THE UNIVERSITY OF NAIROBI.

DETERMINATION OF PHENOLIC CONTENT LEVEL AND FREE RADICAL SCAVENGING ABILITY IN FRESHLY PREPARED AND COMMERCIAL ORANGE JUICE IN NAIROBI COUNTY-KENYA.

BY

DAVID WERE
I56/88938/2016

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY OF THE UNIVERSITY OF NAIROBI.

AUGUST, 2021

DECLARATION

I declare that this thesis is my original work and has not been submitted partially or wholly for the award of the degree to this or any other institution of learning. Where other people's works have been used, have properly been acknowledged and referenced following the University of Nairobi's requirements.

Signature  . Date…23/08/2021.

DAVID WERE.

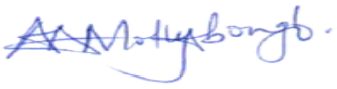
I56/88938/2016.

This thesis has been submitted for examination with our approval as the university supervisors.

DR. JOYCE G. N. KITHURE ……Date…23/08/2021.

Department of Chemistry

University of Nairobi.

DR. DEBORAH A. ABONG'O ……  Date…..23/08/2021.

Department of Chemistry

University of Nairobi.

DEDICATION.

First and foremost, I dedicate this research project to my parents for believing in me and for their encouragement to ensure that I become the best I can be. Secondly, I dedicate this work to my wife, Yvonne, and daughter Michelle for the love, support, and understanding they have given me throughout the program. Thirdly, I also dedicate this work to all my family members for their prayers and support they have given me up to this moment.

ACKNOWLEDGEMENTS.

I am greatly indebted to the Almighty God for his grace and the far he has brought me.

Special thanks to the University of Nairobi for giving me a scholarship that has enabled me to pursue this study.

I also extend my sincere gratitude to my supervisors, Dr. Joyce G. N. Kithure and Dr. Deborah A. Abong'o for their advice, guidance, and support they offered during the research study.

I also thank the Department of Chemistry-Coffee Research Institute for allowing me to use their laboratory for research.

Finally, special thanks to my colleagues for their guidance, encouragement, and support throughout the study.

ABSTRACT.

The orange fruit is widely consumed in the world. They are used to manufacture orange juice which is commercially sold as bottled orange juice. The higher consumption of orange fruits and orange products is because it has an attractive taste, has no cholesterol, and contains potassium, carbohydrates, vitamins A and C as well as different classes of phenolic compounds that help in the protection of the human body system against free oxygen radicals. This study aimed to quantify the total phenolic content and determine the free radical scavenging ability in both the freshly prepared orange juice and commercially bottled orange juice in Nairobi County. The total phenolic content (TPC) was done using the Folin-ciocalteu method. The total phenolic content of the fresh orange extract samples ranged from 129.75±0.39 mgGAE to133.1±0.95 mgGAE while the commercial bottled orange juice ranged between 110.23±0.23 mgGAE to 131.54±0.02 mgGAE. The study further evaluated the scavenging ability of both the freshly prepared orange juice and the commercially bottled orange juice using 2,2-diphenyl-1-picrylhydrazyl as a free radical. The ability to scavenge for free radicals of all the samples was expressed as the percentage scavenging activity which was compared with the standard antioxidant ascorbic acid, it was observed that the percentage scavenging acitivity of the fresh orange extract samples analyzed ranged from 16.40±0.02% to 18.32±0.1% while the percentage scavenging activity of the commercial bottled orange juice samples ranged between 10.20±0.8% to 20.28±0.23%. The higher the total phenolic content the higher the percentage inhibition.

TABLE OF CONTENTS

LIST OF FIGURES.

LIST OF TABLES.

LIST OF ABBREVIATIONS AND SYMBOLS.

µm	Micrometer
Abs	Absorbance.
ATP	Adenosine-5-triphosphate
BHA	Butylated hydroxyanisole
℃	Degrees Celsius.
CAT	Catalase
CBD	Central business district.
CCD	Charge couple device.
DAHP	3-Deoxy-D-arabino-heptulosonic acid-7-phosphate
DHQ	3-dehydroquinate
DNA	Deoxyribonucleic acid.
DPPH	2,2-diphenyl-1-picrylhydrazyl.
EPSP	5-Enolypyruvylshikimate-3-phosphate
HAT	Hydrogen atom transfer.
HOCl	Hypochlorous
HPLC	High-performance liquid chromatography.
LDL	Low-density lipoprotein.
LED	Light-emitting diode.
mL	Milliliters
MPO	Myeloperoxidase
NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NIR	Near-infrared.
nm	Nanometer
NO	Nitric oxide

OH	Hydroxide.
$ONOO^-$	Per nitrite oxide
ORAC	Oxygen radical absorbance capacity.
PEF	Pulse electric field
RNS	Reactive nitrogen species.
ROS	Reactive oxygen species.
SET	Single electron transfer.
SOD	Superoxide dismutase
TPC	Total phenolic content.
UV	Ultraviolet
UV-VIS	Ultraviolet visible

CHAPTER ONE

INTRODUCTION..

1.1 Citrus fruits.

Orange fruits, one of the citrus fruits originated from southern China and is now planted on a small and large scale in humid areas across the world. The common varieties of citrus fruits grown in Kenya include; Sweet oranges *(Citrus sinensis*), Limes (*Citrus aurantifolia*), Grapefruits (*Citrus paradise*), Lemons *(Citrus lemon*), and Mandarins (*Citrus reticulate*).

1.1.1 Sweet Oranges.

Sweet orange is known scientifically as *Citrus sinensis*. This is a fruit that has a small evergreen tree which grows to a height of 8-15ft (Etebu and Nwauzoma, 2014)**.** Table 1.1 shows the classification of the sweet orange fruit.

Table 1.1: Classification of orange fruits.

Kingdom	Plantae
Division	Magnoliophyta
Class	Dicotyledons
Subclass	Sapindales
Order	Rosidae
Family	Rutaceae
Subfamily	Aurantoideae
Genera	Citrus
Sub genera	Papeda
Species	Sinensis

The orange fruit is delicious and very juicy which makes it one of the commonly grown fruit in the world which is estimated to be 120 million tons (Parle and Chaturvedi, 2012). The orange fruit does well in areas that experience an average temperature of 18℃ with plenty of rainfall of about 60 millitres per month for the production of juice and also for its therapeutic value. Figure 1.1

shows the percentage of orange fruit with other citrus fruits (Mann 1987). From Figure 1.1, oranges compose 56% of citrus fruits worldwide followed by tangerines, mandarins and clementine (17%), lemons and limes (11%), grapefruit and pomelo (10%), and others (6%)

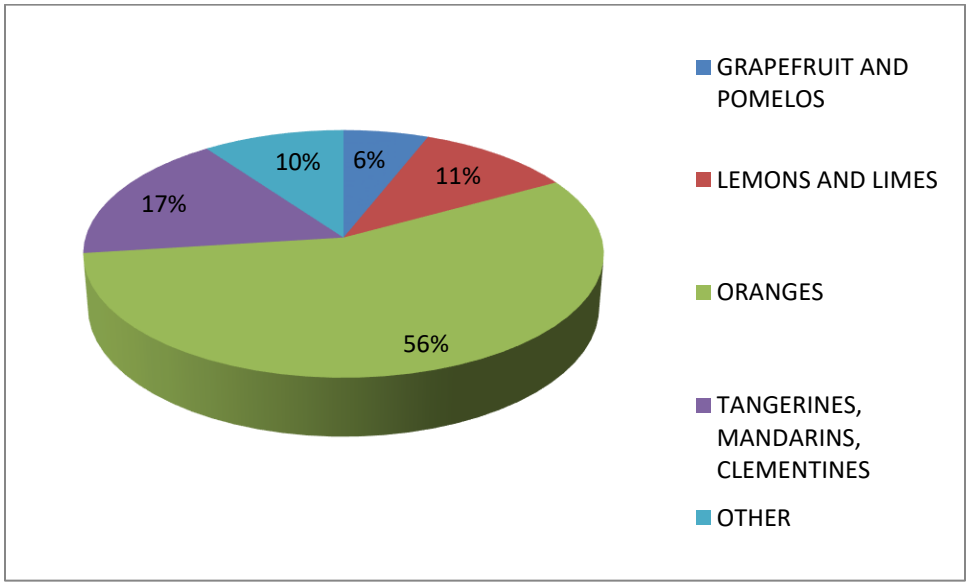


Figure 1.1: World distribution of citrus fruits by 2010.

Source: Mann 1987

The orange fruit is known to possess anti-oxidant property brought about by the fact that it has vitamin C, flavonoids, phenolic compounds, and pectins (Jae-Hee *et al.*, 2014). The orange fruits offer a large percentage of vitamin C per day. Vitamin C is water-soluble and the most common antioxidant which has the ability to inhibit the production of radicals in the body and destruction to the body tissues. Vitamin C also plays a key role in keeping away cold, cough, and recurrent ear infections. Furthermore, Vitamin C aids the body's immune system to function properly (Sudha and Reshman, 2017).

The consumption of orange fruits helps to guard against heart diseases by lowering the amount of homocysteine in the body. The vitamin C in the orange fruit, carotenoids, and the flavonoids are cardioprotective. The orange fruit also contains limonene that can lower the levels of dietary fat.

Furthermore, the orange fruit has been known to possess anti-carcinogenic property since it contains limonene which has the ability to lower the risk of several cancers in the body, for instance, cancer of the mouth, cancer of the skin, lung cancer, breast cancer, and stomach cancer and colon cancer. Its anti-carcinogenic property depends on the antioxidant properties of the molecules and also the ability to control the functionality of cleansing hepatic enzymes (Santa *et al*., 2017).

The orange fruits have also the ability to reduce the chances of developing kidney stones. The consumption of a lot of orange juice per day increases the pH value of the urine and the elimination of citric acid which leads to a reduction in the formation of calcium oxalate stones (Clarita, 2006). The orange fruits are vital in the prevention of stomach ulcers. Ulcers are caused by the presence of helicobacter pylori; the frequent consumption of orange juice daily lowers chances of infection with the helicobacter pylori thereby stopping the growth of ulcers, likewise, the orange fruit plays a crucial role in keeping away typhoid, which is a major problem mainly affecting the developing countries (Giuseppina *et al*., 2017).

The flavonoids in the orange are good in fighting the salmonella typhi liable for the development of typhoid. The orange fruit can also be used to reduce nervousness by the sweet orange oil which acts as an anxiolytic agent (Fahimeh *et al*., 2015). They are also good at relieving fever, when the pulp is roasted it can be compressed for the treatment of skin cancer. Acnes can be treated by rubbing freshly prepared peels on the affected area. The peels of orange contain oil that has a toxic effect on fleas, fire ants, house flies because it has a large percentage of limonene. The orange peels are also used medicinally to kill fungi (Maria *et al*., 2013).

The bioflavonoids present in orange to be precise hesperidin and naringin have anti-diabetic activity. The potential to deal with diabetes is aided through the ant-peroxidation and the inhibition of the activity of the α-amylase enzyme that causes change of the complex carbohydrates to glucose; it increases the amount of hepatic glycogen, stimulates the secretion of insulin, and repairs the pancreatic β-cells which have secretory defects (Franziska *et al*., 2018).

Orange has been used traditionally as a medicine to manage various ailments. This plant has been a source of flavonoids, terpenes, potassium, calcium, and various vitamins like B1, B2, B3, B5, A, B6, and C. It has been useful in the following cases; orange juice is very good in the removal of

toxic substances in the body, it keeps the body hydrated, it has been used in the management of obesity (Carolina and Thais, 2017).

1.1.2 Mandarins, Tangerines, and Clementine.

The orange fruits that have a thin loose peel and are dubbed 'kid glove' are all classified as mandarins. These oranges are considered to be members of distinct species namely *Citrus reticulate* (Usman and Fatima, 2018). They can also be referred to as tangerine instead of mandarin to stand in for the whole group but in the markets, it is used to refer to the oranges with red-orange skin. A clementine also belongs to the mandarin family; the fruit requires low heat for the maturity of its fruits.

The mandarin trees are known to be small; they have spines with slender branches. They have also nicely shaped symmetrical rounded crowns which do not require pruning. The tree starts producing fragrant flowers around March and April that attract hordes of honey bees. The fruits produced by most mandarin oranges contain a lot of juice and are very sweet with loose skin which can be peeled off easily. The oranges are similar to the mandarin fruits only that the mandarin fruits appear smaller and they contain loose skin that can be peeled off easily. The mandarin trees are usually erect, unlike other citrus trees. They also have long willowy branches that make the trees show a dropping habit. It has very brittle stems and the limbs are prone to breakages when they are loaded with a lot of fruits.

The tangerines and mandarins sometimes are confused to be the same fruit. However, the tangerine is a type of mandarin orange. The difference between a mandarin and a tangerine is the skin color (Daemmon *et al*., 2002). The tangerine is the most common mandarin fruit, its name was coined by the Americans for the mandarin fruit that went through the Moroccan port of Tangiers. The consumption of mandarin fruits has very many health benefits. The mandarin fruits help to solve cholesterol problems in the body system. The mandarins have fiber which plays a major role in cleaning the arteries removing bad cholesterol. It has also antioxidants that lower the levels of bad cholesterol and increase the levels of good dietary fat (Karsheva *et al.*, 2013). The cleansing of the arteries leads to a smooth blood flow and thus normal blood pressure is attained. Eating mandarin fruits is also important for people who wish to lose weight. This is facilitated by the fibers that make the stomach stay full for a long time and therefore leading to a reduction in the need to eat

food. The presence of vitamin C is also crucial in keeping away colds and the maintenance of a good immune system.

Furthermore, vitamin C provides the body with the antioxidants which are vital in the reduction of free radicals in the body (Satish and Dilipkumar, 2015). Studies have also shown that consumption of mandarin fruits regularly has a great impact on skin health. When the juice is taken regularly, the skin tends to look much better and healthier thus help to fight against aging. The mandarin fruit oil helps in the development of new cells and tissues; this makes it to be good in the curing wounds. The fruits also contain a lot of vitamins E and B12; these vitamins are good for healthy hair (Hind *et al*., 2018).

1.1.3 Lemons and Limes.

A lemon tree is very small and thorny. The tree grows to a height of about 10feet to 20feet (Christensen, 2018). The tree has leaves which are dark green alternating on the stem. The lemon tree produces white fragrant flowers each containing five petals. The lemon fruits have an oval shape and their skin is porous. The tips of the fruits are either pointed or rounded. Some of the lemon varieties include the bush lemon, Verna lemon, and eureka lemon, etc. The lemon fruits have color ranging from green-yellow to bright yellow.

The lemon fruits resemble limes only that the lemons are larger and appear yellow when they are ripe, unlike the limes which are green in color (Jose *et al*., 2015). The regular intake of limes has a positive impact on the immune system. First, it can be used to cure scurvy; the disease is characterized by the lack of vitamin C. The disease leads to frequent attacks with coughs, colds and cracked lips, etc. limes contain a lot of vitamin C which can be used to cure scurvy. Taking lime juice helps to rejuvenate the skin and makes it have a shining glow appearance. The lime juice also protects the skin from infections and lowers body odor because they contain vitamin C and flavonoids that act as antibiotics and as disinfectants (Mahendra, 2017).

The lime juice has acids which when applied externally on the skin it helps in scrubbing out of dead cells, does away with dandruff and rashes. Lime has also an irresistible scent that makes the mouth watery that eventually aids in the digestion of food. The flavonoids in the lime juice are good in stimulating the digestive system increasing the production of digestive juices, bile, and acids and also initiate the peristaltic motion (Mohanapriya *et al*., 2013). This is why lemon is

pickled with lunch and dinner in some countries. The consumption of lemon fruits is also vital in relieving constipation. This is because it has a roughage that aids in the digestion of food and the acids in it helps in the clearing of the excretory system by washing and cleaning off the tracts. Lime also helps in the fight against peptic ulcers since it contains flavonoids that act as antioxidants, antibiotics, and detoxification of the body and thus help in the healing of peptic ulcers. Lime has also been used to solve respiratory disorders. Lime oil is used to make anti congestive medicines for example the inhalers because they contain kaemferol. When a peel of lime is scratched and inhaled it results in immediate relief in congestion and nausea.

Vitamin C in the lime is also good in the protection of the eyes against aging and the flavonoids ensure that they are protected from infections. Besides, Lime is critical in the treatment of piles. This is brought about by the fact that pile is caused by ulcers, wounds, and constipation and since lime is used to heal ulcers, wound, and relieves constipation it, therefore, eradicates all the predisposing factors of pile keeping the body safe. Lime has a lot of potassium that can remove the harmful substances and the precipitates deposited in the kidneys and urinary bladder. The availability of potassium also helps stop the growth of the prostate and clears the urinary tract due to the deposition of calcium. Studies have also shown that lime juice can reduce weight (Frederick, 2010) due to the presence of citric acid that helps to burn excess fat in the body (Ajugwo, 2012). Other benefits of taking lime include, lime helps to cure arthritis, heart diseases, rheumatism, etc.

1.1.4 Grapefruits and Pomelo.

Grapefruits belong to the class known as *Citrus x paradise* obtained by crossing a pomelo and orange fruits Grapefruit and pomelo trees are very large and are normally green throughout the year. Grapefruits do well in warm areas since temperatures determine the time taken for the trees to produce flowers and also the time taken for the fruits to mature. The tops of grapefruits trees are round and the branches spread out, the leaves have ovate shapes and have lots of tiny oil glands. The flowers of grapefruits are white with four petals. The fruits are round in shape approximately 10 cm to 15 cm wide, they are smooth and with dotted peels (Jawad and Sardar., 2007). The middle of the fruits may be solid or semi-hollow; the colors of the pulps are either yellow or pink-red with 11 to 14 segments with thin membranes (Morton, 1987). The taste of grapefruit is more acidic than that of orange fruit and that of a tangerine fruit. Pummelo fruits are larger than oranges and grapefruits. They are also much sweeter than grapefruits and oranges. The consumption of

grapefruits and pommelo has a lot of health benefits. It has been used by people to manage their body weight; this is because it contains fiber that keeps the stomach full for a long time thus reducing the appetite for food. The fruit also contains vital nutrients such as vitamin A, calcium, phosphorus, potassium, iron, etc. which boost the immune system. The grapefruit juice is used as a beverage, is also used to make vinegar, and can also be fermented to make wine (Sujit *et al*., 2015).

