EFFECT OF HEAT ON ASCORBIC ACID CONTENT OF SOME LOCALLY SOURCED FRUITS (MANGO, TANGERINE, LEMON AND GRAPE)

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ABSTRACT

Ascorbic acid plays a critical role in various metabolic activities such as the absorption of iron from nonheme products and negates the formation of reactive oxygen, a major product responsible for oxidative stress and recent studies has shown that it is thermally labile. The effect of heat on its content in fruit juices has been successfully investigated.

Fresh fruit samples were obtained from New Benin market and the juice extracts were obtained using mechanical press after which temperatures of 40, 60, 80, and 100°C were applied and the ascorbic acid contents of the fresh juice extracts and after treatment were determined using iodimetry at interval of 24 hours for five days.

From the results obtained, the ascorbic acid content was found highest for mango juice extract with a value of 11.40mg. The values obtained for grape, lemon, and tangerine are 10.42, 5.86, and 4.98mg respectively.

From the values, it was obvious that heating played a major role in the ascorbic acid content of the extracts. This was demonstrated by plotting the contents in mg as functions of time from where it was seen that Lemon fruit juice had the highest correlation coefficient of 0.9918 at 60°C while the least was found for grape fruit juice at 40 and 80°C with a value of 0.9205.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Human nutrition, as a field of knowledge, had a great impact at the commencement of the twentieth century. Experiments such as those developed by English biochemist Frederick Hopkins (1861–1947) from 1992 demonstrated the existence of certain organic substances in food that are essential for health. Hopkins called them 'accessory food factors' *(Rosenfeld, 1997; Semba and Bloem, 2002; Mal and Casimer, 2013)*. Not too long after his discoveries, the Polish biochemist Casimir Funk (1884–1967) proposed the term 'vitamins' to identify the substances previously termed 'accessory food factors' *(Varela, 2005; Mal and Casimer, 2013).* The etymology of the term vitamin derives from the Latin 'vita' (life) and 'amina' and Funk drew the conclusion that these substances were necessary for life and most of them contained an amino group *(Rosenfeld, 1997; Mal and Casimer, 2013)*. These researches led to the identification of essential nutrients necessary to support human life and health (macronutrients, micronutrients and trace elements) which was almost concluded in the early sixties *Mal and Casimer, 2013).*

In the latter half of last century, all vitamins were identified with their chemical structures determined and natural sources from which they can be obtained were described in detail. The biological function of each vitamin, their connections with several metabolic pathways and human pathologies and their importance in human nutritional processes were also quickly established *(Semba and Bloem, 2002; Mal and Casimer, 2013).* Advances in chemical analysis/technologies has been made during the last three decades and these have provided the tools to produce vitamins in vitro (even at large scale). Thus, vitamins can be currently obtained via chemical synthesis, by isolation of natural sources (fat-soluble vitamins) or by microbial biotechnology (mainly water-soluble vitamins).

This has paved way for full eradication of several human pathologies based on vitamins deficiency or a substantial decrease in their prevalence. With these understanding, there is promotion of good nutrition practices and use of dietary supplements containing mainly vitamins and trace elements. Even with these results obtained from researches which has promoted the understanding of vitamins, problems associated with malnutrition still persist especially in some geographic regions characterized by poverty, poor understanding of nutrition and practices, and deficient sanitation and food security

1.1.1 Background of Study

The need to satisfy vitamin deficiencies has led to the formulation of various supplements in which cases the vitamins are either synthetized, of extracted from natural products. This has further led to the extraction of fruit juices under varying conditions and their fortification with vitamins and minerals with enrichment when necessary. During juice extraction, some of the natural products may be lost and on storage, degrade and this has led to various studies on the effect of these conditions on the level of vitamins and minerals present in the juice extracts of fruits. The total fruit production in some major countries contributes to their net income and some of these fruits are consumed in the form of juices. For example, the total fruit production in the US as of 2007 was 26.6 million metric tons. Non citrus production accounted for 15.3 while citrus production was million metric tons all giving a net worth of $14.5 and $3.1 billion. 7% of the fruit production in same year was consumed as juice in the United States and the fruits most commonly processed into juice were orange, grape fruit, apple, grape, and peach. Thus, it is necessary to assess the quality of these fruit juices as their nutritional values may not remain the same after extraction and storage under some given set of conditions (*Garcia-Torres et al., 2009*).

1.1.2 Statement of Problem

Ascorbic acid excess can lead to gastric irritation, and the metabolic product of vitamin C (oxalic acid) can cause renal problems *(Hodgkinson, 1977)*. In some cases, excessive quantities of ascorbic acid may result in the inhibition of natural processes occurring in food and can contribute to taste deterioration; added to fruit pulps, vitamin C inhibits oxidation processes responsible for fruit juice aroma *(Wawrzynia et al.,2005)*. Ascorbic acid is thermally labile, as it is easily degraded by enzymes and atmospheric oxygen. Its oxidation can be accelerated by excessive heat, light, and heavy metal cations *(Bhagavan, 2001)* all of which may take place during processing and this may eventually lead to the decrease it its content even on storage.

1.1.3 Justification of Study

Various processing techniques applied in fruit juice production subjects them to a decrease in nutritional value. Vitamin C, an essential vitamin that is synthesized exogenously and thus must be obtained from plants to balance its deficiency in humans. It is thermally labile thus; heat treatment and light predispose it to oxidation. That is why ascorbic acid content of foodstuffs and beverages represents a relevant indicator of quality which has to be carefully monitored, regarding its variation during manufacturing and storage.

1.1.4 Scope of Work

This research work covers the assessment of the ascorbic content of tangerine, lemon, mango, cashew, and pawpaw juice subjected to various processing techniques. The various processing stages to be employed include blending and heat treatment of the juice followed by the use of available analytical tools to determine the ascorbic acid content of the selected fruits before and after processing.

1.1.5 Aim and Objectives

This study aims at assessing the level of degradation of ascorbic acid in some selected fruits juice during extraction and processing.

To achieve this aim, the following objectives has been set;

- Extraction and processing of juice from mango, tangerine, grape, and lemon.
- Quantitative analysis of the ascorbic acid content of juice extract from the selected fruit samples at 24 hours interval for 5 days at different temperatures using redox titration.

1.2 LITERATURE REVIEW

Vitamins have been described as micronutrients essential for growth and the normal functioning of the body *(Onyekere et al., 2020)*. They are organic micronutrients mainly synthesized by plants and microorganisms, which do not provide energy. Animals are not able to synthesize them hence, these essential micronutrients must be supplied by the diet in small amounts or even trace amounts (micrograms or milligrams per day) for the maintenance of the metabolic functions of most animal cells (Uribe *et al.,* 2017).

