit ripening is advisable. Given its natural composition, wood ash may offer a safer alternative to Calcium carbide. However, comprehensive research is essential to assess its efficacy and any potential effects on fruit quality and safety. Moreover, it's crucial to establish appropriate guidelines and regulations to ensure the safe and responsible application of wood ash in fruit ripening methods.

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Plate 1: showing carbide ripened citrus *sinensis*

Plate 2: showing woodash ripened citrus *sinensis*

Plate 3: showing naturally ripened citrus *sinensis*