Studies have shown that fruits, herbs, and vegetables contain phenolic compounds which are vital in scavenging oxygen free radicals in our body system. The free radicals may lead to the oxidation of lipids and DNA as well as the cause of various ailments which include rheumatoid arthritis, cancer, and various neurodegenerative and pulmonary diseases (Lobo *et al*., 2010). The powerful antioxidant ability of phenolic compounds from the oranges can help safeguard the body against the free radicals and hence they are important substances in obtaining and preserving human health (Ashwell *et al*., 2010).

Plants synthesize phenolic compounds as natural products which help them in dealing with physiological and ecological stresses, for example, plants have to protect themselves against insect attack, pathogens, harmful ultra-violet (UV) radiations, and wounding (Mierziak *et al.*, 2014). All phenolic compounds have aromatic rings in their structures with either one or several hydroxyls (OH) groups. These compounds are classified into two broad classes, namely; simple phenols and polyphenols. The different colors of the plants for example, purple, and yellow, amongst others come about because of the flavonoids which are biosynthesized by the plants from phenylalanine as well as tyrosine (Khoddami et *al.*, 2013).

The different classes of phenolic compounds depend on the extent of hydroxylation and the location of those groups on the ring. This leads to two classes that have distinct structures that are hydroxycinnamic derivatives, for example, p-coumaric, and the second class known as hydroxybenzoic derivatives, for example, Gallic (Milena *et al*., 2019).

There is another group of plant phenolic compound that is known as the cell wall phenolic. The compounds are unsolvable as well as exist in different forms within the cell modules. The cell wall phenolic can also be classified into two main classes, namely; hydroxycinnamic acids and lignins.

These compounds are very important as the plant grows because they help in the protection of the plant against infection, harmful ultraviolet radiation, and wounding (Oscar and Monica, 2018).

The process of determination and quantification of phenolic compounds in fresh orange and products has been an area of extensive research. There are several high-performance liquid chromatography (HPLC) methods that can be used to evaluate and quantify the number of compounds having phenol units in the orange fruit and orange processed products (Obafaye *et al*., 2015).

1.2 Statement of the Problem.

Rizvi and Kanti (2009) reported that citrus fruits contain phenolic compounds. The compounds are taken in our daily diet and it's estimated to be about one gram per day.

Phenolic compounds act as natural antioxidants, which scavenge harmful reactive oxygen species that cause degenerative ailments, for example, cancer, in addition to the progressive processes which come about as one grows older (Kumar *et al*, 2014).

The use of phenolic compounds as natural antioxidants forms a basis to investigate and determine their fate in the production of orange juice.

1.3 Hypothesis.

If fresh juice is consumed daily then it results in strong body immunity and lowers the chances of getting infected with several diseases.

1.4 Main Objective.

The main objective of this study was to quantify the total phenolic content and assess the free radical scavenging ability in freshly prepared and commercial bottled orange juice in Nairobi County.

1.4.1 Specific objectives.

The specific objectives were to:

1. Determine the total phenolic content of freshly prepared juice from Ngara, Gikomba, Muthurwa Markets and commercially bottled orange juice from retail stores at the central business district (CBD) in Nairobi City
2. Determine the free radical scavenging ability of freshly prepared orange juice from Ngara, Gikomba, Muthurwa Markets, and commercially bottled orange juice from retail stores at the CBD in the city.

1.5 Justification and the Significance of the Study.

Fruit juice contains phenolic compounds. The compounds provide the body with natural antioxidants, which are capable of preventing the body against ailments, for example, diabetes; this has led to an increase in the intake of fruits (Joanne and Beate, 2012).

The commercially processed orange juice go through various procedures such as, pasteurization and therefore, it is expected that these processes will make a difference in the both the total phenolic content and antioxidant activity from those of fresh orange juice (Savatovic *et al*., 2009). The current study was to provide more information to consumers on different commercial processed orange juice and the homemade fresh orange juice in terms of the total phenolic content and the free radical scavenging ability. This finding contributes a springboard for my study on the total phenolic content and free radical scavenging ability in processed orange juice in Nairobi County. If done for orange juice, for example, the results will form a basis for the study of the juice from other fruits in Nairobi County.

CHAPTER TWO

LITERATURE REVIEW..

2.1 Secondary Metabolites of orange plants.

These are natural compounds that are produced by particular plant species. They are produced to protect the plants against pathogens and for the plant interactions with the environment (Mazid *et al.*, 2011). The plants which manufacture secondary metabolites have been used widely by human beings to help them manage health issues, for instance, health disorders, diseases, and certain infections (Wink, 2012).

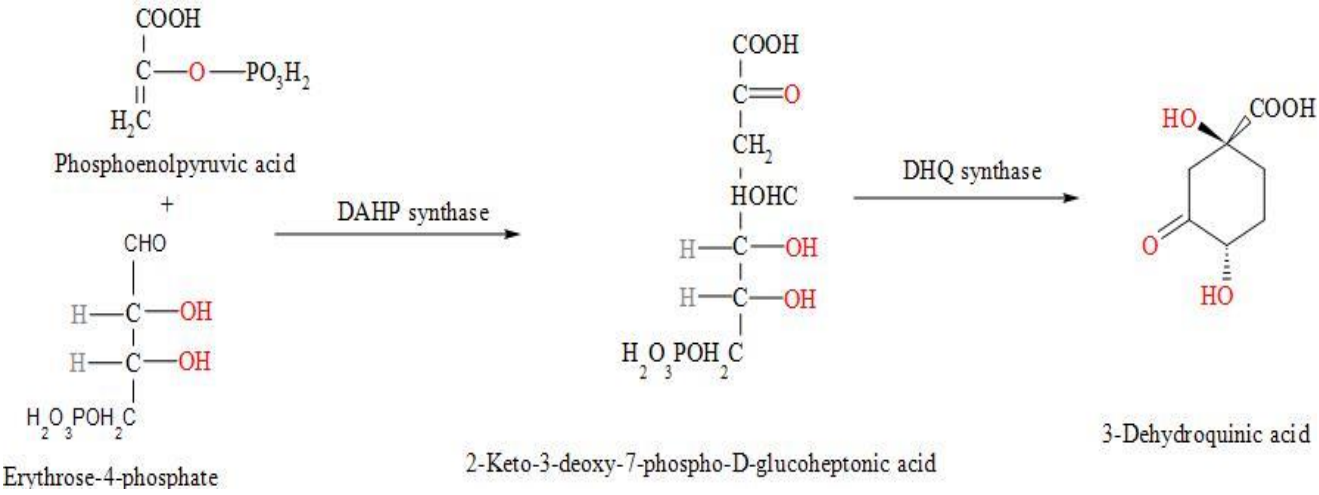
The secondary metabolites are manufactured by plants from the modified primary metabolite synthesis. These compounds are estimated to be more than 30,000 across all the plant species. The wide distribution of the secondary metabolites across the plant species enables us to classify them into three major classes basing on the following parameters such as their chemical composition, chemical structure, and their biosynthesis pathway (Tiwari and Rana, 2015).

The secondary metabolites are divided into three main classes namely; alkaloids, terpenes, and phenolic compounds. Alkaloids comprise all the secondary metabolites that are biosynthesized from amino acids. The alkaloids are composed of either one or several rings of carbon which contains nitrogen. Terpenes are secondary metabolites that are formed from the combination of several skeletons of five-carbon isoprene units (Mandal *et al*., 2010). Mandal (Mandal *et al*., 2010) reported that the most common natural compounds synthesized by plants are phenolic compounds, they are made up of aromatic amino acids phenylalanine and tyrosine synthesized through the shikimic acid pathway.

Shikimic acid was named after the Japanese flower known as shikimi and it's from this flower that the acid was isolated by Johan Fredrick Eykman in 1885 (Ghosh *et al*., 2012). The anionic form of shikimic acid is called shikimate. The shikimic acid pathway involves seven steps which are undertaken by plants, fungi, parasites, bacteria, and algae for the synthesis of tryptophan, phenylalanine and tyrosine which are aromatic amino acids. The shikimic acid pathway is only unique to plants and not found in animals and therefore, the amino acids tryptophan and phenylalanine are essential amino acids which must be provided in the animal's diet. Tyrosine can

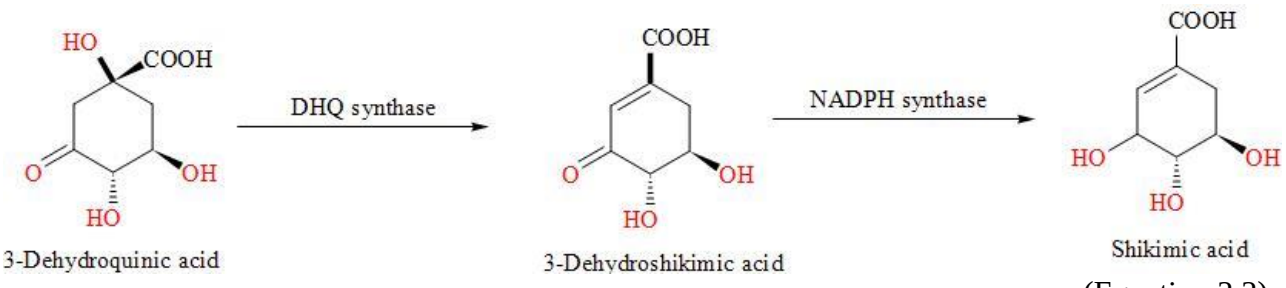
be synthesized by animals from phenylalanine and thus it is not an essential amino acid not unless the person cannot convert phenylalanine to tyrosine.

The seven steps synthesis starts with the reaction between phosphoenolpyruvate with erythrose-4-phosphate to produce 2-keto-3-deoxy-7-phospho-D-glucoheptonic acid. This first reaction is aided by the enzyme DAHP synthase (Equation 2.1).



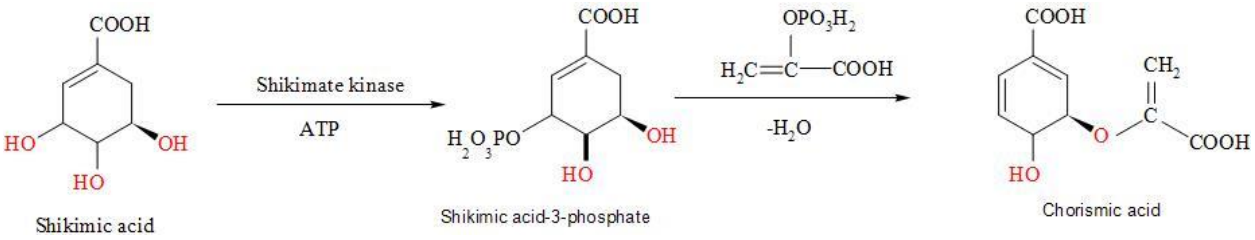
(Equation 2.1)

The second step in the shikimic acid pathway involves the conversion of 2-keto-3-deoxy-7-phospho-D-glucoheptonic acid to 3-dehydroquinate (DHQ) by the enzyme 3-dehydroquinate synthase (DHQ). This process needs a cofactor nicotinamide adenine dinucleotide (NAD) but it ends up not using it because the mechanism of the enzyme produces it leading to no need of nicotinamide adenine dinucleotide (NAD). The next step in the shikimic acid pathway involves the dehydration of 3-dehydroquinate to form 3-dehydroshikimic acid catalyzed by 3-dehydroquinate dehydratase enzyme which is further reduced to produce shikimic acid (Equation 2.2) aided by shikimate dehydrogenase enzyme (NADPH) and nicotinamide adenine dinucleotide phosphate (NADPH) which acts as a cofactor (Coracini and Azevedo, 2014)



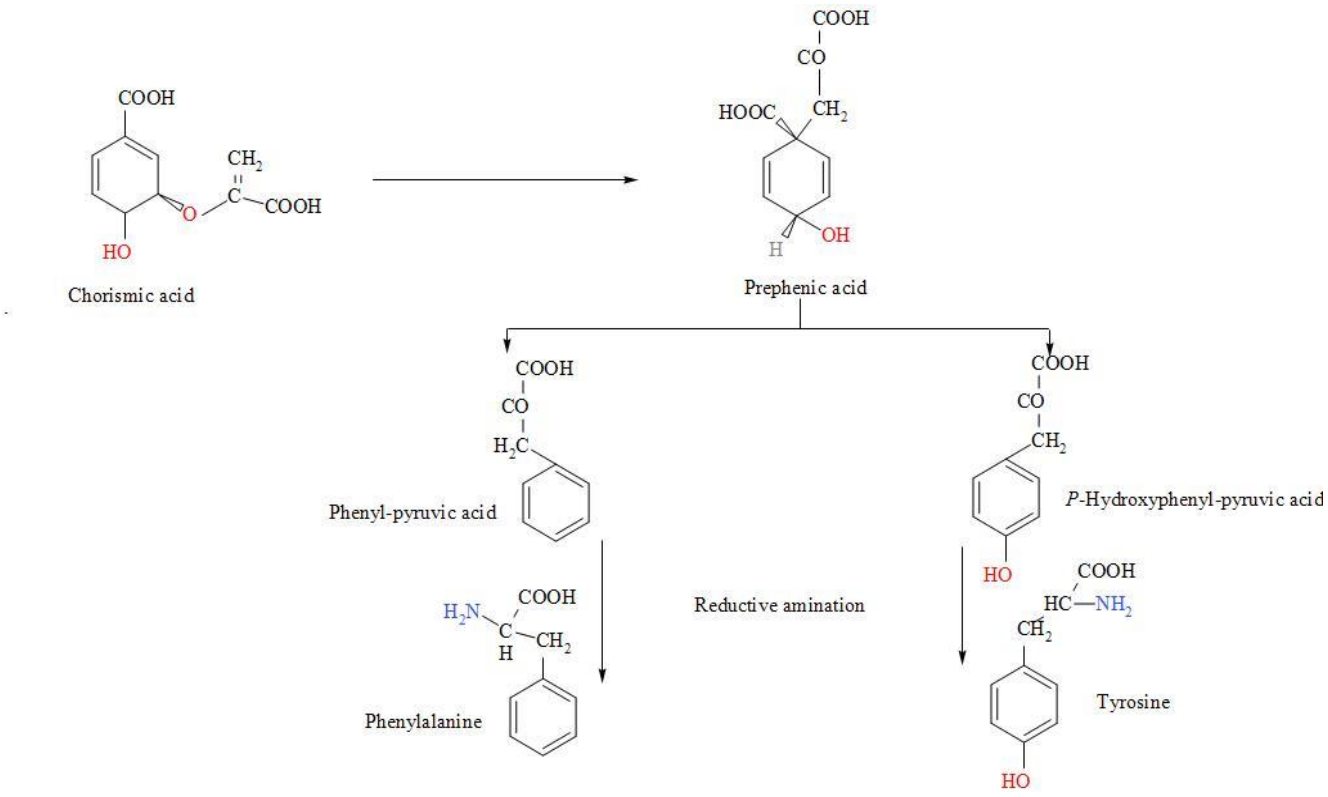
(Equation 2.2)

The fifth step in the shikimic acid pathway involves the phosphorylation of shikimate to produce shikimate-3-phosphate (Equation 2.3). The phosphorylation of shikimate depends on ATP and is catalyzed by the enzyme shikimate kinase.



(Equation 2.3)

Once the shikimate-3-phosphate is formed, is further combined with phosphoenol pyruvate to produce 5-enolpyruvylshikimate-3-phosphate (EPSP), the coupling is aided by the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase. The last step involves the conversion of 5-enolpyruvyl shikimate-3-phosphate to chorismate by the help of the enzyme chorismate synthase (Dewick, 2009)



(Equation 2.4)

Some of the common phenolic compounds and flavonoids found in the orange juice are shown in Figure 2.1.

