EVALUATION OF THE EFFECT OF *Manihot esculenta* EDIBLE COATING INCORPORATED WITH *Ocimum gratissimum* ESSENTIAL OIL ON THE SHELF-LIFE OF FRESH *Mangifera indica*

BY

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DECLARATION PAGE

Declaration by the Candidate

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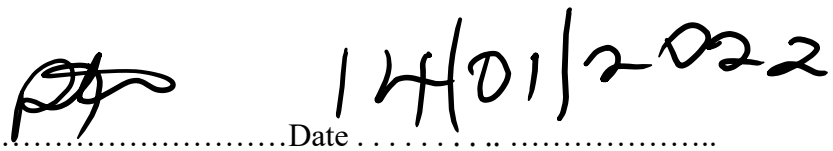
MS/ACH/4312/20

Declaration by Supervisors

This thesis has been submitted for examination with our approval as University Supervisors.

Dr. Phanice Wangila  Date . . . . ……..

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DEDICATION

To Almighty God, most merciful and divine, the ultimate source of strength who keep pouring his blessings on me; I bow my head to thank him for being at my side at every step.

To my beloved family for being a pillar of strength and ocean of love on my life. To my siblings for their positive influence on my life for their love, care, concern and useful advice. May God bless you abundantly.

ABSTRACT

Fresh produce often suffers from post-harvest spoilage losses. From the 2020 post-harvest losses report in Kenya, about 512,000 metric tons of mangoes were lost. Continued use of chemical preservatives for regulation of postharvest deterioration and prolonged shelf-life has led to pathogen-resistant strains, harmful effects on human health and the environment. The general objective of this study was to evaluate the antibacterial and antioxidant efficacy of *M. esculenta* (CS) edible coating modified with *O. gratissimum* (African basil) leaves essential oil for the improvement of the shelf-life of fresh *Mangifera indica* (mango) fruit. The specific objectives of the study were to: investigate the phytochemical composition of *Manihot esculenta* tubers and *O. gratissimum* leaves crude extracts; prepare and modify *M. esculenta* edible coating using 0.5%, 1%, and 1.5% *O. gratissimum* essential oil; characterize the modified and unmodified coating; determine the antioxidant and antibacterial activity of the modified coating, and establish the shelf-life of coated mango at room temperature (23 ± 2 °C). Serial maceration method using hexane, ethyl acetate, acetone, and methanol as solvents were used for extraction. Standard methods were used to determine the phytochemical components. Preparation and modification of the coating were homogenized at 70 ± 2 °C. Ultraviolet-Visible (UV-Vis) Spectrophotometry, Fourier Transform Infrared (FT-IR) Spectroscopy, and Gas Chromatography-Mass Spectrometry (GC-MS) were used for characterization. Physico-chemical parameters including weight loss, titratable acidity (TA), Vitamin C concentration, total soluble solids (TSS), and pH were used to establish the fruits' shelf-life. The antibacterial activity of the modified coating and antioxidant activity were determined by the agar disc diffusion method and a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay respectively. Phytochemical screening results indicated the presence of alkaloids, flavonoids, terpenoids, phenols, and tannins. The UV-Vis spectra of the coatings showed absorbance in the spectral range between 300 nm and 600 nm, while the FT-IR spectra showed peaks at 3400 cm^{-1} (OH), 2929 cm^{-1} (C-H stretch), 1586 cm^{-1} (C=C stretch), and 1150 cm^{-1} (C-O stretch). GC-MS analysis of *O. gratissimum* oil identified a total of 22 compounds with 2-Octen-1-ol, (Z) (26.66%) as the major compound. The % DPPH inhibition of the modified coating at 0.5%, 1.0%, and 1.5% were 24.73 ± 1.2, 39.74 ± 0.4, and 51.72 ± 0.8% respectively. The antibacterial activity of the modified coating (1.5%) showed the most significant ($P<0.05$) inhibitory effect of 22.22 ± 0.03 and 17.53 ± 0.28 mm on *Staphylococcus aureus* and *Escherichia coli* respectively. The modified coating (1.5%) maintained TA (0.66%), reduced weight loss by 27.81%, delayed the increase of TSS (7.50%) and pH 5.20 while retaining the vitamins C (8.13 mg/100g concentration) up to 18 days of storage at room temperature (23 ± 2 °C). The distilled water treatment (control) reduced TA to 0.11%, increased weight loss to 47.12%, TSS to 20.22%, and to pH 8.21 while retaining the vitamin C to 3.74 mg/100g concentration. In conclusion, modified *M. esculenta* coating showed remarkable antibacterial and antioxidant activities with a shelf-life of 18 days for coated fruit as compared to 9 days for the uncoated one. Modified *M. esculenta* edible coating is recommended for preserving fresh produce while maintaining their physico-chemical qualities during storage.

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LIST OF ABBREVIATIONS

ACEII PTRE Africa Center of Excellence in Phytochemicals, Textile and Renewable Energy

ATCC American Type Culture Collection

CS Cassava starch

DPPH 2, 2-Diphenyl-1-1 Picrylhydrazyl

E.I Electron Ionization

EOs Essential oils

FT-IR Fourier-transform Infrared Spectroscopy

GAE Gallic Acid Equivalent

GC-MS Gas Chromatography- Mass Spectrometry

NIST National Institute of Standards & Technology

NTU Nephelometric Turbidity Unit

pH Potential Hydrogen

RH Relative Humidity

TA Titratable Acidity

TFC Total Flavonoid Content

TSS Total Soluble Solids

TPC Total Phenolic Content

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CHAPTER ONE: INTRODUCTION

1.1 Background

Post-harvest food losses have been a concern for quite some time both in the developed and developing world (Botelho *et al*., 2016). Fresh produce is key to human nutrition and minimizing their post-harvest loss may improve the shelf-life of fruits as well as their quality while preserving active constituents for use by its consumers. Typically, losses or wastage in quality and quantity of fresh produce usually occur during production, postharvest, and processing stages in the food supply chain (Andrade *et al*., 2016). Major leading post-harvest factors have been attributed to, physical damage, poor handling, inappropriate packaging, elevated post handling conditions, delay in marketing, fungal and microbial infections (Ambuko, 2020).

Post-harvest application of chemical preservatives to regulate postharvest deterioration and prolong fresh produce shelf-life have been utilized, however, constant application of these treatments have been associated with chemical deposits, or the propagation of resistant pathogenic strains has resulted in serious health and ecological concerns (Sapper & Chiralt, 2018). A transformed curiosity in consumer requirements of natural preservation seems to be stirred up by existing food safety concerns, microbial resistance menace, and an increase in production of minimally processed foodstuffs linked with green picture guidelines of the food industry (Gustavsson *et al*., 2011). As a result, consumers are increasingly interested in food products of high quality especially fruit, vegetable, and other useful foodstuffs that are minimally processed without chemical preservatives (Salgado *et al*.,2015).

The Mango fruit (*Mangifera indica* L.) is among the most imperative tropical fruits and it belongs to the family *Anacardiaceae* (Shah *et al*., 2010). It is rich in nutritive

constituents such as, ascorbic acid, Pectin, fiber content, and phenolic content. It is also a source of iron, calcium, carotene, niacin, and riboflavin which are quite beneficial to the dietary requirements needed by the body (Mirza *et al*., 2020). Ripe mango fruit is considered to be invigorating and freshening. The intake of mango as a fruit, extracted as juice or used in cocktails, is well-known to ease high blood pressure, serum cholesterol, and stomachache. Also, mangoes are highly recommended for those suffering from cancer, arthritis, and the digestive tract condition (Ayele *et al*., 2012) .

Despite these useful applications, mango fruits are still highly susceptible to microbes during ripening process and this leads to spoilage. The climatic condition of mango fruit limits its postharvest life for a period of about 9 days at room temperature conditions of storage (Shah *et al*., 2010). Storage of mango fruits at elevated temperatures results in growth of pathogenic microbes, high-ethylene production, rise in respiration rate, and water and gloss loss. Conversely, storage under cool conditions exacerbates chilling injury symptoms. Research studies on vegetables and fruits including mango postharvest decay have been documented (Adhikari, 2006).