Some vitamins however can be synthesized by humans in varying concentrations an example of which are vitamin D and Niacin which are readily synthesized in the skin by exposure to the sun or from the amino acid tryptophan, respectively) *(Nair and Maseeh, 2012; Bender, 2003).*

Other vitamins viz K, B1, B2, and biotin are synthesized internally by intestinal bacteria but yet, all of these cannot make of for the required daily those of vitamins for man hence the need to obtain them from the consumption of plants and foods that are fortified or enriched with them. In cases where a disease is reported due to lack of a particular vitamin, then the disease is said to have occurred as a result of the vitamin's deficiency and is termed deficiency disease.

They classified based on their solubility as water soluble and fat soluble vitamins. The various vitamins and their class are tabulated below:

Table 1.0 Fat and water soluble vitamins *(Onyekere et al., 2020)*

Water soluble vitamins	Fat soluble vitamins
Vitamin B1 or Thiamine	Vitamin A or Retinol
Vitamin B2 or Riboflavin	Vitamin D or Calciferol
Vitamin B3 or Niacin	Vitamin E or α-Tocopherol
Vitamin B5 or Pantothenic acid	Vitamin K or Phylloquinone
Vitamin B6 or Pyridoxine	
Vitamin B7 or Biotin	
Vitamin B9 or Folic acid	
Vitamin B12 or Cobalamin	
Vitamin C or Ascorbic acid	

The fat soluble vitamins are not excreted via urine and thus, overdose, a case called hypervitaminosis poses a serious health challenge as they are accumulated and may eventually cause toxicity. They are readily absorbed in the presence of fats and bile salts, the basis for their classification. On the other hand, water soluble vitamins are readily absorbed due to their solubility in water and acute toxicity is rare as a result of the fact that they are readily excreted in urine. They contain nitrogen in their structure except ascorbic acid while all fat soluble vitamins lack nitrogen in their structure.

1.2.1 Ascorbic Acid

Ascorbic acid (vitamin C) is a water-soluble vitamin which can be found in many biological systems and foodstuffs (fresh vegetables and fruits, namely, citrus). Ascorbic acid plays an important role in collagen biosynthesis, iron absorption, and immune response activation and is involved in wound healing and osteogenesis. It is the only water soluble vitamin that lacks nitrogen in its structure. It is exogenous to humans; this means that it is supplied by food. One of the most common vitamins whose deficiency is highly pronounced is ascorbic acid and as such attracted researchers.

1.2.1.1 History of Ascorbic Acid

For centuries people, especially sailors and polar investigators, were subjected to the disease scurvy, which is characterized by apathy, weakness, easy bruising with tiny or large skin hemorrhages, friable bleeding gums, and swollen legs

(Mieszczakowska-Frac et al., 2021). This situation came as a result of stress and the fact that these sailors spend longer time in the seas and oceans. This is usually the case because the stress experienced by the sailors predisposed them to more oxidative damage and thus reducing the serum concentration of vitamin C drastically. To compensate for this, the British Navy administered lime juice to their sailors. This sparked the interest of researchers on ascorbic acid and eventually it was isolated in 1932 and was made available commercially in 1934 *(Njoku et al., 2011; Pacier and Martirosyan, 2015).*

The antiscorbutic properties of certain foods were demonstrated in the 18th century by James Lind. Axel Holst and Theodor Frølich in 1907, discovered that the antiscorbutic factor was a water-soluble chemical substance and was distinct from the one that prevented beriberi. Between 1928 and 1932, Albert Szent-Györgyi isolated a candidate for this substance, which he called it "hexuronic acid", first from plants and later from animal adrenal glands. In 1932 Charles Glen King confirmed that it was indeed the antiscorbutic factor.

In 1933, sugar chemist Walter Norman Haworth, working with samples of "hexuronic acid" that Szent-Györgyi had isolated from paprika and sent him in the previous year, arrived at the correct structure and optical-isomeric nature of the compound, and in 1934 reported its first synthesis. In reference to the compound's antiscorbutic properties, Haworth and Szent-Györgyi proposed to rename it "a-scorbic acid" for the compound, and later specifically l-ascorbic acid. Because of their work, in 1937 the Nobel Prizes for chemistry and medicine were awarded to Haworth and Szent-Györgyi, respectively *(Wikipedia, 2021)*.

1.2.1.2 Structural Description of Ascorbic Acid

Ascorbic acid has an enediol structure conjugated with the carbonyl group in the lactone ring. The two enolic hydrogen atoms are the ones that give this compound its acidic character and provide the electrons for its function as an antioxidant by conferring the property of a reducing agent on it. In the presence of oxygen, ascorbic acid is transformed into dehydroascorbic acid, which has the same vitamin activity *(Barba et al., 2014)*.

Ascorbic acid exists as two enantiomers (mirror-image isomers), commonly denoted "l" (for "levo") and "d" (for "dextro"). The l isomer is the one most often encountered: it occurs naturally in many foods, and is one form ("vitamer") of vitamin C, an essential nutrient for humans and many animals. The "d" form can be made via chemical synthesis but has no significant biological role. *(Wikipedia, 2021)*.

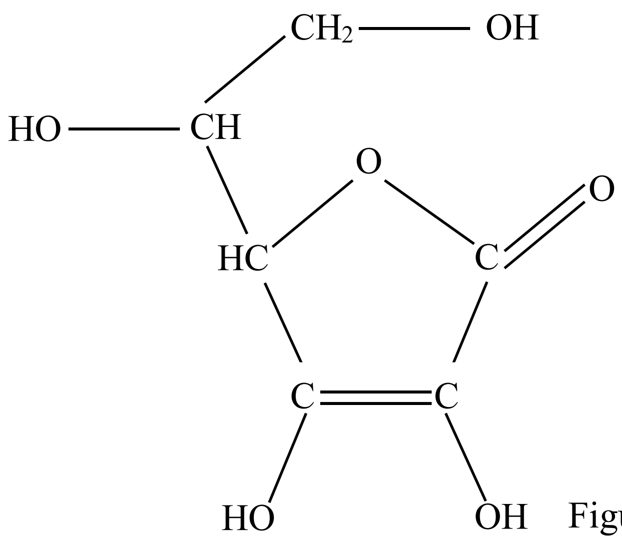


Figure 1.0 Structure of Ascorbic Acid

1.2.1.3 Sources of Vitamin C

The major sources of vitamins are fruits and vegetables. It is vitamin is particularly abundant in rose hips, black currants, sea buckthorn, strawberries, kiwifruit, parsley, oranges, lemons, grapefruit, papaya, pineapple, mango, quince, a variety of cabbages, broccoli, cauliflower, peppers, turnip, kale, and potatoes. Studies has been conducted on the ascorbic content of some fresh fruits applying various analytical techniques.

It is also found synthesized in smaller quantities in animals and this quantity is not enough to make up for man's daily dosage of vitamin C. The source of ascorbic acid in animals is majorly the liver and kidneys.

The table below shows the ascorbic acid content of some fresh fruits that has been analytically determined and documented in research articles.