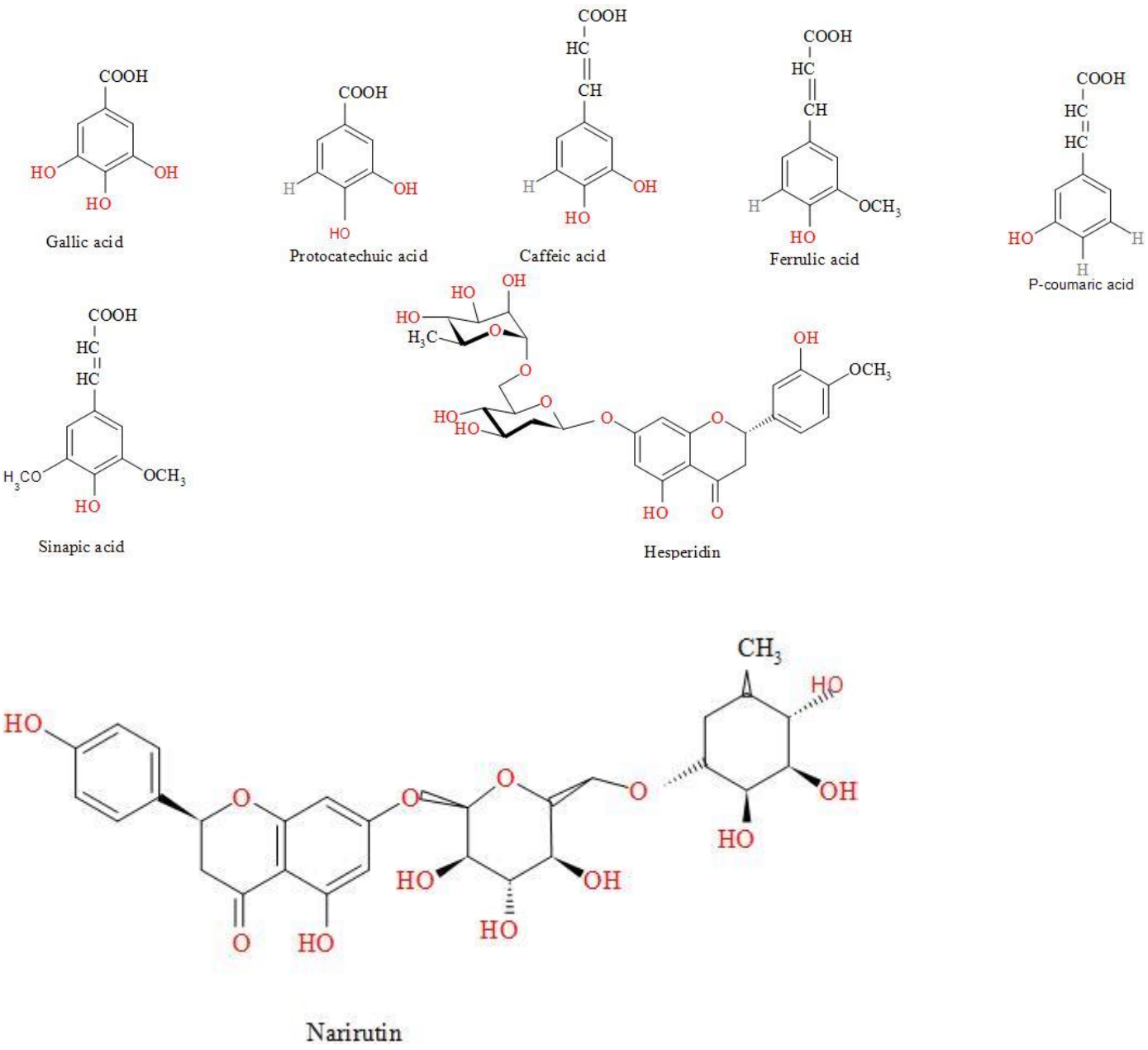
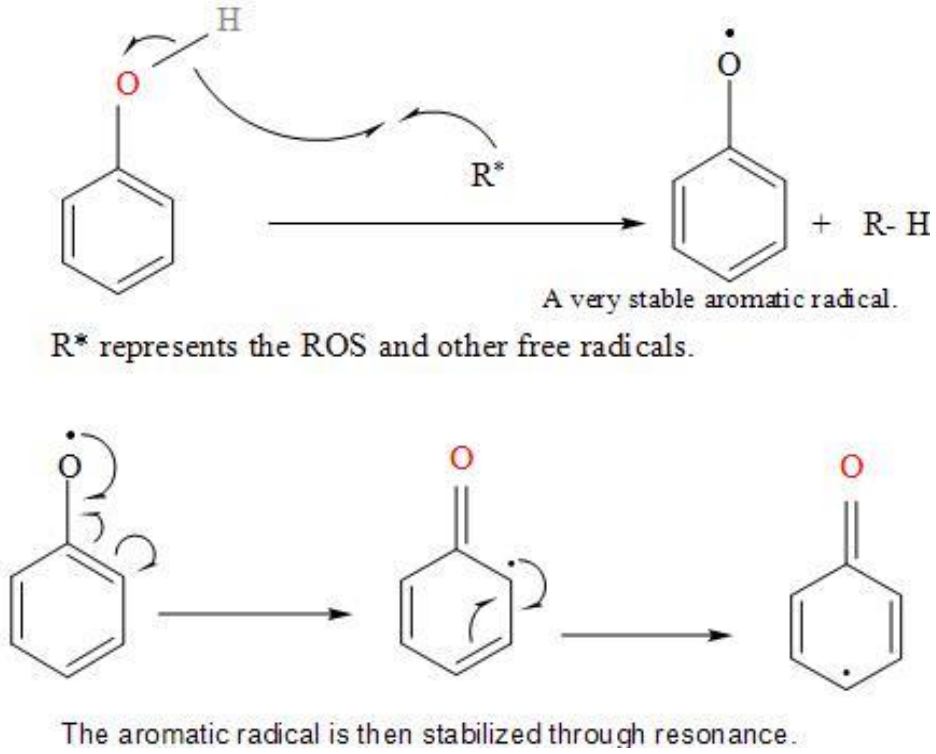


Figure 2.1: Structures of phenolic compounds and flavonoids in orange fruit juice.

The structures of these compounds contain the phenol subunit which is divided into simple phenols and polyphenols. The simple phenols include the phenolic acids (gallic acid, protocatechuic acid, caffeic acid, sinapic acid, ferrulic acid, and p-coumaric acid) while the polyphenols are known to have at least two phenol rings, for instance, the flavonoids, hesperidine and nairutin (Lattanzio, 2013).

Phenolic compounds are recognized as a source of ordinary antioxidants and thus important in food and biological systems because they undergo oxidation which results in sparing of nutrients, cells, and tissues. Lobo *et al*., (2010) noted that phenolic compounds have the potential to combat free radicals that are harmful to the body and food systems, they do so by forming a very stable aromatic radical which is resonance stabilized (Equation 2.5).



(Equation 2.5)

Phenolic compounds play a major role in the activation of the phagocytic cells, they also host-mediated tumor activity. Studies have shown that phenolic compounds were used in the treatment of wounds and management of a running stomach. Phenolic compounds have also been reported to have antimicrobial activities that are performed in the following ways; they interfere with the cell homeostasis that leads to inhibition of cell growth and subsequently cell death, they also deactivate the enzymes and formation of complexes with the cell walls and the metals. The simple phenols work by dispossession of the substrate and interruption of the cell membrane, the hydroxyl groups available on a particular phenol defines its level of toxicity (Malcolm *et al*., 2016).

2.2 Role of phenolic compounds in plants.

Plants manufacture natural products for their protection against disease-causing microorganisms and for physiological activities. The ultraviolet radiations have adverse effects on plants and therefore plants produce flavonoids that absorb the ultraviolet radiation preventing the plant from damage (Lwashina, 2003). Furthermore, the plants manufacture the phytoalexins which are good in fighting the disease-causing microorganisms. The phytoalexins fight the microorganisms by inhibiting the enzymes responsible for different diseases; they also do so by complexing with the extracellular proteins and interference with the cell wall (Mason and Wasserman, 1987).

Nahrstedt (1990) noted that phenolic compounds act as feeding deterrents to insects for instance the flavonoids like the luteolin that has been found to act as feeding deterrents to aphids (Dreyer and Jones, 1981). Besides, phenolic compounds are toxic to the larvae of the fruit worm Heliothis Zea and therefore they prevent the development of the larvae that depend on the leaves which contain these compounds (Isman and Duffey, 1982).

Mammalian herbivore poses a threat to plants and thus the plants produce some phenolic compounds to protect themselves against these animals; the best example is the black rush plant which produces the proanthocyanidins to protect itself from the snowshoe hare from feeding on it (Clausen *et al.,* 1990).

Phenolic compounds also play important roles during pollination and seed dispersal. The anthocyanins which give color to the various leaves and flowers are good in attracting the different insects and therefore help the plant in transferring pollen grains and in seed dispersal (Mazza and Miniati, 1993; Springob *et al.,* 2003).

Phenolic compounds have also been found to be allelopathic which help the plants to reduce competition. For example, the apple tree prevents the growth of other plants around it by producing phlorizin from its roots which are good at killing other plants (Mann, 1987). Plants attain stability and strength by producing the hydroxycinnamic acids that are used in the lignification of plants (Russel *et al.,* 1999).

2.3 Orange juice extraction process.

The orange fruit is a berry consisting of a peel which is aromatic and a fleshy succulent inner part. The outer part of the peel is called the flavedo with an orange colour. The flavedo has a number of oil glands which produce orange oil. The inner part of the peel is called the albedo, the albedo has a white colour and a spongy feeling. The fleshy succulent inner part of an orange is called the endocarp. The endocarp is made up of segments with wedge shapes and is highly filled with a number of fluid filled vesicles. The juice vesicles are most important section of an orange fruit because they are edible and are the ones which are used to produce orange juice (Ibrahim *et al*., 2020).

The orange fruits are harvested from farms and transported to the orange juice processing plant using tractors. On arrival at the plant, they are sorted out and cleaned. Sorting involves removing fruits that are very small, those destroyed by the pests and diseases and those that have withered. Thereafter, the fruits are thoroughly cleaned with water mixed with detergents using washing machines to remove mud and pesticide residues to avoid contamination of the juice.

The cleaned orange fruits are then taken to the extraction chamber where the orange peels are removed using machines; this separates the orange peels and the endocarp, the endocarp is the juicy succulent part of the orange from which the juice is stored. The endocarp is then pressed to obtain the juice which is taken to the filtration chamber to remove the coarse and suspended particles in the juice in order to maintain the colour and flavor of the juice. The process involves removing the unwanted solid substances from the juice. This happens once the juice containing vesicles have been pressed by machines to get out the orange juice. The removal of solid substances from the juice is done by the help of stainless steel screens which trap the solid substances such as embryonic seeds leaving solid free juice.

The solid free orange juice might contain air that has the ability to damage vitamin C and also lead to frothing associated fill problems at the packing step thus is taken through dearation process. De-aeration involves removing air from the juice so that it does not interfere with the quality of the juice. In addition, excess peel oil is also removed to prevent the juice from having a peculiar smell. The process of de-aeration is done just before the juice is thermally treated.

The extracted juice is then kept at a temperature of 91℃-95℃ for about 15 seconds to sterilize the juice; this process is known as pasteurization. The process involves heating of the orange juice with the intention of destroying the microorganisms which can spoil the juice and also denature the enzymes that weaken the juice matrix and lower the quality of juice**.** After pasteurization, the juice is further crushed using machines to make sure that the juice is homogenized, this removes pectin and a cloudy juice is obtained that will never precipitate. Finally, the pasteurized juice is led to a charging container from where the cans and bottles are filled directly.

2.4 Influence of processing of fruit juices on phenolic compounds.

In the production of orange juice for commercial purposes, it is always good to pasteurize it so that its shelf life can be improved. In the study done by Sentandreu et al (2007) on the influence of pasteurization on phenolic levels in the orange juice, it was noted that the process had a very little effect on the total phenolic content. To arrive at that conclusion, they had to prepare various orange juices that were squeezed through industrial processes; the juice was then pasteurized at 90℃ for a period of 30 seconds and then stored at a temperature of 20℃. They further determined the levels and antiradical activities of the flavanones-7-O-glycosides and fully Methoxylated flavones which were then compared to those of freshly prepared orange juice and noted that pasteurization, industrial squeezing or concentration had no effect on both their levels and the antiradical effects.

In another study done by Agcam et al (2014), where the orange juice was processed through pulse electric field (P.E.F) together with thermal pasteurization which was aimed to compare the changes in levels of phenols before and after storage at 4℃ for a period of 180 days. They noted that the orange juice that had the highest levels of phenolic was that one that had been processed through the application of high energy as compared to the orange juice that had been processed through the application of low energy. The reason for the observation made was that pulse electric effect increases the intracellular extraction by enhancing the permeabilisation of plant cells and therefore increasing the production of juice thus the intracellular metabolites are extracted which results in the enhanced extraction efficiency.

The impact of bioactive compounds in orange juice and their antioxidant activity in relation to the traditional thermal processing was done by Sanchez-Moreno et al (2005). In their study, they were

able to show that the concentration of flavonone in the orange juice was enhanced with the pulse electric field and thermal pasteurization. The concentration of the same flavonone was noted to be low when the same juice was treated with high thermal pasteurization at 90℃ for 60 seconds.

2.5 Packaging of orange juice.

There are huge investments in the beverage industry with the aim of expansion and up grading. The process of packaging beverages is a complex technological branch as far as food production is concerned. The traditional packaging of most beverages was the returnable glass bottles which have led to the development of other packaging containers like the plastic bottles and the paper cartons. Most of the packing materials of fruit juice have several layers for instance; the packaging material might have different layers of plastics or sometimes a combination of plastic with paper or plastic with glass. The type of the material used to make a package depends on the food characteristic that ought to be kept. For example, orange juice contains organic substances such as, phenolic acids, polyphenols, flavonoids, these organic substances are very sensitive to light, bacterial contamination and oxygen. For example, the plastic bottles are very economical and very flexible however; the plastic bottles are prone to oxygen permeability (Kumar, 2002)

To prevent entry of oxygen to the juice, a combination of polymer layer with a board is used to make the package. The board provides the package with the strength while the plastic acts as a barrier that contains the juice within the package. To further protect the juice from being spoiled by oxygen and light, an aluminium foil layer is included within a carton to make sure that oxygen does not get in and spoil the juice. The different types of packages used to pack the commercial orange juice are outlined in table 2.1.

Table 2.1: Packaging of commercial orange juice.

Brands	Package
S4	Plastic bottle
S5	Carton- aluminum coating
S6	Carton- aluminum coating
S7	Plastic bottle
S8	Carton- aluminum coating
S9	Plastic bottle
S10	Plastic bottle
S11	Plastic bottle

2.6 Shelf life.

Shelf life can be defined as the period within with the juice substance retains desired chemical qualities, physical characteristics, and biological characteristics and also complies with any information declared on the label under specified storage conditions. The end of the shelf life of juice products is brought about by the oxidative processes. It is expected that the juice will adhere to the information declared on the label within specified storage conditions. It is important to note that the shelf life of juice depends on the method used to process, the composition of the juice, the type of package used and most importantly the storage conditions (Mkandawire, 2016).

Table 2.2: The manufacture and expiry dates of orange juice as claimed on the label.

Brands	Date samples bought	Manufacture date	Expiry Date	Shelf life	Shelf life after opening
Sky food (S4)	4/07/2019	20 /03/2019	23/12/2019	9 months	7 days-refrigerated
Kevian (S5)	4/07/2019	18/05/2019	18/05/2020	12 months	3 days- refrigerated
Delmonte (S6)	4/07/2019	21/03/2019	20/03/2020	12 months	4 days-refrigerated
Excel (S7)	4/07/2019	12/04/2019	12/04/2020	12 months	Not indicated
Bidco (S8)	4/07/2019	18/05/2019	17/05/2020	12 months	Not indicated
Excel (S9)	4/07/2019	04/05/2019	04/02/2020	10 months	Not indicated
Nairobi bottlers (S10)	4/07/2019	24/06/2019	03/03/2020	9 months	4 days-refrigerated
Kevian (S11)	4/07/2019	07/4/19	07/4/2020	12 months	3 days-refrigerated
Fresh Oranges	8/07/2019			2-3 Weeks	1-2 Months-refrigerated
Cut Oranges				Same Day	1-2 Days-refrigerated

2.7 Free Radicals.

These are substances that contain at least an electron that is not paired in their valence shell. The presence of the unpaired electron makes free radicals to be energetically and kinetically unstable and it is also responsible for their reactivity (Phaniendra *et al.,* 2015).

Free radicals can be classified into several classes. Due to their numerous types, they are divided into two main categories namely; reactive oxygen species and those that contain nitrogen species.

Free radicals that contain oxygen atoms include, hydroxyl radical (OH^-), superoxide (O_2^-), peroxyl radical amongst others (Lobo *et al.*, 2010).

Reactive oxygen radicals are produced in the mitochondria during the reduction of oxygen in the electron transport chain during a normal aerobic process (Rahman, 2007). The reactive nitrogen species, on the other hand, are produced by specific biological systems. These nitrogen free radicals are extremely reactive resulting in great damages (Kozlov and Weidinger, 2015). The reactions of free radicals are very harmful, these reactions damage the stable molecules in their surroundings, and for instance, the reactions can damage the lipids, proteins, carbohydrates, and nucleic acids

The human body system produces free radicals as intermediates to assist in the various biological reactions. Also, the body produces free radicals to help in the transfer of signals to various reactions that are controlled by the enzymes. The human immune system has the responsibility of protecting the body against pathogens; the fight against pathogens is achieved by the help of free radicals that are naturally produced by the body (Pham-huy *et al*., 2008).

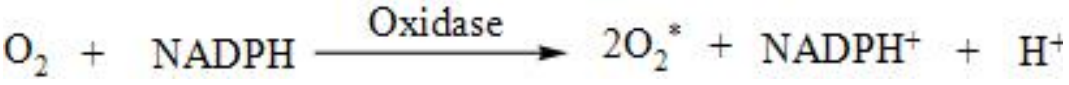
In the process of fighting pathogens, there can be the production of excess free radicals which become a threat to the body resulting in oxidative stress which leads to several biochemical and physiological injuries that interfere with metabolic processes leading to the death of cells (Lien *et al*., 2008)

The damage due to free radicals can result in numerous ailments and degenerative processes (Harrison *et al*., 2007). Due to the negative effects associated with free radicals, there is a need to reduce their level in the body. The reduction of free radicals can be achieved by frequent intake of antioxidants nutrients which can decrease the chances of free radical associated health challenges.

2. 7.1 Types of free radicals.

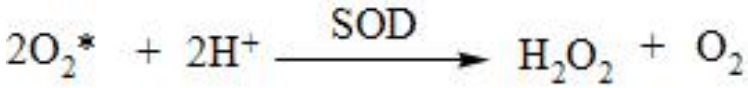
Free radicals are categorized into two main classes. Those comprising oxygen elements are known as the reactive oxygen species (ROS) and those that comprise nitrogen known as the reactive nitrogen species (RNS). Reactive oxygen species (ROS) are created during the ordinary biological processes in the mitochondria during the reduction of oxygen in the electron transport chain (Turrrens, 2003). The production of the free radicals consisting of oxygen starts with the intake of

oxygen, followed by the stimulation of the NADPH oxidase and then production of the superoxide anion radical, O_2* (Equation 2.6).