Preservation during storage and handling in an effort to lengthen the shelf-life of mango among other fruits has been given a lot of attention (Mirza *et al*., 2020). Recent emergence of edible packages has been given attention to control this waste, both synthetic and natural ones (Valencia *et al*., 2011). The application of edible films and coatings for food preservation was first utilized in the 12th century when wax was used to regulate water loss and preserve the quality of fresh produce (Pop *et al*., 2020). Edible coating or film is any substance prepared from food-grade matrices such as; bio-macromolecules, proteins, polysaccharides and lipids (Ju *et al*., 2019) that can be used for wrapping, enrobing or

coating various foods to augment food value and prolong the postharvest life of fresh produce.

The food transnational law considers food coatings as a portion of food (Fuciños *et al*., 2017), which have found an extensive application in food industries because they can be consumed with or within the food and can be degraded (Sapper & Chiralt, 2018). Frédéric (2010) records that in 15th century, the first edible film was prepared from soymilk in Japan for the conservation of fresh produce to obtain shiny surface. However, this kind of edible package did not gain great interest since a narrow variety of materials were used to protect vegetables and fruits at a time. Lipid coatings have also been used since middle ages to preserve meat and cheese against shrinkage (Pascall, 2012). Also sugar coatings have been used in therapeutic industries on drug pills and soft capsules (Pop *et al*., 2020).

Several studies have revealed that many natural constituents of plants such as essences might play a profound role in the host-pathogen association (Salgado *et al*., 2015). Incorporation of convenient extracts with edible coatings may increase synergism for antioxidant and antimicrobial activities which will enhance gloss appearance, color, taste, regulate the release of antimicrobial particulates from the food surface, ethylene production, weight loss, and respiration and transpiration rates. Coating films selectively permits the useful gases for instance, ethylene, oxygen, and carbon dioxide, which are quite necessary in oxygen dependent processes by inhibiting moisture losses between the food and the surrounding environment (Valencia *et al*., 2011).

Moreover, research has indicated that edible coatings can be modified with natural extracts that contain important biological phytochemicals constituents such as, enzymes, antioxidant, antimicrobial agents and mineral salts (Bustos *et al*., 2016). Currently, the utilization of edible packages has gained a lot of interest not only in preserving fruits and

vegetables but also, nuts, candies, and animal products (Anshu Sibbal Chatli & Mehndiratta, 2018). Even though the utilization of this technique is not anticipated to substitute synthetic packing products and use of chemical preservatives, they can be utilized to prolong food stability by monitoring and averting surface contamination maintaining the value of food and packaging efficacy resulting in minimized or controlled utilization of synthetic films and preservative chemicals (Sapper & Chiralt, 2018).

Edible packages range from cellulose, starch, proteins, lipids and fats. Amongst the natural edible biopolymers, starch has gained massive interest in formulations of edible packaging, due to its film-genic capacity, low cost, readily available, and biodegradable. Additionally, they are colorless, tasteless, and odorless, hence they can be utilized to lengthen the postharvest life of perishable fresh product (Iamareerat *et al*., 2018). Starch based edible coatings have the limitation of being highly permeable to water vapor transfer. This is solved using lipid additives, plasticizers, essential oils, and waxes to increase the hydrophobic portion of the film to boost flexibility and stability of film matrix (Luchese *et al*., 2017).

Some of the plants used in starch coatings include cassava, sweet potato, wheat, corn, banana, and rice (Sapper & Chiralt, 2018). Cassava (*M. esculenta*) starch has been reported to be the best candidate for edible package formulations due to its paste clarity, minimized gelatinization temperature, odorless, tasteless, colorless, non-toxic and super gel stability (Piñeros *et al*., 2017).

Essential oils (EOs) is another group of bioactive constituents from natural plant extracts which have been combined with starch polymer packages due to their biologically active compounds such as eugenol, thujene, thyme, D-limonene, among others (Antolak & Kregiel, 2017). Some of the plants that have been utilized in food preservation include;

Cinnamon, cloves, garlic, parsley, rosemary, thyme, basil, cardamom, lemongrass, carrot, tamarind, and nettle plant (Zullaikah *et al*., 2018).

The *O. gratissimum* extract have been used in food preservation but it's utilization on edible coating formulations has not been reported. Therefore, considering bioactive constituents of *O. gratissimum* leaves essential oil and preservative properties of *M. esculenta*, this study focused on the formulation of biodegradable *M. esculenta* edible coating (cassava) starch incorporated with *O. gratissimum* (African basil) leaves essential oil to evaluate its efficacy on the lengthening of postharvest life of fresh mango fruits.

1.2 Statement of the Problem

Fruits and vegetables are easily affected by pathogens during handling and storage (Hammond *et al*., 2015). This loss contributes greatly to poor nutrition as well as food insecurity in Kenya hence the sustainable development goals number 2 and 3 on Zero hunger and Good Health and well-being are becoming a challenge to achieve (L *et al*., 2020). Mangoes are known to help lower blood pressure, serum cholesterol, used in cancer management among other uses. However, the fruits have a short shelf-life due to the damage from microbes. If the storage and handling is not improved, then a great loss is observed (Engineering for Change, 2020). This loss definitely affects its consumers posed by poor-quality fruits or damaged ones, while those preserved by synthetic preservatives pose the challenge of toxicity (Shaikh *et al*., 2016). There is need to improve the postharvest life of the fruits as well as their quality while preserving their constituents for use by the consumers. Although various methods like drying, precooling, pickling, freezing, oil packing and many more have been used to reduce fruits spoilage, there is still a need for improved methods that could enhance their shelf life without altering the

vegetables and fruits' nutritive values whilst preserving the environment from contamination (Kraśniewska & Gniewosz, 2012).

1.3 Objectives of the Study

1.3.1 General Objective

The general objective of this study was to evaluate the antibacterial and antioxidant efficacy of *M. esculenta* (CS) edible coating modified with *O. gratissimum* (African basil) leaves essential oil for the improvement of the shelf-life of fresh *Mangifera indica* (mango) fruit.

1.3.2 Specific Objectives

The specific objectives of this study were to:

1. Determine the phytochemicals components present in *M. esculenta* tubers and *O. gratissimum* leaves crude extracts.
2. Prepare and modify *M. esculenta* starch coating using *O. gratissimum* essential oil.
3. Characterize the *O. gratissimum* EOs, unmodified, and modified, *M. esculenta* coating.
4. Determine antioxidant and antibacterial activity of the *M. esculenta* coating incorporated with *O. gratissimum* EOs.
5. Compare the shelf -life of mango fruits coated with unmodified and modified *M. esculenta* edible coating at 4 ± 2 ℃ and 23 ± 2 ℃.

1.4 Research Questions

1. Which are the major phytochemical components present in *M. esculenta* (cassava) tubers and *O. gratissimum* leaves crude extracts?
2. Can *M. esculenta* coating be prepared and modified with *O. gratissimum* essential oil?
3. How do modified, unmodified *M. esculenta* coating and *O. gratissimum* EOs compare in their characterization?
4. Does *O. gratissimum* EOs incorporation in *M. esculenta* (cassava) starch coating affect the cassava's antioxidant and antibacterial properties?
5. How does the shelf life of mango fruit coated with modified *M. esculenta* (cassava) starch coating compared with that coated with the unmodified cassava starch coating?

1.5 Justification of the Study

Fresh produces are easily affected by pathogens during handling and storage. In Kenya, about 1,024,500 metric tonnes of mangoes are produced annually, but due to a number of factors including poor post-harvest handling techniques, it is estimated that about 512,000 metric tonnes of mangoes are lost (Engineering for Change, 2020). This loss has greatly exacerbated Kenya's food insecurity, hence the sustainable development goal on food security is becoming a challenge that needs to be addressed (L *et al*., 2020). The rationale of this research was to enhance the scientific knowledge on food preservation measures by use of readily available starch-based materials to improve the sensorial attributes of fruits, storability and prolong their postharvest life. The study also enhanced knowledge on the impacts of the coating preservation method and storage conditions of fresh

produce. The data generated in this study is valuable in addressing health concerns to the consumers, and ecological related concerns to the policy makers. The research product solves part of the national government problem on food security and also globally by contributing to sustainable Development Goals 2 and 3 on Zero hunger and Good Health and well- being respectively. Validation of efficiency of the modified cassava starch edible coating will enhance novel strategies in food market industries and food security as well as improve the life style of people.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of Postharvest Losses

In Kenya, about 1,024,500 metric tonnes of mango are produced annually, but due to a number of factors including poor post-harvest handling techniques, it is estimated that about half of mangoes produced are lost (Engineering for Change, 2020). Due to these losses, several preservation treatments have been utilized to maintain the quality of the fruits for longer periods. Some of these methods include, thermal heating, drying, pickling, freezing, synthetic chemicals and use of edible coatings (Dwivedi *et al*., 2017).