Fruit specie	Content (mg/100g)	Fruit specie	Content (mg/100g)
Blackcurrants	181.0	Blackberries	21.0
Green kiwi fruits	92.7	Quinces	15.0
Pummelo	61.0	Cramberries	14.0
Papayas	60.9	Pomegranates	10.2
Oranges, navels	59.1	Apricots	10.0
Strawberries	58.8	Red sour cherries	10.0
Oranges, common variety	53.2	Avocadoes	10.0

Lemon	53.0	Blueberries	9.7
Pineapple	47.8	Plums	9.5
Oranges, Florida	45.0	Bananas	8.7
Red and white currants	41.0	Persimmons, Japanese	7.5
Mango	36.4	Sweet cherries	7.0
Elderberries	36.0	Yellow peaches	6.6
White grapefruit	33.3	Grapes, muscadine	6.5
Pink and red grapefruit	31.2	Nectarines	5.4
Lime	29.1	Apples with skin	4.6
Gooseberries	27.7	Pears	4.3
Tangerine	26.7	Apples without skin	4.0
Raspberries	26.2	Red or green grapes	3.2

Table 1.1 Ascorbic acid content of some common fruits *(Mieszczakowska-Frac et al., 2021)*

Another source of vitamin C is drug formulations, processed food where it is used as preservative, and in food supplements. They are usually used in the synthetic form for enrichment and fortification of food products.

1.2.1.4 Physical Properties of Ascorbic Acid

Vitamin C is white crystalline solid that changes colour gradually on exposure to light. It is soluble in water and possess some ionic character thus making it sparing soluble in ethanol and some other organic solvents basically ether and chloroform. It melts at 190-192°C and is characterized by a sharp pleasant acidic taste. In its natural form, its density is 1.7g/ml with pKa values of 4.2 and 11.6. These pKa values are based on the presence of the hydroxyl groups in different positions of the lactone ring viz positions 3 and positions 2.

1.2.1.5 Chemical Properties of Ascorbic Acid

Based on its structure with the presence of the ketone and hydroxy functional groups, ascorbic acid possesses some chemical properties and undergoes some major reactions. Some of these properties and reactions are;

- **Acidity**: Ascorbic acid is a vinylogous acid and forms the ascorbate anion when deprotonated on one of the hydroxyls. This property is characteristic of reductones: enediols with a carbonyl group adjacent to the enediol group, namely with the group –C(OH)=C(OH)–C(=O)–. The ascorbate anion is stabilized by electron delocalization that results from resonance between two forms:

  For this reason, ascorbic acid is much more acidic than would be expected if the compound contained only isolated hydroxyl groups.
- **Salts Formation**: The ascorbate anion forms salts, such as sodium ascorbate, calcium ascorbate, and potassium ascorbate. This takes place when ascorbic acid is treated with alkali such as sodium hydroxide and potassium hydroxide.

- **Esters**: Ascorbic acid can also react with organic acids as an alcohol forming esters such as ascorbyl palmitate and ascorbyl stearate.
- **Nucleophilic attack:** Nucleophilic attack of ascorbic acid on a proton results in a 1,3-diketone
- **Oxidation:** The ascorbate ion is the predominant anionic specie at typical biological pH values. It is a mild reducing agent and antioxidant. It is oxidized with loss of one electron to form a radical cation and then with loss of a second electron to form dehydroascorbic acid. It typically reacts with oxidants of the reactive oxygen species, such as the hydroxyl radical usually generated from hydrogen peroxide thus reducing the species.

  It can transfer a single electron owing to the resonance stabilized nature of its own radical ion. This radical ion is called semidehydroascorbate and the net reaction is:

$$RO^{\cdot} + C_6H_7O_6^- \rightarrow RO^- + C_6H_7O_6^{\cdot} \rightarrow ROH + C_6H_6O_6$$

  On exposure to oxygen, ascorbic acid will undergo further oxidative decomposition to various products including diketogulonic acid, xylonic acid threonic acid and oxalic acid (*Gaonkar and McPherson, 2016)*.
- **O-Nitrosation:** Ascorbic acid reacts readily with nitrous acid in mildly acidic solution (and also with other nitrosating species) to give dehydroascorbic acid. The reaction follows the scheme

$$C_6H_8O_6 + 2HNO_2 \rightarrow C_6H_6O_6 + 2NO + 2H_2O$$

Under anaerobic conditions the other product is nitric oxide, which will react further in the presence of oxygen *(Williams, 2004)*. The reaction was first reported in 1934. In the laboratory, this reaction is much used to generate solutions of nitric oxide, when great care must be taken to eliminate all traces of oxygen.

1.2.1.6 Ascorbic Acid Deficiency – Individuals at Risk

Smokers tend to consume healthy diet (specifically fruits and vegetables) and thus has led to 16% decrease in vitamin C intake. This poses smokers at risk of vitamin C deficiency and the health challenges associated with it. It has been demonstrated by the United States Department of Agriculture (USDA) that there is a strong correlation between individuals who smoke tobacco and vitamin C deficiency and this body has made specification on their Recommended Dietary Allowances (RDA) that smokers require an additional 35 mg of vitamin C daily to maintain adequate levels *(Institute of Medicine, 2000)*. In addition to the less consumption of healthy diets by smokers, cigarette smoke causes vitamin C to be depleted at a much faster rate, in order to compensate for the oxidative stress *(Alberg, 2002)*. This was proven in one study conducted, were it was observed that ascorbate was completely depleted from human plasma after six puffs of cigarette smoke *(Eiserich et al., 1995).*

An association also exists between lower-income households and vitamin C deficiency. A study conducted on the 15% lowest income households to determine plasma vitamin C levels in the United Kingdom gave some major proof to this *(Mosdøl et al., 2008).* The results indicated that 26% of men and 16% of women were vitamin C deficient (<11 µmol), and 21% of men and 18% of women had depleted vitamin C levels (11–28 µmol) *(Mosdøl et al., 2008).* A main reason for this is that households with lower socioeconomic status tend to consume less fruits and vegetables, which are essential sources of vitamin C. In one study, it was shown that 53% of low-income households spend less than $1.00 a week on fresh fruit and vegetables *(Phipps et al., 2013).*

Vitamin C decreases more quickly with increased oxidative stress, so higher intakes can help to better manage the increased emotional/physical pressure. It is therefore obvious that individuals with more stressful life are prone to increased oxidative stress which reduces the plasma concentration of vitamin C and eventually results in deficiency. It has been shown that helicopter pilots have a more stressful job compared to non-flight staff, based on serum levels of stress indicators, with 21.1% higher malondialdehyde (MDA), 21.7% higher superoxide dismutase (SOD), and 25.1% higher total antioxidant capacity (TAC) *(Taleghani et al., 2014).* Oxidative stress could also be the product of the working environment and not the job itself. For it was reported that workers who have been exposed to lead (73µg of lead/dl of blood) compared to those who haven't (6.7µg of lead/dl) show a 46.2% higher thiobarbituric acid reactive species concentration (TBARS (nmol MDA/ml PG)), 60.9% higher SOD, 70.3% higher chloramphenicol acetyltransferase (CAT) activity, and a 40%

higher TAC workers *(Rendón-Ramírez et at., 2014).* When these lead-exposed workers were given daily oral 1 g vitamin C supplements (in addition to 400 IU vitamin E) for one year, their TBARS decreased by 46.2%, TAC decreased by 36.4%, CAT decreased by 59.5%, and SOD decreased by 48.5%, bringing them back down to levels of their non-lead exposed workers *(Rendón-Ramírez et at., 2014).*