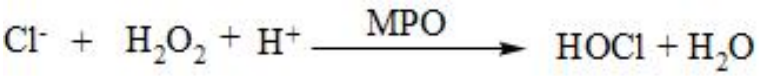
(Equation 2.6)

The superoxide radical formed in the reaction above is normally converted immediately to hydrogen peroxide (Equation 2.7) by the superoxide dismutase (SOD) (Pallavi *et al*., 2012).



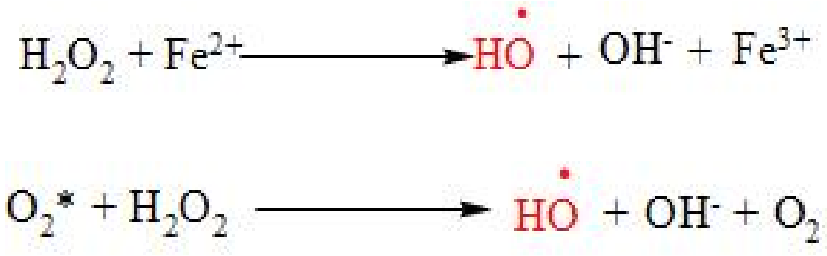
(Equation 2.7)

The reactive oxygen species can also be produced by the activities of myeloperoxidase (MPO) enzyme found in the neutrophil cytoplasmic granules where it converts the hydrogen peroxide to hypochlorous (HOCl) radical (Equation 2.8) in the presence of chloride ions (Ulfig and Leichert, 2021).



(Equation 2.8)

Fenton and Haber reactions also lead to the production of reactive oxygen species from the hydrogen peroxide and the superoxide (Equation 2.9).



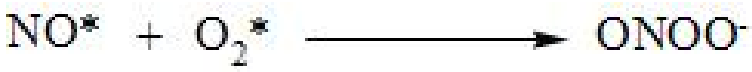
(Equation 2.9)

These radicals containing oxygen help in the transmission of various signals in the body in several enzyme reactions. However, if they are excessively generated they lead to oxidative stress and loss of cell function which results in cell death. The free radicals that contain nitrogen are also generated in biological systems only that their production is done for a particular function. The

reactive nitrogen species for example, the nitric oxide (NO*) are produced from arginine by the nitric oxide synthase (Equation 2.10) (McKinley *et al*., 2015).

(Equation 2.10)

The enzyme nitric oxide synthase keeps on generating good amounts of NO* radical which plays a role of quenching the superoxide radical O_2*. Once the NO* is produced, it further reacts with the superoxide radical forming the peroxynitrite radical $ONOO^-$ which is a very strong oxidant and therefore, the superoxide and the nitric oxide radical can regulate the effects of each other (Equation 2.11).



(Equation 2.11)

The peroxynitrite being a very strong oxidant, it has the ability of attacking a number of biological systems for instance, it combines with the aromatic amino acid residues in the enzyme leading to nitration of the aromatic amino acids, the changes that occur to the amino acids make the enzyme to be deactivated. The reactive nitrogen species are very reactive and therefore can damage the body cells through the oxidative stress and nitration of molecules (Sergio *et al*., 2016).

2.7.2 Benefits of free radicals to the body.

Droge, (2002) noted that manageable levels of free radicals are very important to the body system. They are involved in the biosynthesis of some cell organelles and also used by the body defense system to fight disease causing organisms. For example, phagocytes manufacture and keep free radicals which are then availed in the attack and destruction of disease causing microbes.

The best example to demonstrate how important free radicals are to the body is seen in people suffering from granulomatous disease, these patients cannot release the superoxide radicals (O_2^{*-}) due to malfunctioning NADPH oxidase system and therefore they are exposed to a number of persistent and continuous infections (Valko *et al*., 2007).

Free radicals also act as messengers to various cellular activities. In this case, they are released by the NADPH oxidase system that is non-phagocytic where they are involved in the regulation of intracellular signals. For example, nitric oxide radical (NO) does very well as a messenger in blood flow modulation, involved in thrombosis and very vital in the normal neural activity. The nitric

oxide also defends the host against pathogens within the cell and against tumor cells (Pacher *et al*., 2007)

2.7.3 Dangers of free radicals to the body.

There are a number of diseases associated with oxidative stress for instance, diabetes disease that is brought about by the defect in the metabolism of glucose. When the glycated protein cells produce a lot of the reactive oxygen species they lead to the development of complications in the disease which results in impaired healing and perforations in ulcers (Paolisso & Giugliano, 1996).

The free radicals both the reactive oxygen species and the reactive nitrogen species stimulate the development of cancer by starting it, stimulating and allowing it to grow. The very highly reactive free radicals initiate and promote cancer that ultimately results in the damage of the DNA and end up triggering the procarcinogens and then change the cell defense system (Trueba *et al..,* 2004). The damage that occurs to the DNA has adverse effects to the entire body system which results in the halting of transcription.

Parkinson's disease arises from the death of dopaminergenic cells in substantia nigra. The deaths of the cells lead to the reduced availability of the dopamine to the striatum that regulates movement. Danilson and Anderson, (2008) stated that oxidative stress that consists of peroxidation of lipids is one of the mechanisms that have been suggested as possible causes as dopamine cell degeneration in this disease. The over production of oxidants in the body leads to the damage to the substantia nigra of the brain due to the free radical reactions that depend on iron.

Alzheimer's disease that is characterized by the growth of masses of nerve fibers, senile plagues and loss of brain cells. This is has been enhanced by the oxidative stress that accrues free radicals resulting to excessive perioxidation of lipids and deteriorations of the neurons in the some parts of the brain (Lyketsos *et al*., 2006). The brain cells are susceptible to being damaged by the free radicals because they consume a lot of oxygen at higher rates as compared to other organs in the body (Cai and Yan, 2007).

Atherosclerosis disease caused by the atherosclerotic plaques is dumped in the walls of the arteries that cause their walls to become narrow and hard. There is evidence that oxidized variation of the

low density lipoprotein (LDL) is responsible for the start of the atherosclerosis disease (Singh and Jiala, 2006). The macrophages takes the oxidized low density lipoprotein through their scavenger receptor which results in the formation of lipid laden foam cell that plays a major role in the early stages of this disease (Henriksen *et al..*, 1983).

2.8 Antioxidants.

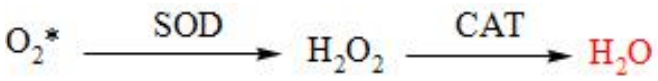
Antioxidants are substances that prevent the oxidation of molecules and therefore, they have various physiological roles in the body. Furthermore, antioxidants are good scavengers of free radicals; they do so by combining with the reactive radicals and destroying them, thus become less harmful. The antioxidants reduces the harmful effect of free radicals by either gaining an electron or electrons from the free radicals or by releasing an electron or electrons to the free radicals, resulting in the stabilization of the free radicals (Lü *et al*., 2009).

Antioxidants can be classified basing on several factors for instance; they can be grouped depending on their activity. This gives us two types of antioxidants namely, the enzyme based antioxidants which are responsible for converting the harmful substances in the body to hydrogen peroxide and then to water which is harmless in the presence of cofactors like copper. The non-enzymatic antioxidants for example vitamin C and E work by stopping the chain reactions brought about by the free radicals (Shahidi and Zhong, 2010).

Antioxidants can also be classified depending on their solubility in either water or lipids. For example, vitamin C dissolves in water and therefore is a water-soluble antioxidant. Furthermore, antioxidants can be categorized as either small or large. In addition, antioxidants can be classified as either natural or synthetic. The natural antioxidants are mainly the phenolic acids that combine with the free radicals and as a result the free radicals are transformed into stable substances. The synthetic antioxidants include the butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT) which is very good at stopping the chain reactions by capturing the free radicals (Mathew *et al*., 2011).

The antioxidants inhibit free radicals from oxidizing molecules, they act through various mechanism, first and foremost, they work through prevention method. This is crucial because some of the radicals are irreversibly produced for example, hydrogen peroxide, superoxide and the hydroxide radical. Therefore, cells produce antioxidants that help to protect the body system against the formation of free radicals. The superoxide dismutase enzyme helps in the conversion of

the superoxide radical to hydrogen peroxide which is further converted to water by the catalase enzyme (Equation 2.12) (Pandey and Rizvi, 2010).



(Equation 2.12)

The Second method which antioxidants work is by scavenging the superoxide radical (O_2*), and other reactive oxygen radicals. The superoxide is a common free radical produced in the cells in the process of killing pathogens and as a byproduct of the mitochondria. It contributes to a huge number of harmful changes which are associated to low levels of oxidants and related with a raise in per oxidative processes. The superoxide radical is not reactive to biological molecules therefore; it helps in the production of hydroxide and per nitrite oxide ($ONOO^-$) radicals which are very strong. Furthermore, the antioxidants scavenge the hydroxyl radical (OH*) and other reactive oxygen species. The hydroxyl radical is known to be very active and also more toxic on biomolecules as compared to other radicals. It is formed through the incubation of iron (ii) sulphate and hydrogen peroxide in aqueous solution in the Fenton reaction, thus the antioxidants prevent the formation of hydroxyl radical by directly scavenging on them and prevent their production by chelation of free metal ions (Krishnamurthy and Wadhwani, 2012)

In addition, the antioxidants lower the effects of free radicals through metal ions chelation. For example, the hydrogen peroxide radical produced as a product of dismutation of the superoxide radical by superoxide dismutase enzyme reacts with copper and iron ions leading to the formation of hydroxide radical which is very active. In the same vain, the iron ions and copper ions react with the hydrogen peroxide forming more singlet oxygen as compared to hydroxide. Therefore, the iron (ii) ions and copper (i) ions are further oxidized to form iron (iii) ions and copper (ii) ions respectively. In the cells, the iron (iii) ions and copper (ii) ions are again reduced which making it possible for their reduced form to react with the hydrogen peroxide to form the hydroxide radical in the presence of vitamin C. The hydroxyl radical being very active, it combines with proteins and lipids which lead to the formation of carbonyls, lipid peroxidation and cross linking. The production and action of hydroxyl radicals can be minimized through metal ion chelation (Krishnamurthy *et al*., 2012).

Several procedures can be used in the laboratory to assess the antioxidant properties of plant samples. The protocols used work based on the production of free radicals. There are several researches that are being done on antioxidants but the only challenge is that it is hard to relate the results obtained from the different protocols. Antioxidant properties of plant samples can be determined using three classes of assays. There are those assays which depend on the transfer of hydrogen atoms (HAT), we have those that rely on the single electron transfer (SET), and finally

those that rely both on the transfer of hydrogen atoms and at the same time involve the transfer of a single electron (Moharram and Youssef, 2014).

The assay that involves the transfer of hydrogen atoms to assess antioxidant properties of plant samples measure the competitive kinetics and are mainly made of man-made radical generators, they also contain molecules that can be oxidized which acts as a probe and an antioxidant compound. During the hydrogen transfer mechanism, the chain reactions brought about by the free radicals are broken down which leads to the stoppage of the reactions *(*Mustafa *et al.,* 2016*).*

Protocols that make use of a single electron transfer mechanism rely on the redox reactions in which the species acting as the probe is the antioxidant that is used to monitor the reactions. This mechanism makes use of two species in the reaction mixture which are the antioxidant and the oxidant. The antioxidant and the oxidant are related in the following equation;

$$Antioxidant + Oxidant = Reduced\ oxidant + Oxidized\ antioxidant$$ ……..(Equation 2.13)

When the reaction takes place the oxidant changes its color due to gaining an electron from the antioxidant. The extent of color change varies directly as the concentration of the antioxidant in the reaction mixture. The end point of the reaction is attained when there is no further change in the color of the mixture (Simona *et al*., 2010).

Huang *et al*., (2010) classified the antioxidant protocols depending on the type of reaction taking place. They include; those that make use of single electron transfer for instance the Folin-ciocalteu, 1,1-diphenyl-2-picrylhydrazine radical scavenging, etc. and those that make use of transfer of hydrogen atoms from the oxidizing agent to the reducing agent, they include; oxygen radical absorbance capacity (ORAC).

2.9 UV-Vis spectrometry.

2.9.1 The basic principle.

It involves the analysis of samples in the liquid, solid, and gas form by the use of radiation energy ranging from 300 nm to 3200 nm. This range of radiation is classified into three categories i.e. first is the ultraviolet radiation that ranges between 300-400 nm, second is the visible radiation that ranges between 400-765nm, and lastly the near-infrared radiation that ranges between 765-3200nm. The equipment used in the analysis is known as the UV-Vis-NIR-Spectrophotometer.

The analysis of the sample is achieved by allowing a beam of light to pass through the sample and then the wavelength of light that reaches the detector is measured. From the measured wavelength of the light very important information of the sample can be obtained, for example, the chemical structure of the sample and the concentration can be determined from the intensity of light (Sibilia, 1998).

The principle of absorption spectroscopy is that light comes in small packets called photons and for a transition to occur the energy level of the photon has to be sufficient enough to make an electron be promoted from one energy state to another for example from the ground state to the first excitation state. When the sample is placed in the machine, light of a particular wavelength and energy is directed to the sample where some of the light is absorbed and the rest which is transmitted light from the sample is recorded by the use of a photodetector as the absorbance of the sample. The human eye is not sensitive to the ultraviolet range and therefore, the UV-Vis-NIR-Spectrophotometer makes it possible for absorbance to be measured from the ultraviolet range to the visible range which the human eye can be able to detect (Kelleher and Podobedova, 2008).

The relationship between the incident light directed to the sample and the transmitted light from the sample can be illustrated as shown in Figure 2.2:

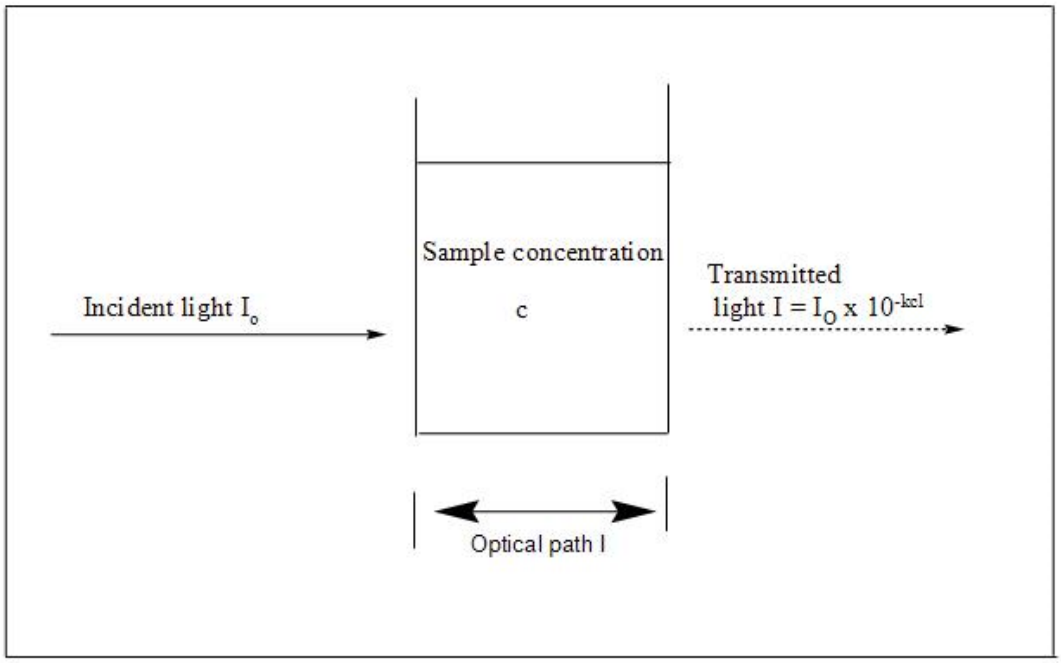


Figure 2.2: The Bouguer-Beer rule (Haim *et al*., 2008).

From Figure 2.7, $Transmittance\ (T) = \frac{I}{I_0}$……..…..Equation 2.14

$Transition\ rate\ (T\%) = \frac{I}{I_0} \times 1000$…….……...…Equation 2.15

The absorbance of the sample is determined as $\log \frac{I}{T} = \log \frac{I_0}{I}$ ……..…Equation 2.16

Leading to the relationship:

$T = \frac{I}{I_0} = 10^{-kcl}$……..Equation 2.17

Therefore; absorbance, $(Abs) = \log \frac{I}{T} = \log \left(\frac{10}{I}\right) = -kcl$…….…..…Equation 2.18

Where, k = proportionality constant and l = length of the light path in centimeters (cm).

From the formulas it can be seen that absorbance is directly proportional to the sample concentration and the length of light. If the concentration of the sample under analysis is 1 mol/L and the length of the cuvette is 1 cm then the proportionality constant is called the molar absorption coefficient and its normally expressed using the symbol ε (Kalantar-zadeh and Fry, 2008). The molar absorption coefficient provides information on the features of the sample under certain and specific conditions.

2.9.2 UV-Vis-NIR-Spectrophotometer experimental set-up.

A UV-Vis-NIR-Spectrophotometer is made of a light source producing visible light or ultraviolet light, a monochromator that helps to select light a particular wavelength, a sample holder, radiation sensor, signal processor, and a reader. The source of radiation comes from either a tungsten filament, a deuterium arc lamp that is continuous over the ultraviolet region or from light-emitting diodes (LED), or xenon arc lamps for the visible wavelengths (Kalantar-zadeh and Fry, 2008).

The detectors are usually photodiodes or sometimes CCD. The photodiodes are used with the monochromator to enable the filtering of light so that only light of a particular wavelength reaches the detector. When analyzing a sample within the ultraviolet range then the lamp producing the visible light has to be put off and the same is done when analyzing the sample within the visible region. The diagram below shows a schematic arrangement of a UV-Vis-NIR-Spectrophotometer (Pavia *et al*., 2008).