2.2 Food Preservation Methods

2.2.1 Thermal or Heating Processing

Thermal or heating processing is one of the methods that has been applied in preserving dairy products, bakeries, fruits and vegetables (Prieto *et al*., 2020). The process entails applying heat to foods in containers such as canning, between 75 and 90 ^{o}C higher with a holding time of 25-30 s. Also this process has widely been utilized for the production of jams and jellies, canned and bottled fruits and vegetables (Barrett & Lloyd, 2012). This is because the technique effectively reduces microbial population growth and pulverizes natural enzymes. However, research has also established nutrient losses, energy waste, flavor alterations and reduction in the food matrix (Roselló-Soto *et al*., 2018). A study conducted on mango fruits preservation using this method, exhibited undesired chemical effects, physical, loss of phytochemicals, and sensorial changes in the fruit (Codina - Torella *et al*., 2017).

2.2.2 Drying

Drying is one of the oldest preservation methods that has been utilized since the ancient times for preservation of cereals, meat, fish, fruits and vegetables. Foodstuffs can be dried in a variety of ways, such as drying in the sun (natural drying) or by using simulated heat under controlled temperature, in specifically designed chambers known as dehydrators or dryers (Devi *et al*., 2015). Consequently, the moisture content of dried fruits and vegetables is reduced to 10-15%, inactivating and inhibiting growth of present pathogens (Ingham, 2008). Also, the dried food products get lighter in weight, thus reducing the cost of delivery. However, the dehydration of fruits and vegetables is not recommended because the process makes them too brittle for consumption. A study conducted on dried mango slices revealed that, drying leads to degradation and loss of nutrients (Sharif *et al*., 2017).

2.2.3 Pickling

Pickling is another old technique which uses salt and vinegar for preservation of fruits and vegetables (Dwivedi *et al*., 2017). This process involves addition of 8 to 10% of salt and 3% vinegar to fruits and vegetables. Following that, they will be maintained under the sun for 6 to 8 days at a temperature of 29 ^{o}C to 30 ^{o}C for fermentation with the help of lactic acid bacteria. After that, it will be combined together with coarsely powdered spies in the final step, and then entirely covered with moisture-free edible oil. Pickles should be kept in the sun for another three (3) weeks (Devi *et al*., 2015). Brine, vinegar, alcohol, and vegetable oil are common pickling agents. A pH of less than 4.6 is another differentiating feature, which is adequate to kill most microorganisms (Dwivedi *et al*., 2017). Because pickling permanently affects the flavor of foods, the pickled item may not

be a viable substitute for a fresh ingredient in a recipe. Research work on sweet mango pickles claimed that, the pickles had high content of sodium which resulted to increased blood pressure and cholesterol levels and should be avoided (Haidar *et al*., 2016).

2.2.4 Freezing

Cooling and freezing are techniques which have been widely utilized for preservation of leafy vegetables, fruits, spices, meat, poultry, fish and milk products to conserve the sensorial properties and nutrition qualities (Cheng *et al*., 2017). Freezing techniques involve air blast, cryogenic, direct contact and immersion. Freezing involves reduction of water level activities in various food products hence inhibiting the microbial activities and decrease the chemical reaction rates (Gambuteanu *et al*., 2013). Although, it is the easiest method of preservation, research studies have also claimed that, the process is too complex due to the involvement of heat transfer and also the changes in chemical and physical aspects that impacts the food products (Barrett & Lloyd, 2012). Additionally, in the course of freezing and defrosting process the food's tissue structure gets damaged as a result of the rate and temperature used (Ingham, 2008). Food that has been frozen loses more nutrients during storage the oxidation process. According to Kim *et al*., (2015), frozen mango fruits resulted to deterioration of texture, color and flavor

2.2.5 Synthetic Chemicals

Most of the cosmetic, pharmaceutical and food sectors employ the use of synthetic preservatives to maintain and prolong the longevity of the products (Shaikh *et al*., 2016). Some of the chemicals which have been used for food preservation include, sulfites, nitrates, and nitrites, benzoates, sorbates, saccharin, and caffeine. Many of the synthetic food additives are considered to be safe, but some of them have been reported to be

carcinogenic and very toxic (Dwivedi *et al*., 2017). For instance, the use of sulfites for preservation of fruits including mangoes, have been reported to cause side effects in form of allergic reactions, headaches, asthma, palpitations and cancer. Also, benzoates can initiate skin rashes, asthma and brain damage (Sharma, 2015). Generally, the use of synthetic chemical additives and preservatives should be avoided because many of them have not been tested appropriately (Shaikh *et al*., 2016).

2.2.6 Edible Coatings

Edible coating is a novel technique which has been extensively considered for food preservation including fruits and vegetables (Sharif *et al*., 2017). Edible coatings are derived from natural resources hence environmentally friendly, this has triggered growing of public demand for safe and healthy products. Edible coatings can be formulated from a wide array of food-grade polymer matrices. These polymers are based on proteins, waxes, lipids, polysaccharides, and resins (Han, 2013) which may be used as capsules, wraps, pouches, or casing after being subjected to further modifications (Baldwin *et al*., 2011).

The edible films designed for particular product must be well-suited for them (Šuput *et al*., 2016). For the film-forming process to auger well with the intended functions, certain fabrication conditions have to be considered; pH adjustment, application of heat, additive addition, enzymatic adjustment, drying, use of water or alcohol-based solvents and food-based reactions (Han, 2013).

Coatings are quite beneficial since they are consumable and easily biodegradable (Ju *et al*., 2019). Also they improve sensorial properties of foodstuffs, guarding them from mechanical and physical injuries, invasion of pathogens, moisture migration and

importantly lengthening the shelf life of foodstuffs (Riva *et al*., 2020). Furthermore, coating packages act as potential carriers of significant agents such as antimicrobial, antioxidants and more importantly act as barriers against gases, and oils surrounding the package (Bustos *et al*., 2016).

Edible coating or film is any substance prepared from food-grade polymers for instance; bio-macromolecules, polysaccharides, lipids, and proteins (Ju *et al*., 2019) that can be used for wrapping, enrobing or coating various food to enhance food quality and prolong the postharvest life of fresh produce. The food transnational law considers food coatings as a portion of food (Fuciños *et al*., 2017) which have found an extensive application in food industries because they can be consumed with or within the food and can be degraded (Sapper & Chiralt, 2018).

Frédéric, (2010) records that in 15th century, the first edible film was prepared from soymilk in Japan for the conservation of fresh produce to obtain shiny surface. However, this kind of edible package did not gain great interest since a narrow range of materials were used to protect vegetables and fruits at a time. Lipid coatings have also been used since middle age to preserve meat and cheese against shrinkage (Aydin *et al*., 2017). Additionally, sugar coatings have been used in therapeutic industries on drug pills and soft capsules (Pop *et al*., 2020).

2.3 Building Blocks of Edible Coatings/Films

Edible coatings can be formulated from a variety of food-grade additives such as proteins (gelatin, casein, albumins), lipids (beeswax, fatty acids), and polysaccharides (starch, chitosan, cellulose, pectin, guar gum, alginate) or their combination. These constituents are usually obtained from plants and animals hence they are eco-friendly and sustainable

to the consumers and environment (Films & Applications, 2009). Generally, the combination of these additives results to a more enhanced end product. Moreover, polysaccharides and proteins-based edible coatings forms cohesive molecular networks by strong interactions between molecules, which delivers good mechanical and barrier attributes to gases such as oxygen and carbon (iv) oxide (Vargas *et al*., 2008).

Usually, these polymers are polar leading to matrixes with high water permeability and low cohesion, hence various alternatives such as the addition of plasticizers, emulsifiers, lipids and essential oils have been utilized to improve and modify the polymer matrix (Andrade *et al*., 2015). Presently, the use of edible coatings has massively gained a lot of curiosity in preserving a wide array of fruits and vegetables including grapefruits, cherries, cucumber, strawberry, tomatoes, apples, oranges, melons, guavas, bananas and mangoes, (Sapper & Chiralt, 2018).