Elderly individuals are at risk for malnutrition from multiple factors, including disease, medications, diminished senses (taste/smell), physical limitations (reaching/bending), depression, oral issues (difficult chewing/swallowing, mouth pain, tooth loss, poorly fit dentures), financial issues, and stress (death of a loved one) *(Sharkey et al., 2002).*

Another group of individuals who are at risk include those who suffer from certain addictions. It has been shown that increased alcohol consumption leads to a decrease in plasma vitamin C due to the action of ethanol and/or poor diet: low alcohol consumers (~10.6 g/d) have 41.4 µmol/L, moderate consumers (~59 g/d) have 32.4 µmol/L, and alcoholics (~194.3 g/d) have 24.2 µmol/L *(Lecomte et al., 1994)*. This relationship has also been demonstrated in heroin addicts, where increased use led to a further decrease in vitamin C levels *(Lecomte et al., 1994)*. In another study, various drug addicts were shown to have a 43.6% lower vitamin C level than control subjects *(Nazrul et al., 2001).*

Women who are pregnant suffers oxidative stress and this puts them at risk of ascorbic acid deficiency. Pregnant women who are obese (BMI >30 kg m−2) have a greater risk of low vitamin C levels than pregnant women of a healthy weight (BMI 18–25 kg m−2), because their oxidative stress has been shown to be greater *(Sen et al., 2014).* Low vitamin C levels during pregnancy has the disadvantage of possibly leading to various health issues to the fetus, such as low birth weight *(Saker et al., 2008).* Many women take iron supplements during pregnancy, which have been shown to decrease vitamin C levels. In one study, pregnant women who were taking iron supplements had a 24% decrease in vitamin C levels from the 1st to the 3rd trimester, while women who were not taking iron supplements only had a 3.7% loss. This could be the possible reason why pregnant women are placed on routine pills of which vitamin is a major requirement.

Individuals who are being treated in hospitals may also suffer from vitamin C deficiency. This is especially true for maintenance haemodialysis (MHD) patients. One study determined that 44.1% of MHD patients are vitamin C deficient with <2 µg/mL (or <11 µmol/L) *(Zhang et al., 2012)*. This is due to multiple factors, one being that MHD patients need to avoid foods that are high in potassium in order to help prevent hyperkalemia. Potassium-rich foods tend to be high in vitamin C; therefore, a decrease in potassium also causes a decrease in vitamin C *(Zhang et al., 2012).* Another reason these patients are at risk is that in each dialysis treatment, an average of 66 mg of vitamin C can be removed from their plasma levels *(Morena et al., 2002).*

1.2.1.7 Ascorbic Acid Dosage and Overdose Effects

As with most nutrients, there are always questions about optimal intake. This can vary dramatically when considering different factors of age, health, lifestyle and gender. In regards to determining a daily amount best suited for the general population, the most recent RDA has been calculated at 90 mg for men and 75 mg for women, daily *(Institute of Medicine, 2000)*. Many experiments reviews have been conducted by researchers who believed that this amount is too low to explain why a higher daily intake would better benefit our health.

Levine, Wang, Padayatty, and Morrow (2001) conducted their own depletion-repletion study specifically for women and determined that both sexes should have the same 90 mg daily intake. Some studies and reviews have asserted that 200 mg daily is the optimal intake, with others claiming that 1,000 mg (500 mg twice daily) provides the best health effects *(Ordman, 2010)*. The absorption efficiency of vitamin C is 89% for 15mg/day, 87% for 30mg/day, 85% for 50mg/day, 80% for 100mg/day, 72% for 200mg/day, 63% for 500mg/day, and 46% for 1250mg/day in terms of bioavailability *(Graumlich et al., 1997)*. From these data, it seems that although all the specified dosages are readily absorbed, the absorption efficiency is at its peak at a daily dosage of 500mg/day. Though vitamin C tends to be well tolerated, even with high doses, maximum limits have been established. The maximum tolerated single dose is 3g and the maximum tolerated daily dose is 18g *(Padayatty et al., 2006)*. These dosages cannot be achieved from the daily consumption of meals hence, must be compensated for by the consumption of food supplements and administered via syrups, pills, and injections.

There have been some reports of negative effects in larger doses of ascorbic acid. To help curb the negative side effects of ascorbic acid overdose, the USDA set the upper tolerable limit (UL) for vitamin C at 2g *(Institute of Medicine, 2000)*. The most common side effects are gastrointestinal distress and diarrhea and have been shown in single oral doses of 5-10 g or greater than 2 g daily, with symptoms disappearing within 1-2 weeks *(Fukushima and Yamazaki, 2010; Deruelle and Baron, 2008)*. However, there has been evidence to suggest there are a few more severe side effects with high-dose vitamin C. The most note-worthy of these is the production of calcium oxalate stones in patients with renal issues (though some healthy individuals can also produce excessive oxalate at doses greater than 1g daily *(Levine et al., 1999)*. This case is known because vitamin C converts to oxalate during the elimination process, which can cause formation of stones at high doses. In a fairly recent study, there was evidence to support limited, but still statistically significant, oxalate formation with high dose vitamin C. In this both groups were administered 1g vitamin C supplement orally twice daily for 6 days with the completion of two trials. The healthy group reported a 20% increase in oxalate, while the group with renal issues had a 33% increase *(Traxer et al., 2003)*. This study helps explain the controversy of whether high dose vitamin C is dangerous or not. There are documented cases of oxalate stone formation in subjects with renal issues, but the incidence rate is low. Vitamin C has an advantage of increasing iron absorption by helping to transport iron across the epithelium in the small intestine *(Lykkesfeldt et al., 2014).* This could be an addition concern with respect to vitamin C overdose as there is increased potential for an iron overload in some individuals with diseases such as hemochromatosis, sideroblastic anemia, beta-thalassemia major and sickle cell anemia *(Nienhuis, 1981).*