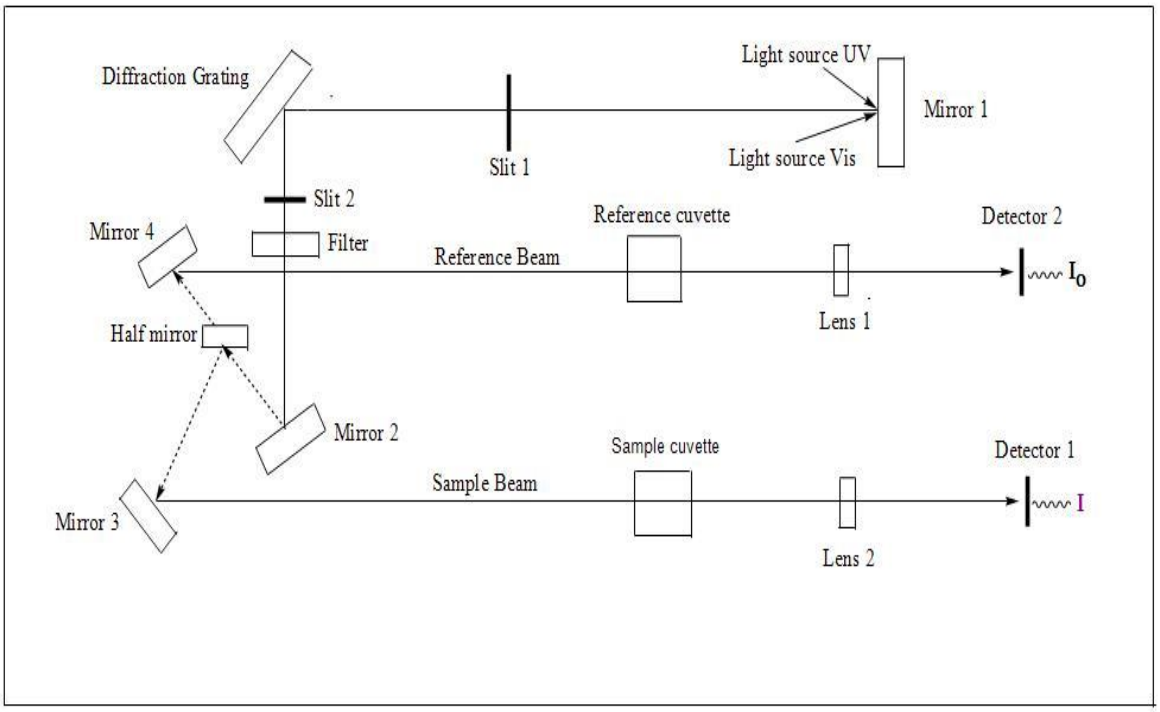


Figure 2.3: Schematic arrangement of UV-Vis-NIR-Spectrophotometer.

From the figure, the light from the source is split into two equal beams by a half mirror before it is illuminated to the sample. The sample beam is made to go through a transparent cuvette which contains the sample dissolved in a solvent while the reference beam goes through another transparent cuvette that contains only the solvent. The detector is light sensitive and therefore it follows the chamber holding the sample so that the transmitted light from the sample in the cuvettes is measured and the value is displayed on the screen (Kalantar-zadeh and Fry, 2008).

The two light beams from the reference and the sample are electronically measured and compared. The UV-Vis spectrophotometer measures the sample beam and that of the reference at the same time. The intensity of the reference is normally denoted as I_0 and that of the sample is denoted as I. the machine automatically scans the sample wavelength within a short time i.e. the visible region is scanned between 400-800 nm and the ultraviolet region is scanned between 200-400 nm. UV-Vis-spectrophotometry can be used to determine the concentration of the sample, the changes in the structure, and also the changes in the vibration and conformation energy levels after and interaction with another molecule (Pavia *et al*., 2008).

CHAPTER THREE

MATERIALS AND METHODS.

3.1 Chemicals.

The chemicals that were used in this study were of analytical grade and they were all locally acquired from Vision Scientific Laboratory Chemicals within Nairobi city. They included, gallic acid, Folin-ciocalteu, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, ascorbic acid, nitric acid, chromic acid, and distilled water.

3.2 Apparatus and equipment.

The apparatus and equipment that were used were; 100 ml volumetric flasks, 250 ml glass beakers, 20 ml pipettes, wash bottles, plastic basins, knife, filter funnels, 250 ml conical flasks, refrigerator, Perkin Elmer UV-Visible spectrophotometer, 0.45 µm pore filter membranes, sonicator, and analytical balance.

3.3 Cleaning of glass and plastic containers.

The glass apparatus that was used in the study were soaked for 24 hours in freshly prepared chromic acid solution. Thereafter, they were rinsed using distilled water, which was followed by soaking them again in distilled water for another 6 hours to remove any absorbed chromic ions. All the glassware was then dried in the oven after having rinsed with distilled water. The plastic apparatus were first cleaned with a detergent and then rinsed with distilled water and finally dried in the open rack.

3.4 Sample collection and transportation to the laboratory.

Triplicate orange fruit samples were each purchased from Ngara, Gikomba, and Muthurwa markets in Nairobi County. The samples were packed in self-sealing polythene bags labeled and stored in polyurethane cooler boxes and transported to the laboratory at the Department of Chemistry, Coffee Research Institute. In the laboratory, the fruits samples were thoroughly washed with running water from the tap and then rinsed using distilled deionized water and kept in the fridge at 4.2℃ awaiting extraction and analysis. Commercial Orange juices were purchased in triplicate from retail stores from the central business district of Nairobi city.

Table 3.1: Composition of Orange juices as claimed on the label.

Brands	**Ingredients**
Sky food (S4)	Water, concentrated juice of oranges, Ascorbic acid(vitamin C) Natural flavor, Beta Carotene
Kevian (S5)	Orange juice concentrates
Delmonte (S6)	Water, pure orange juice and natural orange flavors
Excel (S7)	10% orange juice, water, sugar, stabilizer (soadium carboxymethyl cellulose) acidity regulator (citric acid), antioxidant (ascorbic acid), preservatives (potassium sorbate, sodium benzoate)
Bidco (S8)	Water, sugar, Orange juice Concentrate (10%), Acidity regulators (Citric Acid and potassium citrate) stabilizer (Cellulose gum), Natural flavourings, colour,Beta carotene.
Excel (S9)	Purified water, sugar, orange emulsion, citric acid, stabilizer, permitted food colours and flavours, preserved with sodium benzoate.
Nairobi bottlers (S10)	Water, sugar, orange pulp, orange juice concentrate, acidulants(citric acids and malic acid, stabilizers E468, E414, E445, Trisodiumcitrate, ascorbic acid, nonnutritive sweeteners (sodium saccharin, glucose syrup and color beta carotene.
Kevian (S11)	10% Orange juice concentrate,, purified spring water, sugar and citric acid.

Table 3.2: Nutritional facts as claimed on the label.

Brands	Calories	Total Fat	Na	Total Carbohydrate	Total Sugars	Vitamin C	K	Protein	Ca
Sky food (S4)	120 kJ	0	10 mg	29 mg	25 g	72 mg	244 mg	0 g	30 mg
Kevian (S5)	46 KCL (194K)	< 0.2 g	Not indicated	10 mg	Not indicated	Not indicated	Not Indicate	0.5 g	Not indicated
Delmonte (S6)	239 KJ /56KCal	trace	Not indicated	13.5 g	Not indicated	33.4 mg	Not Indicate	0.3 g	Not indicated
Excel (S7)	217 kJ	< 1 g	Not indicated	12.4 g	Not indicsted	Not indicated	Not indicated	< 1g	Not indicated
Bidco (S8)	210 KJ	0	<0.01 g	12 gm	12 gm	9 mg	Not indicated	<0.1 g	Not indicated
Excel (S9)	Not indicated	Not indicated	Not indicated	Not indicated	Not indicated	Not indicated	Not Indicate	Not Indicate	Not indicated
Nairobi bottlers (S10)	193 KJ /45KCAL	0	12mg	10.6 mg	9.3 mg	2.5 mg	Not Indicate	0	Not indicated indicated
Kevian (S11)	294 K /58.7KCal	<1 gm	Not indicated	14.3 gm	Not indicated	Not indicated	Not Indicate	<1 gm	Not indicated

3.5 Sample preparation.

3.5.1 Extraction and clean-up of orange juice.

The orange juice used for analysis was obtained using the modified procedure as described by Bates et al (2001). The orange fruits were first washed in running tap water and then rinsed using distilled water. The oranges were then sliced into halves using a sterilized stainless steel knife; seeds were removed and squeezed by hand into glass beakers to obtain the juice. The juice was then filtered through number one size filter papers to obtain clear juice and kept in the refrigerator at a temperature of 4.2℃ to avoid degradation awaiting analysis. The commercially processed orange juice samples were also filtered using number one size filter papers and then kept under the same conditions as the extracted fresh orange juice waiting for analysis.

3.5.2 Preparation of gallic acid and ascorbic acid stock standard solutions.

The stock standard solutions used in the study were prepared by dissolving accurately weighed 300 mg of gallic acid and then transferred into 100 ml volumetric flask, 100 ml of methanol was added up to the mark. The mixture was ultrasonicated to obtain a homogeneous solution. This stock solution was labeled standard solution 1 (one) with a concentration of 3000 ppm. While 3000 ppm of the ascorbic acid stock standard was also prepared by accurately weighing 300 mg of the acid, transferring to 100 ml volumetric flask, and following procedures as was done with gallic acid. This stock ascorbic acid solution was labeled standard solution 2 (two).

3.5.3 Preparation of gallic acid and ascorbic acid calibration curves.

Using the stock solutions of gallic acid and ascorbic acids solution prepared in section 3.6.2 above, various calibrating standard solutions of 5 ppm, 10 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm were prepared from the stock solution using the equation 3.1

$$C_1V_1 = C_2V_2 \quad \text{.....Equation 3.1}$$

Where; C_1 and V_1 are respectively initial concentration and volume while C_2 and V_2 are respectively the final concentration and volume.

The calibration curves for Gallic and ascorbic acids were prepared by using 10 ml cuvette as sample vials and methanol as a blank sample and scanning the standards using UV spectrophotometer at ƛ max = 515 nm and 730 nm for Gallic and ascorbic acids respectively

3.5.4 The Folin-Ciocalteu Assay.

Lopez et al, (2003). Noted that for a very long time, phenolic compounds were used as natural antioxidants. This is because phenolic compounds are capable of releasing electrons and also have the ability to stabilize free radical intermediates in the prevention of oxidation of cellular and physiological level. Phenolic compounds and polyphenols have a higher percentage composition in various parts of the plant and therefore it is important to work out the number of phenols in fruit juices. In this study, the quantity of phenolic compounds in the orange juice samples was determined by the use of the Folin-ciocalteu assay (Kujala *et al*., 2000). The Folin-ciocalteu method was used because this is one of the oldest methods used to quantify phenols in plants and it's also known to be sensitive since it requires a small amount of plant samples.

This protocol of assessing the total phenolic content of plant samples was first developed by Folin and Dennis (1927). The protocol was used in the determination of an amino acid that contains a phenol group i.e. tyrosine in proteins. The protocol was further modified by Singleton and Rossi Jr (1965) for the analysis of phenolic compounds. The assay is based on the redox reactions and it has been used for many years for the determination of the levels of phenolic compounds in the plant samples by making use of gallic acid as a reference.

The major reaction that takes place involves the reduction of phenols by a phosphomolybdic tungstic acid reagent. The phenolic compounds are oxidized in a basic medium forming the superoxide ion which then reacts with the molybdate to form molybdenum oxide which has absorption at 725 nm. The plant phenols are determined by the reduction of the Folin-Ciocalteu reagent. The process involves taking the suspected sample and adding distilled water to it and then mixing with the Folin-Ciocalteu reagent with 2- 40% sodium carbonate. The reaction mixture is then incubated at room temperature for a period of 40 min and thereafter the absorbance is monitored at 725 nm. The control in this procedure is a mixture that contains the extracting solvent which is usually methanol.

The levels of phenolic compounds in the plant sample are estimated as gallic acid equivalents (GAE). The challenge with using Folin-Ciocalteu to determine the levels of phenolic compounds in plant samples is that apart from the phenols in the samples there are other non-phenolic compounds for example ascorbic acid, sugars, aromatic amines, organic acid, and proteins which also react with the phenol reagent leading to the overestimation of the phenolic compounds in the plant samples.

To prepare the Folin-ciocalteu reagent used in this study, 10 ml of the Folin-ciocalteu reagent from the stock solution was measured accurately using a 10 ml pipette and was transferred into a 100 ml volumetric flask. 90 ml of distilled water was then added to the mark and was shaken well to make a concentration of 0.2 N.

3.5.5 The Prussian-Blue assay.

Grupta and Verma (2011) developed the Prussian–blue assay for determining the total phenolic content. The method involves diluting 1 ml of the sample analyte with 50 ml of distilled water. The diluted sample is then added 3 ml of 0.5M ferric chloride mixed with 0.1N hydrochloric acid together with 3.0 ml of potassium ferricyanide. The mixture is then allowed to stand for about 15 minutes to allow for color development before the absorbance readings are taken at 725 nm using a UV-Vis spectrophotometer with gallic acid as standard reference.

Azlim et al (2010) noted that the most common methods of determining the total phenolic content in plants are the Folin-ciocalteu assay and the Prussian-blue assay. The two methods mainly focus on changes in color which develops due to oxidation of the phenolic compounds by the specific reagents. For instance, in the Folin-ciocalteu assay, it is made up of phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic ($H_3PMO_{12}O_{40}$) acids that are responsible for the oxidation of the phenolic compounds to tungsten (W_8O_{23}) and molybdene (Mo_8O_{23}) which are blue in color. In the Prussian-Blue assay the method is pegged on the reduction of ferric ion (Fe^{3+}) with is red in color to ferrous ion (Fe^{2+}) which is blue by the phenolic compounds. The development of the blue color in both assays shows the amount of phenolic compounds which can be determined by the use of a UV-Vis spectrophotometer at specified wavelength (Stratil *et al*., 2008).

Nur Huda et al (2015) observed that the Folin-ciocalteu assay is the best in determining the phenolic content in plants because it's highly stable and reproducible when compared to the

Prussian-Blue assay which is not stable because the extract forms undesirable precipitates with the reagent which keeps on increasing as the incubation time increases.

3.5.6 Preparation of the 20% sodium carbonate.

To prepare 20% Sodium carbonate, 2.12 grams of sodium carbonate were accurately weighed using an analytical balance and transferred into a 100 ml glass beaker. 40 ml of distilled deionized water was then added and the mixture was thoroughly stirred using a glass rod to dissolve the sodium carbonate. The solution was then transferred to a 100 ml volumetric flask and 60 ml of distilled deionized water was then added to the mark to make 20% sodium carbonate.

3.5.7 The 2,2-diphenyl-1-picrylhydrazyl Assay.

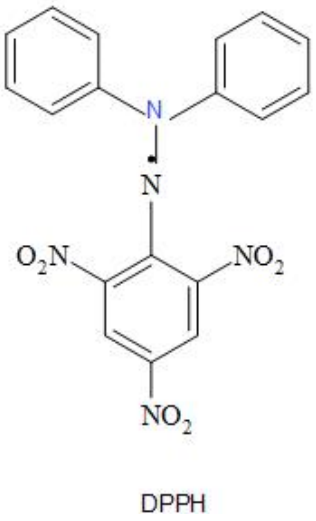
The assay was first described by Blois (1958), and was further improved by several researchers. It is the most commonly used assay to determine the ability of phenols to scavenge for free radicals (Sendra *et al*., 2006). The DPPH radical is very stable and reacts with compounds that can donate hydrogen atoms with an absorbance at 515nm. The reduction of the DPPH free radical by the antioxidants leads to the decolorization of the deep blue color of the methanol solution. The reaction that takes place is the single-electron transfer which is then monitored using a UV-vis spectrophotometer. The extent of decolorization of the reaction mixture depends on the amount of the antioxidants available in that the higher the antioxidants the higher the decolorization of the deep purple methanol solution which eventually leads to lower absorbance signals of the reaction mixture. The results obtained are affected by the amount of the DPPH used, the time given to the reaction mixture at incubation, and the nature of the solvent used in terms of the pH (Sharma and Bhat, 2009). Ascorbic acid is used as a control and antioxidant activity of the samples is estimated using the equation;

$$\%\ scavenging\ activity\ = \frac{Signal\ of\ the\ reference - Signal\ of\ the\ sample}{Signal\ of\ the\ refernce} \times 100 \quad \text{.....(Equation 3.2)}$$

The assay is good in determining the antioxidant activity of phenolic compounds because it is simple, gives accurate and repeatable results (Prakash, 2001).

However, despite its simplicity and giving accurate results, the protocol has some setbacks in that it responds slowly to the antioxidants that act very fast to the peroxyl radicals due to its stability,

furthermore, there are compounds for instance the carotenoids which may have absorbance signals merging with those of the DPPH at 515nm. Furthermore, the results obtained also depend on the nature of the antioxidants present in the reaction mixture in that the small molecules are easily accessed by the DPPH free radical, unlike the large molecules which have a high steric effect and therefore making it difficult to be accessed (Sagar and Singh, 2011). The aptitude of an antioxidant to eliminate free radicals is based on the radical system and testing conditions. The antioxidants inhibit the oxidation of lipids by scavenging the free radicals. Several methods can be used to determine the free radical elimination capacity of a substance. The most common involves the use of 2,2-diphenyl-1-picrylhydrazyl (DPPH) whose structure is shown in Figure 2.3.