2.3.1 Protein Coating Forming Materials

Proteins are polymers which comprises of more than 100 amino acids which must undergo denaturation by heat, acid, alkali or solvent to form the more protracted structures which are essential for film formation (Nur Hanani *et al*., 2014). The protein films and coatings are mostly prepared from animal and plant produce for instance milk, eggs, oil seeds, whey and soy. According to Dhall *et al*., (2013), protein coatings are comprised of macromolecules with a chain of amino acids and molecular structures.

Protein based structures are highly prone to extreme conditions such as pressure, heat, chemical additives, enzymatic reactions, chemical cross-linking among others. As a result of this linkage, protein coating packages are well-thought-out to serve well as good oxygen blockers at limited Relative humidity (RH) levels. Protein based edible films

demonstrate good sensorial attributes, appreciable aroma, and oil-free when applied on various foodstuff (Anshu *et al*., 2018). Most of the edible proteins which have been utilized for coating and film formulation include collagen, gelatin, casein, gluten and Zein (Zhou *et al*., 2020).

2.3.2 Lipid Coating Forming Materials

Lipids derived substances have been applied in food packaging industries even though they are not polymer components (Aydin *et al*., 2017). Naturally, lipids are soft-solids at ambient conditions. They are biodegradable, and cohesive with phase shift temperatures hence they can be molded to any shape by casting schemes after applying heat, resulting to reversible phase transitions between fluid. Due to their hydrophobicity, they are normally combined with polysaccharide matrices during coating formulation in order to obtain an optimized water vapor barrier (Zhou *et al*., 2018). Numerous lipid components for instance, bee waxes, resins, paraffin, are generally utilized to provide palatable surfaces for preserving the sensorial attributes of coated foodstuffs including fresh fruits. Figure 2.2 shows fatty alcohols (R length is 12-34 carbon atoms) and fatty acids (R length is 12-34 carbon atoms) used in edible preparation (Baldwin *et al*., 2011).

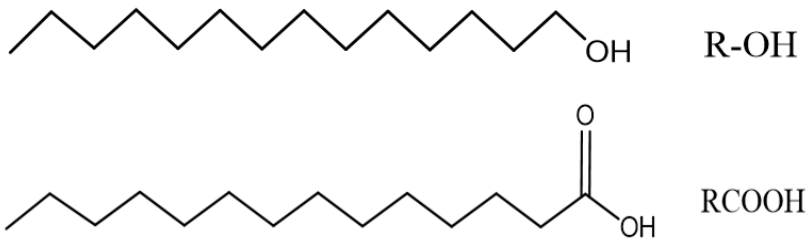


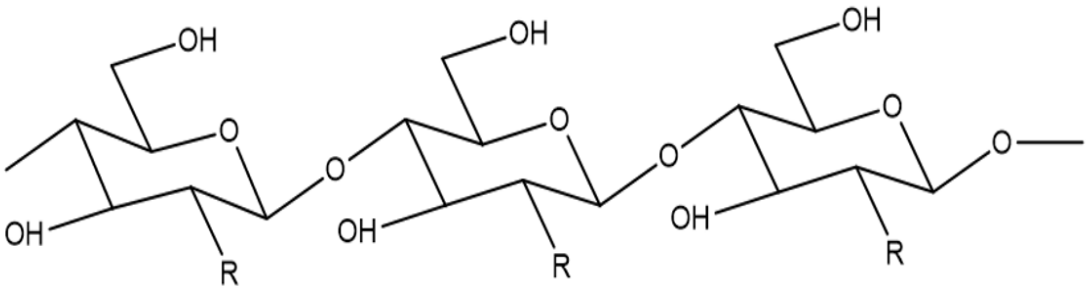
Figure 2.1. Fatty alcohols, R-OH (R length is 12-34 carbon atoms) and fatty acids RCOOH, (R length is 12-34 carbon atoms) used in food preservation.

2.3.3 Polysaccharides Film Forming Material

Polysaccharides are natural polymers with a range of components such as starch, gums, cellulose, carrageenan, chitosan, pectin, alginates, among others (Han, 2013). Coatings formulated from polysaccharides are considered as effective oxygen barriers due to their well-linked hydrogen bonded structures. Carbohydrates are neutral with large numbers of hydroxyl groups and hydrophilic moieties on their structures, the hydrogen bonds participate greatly in coating formation and characteristics properties (Dhall *et al*., 2013). Some polysaccharides such as gums and cellulose are negatively charged hence, they exhibits acidic properties as compared to alkaline and neutral ones, along with the presence of multivalent cations (Kumar & Neeraj, 2019).

Polysaccharides are extensively applied in food preservation field due to their film-genic capacity, good viscosity, thickening parameter, viscosity and ability to adhere to food surfaces during application. Additionally, the hydrogen bond forming capacity makes it efficient to selectively permit important gases between the food and the surrounding, resulting in desired modified atmosphere that enhances the value of foodstuffs including fruits (Baldwin *et al*., 2011).

Figure 2.2 shows some chemical structure of polysaccharides such as chitin, chitosan, and cellulose used in edible coating preparation (Zhang *et al*., 2017).



1.Chitin: R = $NHCOCH_3$
2.Chitosan: R = NH_2
3.Cellulose: R = OH

Figure 2.2. Chemical structures of chitin, chitosan, and cellulose.

2.3.3.1 Cellulose Derivatives

Cellulose is the most abundant polymer resource mainly derived from plant cell wall and it has been widely reported as a raw material for formulation of edible films and coating mainly because of its low-toxicity, biocompatibility, biodegradability, low cost, and chemical stability (Cazón *et al*., 2017). Cellulose and its derivatives are water insoluble owing to their large number of intra-molecular hydrogen bonding, see (Figure 2.3). Thus, they undergo etherification to form water soluble ethers such as; methyl-cellulose (MC), hydroxy-propyl methylcellulose (HPMC), hydroxy-propyl cellulose (HPC) and carboxy-methylcellulose (CMC), which have good film forming properties. Coatings and films formulated from cellulose and its derivatives are extensively utilized in packaging industries because of their flexibility, water solubility, transparent, odorless, and resistant to moisture, gases such as oxygen and carbon (iv) oxide (Singh *et al*., 2019).

2.3.3.2 Chitosan

Chitosan is a linear polysaccharide comprising of (1,4)-linked 2- amino-deoxy-b-D-glucan, see (Figure 2.3). It is biodegradable, non-toxic, and biocompatible. Chitosan is

obtained by alkaline N-deacetylation of chitin, which is derived from fish waste and fungal cell walls (Li *et al*., 2019). Chitosan has the capability to disrupt the negatively charged residues of macromolecules visible on the fungal cell surface and alters the permeability of the plasma membrane. Also, chitosan has fatty acids which enhances antimicrobial properties. As a result of vast potential properties, chitosan has been extensively utilized in edible coatings and films for food preservation to inhibit pathogen growth extending the shelf-life of foodstuffs including fruits (Cazón *et al*., 2017).

2.3.3.4 Starch-Based Edible Films

Starches can be categorized into potato, corn, and cassava (Luchese *et al*., 2017). Starch has gained massive interest in formulations of edible packaging because of its film-genic capacity, low cost, readily available and it is biodegradable. Furthermore, they are colorless, tasteless and odorless; hence they can be utilized in extending the shelf-life of perishable fresh produce. However, the greatest limit is their high porousness to water vapor transmission, which can be solved by the addition of various essences such as plasticizers, plant extracts, and waxes to increase the hydrophobicity, flexibility and stability of the coating matrix (Luchese *et al*., 2017). Starch, especially from *M. esculenta* (cassava) has been reported to be the best candidate for edible package formulations due to its paste clarity, minimized gelatinization temperature, odorless, tasteless, colorless, non-toxic, and super gel stability (Piñeros *et al*., 2017).