1.2.1.8 Industrial Synthesis of Ascorbic Acid

Eighty percent of the world's supply of ascorbic acid is produced in China *(Weiss, 2007)*. Ascorbic acid is prepared industrially from glucose via a method based on the historical Reichstein process. Glucose is catalytically hydrogenated to sorbitol, followed by oxidation in the presence of the microorganism *Acetobacter suboxydans* to sorbose in the first of a five-step process. In this enzymatic action, only one of the six hydroxy groups is oxidized. Two routes are made available from this point. Four of the remaining hydroxy groups are converted to acetals on treatment with acetone in the presence of an acid catalyst leaving an unprotected hydroxyl group which is then oxidized to the carboxylic acid by reaction with the catalytic oxidant TEMPO (regenerated by sodium hypochlorite — bleaching solution). Acid-catalyzed hydrolysis of this product performs the dual function of removing the two acetal groups and ring-closing lactonization. This step yields ascorbic acid. Each of the five steps has a yield larger than 90% *(Eggersdorfer et al., 2012).*

A more biotechnological process, first developed in China in the 1960s, but further developed in the 1990s, bypasses the use of acetone-protecting groups. A second genetically modified microbe species, such as mutant Erwinia, among others, oxidizes sorbose into 2-ketogluconic acid (2-KGA), which via dehydration can then undergo ring-closing lactonization. This method is used in the predominant process used by the ascorbic acid industry in China, which supplies 80% of world's ascorbic acid. The need to carry out on-pot fermentation directly from glucose to 2-KGA has sparked interest of American and Chinese researchers. They are in competition to engineer a mutant that can carry out a one-pot fermentation directly from glucose to, bypassing both the need for a second fermentation and the need to reduce glucose to sorbitol.

1.2.1.9 Biological Relevance and Other Uses of Ascorbic Acid

The biological functions of ascorbic acid stem from its ability to provide reducing equivalents for a variety of biochemical reactions. Based on its reducing power, it can reduce physiologically relevant reactive oxygen species. The biological functions of ascorbic acid are;

- Cofactor for reactions that require a reduced iron or copper metalloenzyme. In this case it maintains the reduced state of metal cofactors such as Cu^{+} and Fe^{2+} at monooxygenase and dioxygenase respectively *(Linster and Van, 2007).*

- It acts as a protective antioxidant that operates in the aqueous phase both intra- and extra-cellular *(Shilpi et al., 2018)*. In this case when Fe and Cu are reduced, the reduction of hydrogen peroxide occurs and the product such as reactive hydroxy radical destroys cancerous cells *(Linster and Van 2007; Shilpi et al., 2018).* But in case of normal cells, H_2O_2 destroyed immediately due to the presence of catalase and Glutathione peroxidase (GP) (both are a class of antioxidant enzymes) in high concentration due to high blood flow. This helps to preserve cells towards reactive oxygen *(Gazdik et al., 2008).*
- In combination with anticancer drugs, it improves the immunity, tissue repair, detoxification process of some drugs. It can synergize the anticancer activity of some drugs such as cisplatin, dacarbazine, tamoxifen, doxorubicin and paclitaxel.
- Vitamin-C increase the production of lymphocytes in the body resulting in the enhancing the immune system *(Gorkom et al., 2018)*. It has been observed in cancer patients that the lymphocytes ascorbate level is low. Lymphocytes are necessary to prevent initiating phase of the cancer cell growth and their production and effectiveness is enhanced by adequate levels of ascorbic acid in the plasma. Vitamin-C increase the immune

reconstitution of cancer patients treated with immunotoxic drugs *(Shilpi et al., 2018)*.

- It increases the hydroxylation of proline and lysine to hydroxyproline and hydroxylysine, respectively. A proper cross-linking of these amino acids results in the formation of a stable triple helix form of collagen. The triple helix collagens provide stability to for stable barrier surrounding the tumor mass that prevent the metastasis of tumor *(Buettner and Jurkiewicz, 1993; Whiteman et al., 1997)*.

Other uses of ascorbic acid are;

- Reductant in photographic developer solutions. This is based on the fact that ascorbic acid is easily oxidized (among others). This chemical character has also made it useful as a preservative.
- It is used as an antioxidant to increase fluorescent signal and chemically retard dye photobleaching in fluorescence microscopy and related fluorescence-based techniques *(Widengren et al.,2007)*.
- It has also found application in the removal of dissolved metal stains, such as iron, from fiberglass swimming pool surfaces.
- In plastic manufacturing, ascorbic acid can be used to assemble molecular chains more quickly and with less waste than traditional synthesis methods.

- Heroin users are known to use ascorbic acid as a means to convert heroin base to a water-soluble salt so that it can be injected *(Beynon et al., 2007).*
- It is used to negate the effects of iodine tablets in water purification based on the justification that it reacts with iodine. It reacts with the sterilized water, removing the taste, color, and smell of the iodine. This is why it is often sold as a second set of tablets in most sporting goods stores as Potable Aqua-Neutralizing Tablets, along with the potassium iodide tablets.
- High-dose ascorbate is being used as a chemotherapeutic and biological response modifying agent that is currently it is still under clinical trials *(NCI, 2014).*
- Ascorbic acid and its sodium, potassium, and calcium salts are commonly used as antioxidant food additives. These compounds are water-soluble and, thus, cannot protect fats from oxidation: For this purpose, the fat-soluble esters of ascorbic acid with long-chain fatty acids (ascorbyl palmitate or ascorbyl stearate) can be used as food antioxidants.

1.2.2 Methods for Quantification of Ascorbic Acid

Several analytical methods have been used for quantification of vitamin C in food and drug samples. Examples of these analytical methods are titrimetric, spectrophotometry, chromatography, voltammetry, fluorometry, and potentiometry *(Anal and Shuchi, 2019).* The commonly used of these is the spectrophotometric and titrimetric methods which offers simplicity although the spectrophotometric method offers more precise results compared to the titrimetric method.

1.2.2.1 Spectrophotometric Method

The spectrophotometric method is based on the principle that certain organin molecules absorb light in the ultraviolet and visible region of light. It is usually employed for molecules in which electronic transitions can occur within bonding and antibonding orbitals that possesses certain energy differences. Molecules with lone electron pairs and pi-bonds (unsaturation) absorb light within this region of light and the energy corresponding to the wavelength at which maximum absorption takes place depends on the type of bonds found in the molecule. Thus, this wavelength often referred to as λ_{max} is characteristic of the molecule. The electronic transitions which are permitted in the UV-Vis region of light are $\pi \rightarrow \delta^*$, $\pi \rightarrow \pi^*$, and $\eta \rightarrow \pi^*$. Ascorbic acid and its oxidized product absorb light in this region since it possesses these orbitals and allows electronic transition within them.

In the spectrophotometric method for quantification of ascorbic acid and 2,4-Dinitrophenylhydrazine solution (2,4 DNPH) undergoes a coupling reaction. This method is used in determining ascorbic acid content in different fruits and vegetables. 2,4 DNPH act as a dye in this method. This method is suitable for the determination of the total amount of vitamin C (Ascorbic acid +

Dehydroascorbic acid) in fruits by using UV spectrophotometer. Prior to the coupling reaction, bromine water is used to oxidize the ascorbic acid into dehydroascorbic acid in the presence of acetic acid. Then known amount of 2,4 DNPH is added which gives coupling reaction. Solutions are kept for 3 hours after which 85% H_2SO_4 is added which gives coloured solution *(Anal and Shuchi, 2019).* These solutions are then read at a determined wavelength using a spectrophotometer. In this case, a standard calibration cure is prepared first by plotting the absorbance of various standards as a function of concentration from which the analyte can be quantified.