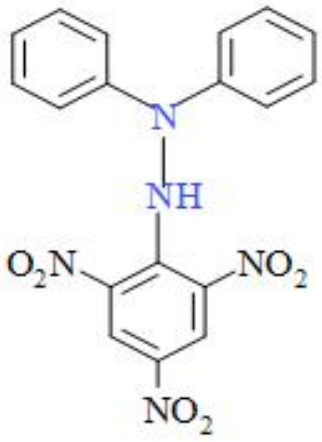


DPPH

Figure 3.1: The structure of diphenyl-picrylhydrazine (free radical) (Molyneux, 2004).

The determination of the scavenging ability of antioxidants by the use of DPPH is because the DPPH can donate hydrogen (Singh and Sagar, 2011). The DPPH free radical is resonance stabilized by the spare electron which makes it have a deep violet color. The free-electron which is delocalized throughout the molecule makes the radical to be paramagnetic.

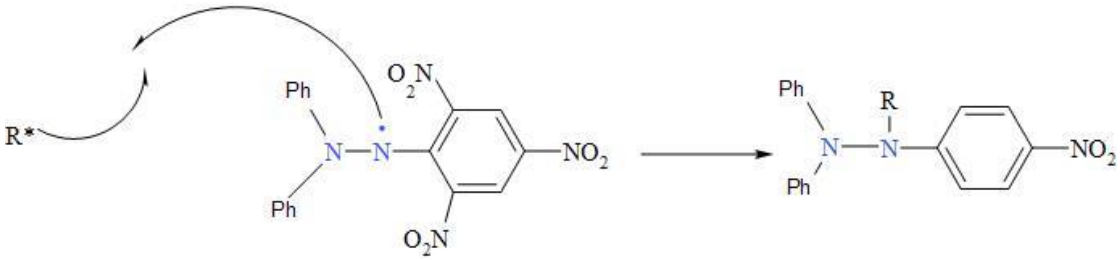
The paramagnetic property is lost once the radical becomes stable through acceptance of an electron or acceptance of hydrogen radical and thus becomes diamagnetic as shown in Figure 2.4 below:



DPPH Non-radical.

Figure 3.2: The structure of diphenyl-picrylhydrazine (non-radical) (Molyneux, 2004).

The moment the free radical attains stability (Equation 3.3), the deep violet color turns to pale yellow due to the formation of a picryl group (Akar *et al*., 2017).



(Equation 3.3)

Inhibition of a polymer chain, R, by DPPH (Akar *et al*., 2017).

The absorbance of the stable radical is measured using the UV-VIS spectrophotometer at a particular wavelength. The maximum absorption of the DPPH takes place at around 515-520nm and starts to disappear when the free electron has been stabilized (Tailor and Goyal, 2014).

In the Preparation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent, 19.7 mg (milligrams) of 2,2-diphenyl-1-picrylhydrazyl was accurately weighed using an analytical balance and transferred into a 50 ml glass beaker. 10 ml methanol was then added and the mixture was sonicated for two

minutes to dissolve the DPPH reagent. The solution was then transferred to a 500 ml volumetric flask and methanol was then added to the mark to make a 0.1 mM solution.

3.6 Determination of total phenolic compound levels in orange samples from the markets.

The quantification of the total phenolics acid of orange juice samples from the Ngara, Gikomba, and Muthurwa Markets were done using the Folin-ciocalteu reagent. This was achieved by making use of the stock Gallic acid. The Gallic calibration curve was prepared by measuring the absorbance of solutions of 3, 6, 9, 12, 15, 18, 21, 24, and 30 ppm in methanol from the stock standard 1 (one) solution of the gallic acid solution prepared in section 3.6.2.

The concentrations of the standard solutions were calculated using the procedure in section 3.6.3. From each of the dilutions, 200 µl of the solution was mixed with 400 µl of distilled water and followed by the addition of 100 µl of Folin-ciocalteu reagent, and allowed to stand for 10 minutes. Thereafter, 1 ml of 20% sodium carbonate was added to the reaction mixture and their absorbance was recorded after one hour against methanol blank at 730 nm using a UV spectrophotometer to obtain a calibration curve. The same procedure was used to prepare the orange juice samples from Ngara, Gikomba, and Muthurwa Markets, for analysis and recording the absorbance for each sample. The calculation of the amount of the total phenolics in the orange samples was quantified as gallic acid equivalents (mg/gGAE). This was obtained by using the equation:

$$Total\ Phenolic\ Content\ (TPC) = \frac{cV}{m} \quad \text{.....Equation 3.4}$$

Where, c = concentration from the standard calibration curve of Gallic acid, V= total volume of the extract, and m = mass of the extract.

3.7 Determination of total phenolic compound levels in commercial orange juice from the supermarkets.

The quantification of the total phenolics acid of commercial orange juice from the Tuskys and Uchumi supermarkets were done using the Folin-ciocalteu reagent following the procedures outlined in section 3.7 above. The calibration curve standards and total phenolic compounds from the fresh orange juice extracts from the markets were obtained. The calculation of the amount of total phenolic was obtained by using equation 3.3 above

3.8 Determination of the Antioxidant activity of orange juice from markets.

The standard calibration curve of ascorbic acid for determining the antioxidant activity of the samples was obtained by making calibration standards of 5, 10, 20, 40, 60, 80, and 100 ppm in methanol from the stock standard solution 2 (two) of ascorbic acid solution. 200 µl of each of these standard solutions was put in 5 ml sample cuvette mixed with 3.9 ml of the DPPH which was dissolved in methanol and the absorbance of each standard solution with DPPH was measured after one hour with UV spectrophotometer at ƛ max = 515 nm using methanol as the blank.

Similarly, the antioxidant of the orange juice samples from the Ngara, Gikomba, and Muthurwa Markets were analyzed by pipetting 1 ml of each juice sample into 10 ml volumetric flasks; 9 ml of methanol was added to the mark. 1 ml of each diluted orange juice sample transferred to 5 ml sample cuvette and 3.9 ml of DPPH solution in methanol was added. This was followed by stirring each mixture thoroughly and then given time to settle for 60 minutes at 25℃ in the dark, after which the absorbance of the remaining DPPH was measured and recorded after one hour against methanol blank with UV spectrophotometer at ƛ max = 515 nm. The analyses were done in triplicates and the mean absorbance for each sample was determined and recorded. The free radical scavenging ability of the orange juice samples was determined as follows:

$$\%\ scavenging\ activity\ = \frac{A_0 - A_1}{A_0} \times 100$$----------Equation 3.5

Where A_0 and A_1 are the signal of the reference and the sample respectively.

3.9 Determination of the Antioxidant activity of commercial orange juice.

To determine the antioxidant activity of commercial orange juice from the supermarkets, the standard calibration curve of ascorbic acid for determining the antioxidant activity of the samples was obtained by making the calibration standard as was done in section 3.9 above.

200 µl of each of these standard solutions mixed with 3.9 ml of the DPPH which was dissolved in methanol and the absorbance of each standard solution with DPPH was measured after one hour with methanol used as blank with UV spectrophotometer at ƛ max = 515nm was used to obtain a calibration curve. Similarly, the antioxidant of the commercial orange juice samples from the

Tuskys and Uchumi Supermarkets were analyzed following the procedures outline in section 3.9 above. The analyses were done in triplicates and the mean absorbance for each sample was determined and recorded.

The free radical scavenging ability of the orange juice samples was determined and calculated using equation 3.4 above

3.10 Data analysis.

The data in this study are presented as the mean $\pm$ standard deviation of the triplicate measurements. Also, the data was presented using bar graphs, the correlation between the total phenolic content and the percentage inhibition was carried out using the Pearson correlation coefficient and the linear regression between the total phenolic content and the percentage inhibition was done using Stata software version 14.2.

CHAPTER FOUR

RESULTS AND DISCUSSION.

4.1 The total phenolic contents (TPC) of fresh orange juice samples.

The TPC of the juice samples was determined using the Folin-Ciocalteu method. The method depends on the reduction of phenolic compounds in an alkaline medium. The calibration curve of gallic acid with $R^2 = 0.9929$ was used to work out the TPC. TPC of the fresh juice ranged from 129.75±0.39 mgGAE to 133.10±0.95 mgGAE. The fresh orange juice samples were identified as S1, S2, and S3. The highest TPC value in the fresh orange juice was detected in sample S2 and the lowest was detected in sample S1 (Table 4.1(a)). Therefore, the TPC of the fresh orange juice were detected as follows, S2 > S3 > S1. The difference in the total phenolic content of the orange juice samples can be attributed to the difference in the climatic and geographical conditions from which the orange fruits used to process the juice were grown (Zarei *et al* 2010).

Table 4.1(a): The total phenolic content (TPC) of fresh orange juice samples.

Sample	Source	Weight used (mg)	Mean absorbance (nm)	TPC (mgGAE)
S1	Ngara	962.9±5.9	0.5160±0.0016	129.75±0.39
S2	Gikomba	950.7±11.76	0.5220±0.0022	133.1±0.95
S3	Muthurwa	953.1±4.08	0.5127±0.0033	130.24±0.28

4.2 Total phenolic content (TPC) of commercial orange juice samples.

The TPC of commercial orange juice samples was also determined using the Folin-Ciocalteu method. The calibration curve of gallic acid with $R^2 = 0.9929$ was used to work out the TPC. The TPC of commercial orange juice ranged from 110.23±0.25 mgGAE to 131.54±0.02 mgGAE. The commercial orange juice samples were identified as S4, S5, S6, S7, S8, S9, S10, and S11. The highest TPC value in the commercial orange juice was detected in sample S6 with 131.54±0.02 mgGAE and the lowest was detected in sample S10 with 110.23±0.25 mgGAE (Table 4.2). Therefore, the (TPC) in the commercial orange juice were detected as S6 > S5 > S7 > S4 > S9 > S8 > S11 > S10.

Frohling et al (2012) worked on the TPC and the antioxidant activities of commercial nectars. In their study, they observed that the TPC in fruit juices was affected by a number of factors for instance, type of fruit chosen to produce juice, the methods used to process the juice and also the storage conditions of the juice. Commercial orange juice sample S6 had 33.4 mg of vitamin C per 100 ml of the juice, it was majorly composed of pure orange juice according to the label and had been packed in a carton with aluminium coating. The high levels of total phenols could be due to the pure orange used to make the juice and also the aluminium coating in the carton package protected the phenolic compounds in the juice from destruction by light.

The commercial orange juice sample S10 had the lowest total phenolic content. The composition of the juice had sugar, orange pulp, orange juice concentrate, acidulants, ascorbic acid, sodium saccharin, glucose syrup, and color beta carotene. From its composition, it would been expected to have high levels of total phenols because in addition to the phenolic compounds in the juice, other substances like ascorbic acid, sugars and beta carotene also affect the total phenolic content. This was not the case instead it recorded the lowest phenolic content. This could have been brought about maybe by the kind of package that was used to hold the juice. Plastic bottle packages are prone to permeability to oxygen that causes oxidative reactions especially with vitamin C leading to low levels of total phenolic content.

Furthermore, the differences in the total phenolic content noted in the commercial orange juice could be due to the processing methods used to produce the various brands of juice, for example, filtration, clarification and pasteurization would greatly affect the phenolic content. During

filtration and clarification of commercial juice to obtain clear juice results in the removal of phenolic content bound to fibre and pectin and thus low levels of total phenolic content (Candrawinata *et al* 2010).

Besides, the total phenolic content of the orange juice is also affected by the differences in the climatic and geographical conditions from which the orange fruits used to process the juice were grown (Zarei *et al* 2010). This factor was not considered in this study. Table 4.1(b) gives the total phenolic content of commercial orange juice.

Table 4.1(b): Total phenolic content levels of commercial orange juice samples.

Sample	Weight used (mg)	Mean absorbance (nm)	TPC (mg/g GAE)
S4	883.6±3.33	0.4388±0.0023	120.24±0.18
S5	1003.3±3.32	0.5355±0.0040	129.23±0.54
S6	1001.5±2.62	0.5441±0.0015	131.54±0.02
S7	990.7±2.49	0.4931±0.0009	120.51±0.09
S8	1008.4±2.78	0.4840±0.00013	116.22±0.007
S9	999.6±1.53	0.4848±0.00058	117.43±0.04
S10	1004±3.45	0.4571±0.00057	110.23±0.25
S11	1009.5±4.37	0.4619±0.00029	110.79±0.41

4.3 The free radical scavenging activity of fresh orange juice samples.

The ability to scavenge for free radicals of all the samples was determined using the DPPH assay and expressed as a percentage which was compared with the standard antioxidant ascorbic acid. The percentage to scavenge for free radicals in the fresh orange juice samples ranged from 16.40±0.02% to 18.32±0.1% (Table 4.2(a)). The sample with the highest percentage was sample S1 with 18.32±0.1% and the lowest was sample S3 with 16.40±0.02%. Therefore, the free radical scavenging activity was detected as S1 > S2 > S3.

Table 4.2(a): The percentage scavenging activity of fresh orange juice samples.

Sample	Source	Mean absorbance (nm)	% scavenging activity
S1	Ngara	0.555±0.00082	18.32±0.01
S2	Gikomba	0.5390±0.017	16.83±1.56
S3	Muthurwa	0.5668±0.0026	16.40±0.02

4.4 The free radical scavenging activity of the commercial orange juice samples.

The free radical scavenging activity of all the commercial orange juice samples was also carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The percentage to scavenge for free radicals in the commercial orange juice samples ranged from 10.20±0.80% to 20.28±0.23% (Table 4.2(b)). The sample with the highest percentage was sample S6 with 20.28±0.23% and the lowest was sample S10 with 10.20±0.80%. Therefore, the free radical scavenging activity was detected as S6 > S5 > S8 > S4 > S9 > S7 > S11 > S10. The commercial orange juice sample S6 had the highest total phenolic content amongst the commercial orange juices that were analyzed. The high levels of the total phenols could be ones responsible for the highest percentage scavenging ability recorded. Similarly, the lowest percentage scavenging ability in commercial orange juice sample S10 could be due to the lowest total phenolic levels recorded in the sample. The commercial orange juice sample S5 had the second highest percentage scavenging ability and it had also the

second highest total phenolic content, therefore, its free radical scavenging ability can be attributed to the presence of phenolic compounds in the juice.

The variation in the percentage scavenging activity of the different samples of orange juice may be due to the difference in the number of total phenols contained in each juice as well as the difference in the composition of the phenols present, small phenol molecules are easily accessed by the DPPH free radical, unlike the large molecules which have a high steric effect and therefore making it difficult to be accessed, this results in lower levels of free scavenging ability (Sagar and Singh, 2011). Table 4.2(b) shows the percentage scavenging ability of the commercial orange juice.

Table 4.2(b): The percentage scavenging activity of commercial orange juice.

Sample	Mean absorbance (nm)	% scavenging activity
S4	0.5753±0.0037	15.15±0.13
S5	0.5476±0.058	19.28±0.816
S6	0.5413±0.16	20.28±0.23
S7	0.5730±0.0061	12.89±2.11
S8	0.5573±0.057	17.84±0.80
S9	0.5847±0.09	13.84±0.12
S10	0.6092±0.063	10.20±0.80
S11	0.6022±0.08	11.35±0.11

4.5 Comparison of the total phenolic content levels of fresh and commercial orange juice samples.

From the graphical representation (Figure 4.1), the total phenolic content of the orange juice samples ranged from 110.23±0.25 mgGAE to 133.1±0.95 mgGAE. The sample with the highest amount of phenolics was detected in a fresh orange juice sample S2 which had phenolic content of 133.1±0.95 mgGAE. It was followed by the commercial orange juice sample S6 with phenolic content of 131.54±0.02 mgGAE.

The orange juice sample with the least amount of phenolic content of 110.23±0.25 mg/GAE) was sample S10. Thus, the total phenolic content (TPC) of the orange juice samples were detected as S2 > S6 > S3 > S1 > S5 > S7 > S4 > S9 > S8 > S11 > S10.

Generally, apart from commercial orange juice samples S5 and S6, the fresh orange juice samples had higher levels of total phenolic content compared to most commercial orange juice samples (Figure 4.1). This could be because the commercial orange juice go through several process in the cause of producing the juice that might lead to loss of some phenolic compounds thus resulting to low levels of total phenolic content (Candrawinata *et al* 2010). .

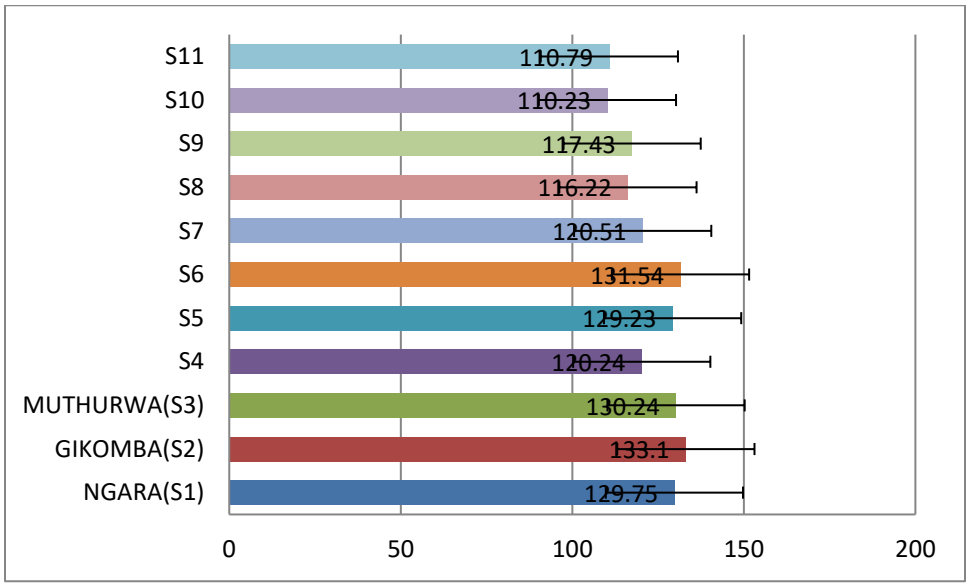


Figure 4.1: Comparison of TPC (mg/gGAE) of the orange juice samples.