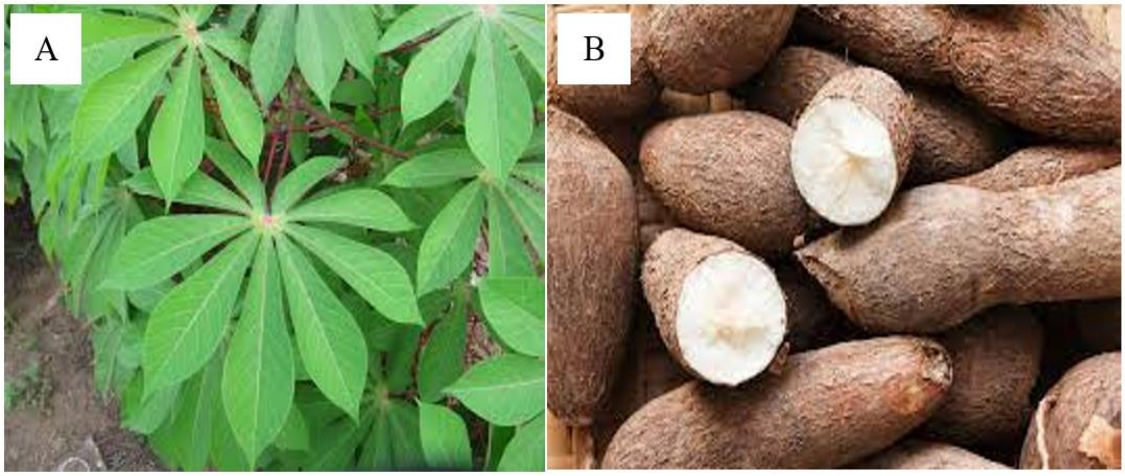


Figure 2.3. *Manihot esculenta* (cassava) leaves (A) and tubers (B).

2.4 Plants that Have Been Used for Food Preservation

Plant extracts and products such as essential oils are secondary metabolites which are quite beneficial in various aspects of life. The extracts are usually applied in edible packages to improve their efficacy (Nanasombat & Wimuttigosol, 2011). Essential oils contain bioactive constituents with antioxidants and antimicrobial activities which when added to foodstuff coating-formulations prolong postharvest life and thus maintain the quality attributes of foodstuffs (Medina *et al*., 2017).

The natural extracts contain bioactive constituents such as phenolic acids, alkaloids, flavonoids, terpenoid, isoflavones, carotenoids, and aldehydes. Moreover, research indicates that coatings containing antioxidant extracts can be utilized to deter the oxidation deterioration of foods which are oily (Murmu & Mishra, 2018). For this specific purpose, natural extracts are considered instead of conventional synthetic compounds due to their environmental and health benefits (Souza *et al*., 2019). Natural extracts such EOs are regarded as effective due to their potency to inhibit lipid oxidation and delay the growth of pathogens on food (Piñeros *et al*., 2017).

2.4.1 Thyme (*Thymus vulgaris*)

Thymus vulgaris (*T. vulgaris*) is an evergreen plant with scented green leaves and pink flowers in the family *Lamiaceae* (Sangsuwan *et al*.,2016). The plant and its functional parts have been used since time immemorial as an additive or infusion bath for body applications. It has also been used in the food industry as an additive since it has been proven to contain bioactive compounds in its extracts or essential oils such as phenolic acids, flavonoids, alkaloids, tannins, saponins, steroids and many more (Chen *et al*., 2012). Studies have reported bioactive compounds in thyme essential oils have strong antifungal, antibacterial, antiseptic, and antioxidant activities which when incorporated in food preservation significantly slows down food deterioration and growth of pathogens (Fachini *et al*., 2012).

Research that was done by Karami *et al*., (2010) reported that thyme EOs significantly prohibited the development of *Erwinia amylovora* bacteria which causes fire blight infections of apples and pear. Bosquez *et al*., (2010) also validated strong repressive impacts against *Rhizopus stolonifera* and *Colletotrichum gloeosporioides* and accountable for the decay of stored papaya produce.

Extracts from *T. vulgaris* also exhibited good antimicrobial activity against food pathogens for instance *Escherichia coli, Staphylococcus aureus, Enterobacter cloacae, Bacillus subtilis, Pseudomona aeruginosa, Salmonella typhimurium, Staphylococcus epidermidis,* and *Mariniluteicoccus flavus* (Soković *et al*., 2010).

2.4.2 Peppermint (*Mentha piperita*)

Mentha piperita (*M. piperita*) is a natural herb kind of water mint hybrid belonging to the *Lamiaceae* family. All over the world, it's been used for pharmaceutical and cosmetic

products, and mainly as a food additive to enhance freshness in food products, flavor and sweet aroma (Chen*et al*., 2012). Peppermint extracts have been used as beverages and most commonly peppermint essential oils have been utilized in pharmaceutical, cosmetic and food industries due to its abundant presence of bioactive compounds. The active phytochemicals that have been detected in peppermint EOs include menthone 30% and menthol 50%.

Other bioactive compounds in EOs include phenolic acids, flavonoids, caffeic acid, vanillic acid, rosmarinic acid, ferulic acid, among others (Riachi & De Maria, 2015). Burt, (2004) reported that mint oil was effective in inhibiting the growth of *Salmonella enteritidis* cucumber salads, tzatziki yogurts, along with cod's roe salad. Additionally, research done by (Saad *et al*., 2014) showed that peppermint oil was effective for anti-adhesive and anti-biofilm property against food decay pathogen *Pseudomonas aeruginosa,* and *Candida albicans.*

2.4.3 Rosemary (*Rosmarinus officinalis*)

This is a perennial herb with green leaves and white flowers which belongs to the family *Lamiaceae*. Globally, rosemary has found wide application in traditional medicine, cosmetics and as a food flavoring due to its pleasant aromatic smell (Nassazi et al., 2020). Studies have indicated that rosemary EOs are rich in bioactive compounds such as monoterpenes which contain borneol, camphor, limonene, plus β-pinene (Teodoro *et al*., 2014). Additionally, *R. officinalis* plant extracts have been stated to comprise phenolic acids, and flavonoids (Szumny *et al*., 2010).

The bioactive compounds of *R. officinalis* have been reported for their antioxidant, antifungal, and antimicrobial activities (Teodoro *et al*., 2014). Vasile *et al*., (2017) also

showed that rosemary essential oils contain antifungal activity against *Fusarium graminearum,* and *Aspergillus niger*. Studies that were carried out by Teodoro *et al*., (2014) on antifungal property of fresh dough spoilage found that *R. officinalis* EOs minimized the decay rate of fresh dough.

2.4.4 Cinnamon Bark (*Czeinnamomum zeylanicum*)

Czeinnamomu zeylanicum (*C. zeylanicum*) is a perennial tree in the family *Lauraceae*. All around the world, cinnamon bark is being used mainly in ethnomedicine and as a flavoring for foods (Antolak & Kregiel, 2017). Cinnamon bark is believed to contain more than 70 phytochemicals, the main components that have been extracted from cinnamon bark include, linalool, coumarin, (E)-cinnamaldehyde, terpinen-4-ol, and eugenol, *C. zeylanicum* extracts and EOs have been characterized to poses strong activity against food spoilage pathogens and deterioration. Some of the pathogens that have been inhibited by *C. zeylanicum* include *Streptococcus aureus, E. coli, Bacillus subtilis, Klebsiella pneumonia, Salmonella typhi,* and *Pseudomonas aeruginosa* (Chen *et al*., 2012).

2.4.5 Cloves (*Syzygium aromaticum*)

Syzygium aromaticum (*S. aromaticum*) is evergreen tropic aromatic spice popular in all parts of the world and belonging to the *Myrtaceae* family. *Syzygium aromaticum* has been characterized with bioactive compounds such as phenolic acids, flavonoids (kaempferol, quercetin and its products). Clove oil comprise of several bioactive compounds which have potentiality for antimicrobial activities, food flavoring and preservation. Studies have documented that clove water extracts exhibits antibacterial impacts against microbial contaminants such as *Bacillus cereus* and *E. coli* while eugenol from the *S.*

aromaticum can deter the development of *Heliobacter pylori* (Hill *et al*., 2013). Moreover, clove oil has been proven to have antifungal property against *Fusarium oxysporum, Trichophyton rubrum,* and *Aspergillus strains* (Park *et al*., 2007).

2.4.6 Tamarind (*Tamarindus indica*)

It is a tropical or subtropical evergreen woody-plant belonging to the family *Leguminosae* (Altemimi *et al*., 2017). *Tamarindus indica* plant parts have been reported to have useful numerous applications in traditional medicine, pharmaceutical industries as well as a food additive to enhance aromatic flavor and as an agent to inhibit food spoilage (Umaraw *et al*., 2020). The leaves and other plant parts have been used mostly for therapeutic uses since it is believed to contain active phytochemical constituents (Adeniyi *et al*., 2017). Consequently, the fruit pulp is edible with sweetish acidic taste hence it is extensively used in various foods seasoning. Additionally, the fruit pulp has been used in cocktails, sauces, curries, and pickles (Daniyan & Muhammad, 2008).