1.2.2.2 Titrimetric Method (Iodimetry)

The fact that ascorbic acid is a reducing agent that readily reacts with molecular iodine and reduce it to iodide in solution provides the basis for the volumetric determination of it in solutions. The reaction scheme is shown below.

$$C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2HI$$

It therefore noteworthy that the iodine solution is decolourized on treatment with ascorbic acid but the reaction does not take place with dehydroascorbic acid.

Once all the ascorbic acid has been oxidized, the excess iodine is free to react with the starch indicator, forming the blue-black starch-iodine complex *(Wikipedia, 2021).* This is the endpoint of the titration.

Another alternative to this involves using potassium iodide to prepare the primary standard which is more stable than the iodine primary standard making it more reliable but it is not straight forward. This method involves direct titration of the titrant against the analyte (ascorbic acid).

In the back (indirect) titration method, the molecular iodine solution is treated inn excess with the analyte. The excess and unreacted iodine is then titrated with standard sodium thiosulphate solution usually 0.1N. The endpoint is noted when the blue-black colouration of the starch-iodine complex disappears.

The method is suitable for use with vitamin C tablets, fresh or packaged fruit juices and solid fruits and vegetables.

1.2.3 Fruit Juice Extraction and Processing

Prior to extraction of juice, fruits are first inspected to remove any spoilt or infected fruits. They are thoroughly washed with clean water to remove dirt or sand particles. The fruits are then peeled manually in preparation for extraction using a manual screw type hand operated juice extractor or power operated commercial extractor *(Pareek et al., 2010)*. For home extraction, a fruit blender can also be used.

The extractor separates the juice from the pomace and both are collected separately. The juice extracted is then filtered using a clean muslin cloth or any other clean material.

In processing, the filtrate (juice) is refrigerated for some hours depending on the fruit type. The juices are further subjected to heat processing to destroy traces of bacteria that may still be present after which they are sealed in pre-sterilized containers *(Pareek et al., 2010)*.

It should however be noted that there are extractors that can carry out all these process in an industrial scale after selection and washing of fruits has been achieved.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

Volumetric flasks

Measuring cylinder

Burette

Pipette

Retort stand

Glass stirring rod

Glass funnel

Beakers

Conical flasks

Spatula

Aluminum foil

Syringe

Hand gloves

Face mask

Weighing balance

Water bath

2.2 REAGENTS

Ascorbic acid

Iodine

Potassium Iodide

Starch

Potassium Bromate

Potassium dichromate

Sodium Thiosulphate

Hydrochloric acid

Sulphuric acid

2.3 METHODOLOGY

2.3.1 Collection and Preparation of Fruit Samples

The fresh fruit samples viz mango, lemon, grape, and tangerine were bought from New Benin market, Oredo Local Government Area, Benin City, Edo State and were washed, peeled, and the juice were extracted using a blender. The extracts were filtered using clean handkerchief to remove particles and seeds.

2.3.2 Preparation of Standard Solutions

All reagents used were of analytical grades and the standard procedures were followed for the preparation of standard solutions.

2.3.2.2 Preparation of Starch Solution

0.25g of soluble industrial starch was weighed into 50ml standard flask followed by its dissolution with distilled water up to mark. It was stoppered and labelled starch indicator.

2.3.3.4 Preparation of 1M Sulphuric acid

54.0ml of sulphuric was pipetted from the 98.80% stock into a 1000ml standard flask containing some amount of distilled water. The solution was then gradually made up to the 1000ml mark.

2.3.1.5 Preparation of 0.1M Sodium Thiosulphate Solution

25g of sodium thiosulphate was weighed into 1000ml volumetric flask, distilled water was added up to mark followed by vigorous agitation to ensure complete dissolution of the solutes.

The resulting solution was then standardized by measuring 10ml of 0.025M iodine solution prepared by mixing 0.15g and 2g of potassium iodate and iodide respectively followed by the addition of 25ml of distilled water and 5ml of sulphuric acid in a conical flask and titrating this mixture against the sodium thiosulphate using starch indicator in duplicates.

2.3.1.6 Preparation of 0.05M Iodine Solution

10 of potassium iodide was weighed into a 500ml standard flask followed by the addition of 6.3452g molecular iodine. Distilled water was then added with vigorous agitation and the solution was made up to mark.

The resulting solution was standardized by withdrawing 10ml aliquots in duplicates into Erlenmeyer flasks followed by the addition of 1ml starch solution and titration with 0.1M sodium thiosulphate.

2.3.2 Quantitative Determination of Ascorbic Acid in Juice Extracts

10ml aliquots were withdrawn from the fresh juice extracts into 250ml Erlenmeyer flasks followed by the addition of 1ml starch indicator. 0.5M iodine was titrated against the extracts while swirling with gradual addition of 1ml of sulphuric acid. 2ml of sulphuric acid was further added and gradually up to 10ml to sharpen the end point and the titration was ran in triplicates.

The procedure was repeated for the juice extracts stored at 40, 60, 80, and 100°C at intervals of 24 hours for five days.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 RESULTS

Table 3.1 shows in the initial ascorbic acid content of the selected fruits (fresh)

Table 3.1 Ascorbic acid content of fresh fruit juice

Sample	Mass (mg)
Lemon	5.86
Tangerine	4.98
Grape	10.42
Mango	11.44

Table 3.2 shows the ascorbic acid content in the fruits after subjecting to heat at 40°C

Table 3.2 Ascorbic acid content (mg) of heat treated fruit juice at 40°C

Days	Mango	Tangerine	Lemon	Grape
1	9.24	4.40	5.72	7.48
2	7.26	4.40	4.84	5.72
3	6.16	3.96	4.62	5.28
4	5.28	3.52	3.96	4.84
5	4.84	3.08	3.74	3.96

Table 3.3 shows the ascorbic acid content of the selected fruits after subjecting to heat at 60°C

Table 3.3 Ascorbic acid content (mg) of heat treated fruit juice at 60°C

Days	Mango	Tangerine	Lemon	Grape
1	8.80	4.40	5.06	7.04
2	7.92	3.96	4.40	5.28
3	7.04	3.52	3.96	4.84
4	6.60	2.64	3.52	3.96
5	4.84	2.20	3.08	3.52

Table 3.4 showing the ascorbic acid content in the selected fruits after subjecting to heat at 80°C

Table 3.4 Ascorbic acid content (mg) of heat treated fruit juice at 80°C

Days	Mango	Tangerine	Lemon	Grape
1	7.92	3.96	4.84	6.60
2	7.04	3.30	3.96	4.84
3	6.16	3.08	3.52	4.40
4	5.72	2.20	3.08	3.96
5	3.96	1.76	2.64	3.08

Table 3.5 shows the ascorbic acid content in the selected fruits after subjecting to heat at 100°C.