4.6 Comparison of the antioxidant activities of fresh and commercial orange juice samples.

The free radical scavenging activities of the orange juice samples ranged from 10.20±0.80% to 20.28±0.23%. The sample with the highest percentage scavenging activity was commercial orange juice sample S6 with 20.28±0.23%. It was followed by sample S5 with 19.28±0.80%. The higher levels of percentage scavenging ability noted in sample S6 and S5 could be in addition to the phenolic compounds in the juice, the ascorbic acid that is normally added in the production of the juice also contributes to the free radical scavenging ability of the juices (Sagar and Singh, 2011).

The orange juice sample with the least % scavenging activity was sample S10 with 10.20±0.8%, the same sample was packaged in a plastic bottle that is known to have poor oxygen barrier and therefore, the phenolic compounds and ascorbic acid present in the juice might have been destroyed by the entry of oxygen resulting to lower levels of percentage scavenging ability.

Generally, apart from the commercial orange juice samples S5, S6, and S8 the fresh orange juice samples had higher levels of percentage scavenging activities compared to most commercial orange juice samples (Figure 4.2).

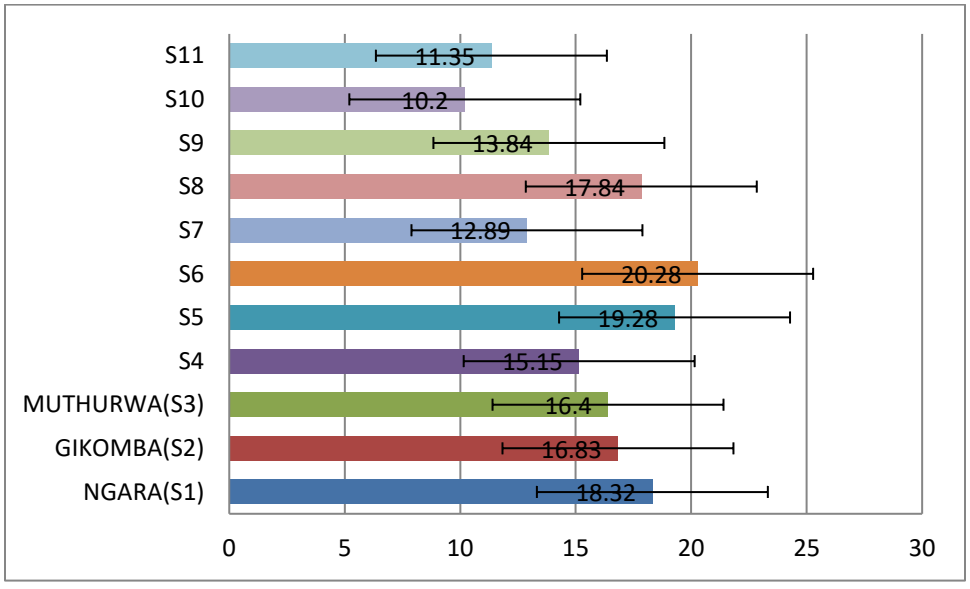


Figure 4.2: The % scavenging activities of fresh and commercial orange juice samples.

4.7 The correlation between the total phenolic content (TPC) and antioxidant activity of orange juice.

The correlation between the total phenolic content and the percentage scavenging activity was performed using the Pearson correlation coefficient (Table 4.3).

Table 4.3: The correlation between the TPC and the antioxidant activity.

	TPC	DPPH
TPC	0.8047	1.000
DPPH	1.000	0.8047

Furthermore, the linear regression of TPC and the antioxidant activity was also carried out using the Stata software version 14.0 (Table 4.4).

Table 4.4: Regression analysis of TPC and antioxidant activity.

DPPH	Coef	std. Err.	t	P>|t|	95% Conf	R^2	Prob>F
TPC	.3128	.0769	4.0700	0.003	.1388	0.6475	0.0028

The results of the study show that there was a strong and positive correlation between the (TPC) and the free radical scavenging ability of the orange juice where the r-squared value was 64%. From the regression results, it was noted that a unit increase in the total phenolic content leads to a 0.31% change in the percentage scavenging activity at a 1% level of significance that is; the p-value was 0.003. This is in agreement with a similar study which was done by Rapisarda et al, 1999.

The strong relationship between TPC and % scavenging ability show that phenolic compounds are responsible for the antioxidant activity of the orange juice. However, the antioxidant activities of orange juice cannot be pegged on the phenolic compounds alone. This is because some of the juices analyzed contained ascorbic acid which is a natural antioxidant and beta carotene that also

contribute to the overall antioxidant activities of the juices (Almeida *et al*., 2011). These compounds (ascorbic acid and beta carotene) were not considered in this study.

Some of the samples analyzed showed low TPC levels but having high antioxidant activities (sample S1), this is so because there might be some soluble compounds in the solvent used for instance, methyl xanthine which can combine with DPPH radical leading to high values of percentage scavenging ability (Belsack *et al*., 2009).

Besides, the presence of other substances like sugars, organic acids and ascorbic acid in some of the commercial orange juices can lead to the overestimation of the TPC by the Folin-ciocalteu and thus, it is crucial to quantify the individual phenolic compounds in the juice using other analytical methods.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS.

5.1 Conclusion and recommendations

From the study, we can note that it's good to take both the fresh orange juice and the commercial processed orange juice because there was no much difference in the TPC and the free radical scavenging ability of these juices. This study gives very important information on the choice of various types of orange juice in order to maintain good health. In addition, the information provided in this study, allows consumers to have alternatives when it comes to choosing orange juice for consumption and also help the consumers to be aware of various composition of these juices thus avoiding misinformation especially on labels of juices. It's recommended that studies have to be carried out in future to analyze the specific phenolic compounds and polyphenols in orange juice to ascertain the ones responsible for the antioxidant activities of orange juice.

REFERENCES.

Agcam, E., Akyldz, A. and Akdemir, G. (2014). Comparison of phenolic compounds of orange juice processed by pulsed electric fields (PEP) and conventional thermal pasteurization. Food Chemistry, 143: 354-361.

Ajugwo, A. (2012). Nutritional value of lime and lemon in hypercholesterolaemic induced rats. *Asian journal of medicinal science*, vol 3: 13-16.

Akar, Z., Murat, K., and Hacer, D. (2017). A new colorimetric DPPH scavenging activity method with no need for a spectrophotometer applied on synthetic and natural antioxidants and medicinal herbs. *Journal of enzyme inhibition and medicinal chemistry*, 32(1): 640-647.

Almeida, M.M.B., de Sousa, P.H.M., Arriaga, Â.M.C., Do Prado, G.M., de Calvalho Magalhães, C.E., Maia, G.A. and de Lemos, T.L.G. (2011). Bioactive compounds and antioxidant activity of fresh exotic fruits from Northeastern Brazil. Food Research International 44(7): 2155-2159.

Ashwell, R.N., Moyo, M. and Staden, J.V. (2010). Natural antioxidants: fascinating or mythical biomolecules. *Journal of molecules*, 2010 (15): 6905-6930.

Azlim, A. A., Ahmed, C., Syed, L., Mustapha, K., Aisyah, M. R., and Kamarul, K. (2010). Total phenolic content and primary antioxidant activity of methanolic and ethanolic extracts of aromatic plants leaves. *International food reaserch journal*, 17: 1077-1084.

Bates, R.P., Morris, J.R. and Crandall, P.G. (2001). Principles and practices of small and medium scale fruit juice processing. FAO Agricultural services Bulletin 146. Retrieved from. http://www.fao.org./3/a-y251e.

Belščak, A., Komes, D., Horžić, D., Ganić, K.K. and Karlović, D. (2009). Comparative study of commercially available cocoa products in terms of their bioactive composition. Food Research International 42(5): 707-716.

Cai J., Ying C., Wen-Hui C., Edward C.H., Heng W., Steven G.K., Luis F.P. and Richard Q. L. (2007). A crucial role for olig2 in white matter astrocyte development. Development 134: 1887-1899.

Candrawinata, V.I., Blades, B., Golding, J., Stathopoulos, C. and Roach, P. (2012). Effect of clarification on the polyphenolic compound content and antioxidant activity of commercial apple juices. *International Food Research Journal* 19(3): 1055-1061.

Carolina, R., and Thais, C. (2017). Orange juice allied to a reduced calorie diet results in weight loss and ameliorates obesity-related biomarkers. A randomized controlled trial. Nutrition vol 38, pg. 13-19.

Christennsen and Julie. (2018). The best kind of lemon tree. Home guides/ SF Gate. Retrieved from http://homeguides.sfgate.com/kind-lemon-tree-63335.html.

Clarita, V.O. (2006). Comparative value of orange juice versus lemonade in reducing stone forming risk. *Clinical Journal of American society of Nephrology*, 2006, 1(6): 1269-1274.

Clausen, T. P., Provenza, F. D., Burritt, E. A., Reichardt, P. B. and Bryant, J. P. (1990). Ecological implications of condensed tannin structure: a case study. *Journal of Chemical Ecology*. 16: 2381 - 2391.

Coracini, J. D. and de Azevedo, W.F Jr. (2014). Shikimate kinase, a protein target for drug design. Current Medicinal Chemistry. 2014;21:592-604. DOI: 10.2174/09298673113206660299

Daemmon, R., Treatt, R., David, A., and Florida, T. (2002). Riding the Citrus Trail: When is a Mandarin a Tangerine? Perfumer and flavorist, vol 27(22).

Danielson, S.R. and Andersen, J.K. (2008). Oxidative and nitrative protein modifications in Parkinson's disease. Free Radic Biol Med, 44(10): 1787-1794.

Dewick, P. M. (2009). Medicinal Natural Products: A Biosynthetic Approach. 3rd ed. United Kingdom: John Wiley and Sons Ltd.; 2009. p. 539. DOI: 10.1002/9780470742761

Dreyer, D. L. and Jones, K. C. (1981). Feeding deterrency of flavonoids and related phenolics towards Schizaphis graminumand Myzus persicae: aphid feeding deterrents in wheat. Phytochemistry. 20: 2489 - 2493.

Droge, W. (2002). Free radicals in the physiological control of cellfunction. Physiological Reviews.2002; 82: 47–95.

Etebu, E and Nwauzoma, A.B. (2014). A review on sweet orange (Citrus sinensis Osbeck). Health, disease, and management. *American journal of research*, 2(2): 33-70.

Fahimeh, R.F., Mahbubeh, J., and Hamed, M. (2015). The effect of aromatherapy by essential oil of orange on anxiety during labor. A randomized clinical trial. *Iranian Journal of Nursing and Midwifery Research*, 20(6): 661-664.

Folin O., and Denis W. (1965). On phosphotungstic-phosphomolybdic compounds as colour reagents. *J. Biol. Chem*.12, 239-243.

Franziska, A.H., Franziska, B., Alessa, N., Julian, A., Lena, G., Ralf, S., Reinhold, C., and Anja, B.W. (2018). High orange juice consumption with or in between three meals a day differently affects energy balance in healthy subjects. Nutr. Diabetes 2018; 8:19.

Frederick, G. (2010). Origin, Evolution and Breeding of grapefruit. Plant Breeding reviews, vol 13: 345-363.

Fröhling, B., Patz, C., Dietrich, H. and Will, F. (2012). Anthocyanins, total phenolics and antioxidant capacities of commercial red grape juices, black currant and sour cherry nectars. Fruit Process 3: 100-104.

Ghosh, S., Chisti, Y. and Banerjee, U. (2012). Production of shikimic acid. Biotechnology advances, 2012; 30: 1425-1431.

Giuseppina, M., Carl, B., Santa, C., and Michele, N. (2017). Effectiveness of citrus fruits on Helicobactor pylori. Evid Based complement Alternat Med, 20187: 8379262.

Gupta, C. and Verma, H. (2011). Visual estimation and spectrophotometric determination of tannin content and antioxidant activity of three common vegetables. *International journal of pharmaceutical sceiences and research*, 2(1):189-196.

Haim, A., Henrik, B., and Preben, B. (2008). Correction to the Beer-Lambert-Bouguer law for optical absorption. Applied optics, vol 47, issue 29, pp.5354-5357.

Harrison, D.G., Gongora, M.C., Guzik, T.J. and Widder, J. (2007). Oxidative stress and hypertension. J.Am. Soc. Hypertens. 1, 30-44.

Henriksen, T., Mahoney, E.M., Steinberg, D. (1983). Enhanced macrophage degradation of biologically modified low density lipoprotein. Arteriosclerosis, 3(2): 149-159.

Hind, M., Azhar, A.A., John, P. and Antonella, T. (2018). The role of vitamins and minerals in hair loss. A review Doi: 1007/s 13555-018-0278-6.

Huang D., Ou, B., Hampsch-Woodill M., Flanagan J., and Prior R.L. (2010). High-Throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled Molecules 15

Ibrahim, K., Chuying. C., Zengyu, G., Jinyin, C. and Chunpeng, W. (2020). The effects of Edible Coatings on the Postharvest Quality of Citrus Fruits as Affected by Granulation, Journal of Food Quality. Vol. 2020, Article ID 8819233. https://doi.org/10.1155/2020/8819233.

Isman, M. B. and Duffey, S. S. (1982). Toxicity of tomato phenolic compounds to the fruitworm, Heliothis zea. Entomologica Experimentalis et Applicata. 31: 370 - 376.

Jae-Hee, P., Minhee, L., and Eunju, P. (2014). Antioxidant activity of orange flesh and peel extracted with various solvents. Preventive nutrition and food science, 19(4):291-298.

Jawad, K. and Sardar, Z. (2007). Medical importance of grape fruit juice and its interactions with various drugs. *Nutritional Journal*, vol 6 : (1): 33.

Joanne, L.S. and Beate, L. (2012). Health benefits of fruits and vegetables. *Advances in nutrition an international review journal,* 3:506-516.

Jose, M., Ignacio, P., Manera, F.J., and Agustin, C. (2015). Quality and fruit colour change in Verna lemon. *Journal of Applied Botany and Food Quality*, Vol 1(1): 1. Doi: 10.5073/JABFQ.2015.088.031.

Kalantar-zadeh, K. and Fry, B. (2008). Nano fabrication and patterning techniques. Nano technology-enabled sensors. Boston, MA: Springer US. Pp.135-210.

Karsheva, M., Kivova, E., and Alexandrova, S. (2013). Natural antioxidants from citrus mandarinpeels, extraction of polyphenols; effect of operational conditions on total

polyphenols content and antioxidant activity. *Journal of chemical technology and metallurgy*, 48: 2013 35-41.

Kelleher, D. E., and Podobedova L. J. (2008). Atomic transition probabilities of sodium and magnesium. A critical compilation. Atomic physics division, national institute of standards and technology, Gaithersburg, Maryland 20899-8422.

Khoddami, A., Wilkes, M.A., and Roberts, T. H. (2013). Techniques of analysis of plant phenolic compounds. *Journal of molecules*, 2013 (18): 2328-2375.

Kozlov, V. A., and Weidinger, A. (2015). Biological activities of reactive oxygen and nitrogen species: oxidative stress versus signal transduction. *Journal of biomolecules*, 5(2): 472-484.

Krishnamurthy, P. and Wadhwani, A. (2015). Antioxidant enzymes and human health. In: El-Missiry MA, editor. Antioxidant Enzyme. Croatia: In Tech; 2012. pp. 3-18. DOI: 10.3109/0886022X.2015.1103654

Kujala T.S., Loponen J.M., Klika K.D. and Pihlaja K. (2000). Phenolics and betacyanins in red Beetroot (Beta vulgaris) root. Distribution and effect of cold storage on content of total phenolics and three individual compounds. *Journal of Agriculture food Chemistry*. 48(11): 5338-42.

Kumar, R.K. (2002). Packaging Aspects of Fruits Beverages. Beverage and Food world, 2002(2): 30-32.

Kumar, S., Rajat, S. and Sudarshan, O. (2014). Evaluation of antioxidant activity and total phenol in different varieties of *Lantana Camara* leaves. BMC Res Notes 7, 560(2014)

Lattanzio, V. (2013). Phenolic compounds. *Natural products*, 50:1543-158.

Lien A.P., Hua, H., and Chuong, P. (2008). Free radicals antioxidants in disease and health. *International journal of biomedical science*, 4(2): 89-96.

Lobo, V., Patil, A., Phatak, A., and Chandra, N. (2010). Free radicals, antioxidants, and functional foods: Impact on human health. *Journal of pharmacology review*, 4(8):118-126.

Lopez, M., Martinez, F., Valle, D., Ferrit, M., and Luque, R. (2003). Study of phenolic compounds as natural antioxidants by a fluorescence method. Talanta, vol 60, issue 2-3, pg. 609-616.

Lü, J.M., Lin, P.H. and Yao, Q. (2009). Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems. *Journal of Cellular and Molecular Medicine.* 2009; 14(4):840-860.

Lwashina, T. (2003). Flavonoid function and activity to plants and other organisms. Biological Sciences in Space. 17: 24 – 44

Lyketsos, C.G., Khachaturian, A.S., Bastian, L.A., Charoonruk, G., Tschanz, J.T., Norton, M.C., Piepper, C.F., Munger, R.G., Breitner, J.C., Welsh-Bohmer, K.A., Zandi, P.P., Hayden, K.M. (2006). Vascular risk factors for incident Alzheimer disease and vascular dementia. The Cache county study. Alzheimer Dis Assoc Discord, 20(2): 93-100.

Mahendra, Pal. (2017). Lemon: a versatile fruit of multiple uses. *Journal of agriculture world* vol (3): 52-54.