2.4.7 African/Clove Basil (*Ocimum gratissumum*)

It is an aromatic plant with elliptic-lanceolate leaves and white to pinkish florae like spikes belonging to the family *Lamiceae* (Nweze & Eze, 2009). It is an important herbal medicinal plant not only in sub-Saharan Africa including Kenyan communities (Matasyoh *et al*., 2008), see (Figure 2.4). Other plant species in the family *Lamiceae* include *Ocimum sanctum* (holy basil), *Ocimum canum* (African mint), *Ocimum basilicum* (sweet basil) among others (Gupta *et al*., 2014).

Figure 2.4. *Ocimum gratissimum* (African basil) leaves.

Traditionally, *O. gratissimum* has been recorded to have numerous application in medicinal field to alleviate stomach upset, fever, headache, dried leaves in (Attah *et al*., 2012) food flavorings, feedstuff additive and fibers due to their bioactive compounds such alkaloids, flavonoids, terpenoids, phenolics and other phytochemical constituents (Pandey *et al*., 2015). As such, *O. gratissimum* oil has been validated to contain antimicrobial, antioxidant and antifungal activities (Muráriková *et al*., 2017).

Some of the compounds that have been characterized in *O. gratissimum* include; Eugenol, thymol, linalool, p-cymene, and limonene among others (Mahajan *et al*., 2020), see (Figure 2.6). Studies have shown that *O. gratissimum* extracts exhibit antibacterial activity against microbial contaminants for instance, *Bacillus cereus, Staphylococcus pneumonia, E. coli, S. aureus,* in addition to antifungal activity against *Candida albicans* among others (Nascimento *et al*., 2016). Although *O. gratissimum* extracts have been studied for some therapeutic uses as well as food additives, research on formulation of edible coating had not been given much attention. As such this research focused on the formulation of biodegradable *M. esculenta* (cassava starch) coating incorporated with *O. gratissimum* (African basil) leaves essential oil to evaluate its efficacy on the lengthening

of postharvest life of fresh *M. indica* (mango fruit). Figure 2.5 shows structures of major compounds previously identified in *O. gratissimum* leaves Eos.

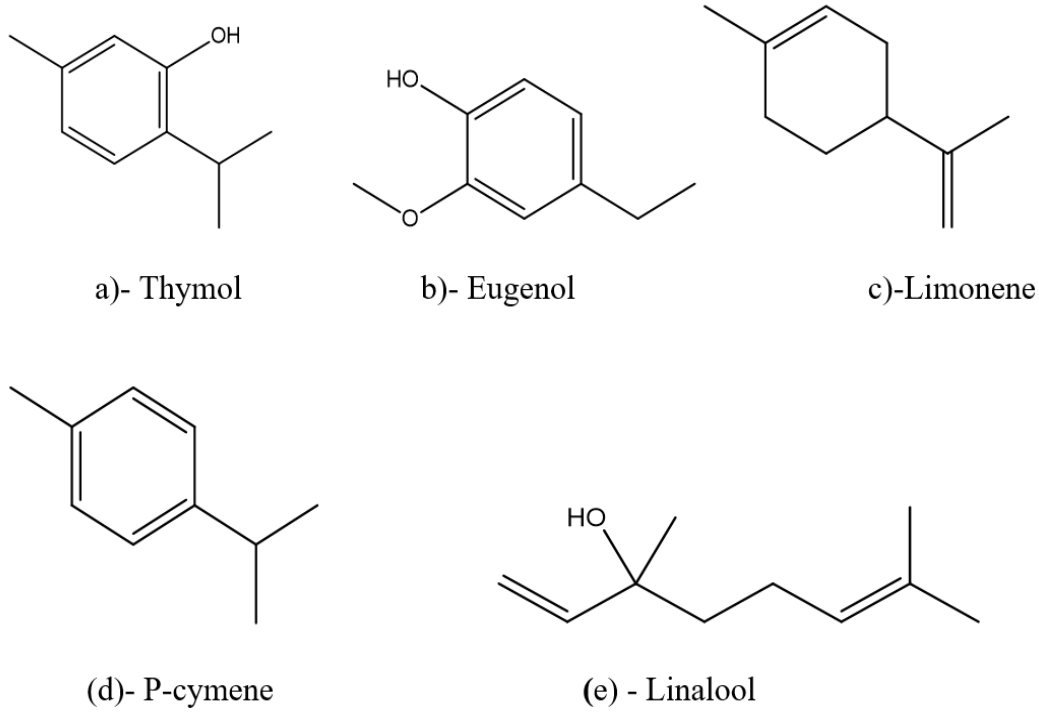


Figure 2.5. Structures of the major compounds previously identified in EOs from *O. gratissimum* leaves (Joshi, 2017).

2.5 Essential Oils

Essential oils (EOs) are volatile secondary metabolites produced by various aromatic plants for instance barks, seeds, flowers, peel, fruit, roots, leaves, wood, fruits, whole plants (Kim & Rhee, 2016). Typically, EOs are referred to as volatile compounds due to their tendency to evaporate even at ambient conditions when exposed (Aziz *et al*., 2018). Essential oils are secreted either directly by the plant protoplasm or by the hydrolysis of some glycosides and plant structures (Abd-Elsalam & Khokhlov, 2015). Essential oils are normally obtained from plant parts through various methods such as direct steam

distillation, hydro-distillation and micro-assisted extraction depending on the nature of plant source.

Some of the major bio-active constituents of EOs include eugenol, limonene, pinene, thymol, linalool, and cymene (Aitboulahsen *et al*., 2018). Chemically, a single volatile oil comprises of more than 200 different chemical constituents, and mostly the trace components are solely responsible for its characteristic flavor and odor (Ekunwe *et al*., 2010). These bioactive compounds have strong antibacterial, antioxidant and antifungal properties which enables them to be used in the various therapeutic and food industries. So far, EOs has been established as a safe additive for many products, such as food, including edible coatings and films to improve their efficiency on food package (Ju *et al*., 2019).

2.6 Current Application of Starch-Based Edible Coatings with Essential Oils in Fruits Preservation

Essential oils can be blended within the edible film matrix by using diverse techniques such as emulsification or homogenization. In order to improve or maintain the quality of food package material natural bioactive constituents such as essential oils have to be applied appropriately either by the aqueous phase or lipid droplets (Sharma *et al*., 2021). The application of essential oils has been widely utilized in the food industries owing to their natural bio preservative impact, which aids to extend the shelf-life in foodstuffs (Herman *et al*., 2019).

Fresh produces are the most common types of foods where essential oils are utilized, including other groups such as dairy products, meat, fish, bread and baked foods. However beneficial, essential oils have a limitation when applied directly to the food

matrix due to their hydrophobicity and instability (Rizzo & Muratore, 2020). In order to improve biological activity, increase the effective utilization rate and reduce the effect on food organoleptic properties of EOs, they need to be incorporated with in a conveying system such the use of edible coating and films which are compatible with food applications (Fernández-López & Viuda-Martos, 2018).

Essential oils result in improved functionality of biopolymer films (Sapper & Chiralt, 2018). Perdones *et al*., (2012) examined the impacts of chitosan–lemon oil blend on strawberry fruits. The authors found that chitosan blend significantly slowed down the percentage of decay on treated strawberries as compared to untreated ones. Perdones *et al*., (2012) evaluated the effect of cinnamon leaf oil films incorporated with oleic acid on antioxidant, antimicrobial, and physical parameters. The authors found that the coatings were effective on lengthening the postharvest life fresh strawberries stored on cold condition.

Incorporation of lemongrass essential oils with chitosan matrix on green bell pepper coating is reported to be less efficient in vivo than in vitro but the blend of EOs and chitosan improved the antimicrobial property of the coating (Ali *et al*., 2015). Vieira *et al*., (2016) performed a test on the impact of chitosan- Aloe vera coating on shelf-life value of blueberry (*Vaccinium corymbosum*) fruit and observed a fungistatic effect during the storage period. The study that was carried out by Ortega *et al*., (2017) regarding the blend of starch and aloe-vera on cherry tomatoes, indicates that coating maintained the quality of the tomatoes by minimizing deterioration.