Table 3.5 Ascorbic acid content (mg) of heat treated fruit juice at 100°C

Days	Mango	Tangerine	Lemon	Grape
1	7.04	2.64	3.96	5.28
2	6.16	2.64	3.30	4.84
3	5.72	2.20	2.64	3.96
4	4.84	1.76	2.20	3.30
5	3.52	1.32	1.75	3.08

3.2 DISCUSSION

From the tabulated values above, the ascorbic acid in the selected fruit samples were observed to decrease as the temperature applied during heat treatment increased from 40°C to 100°C indicating that ascorbic acid is a thermally labile vitamin (Bhagavan, 2001). From quantification of the ascorbic acid content in the fresh fruit samples prior to heat treatment, a highest value of 11.44mg was found for mango while the least was found for tangerine with a value of 4.98mg. These values are represented on figure 3.1 below

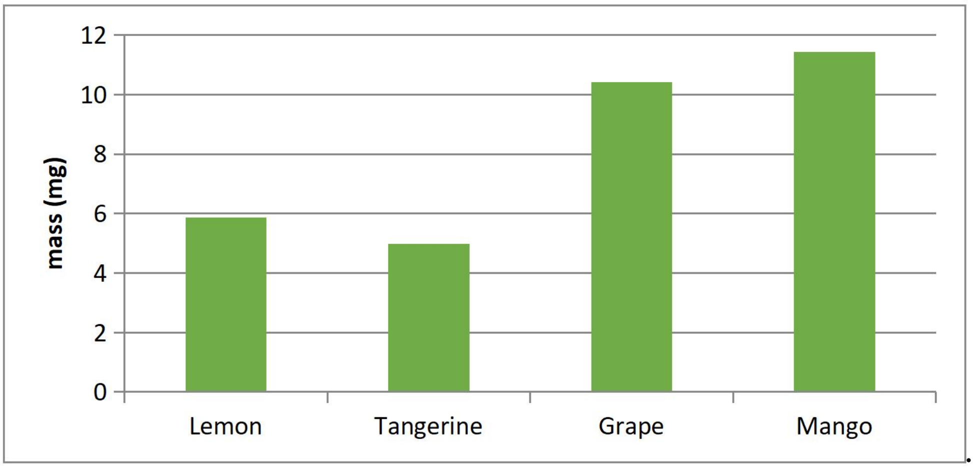


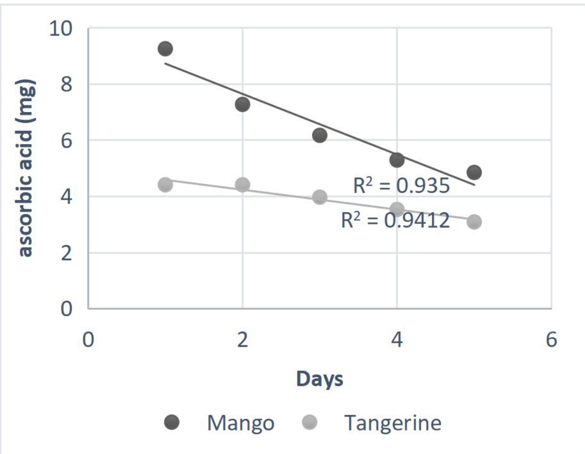
Figure 3.1 Representation ascorbic acid content of fresh fruits on a histogram

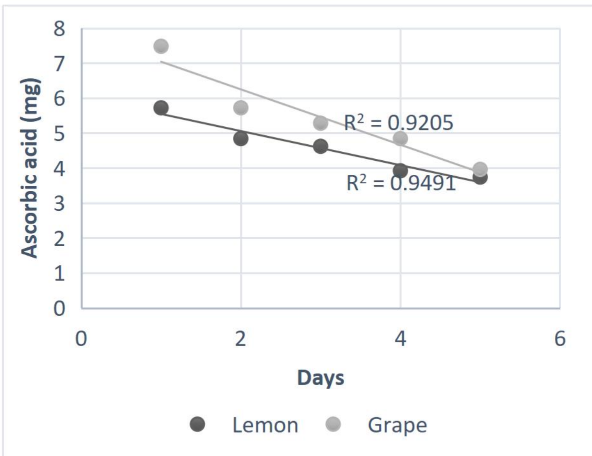
To establish a linear relationship between the duration of heating and the ascorbic acid content of the fresh fruit juice samples, the ascorbic acid contents were plotted as functions of the days for the different temperature.

Figures 3.2 and 3.2b below represent the plots of fruit juice samples from which the best linear relationship was found for Lemon on heat treatment at 40°C for five days

Figure 3.2a Plots of AA contents for Mango and Tangerine at 40°C

Figure 3.2b Plots of AA content for Lemon and Grape at 40°C





The values obtained from heating at 60°C shows that an increase in temperature further reduced the ascorbic acid content of the juice. This was observed in comparison to heat treatment at 40°C as shown on the plots below

Figure 3.3a Plots of AA content for Mango and Tangerine at 60°C

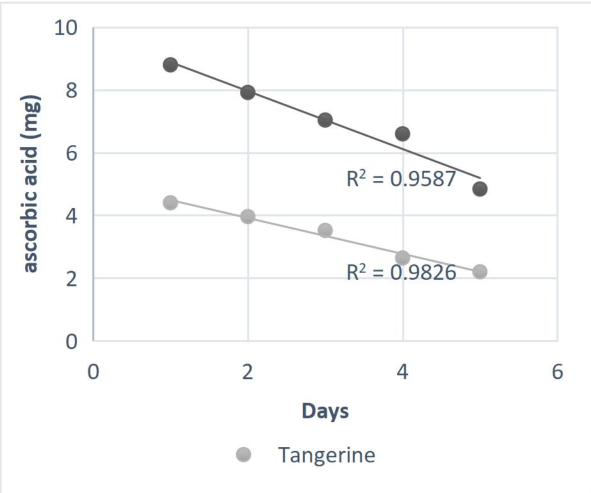
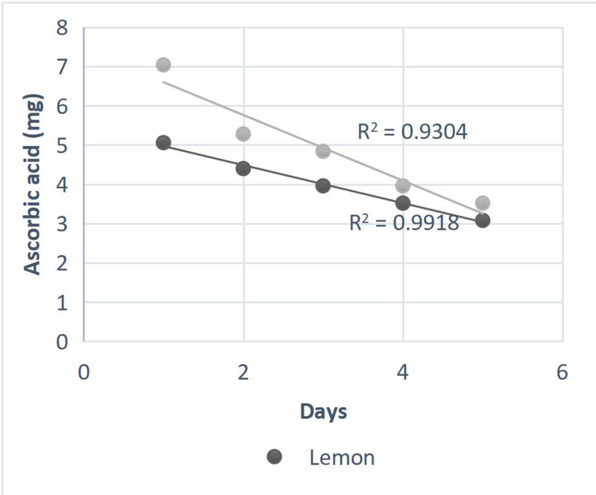