Malcom, J.B., Michael, K.J., Andrew, J.E., and Don, D.R. (2016). Microbiology and biology of water. Tworts water supply (seventh edition), pg. 235-321.

Mandal, M.S., Chakraborty, D., and Dey, S., (2010). Phenolic acids act as signaling molecules in plant-microbe symbioses. *Journal of plant signaling and behavior*, 5(4): 359-368.

Mann, J. (1987). Secondary Metabolism. Oxford University Press. Toronto, ON.

Maria, J.V., Raul, A.S., Enrique, P., Aurelio, L.P. (2013). Antifungal activity of orange (*Citrus sinensis*) peels essential oil applied by direct addition or vapor contact. Food control, 31(1): 1-4.

Mason, T. L. and Wasserman, B. P. (1987). Inactivation of red beet beta-glucan synthase by native and oxidized phenolic compounds. Phytochemistry. 26: 2197 - 2202.

Mathew, B. B., Tiwari, A. and Jatawa, S. K. (2011). Free radicals and antioxidants: A review. *Journal of Pharmacy Research*. 2011; 4(12):4340-4343

Mazid, M., Khan, T.A., and Mohammad, F. (2011). Role of secondary metabolites in defence mechanisms of plants. *Biology and medicine*, 3(2): 232-249.

Mazza, G. and Miniati, E. (1993). Anthocyanins in Fruits, Vegetables and Grains. CRC Press, London, UK.

McKinley, S., Andre, T., and Morita, M. (2015). Combined L-citrulline and glutathione supplementation increases the concentration of markers indicative of nitric oxide synthesis. Internal journal of social sports Nutrition. 2015: **12,** 27.

Mierziak, J., Kostyn, K., and Kulma, A. (2014). Flavonoids as important molecules of plant interactions with the environment. *Journal of molecules*, 2014(19): 16240-16265.

Milena, M.V., Mario, R., Marostica, J. (2019). Phenolic compounds: Structure, classification and antioxidant properties. Bioactive compounds, health benefits and potential applications, 2019: 33-50.

Mitullah, W. (2003). Understanding Slums: Case Studies for the Global Report on Human Settlements 2003: The Case of Nairobi, Kenya, UN-habitat, Nairobi, Kenya.

Mkandawire, W., Tinna, A., Orpa, M. and Kampanje, J. (2016). Estimation of shelf life of mango juice produced using small scale processing techniques. Lilongwe University of Agriculuture, http://dx.doi.org/10.5539/jfr.v5n6p13

Mohanapriya, M., Lalitha, R., and Rajendarn, R. (2013). Health and medical properties of lemon (citrus lemonum). *International journal of Ayurredic and herbal medicine*, 3:1 (2013) 1095-1100.

Moharram, H.A., and Yossef, M.M. (2014). Methods for determining the antioxidant activity. *Journal of food science and technology* vol 11, No, 1, pp.31-42.

Molyneux, P. (2004) the Use of Stable Free Radical Diphenylpicrylhydrazyl (DPPH) for estimating Antioxidant Activity. *Songklanakarin Journal of Science and Technology,* 26, 211-219.

Morton, J. (1987). Grapefruit. Fruits of the warm climates, pp.152-153.

Mustafa, O., Resat, A., Kubilay, G., and Esra, C. (2016). Antioxidant capacity measurement. 2. Hydrogen transfer (HAT). Based mixed-mode (electron transfer (ET)/(HAT) and lipid perioxidation assays. *Journal of agricultural food chemistry,* 64, (5) :1028-1045.

Nahrstedt, A. (1990). The significance of secondary metabolites for interaction between plants and insects. Planta Medica. 55: 333 - 338.

Nur Huda, F., Zlalkha, A. R., Maryam, M. R., and Faujan, B. H. (2015). Comparative analysis of phenolic content and antioxidant activities of eight Malaysian traditional vegetables. *The Malaysian journal of analytical sciences*, vol 19(3):611-624.

Obafaye, O.R., Omoba, O.S., Salawu, O.S., Boligon, A.A. and Athayde, L.M. (2015). HPLC-DAD Phenolic characterization and Antioxidant Activities of Ripe and Unripe Sweet Orange Peels. Antioxidants, 4(3): 498-512.

Oscar, V and Monica, B. (2018). Flavonoids: Antioxidant compounds for plant defence and for healthy human diet. Not Bot Horti Agrobo, 2018, 46(1): 14-21.

Pacher, P., Beckman, S. and Liaudet, L. (2007). Nitric oxide and peroxynitrite in health and disease. Physiological Reviews, 2007; 87: 315–424.

Pallavi, S., Ambuj, B., Rama, S. and Mohammad, P. (2012). Reactive oxygen species, Oxidatve damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. Journal of Botany, vol. 2012, Article ID 217037. https://doi.org/10.1155/2012/217037.

Pandey, K.B. and Rizvi, S.I. (2010). Markers of oxidative stress in erythrocytes and plasma during aging in humans. Oxidative Medicine and Cellular Longevity. 2010; 3(1):2-12.

Paoliso, G., Ceriello, A. and Giugliano, D. (1996). Oxidative stress and diabetic vascular complications. Diabetes care, 19(3): 257-267.

Parle, M. and Chaturvedi, D. (2012). Orange: range of benefits. *International journal of pharmacy*; 3(7): 59-63.

Pavia, D. L., Lampman, G. M., Kriz, G. S., Vyvyan, J. A. (2008). Introduction to spectroscopy: Cengage Learning. United States of America. Kalantar-zadeh, K., and Fry, B. 2008. Nano fabrication and patterning techniques. In: Kalantar-zadeh, K., Fry, B. (Eds). Nanotechnology-Enabled Sensors. Boston, MA: Springer US. pp. 135-210

Pham-Huy, A.L., He, H., and Pham-Huy, C. (2008, June). Free radicals, antioxidants in disease and health. *International journal of biomedical science*, 4(2): 89-96.

Phaniendra A., Jestadi D.B., and Periyasamy, L. (2015). Free radicals; properties, sources, targets and their implication in various diseases. *Indian Journal of Clinical Biochem*. 30(1): 11-26.

Prakash, A. (2001). Antioxidant activity. Med Lab Anal prog, 19(2):1-6.

Rahman, K. (2007). Studies on free radicals, antioxidants, and co-factors. *Clinical* i*nterventions in aging*, 2(2): 219-236.

Rapisarda P., Tomaino A., Lo Cascio, R., Bonina, F., De Pasquale, A., and Saija, A. (1999). Antioxidant effectiveness as influenced by phenolic content of fresh Orange juice. *Journal of Agriculture Food Chemistry;* 47:4718–23.

Rizvi, I.S., and Kanti, B.P. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Journal of oxidative medicine cellular longevity*, 2(5): 270-278.

Russell, W. R., Burkitt, M. J., Provan, G. J. and Chesson, A. (1999). Structurespecific functionality of plant cell wall hydroxycinnamates. *Journal of the Science of Food and Agriculture*. 79: 408 - 410.

Saggerson, E. P. (1991). Geology of the Nairobi Area. Degree Sheet 51, NE QUARTER, Mines and Geology Department, English Press, Nairobi, Kenya.

Sánchez-Moreno, C., Plaza, L., Elez-Martínez, P., De Ancos, B., Martín-Belloso, O., and Cano, M. P. (2005). Impact of high pressure and pulsed electric fields on bioactive compounds and antioxidant activity of orange juice in comparison with traditional thermal processing. *Journal of Agricultural and Food Chemistry*, 53(11), 4403–4409. doi:10.1021/jf048839b.

Santa, C., Alessandro, M., Nadia, F., Sebastiano, G., Gioacchino, C., Udo, S., and Michele, N. (2017). Anticancer potential of citrus juices and their extracts: A systematic review of both preclinical and clinical studies. Front Pharmacolo. 2017; 8:420.

Satish, B. N. and Dilipkumar Pal. (2015). Free radicals, natural antioxidant and their reaction mechanisms. The royal society of chemistry 2015; (5) 27986-28006.

Savatović, S.M., Tepić, A.N., Šumić, Z.M. and Nikolić, M.S. (2009). Antioxidant activity of polyphenol-enriched apple juice. *Acta Periodica Technologica* 40: 95-102.

Sendra, J., Sentandreu, E. and Navarro, J. (2006). Reduction kinetics of the free stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) for the determination of the antiradical activity of citrus juices. Eu. Food Res Technol.223:615-624 doi.10.1007/s00217-005-0243-3.

Sentandreu, E., Navarro, J. L., and Sendra, J. M. (2007). Effect of technological processes and storage on flavonoids content and total, cumulative fast-kinetics and cumulative slowkinetics antiradical activities of citrus juices. European Food Research and Technology, 225(5-6), 905–912. doi:10.1007/s00217-006-0500-0.

Sergio, D., Tanea, T., Paola, V. and Victor, M. (2016). Role of ROS and RNS sources in Physiological and Pathological Conditions. Oxidative Medicine and Cellular Longevity, vol. 2016, Article ID 1245049, https://doi.org/10.1155/2016/1245049.

Shahidi, F. and Zhong, Y. (2010). Novel antioxidants in food quality preservation and health promotion. *European Journal of Lipid Science Technology*. 2010;112:930-940.

Sharma O. P. and Bhat, T.K. (2009). DPPH antioxidant assay revisited. Food Chem.113, 1202-1205.

Sibilia, J. P. (1998). A Guide to Materials Characterization and Chemical Analysis, VCH Publishers (1998).

Simona, C., candra, E., and Gabriel, L. (2010). Methods for the determination of antioxidant capacity in food and raw materials. Bio-farms for Nutraceuticals pp241-259.

Singh, R.P. and Sagar, B. K. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food science technology*, 48(4): 412-422.

Singh, U. and Jiala, I. (2006). Oxidative stress and atherosclerosis pathophysiology, 13(3): 129-142.

Singleton, V.L., and Rossi J.A., Jr. (1965). Colorimetry of total phenolics with phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144-158.

Springob, K., Nakajima, J., Yamazaki, M. and Saito, K. (2003). Recent advances in the biosynthesis and accumulation of anthocyanins. Natural Products Reports. 20: 288 – 303.

Stratil, P., Kuban, V., and Fojtova, J. (2008). Comparison of phenolic content and total antioxidant activity in wines as determined by spectrophotometer methods. *Czech Journal of Food Sciences*, 26(4):242-253.

Sudha, J.D and Reshma, L.R. (2017). Vitamin C: Sources, functions, sensing and analysis, vitamin C, Amal H. Hamza Intechopen, Doi: 10.5772/Intechopen.70162.

Sujit, K., Khindarli, T., Elijah, O., and Ratna, P. (2015). Grapefruit: Nutritional values and drug interactions. *International Journal of Integrative Medical Sciences*, vol 2: 186-189.

Tailor, C., and Goyal, A. (2014). Antioxidant activity by DPPH radical scavenging method of ageratum conyzoides Linn. Leaves. *American journal of Ethno-medicine*, vol 1, No.4, 244-249.

Tiwari, R. and Rana, C.S. (2015). Plant secondary metabolites. *International journal of engineering research and general science*, 3(5): 2091-2730.

Trueba, G.P., Sanchez, G.M. and Giuliani, A. (2004). Oxygen free radical and antioxidant defense mechanism in cancer. Front Biosci, 1(9): 2029-2044.

Turrens J.F. (2003). Mitochondrial formation of reactive oxygen species. 552(pt2): 335-44.

Ulfig, A. and Leichert, L. I. (2021). The effects of neutrophil-generated hypochlorous acid and other hypohalous acids on host and pathogens. *Cell. Mol. Life Sci.* **78,** 385–414

Usman M. and Fatima, B. (2018). Mandarin (*Citrus reticulata* Blanco) Breeding. In: Al-Khayri J., Jain S., Johnson D. (eds) Advances in Plant Breeding Strategies: Fruits. Springer, Cham Christensen, Julie. (2018, December 14). The Best Kind of Lemon Tree. Home Guides | SF Gate. Retrieved from http://homeguides.sfgate.com/kind-lemon-tree-63335.html.

Valko, M., Leibfritz, D., Moncola, J., Cronin, D.,Mazur, M. and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*; 2007; 39: 44–84.

Wink, M. (2012). Medicinal plants: A source of anti-parasitic secondary metabolites. *Journal of molecules*, 17: 12771-12791.

Zarei, M., Azizi, M. and Bashiri-Sadr, Z. (2010). Studies on physico-chemical properties and bioactive compounds of six pomegranate cultivars grown in Iran. *Journal of Food Technology* 8(3): 112-117.

CHAPTER SIX: APPENDIX.

Table A: Mean absorbance of gallic acid standard solution.

Concentration (ppm)	**Absorbance (mean) ƛ max = 730 nm**
3	0.096 ± 0.001
6	0.204± 0.004
9	0.346± 0.002
12	0.436± 0.003
15	0.61± 0.005
18	0.74± 0.001
21	0.867± 0.001
24	1.01± 0.004
30	1.275± 0.003

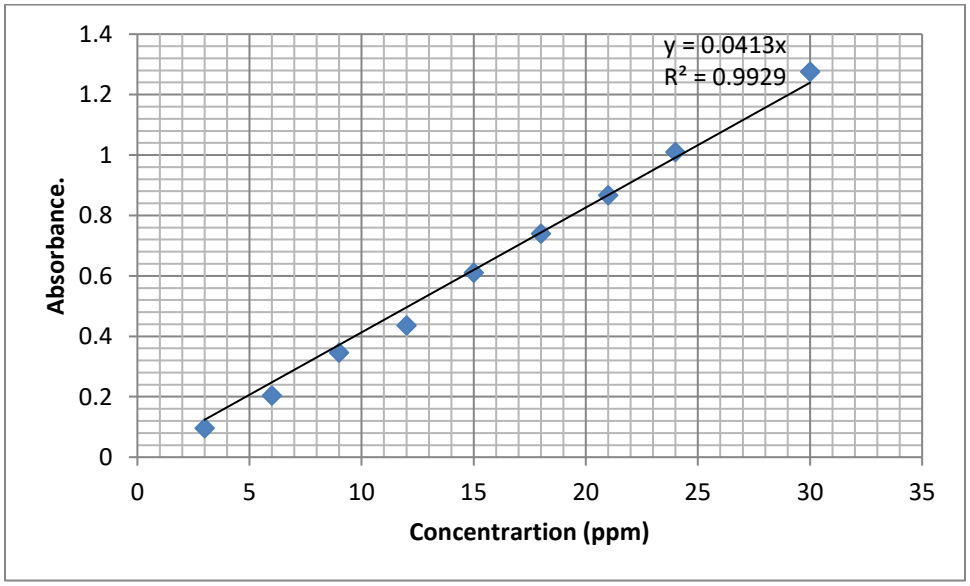


Figure A: Gallic acid calibration curve.

Table B: The mean absorbance of standard ascorbic acid solution.

Concentration (ppm)	Mean absorbance (ƛ max = 515 nm)
5	0.162±0.003
10	0.23± 0.006
20	0.433± 0.002
40	0.812± 0.001
60	1.193± 0.004
80	1.611± 0.003
100	1.932± 0.001

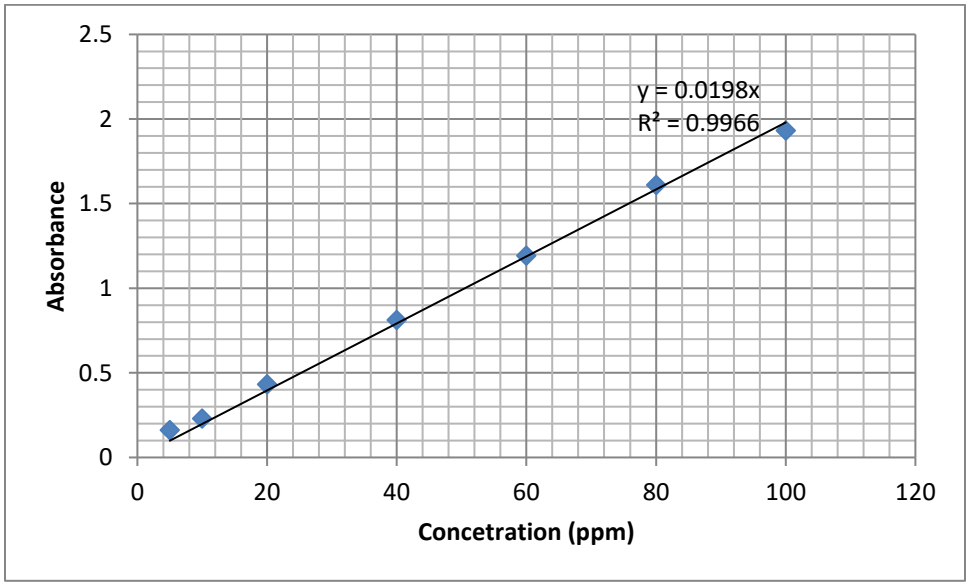


Figure B: The Ascorbic acid standard solution calibration curve.

Table C: Summary statistics.

Variable	Obs	Mean	Std. Dev.	Min	Max
sn	11	6	3.316625	1	11
source	0				
tpc	11	122.6618	8.446565	110.23	133.1
inhibition	11	15.67091	3.283673	10.2	20.28

Table D: The correlation matrix.

	inhibi~n	tpc
inhibition	1.0000	
tpc	0.8047	1.0000

Table E: The regression analysis

Source	SS	df	MS		
Model	69.8207469	1	69.8207469	Number of obs	= 11
Residual	38.004344	9	4.22270489	F(1, 9)	= 16.53
				Prob > F	= 0.0028
Total	107.825091	10	10.7825091	R-squared	= 0.6475
				Adj R-squared	= 0.6084
				Root MSE	= 2.0549

inhibition	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
tpc	.3128327	.0769335	4.07	0.003	.1387971	.4868683
_cons	-22.70172	9.457116	-2.40	0.040	-44.0952	-1.308236