Cassava starch incorporated with cinnamon EOs on guava fruits was efficient in maintaining the fruit quality by regulating anthracnose. The fruits that were treated with cinnamon EOs maintained freshness up to the eighth-day free of anthracnose (Botelho *et*

al., 2016). Although *O. gratissimum* plant has been utilized in food industry, the use of *O. gratissimum* leaves EOs in edible coating formulations has not been reported. Therefore, considering the antioxidant and antimicrobial activities of *O. gratissimum* leaves EOs and the preservative properties of cassava (*M. esculenta*) starch, this study focused on the formulation of biodegradable cassava starch coating incorporated with *O. gratissimum* leaves EOs to evaluate its efficacy in prolonging the shelf-life of fresh mango fruits.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Sampling Area

The mango fruits were collected from Elgeyo-Marakwet County, it lies between 0°58'56.0" N35°35'16.5E coordinates. Cassava tubers and *Ocimum gratissimum* leaves were collected from Nyansiongo settlement scheme, Nyamira County. It lies between 0.754 ^{o}S, 35.0168 ^{o}E coordinates.

3.2 Reagents and Chemicals

The reagents and chemicals used in this study were: sodium hypochlorite (1%), sodium carbonate, sodium hydrogen carbonate, sodium nitrate, aluminum chloride, quercetin, ferric chloride, potassium iodide, sodium sulphate, sodium hydroxide, glacial acetic acid, sodium thiosulphate, Gallic acid (≥ 99.9%), ciprofloxacin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium bromide, potassium iodide, potassium iodate, Muller-Hilton media, phenolphthalein indicator, starch indicator, lead acetate, ascorbic acid, McFarland (Turbidity 0.5 NTU Standard), were purchased from Sigma Aldrich. They were of analytical grade and were used without further purification.

Chloroform, methanol, distilled water, hydrochloric acid, magnesium metal, folin-ciocalteu reagent sulphuric acid, bromine water, glycerol plasticizer, ethyl acetate, peptone water phosphate buffered, Wagner's reagent, and acetone were purchased from Sigma Aldrich.

3.3 Equipment

The equipment used in this study were: digital pH meter (Sr. No. 1630), weighing balance (Mettler-Toledo), clevenger apparatus, bench refractometer, electric blender (NutriBullet-900W), UV-Vis (Beckman Coulter DU^{R}720 model), FT-IR- 8400

spectrometer (Shimadzu Thermo Fisher Science Co., Ltd., MA, USA), and GC-MS (Shimadzu QP 2010-SE).

3.4 Sample Collection

The mature mango fruits at green stage were harvested in the morning hours from small holder farmers in Elgeyo–Marakwet County, Kenya. They were packed in a large container lined with pricked paper and transported to Moi University Chemistry laboratory for preparation.

Ocimum gratissimum leaves were hand-picked from stems and cassava tubers were obtained by digging them up from the ground with a sterilized hoe from Nyansiongo location, Nyamira County. The collected parts were kept in a clean and sterilized sample collecting bag after which they were carried to the Chemistry laboratory, Moi University for sample preparation.

3.5 Sample Preparation

Ocimum gratissimum (African basil) leaves were washed under running tap water followed by distilled water, cut into sizable pieces for extraction.

Manihot esculenta (Cassava) starch tubers for preparation of edible coating, were washed, peeled, chopped into sizable pieces, pulverized, starch slurry washed, refined, dehydrated and dried.

Mangifera indica (Mango) fruits were selected according to uniform size, color and free from visual symptoms, they were cleaned with 1% neutral detergent solution, rinsed, sanitized with sodium hypochlorite solution for 1 minute and sorted randomly into six batches for application of coating treatment.

3.6 Sample Extraction

3.6.1 Extraction *Ocimum gratissimum* Leaves Crude Extract

This was done as per Ali *et al*., (2017). About 100 g of powdered *O. gratissimum* leaves were extracted exhaustively with (cold serial maceration) using hexane, ethyl acetate, acetone and methanol for 3 days as shown in (Appendix I). The extracts were filtered with Whatman No .1 filter paper and concentrated using rotary evaporator at 45 ± 2 ºC and further concentrated to dryness on water bath. The percentage yield of the extract was calculated using (Equation 3.1).

$$\text{Percentage yield} = \left(\frac{A}{A0}\right) \times 100 \quad \text{..... (Equation 3.1)}$$

Where A = mass of crude extract, and A_0 = mass of powder macerated.

3.6.2 Extraction Process of *M. esculenta* Tubers

About hundred (100) g of the pulverized and sieved starch of *M. esculenta* was extracted exhaustively with cold serial maceration using hexane, ethyl-acetate, acetone and methanol for 3 days. The extracts were filtered with Whatman No.1 filter paper and concentrated using rotary evaporator at 45 ± 2 ºC and further concentrated to dryness on water bath. The percentage yield of the extracts was calculated using (Equation 3.1).

3.6.3 Extraction of *O. gratissimum* Leaves Essential Oils

Ocimum gratissimum leaves EOs was obtained by hydro-distillation method using Clevenger apparatus as shown in (Appendix II). About 200 g of fresh pulverized *O. gratissimum* leaves was placed into the hydro-distillation unit and distilled for two hours, the obtained essential oil was dried over anhydrous sodium sulphate, and stored in amber

vials at 4 ^{o}C until use and analysis. The percentage yield of essential oil was calculated using (Equation 3.2) (Nour *et al*., 2013).

$$\text{Yield of EOs } (\%) = \frac{\text{amount of essential oil(g)obtained}}{\text{amount of raw material (g)used}} X100\% \quad \ldots\ldots\ldots\ldots \text{ (Equation3.2)}$$

3.7 Phytochemical Screening

This was done on different extracts to determine the presence of bioactive components present in *Manihot esculenta* tubers and *Ocimum gratissimum* leaves crude extracts following standard protocol as per (Kumar, 2015) The presence of alkaloid, saponin, steroids, tannins, flavonoids, terpenes, terpenoid and phenols were investigated.

3.7.1 Test for Flavonoids

Two (2) mL of the plant extract was taken and put in a test tube and two drops of dilute NaOH added. The appearance of a yellow color which disappeared or became colorless after two drops of dilute sulphuric acid was added confirmed the presence of flavonoids in the plant extract.

3.7.2 Test for Alkaloids

Two (2) mL of the extract was taken and dissolved in 2 mL of the Wagner's reagent. After dissolving the extract and the Wagner's reagent, the appearance of reddish-brown colored precipitate confirmed the presence of alkaloids in the plant extract.

3.7.3 Test for Terpenoids

Two (2) mL of each organic extract was dissolved in 2 mL of chloroform and evaporated to dryness. Two (20) mL of conc. H_2SO_4 was then added to each test tube and heated for about 2 min, formation of a greyish color indicated the presence of terpenoids.

3.7.4 Test for Tannins

Two (2) mL of each extract in separate test tubes was stirred with 2 mL of distilled water and few drops of ferric chloride ($FeCl_3$) solutions was added. Formation of green precipitate indicated presence of tannins.

3.7.5 Test for Saponins

Five (5) mL of each extract was shaken vigorously with 5 mL of distilled water in a test tube and warmed. The formation of stable foam indicated the presence of saponins.

3.7.6 Test for Steroids

Two (2) mL of the plant extract was taken and put in a test tube and 1ml of concentrated sulphuric acid was added by the side of the test tube. The appearance of a dark reddish color confirmed the presence of steroids in the plant extract.

3.7.7 Test for Glycosides

Two (2) mL of concentrated sulphuric acid was added to the extract. Formation of a reddish-brown color indicated the presence steroidal aglycone, which is a part of glycoside.

3.7.8 Test for Phenols

For each extract (500 mg) was dissolved in 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green color indicated the presence of phenolic compounds.

3.8 Preparation and Modification of *M. esculenta* edible coating

3.8.1 Preparation of Unmodified *M. esculenta* (cassava) Starch Coating

This was done as per (Botelho *et al*., 2016). Cassava-starch coating suspension was prepared by dissolving 30 grams of cassava-starch into 1L of distilled water. The starch solution was stirred at 70 ^{o}C until it completely dissolved. Afterwards, the homogenous solution was left to cool at room temperature and then 5mL of glycerol was added as a plasticizer. The formed gel was used without modification. Figure 3.1 shows ground *M. esculenta* tubers (A), dried and sieved starch (B), and prepared edible coating (C).