Figure 3.3b Plots of AA content for Lemon and Grape 60°C



At 80°C a marked decrease was observed from the values obtained at 60°C indicating a strong relationship between heat and ascorbic acid content of the juice extracts as shown on figures 3.4a and 3.4b on the next page

Figure 3.4a Plots of AA content for Mango and Tangerine at 80°C

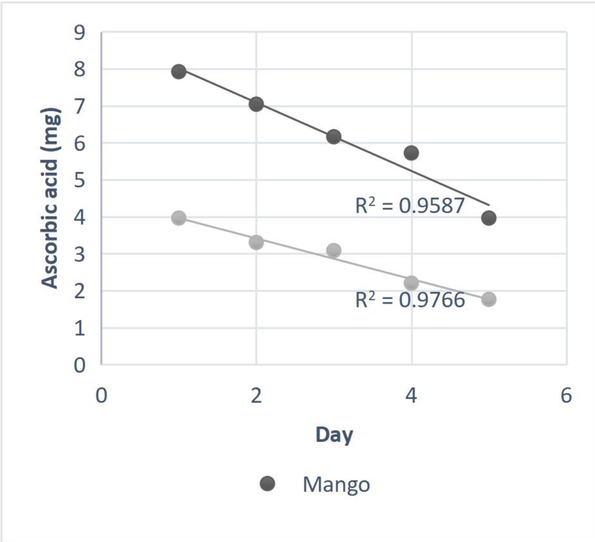
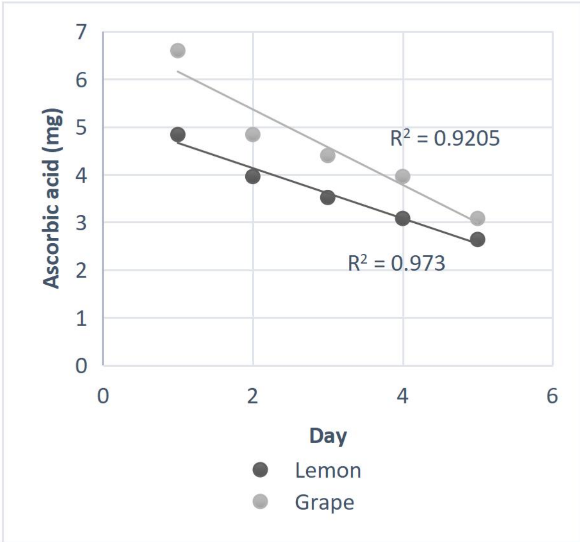


Figure 3.4b Plots of AA content for Lemon and Grape 80°C



A similar trend was observed for the results from quantification of ascorbic acid contents in the juice extracts at 100°C.

A linear relationship was established between these values as shown on figures 3.5a and 3.5b

Figure 3.5a Plots of AA content for Mango and Tangerine at 100°C

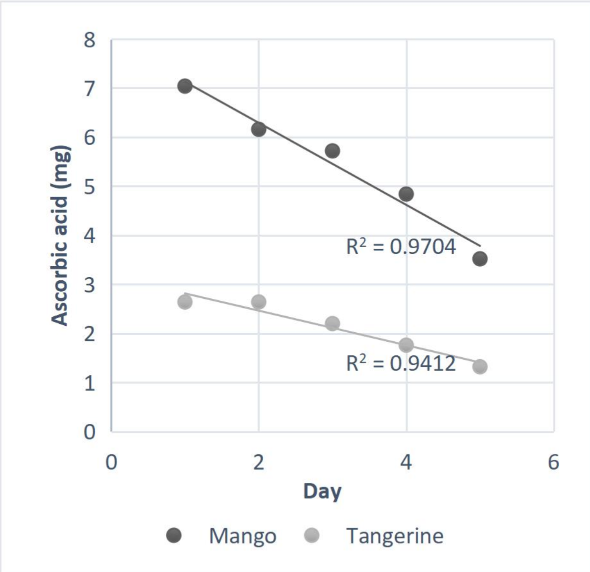
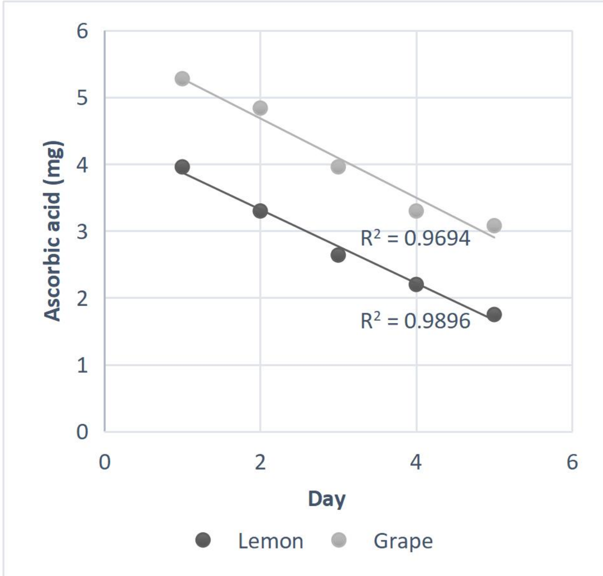


Figure 3.5b Plots of AA content for Lemon and Grape 100°C



From all the plots, a strong correlation exists between the time of heating at constant temperatures establishing that ascorbic acid content in fruit juice during heat processing is a function of temperature and time. Mango fruit still had the highest value all through and thus is a good source of ascorbic acid.

3.3 CONCLUSION

From the results obtained, the ascorbic acid content of the juice extracts from the selected fruit samples degrades with time and at elevated temperature, the decrease is hastened. This indicates that ascorbic acid is a thermally labile vitamin and its quantity in fruit juice extracts would gradually degrade as its shelf life increases and at higher temperatures (Bhagavan, 2001). A good correlation was established between the values as expressed in terms of the correlation coefficients and they can help determine the shelf life of the fruits with respect to their ascorbic acid content if temperature is kept constant and would help in determining the methods needed to preserve them without reducing their ascorbic acid contents over time.

3.4 RECOMMENDATION

The thermal lability of ascorbic acid has been demonstrated by this study thus when subjecting fruit juice extracts to heat on treatment, it is recommended that the ascorbic acid content of the fruit juice extracts are quantified first and if necessary, enrichment should be done to meet the daily dietary intake as recommended depending on the individual (Padayatti *et al.,* 2001; Ordman, 2010). But for best results, the cold treatment would be recommended if it is able to serve same purpose as that of the heat treatment which is majorly to curb the growth of microbes.