Figure 3.1. Ground *M. esculenta* tubers (A), dried and sieved starch (B), and edible coating (C).

3.8.2 Preparation of Modified *M. esculenta* (cassava) Starch Coating

This was done as per (Botelho *et al*., 2016), cassava-starch coating suspension was prepared by dissolving 30 grams of cassava-starch into 1L of distilled water. The starch solution was stirred at 70 ^{o}C until was completely dissolved. Afterwards, the viscous solution was left to cool at room temperature and then 5g of glycerol was added as a plasticizer, see (Appendix iii). The formed gel was divided into two parts and cooled at room temperature. The first part was used without addition of essential oils and the second part of the gel was incorporated with 1.5% of *O. gratissimum* leaves essential oil.

3.9 Determination of Total Phenolic and Total Flavonoid Contents of Modified *M. esculenta* (cassava starch) Coating

3.9.1 Total Phenolic Content (TPC)

Total phenolic content (TPC) of the modified coating sample was quantified using the Folin- Coicalteu method (Şengül *et al*., 2014). This was done by mixing 0.5 mL of the coating sample with 2.5 mL of 10% Folin-Coicalteu reagent dissolved in distilled water with 2.5 mL of 7.5% $NaHCO_3$. The absorbance was measured at 760 nm on a Beckman Coulter Spectrophotometer after 45 ^oC minutes of incubation in the dark. The analyses were done in triplicates. Standard solutions of gallic acid were used for the calibration curve. The mean of 3 readings was obtained, and the total phenol content was expressed in mg of gallic acid equivalents (GAE)/ mL of coating. This was calculated using the Equation 3.3.

$$TPC = DF\ (c\ V)/M \quad \text{(Equation3.3)}$$

Where;

C = concentration of gallic acid established from the calibration curve in mg/mL

V = the volume of the coating

M = the mass of the coating

DF= Dilution factor.

3.9.2 Total Flavonoid Content (TFC)

The TFC of modified edible coating was determined using aluminium chloride assay according to (Baba & Malik, 2015). The aluminium chloride colorimetric assay distinguishes flavonoids in the flavone and flavonol groups as in quercetin. In this study, a calibration curve prepared using quercetin as a standard was used to quantify the TFC of the modified coating sample. Linearity for the quercetin standard calibration curve was established from 10, 20, 40, 80 and 160 mg/mL. Approximately 0.25 grams of the coating samples was dissolved in 1.25 mL distilled water and 75 µL of 6% $NaNO_3$ added and the solution shaken to mix. It was then incubated in the dark oven at room temperature for approximately six minutes. A solution of 10% $AlCl_3$, was added and the mixture further incubated in a dark cabinet for 5 minutes. The absorbance was measured at 510 nm λ max using UV-Visible spectroscopy and calibration curve of the standard quercetin was established. Results were obtained in triplicates and total flavonoid content calculated using (Equation 3.4).

TFC = (c V)/M …… …….....(Equation 3.4)

3.10 Characterization of Modified and Unmodified Coating Samples

3.10.1 UV-Visible Spectroscopy

UV-Visible spectroscopy analysis for the modified and unmodified samples was performed by diluting one gram of the prepared samples with 10 mL of the extraction solvent. The absorbance spectra of both samples were scanned from 200-800 nm.

3.10.2 Fourier Transform Infrared Spectroscopy

FT-IR spectrometer was used to obtain the FT-IR spectra of samples. The functional groups of *O. gratissimum* EOs, modified and unmodified *M. esculenta* coating were analyzed using FT-IR at wave number of 4000-400 cm^{-1} (Sondari *et al*., 2018).

3.10.3 Gas Chromatography-Mass Spectrometry Analysis of *O. gratissimum* leaves EOs

Gas chromatography–mass spectrometry (GC–MS) analysis was carried out using Thermo Scientific TSQ 9000 coupled with a Thermo Scientific 1310 GC fitted with a TG-5 fused silica capillary column (30 m × 0.25 mm: 0.25-µm film thickness). The oven temperature range was programmed from 60 to 220 °C at 3 °C min^{-1}, and helium was used as carrier gas at 1.0 mL mins flow rate for analysis. The injector temperature was set at 230 °C, and the injection volume was 0.1 µL in *n*-hexane, with a split ratio of 1:50. Mass Spectra (MS) was taken at 70 eV with a mass range of *m*/*z* 50–530. Identification of constituents was done based on retention index (RI, determined with reference to homologous series of *n*-alkanes C8–C25 under identical experimental conditions on BP-1 column), National Institute Standards Technology (NIST) 2014 MS Library and Wiley Registry of Mass Spectral Data, 11^{th} Edition, Full Version (947,134 spectra, 707,501 compounds), and by comparing with the MS literature data from injection of commercial samples from VWR, UK (≥98% purity). Mass analysis was done in full scan mode within the ranges 50-500 m/z and the detected peaks matched against the NIST library for possible identification. Both Fragmentation patterns and retention index were used for matching.

3.11 Antioxidant and Antibacterial Activities of Modified Coating

3.11.1 Antioxidant Activity

The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay was carried out using the procedure described by (Alara *et al*., 2019). Briefly, 0.1M solution of DPPH was prepared by dissolving 0.004 g of DPPH crystalline solid in 100 mL of analytical grade methanol and stored at 4 °C. About 0.2 mL of DPPH solution was added to 2 mL of the coating sample. After 30 min of incubation in the dark, the absorbance at 517 nm using a UV-Vis Spectrophotometer was recorded. Methanol was used as the blank. The analysis was performed three times. The percentage of DPPH radical inhibition was determined using (Equation 3.5).

$$\text{Inhibition } (\%) = \frac{(\text{Ac}-\text{As})}{\text{Ac}} x\ 100 \quad \text{.....(Equation 3.5)}$$

Where;

As is the absorbance of the sample

Ac is the absorbance of the control.

3.11.2 Antibacterial Activity

Pure isolate of *E. coli* (ATCC 29522) and *S. aureus* (ATCC 25923) were obtained from the laboratory of Kenya Medical Research Institute (KEMRI) Nairobi, Kenya. The organisms were sub-cultured on nutrient agar in plates within 24 hours at 37 °C and thereafter the isolate was grown on nutrient agar slants and preserved in refrigerator at 4 °C during the study.

Agar disc diffusion method with some modifications was used to assess the antibacterial behavior of modified edible coating (Mada *et al*., 2013). The modified, unmodified coating suspension and *O. gratissimum* leaves EOs (0.5%, 1%, and 1.5%) were placed on Muller Hinton Agar plate media, which had been previously swapped with inoculums suspension. Commercially available ciprofloxacin discs were also used as positive control. Then the plates were incubated at 37 °C. Antibacterial activity was determined by measuring the diameter of the inhibition zone (IZ) around the discs using a 30 cm ruler. The assay was repeated three times and the antibacterial activity was expressed as the mean zone of inhibition diameters (mm).

3.12 Fruit Coating process

The fruit coating process was carried out following the procedure described by (Botelho *et al*., 2016). The selected fruits were randomly divided into six batches (180 fruits), 30 fruits per each batch to embrace the treatments: 2 Controls (C), 2 cassava *M. esculenta* starch (CS) treatments, and 2 *M. esculenta* starch treatments modified with 1.5% *O. gratissimum* oil (CS+EO) subsequently, the fruits of treatments CS and CS + EOs were immersed in the coating gels for 30 minutes; the excess was removed and, after drying for 10 min, the procedure was repeated in all batches, see (Appendix IV). The fruits were then air-dried on trays, keeping a minimum distance of 2cm between the fruits. After drying, 3 batches of fruits were stored at room temperature condition of (23 ± 2 °C), and 3 other batches at low temperature condition of (4 ± 2 °C), and evaluations of Physico-chemical parameters was performed/monitored at intervals of 3 days until spoilage. Figure 3.2 shows Prepared edible coating (A), dipping process (B), and coated mango fruits (C).

Figure 3.2. Prepared edible coating (A), dipping process (B), and the coated mango fruits (C).

3.13 Evaluation of -Chemical Properties of coated Mango Fruits

3.13.1 Preparation of Homogenous Solution of Mango (*Mangifera indica*) Fruits

This was done as per Daisy *et al*., (2020), three pieces of mangoes from each batch subjected to the control (uncoated), unmodified coating, and modified *M. esculenta* (cassava) treatments were randomly picked, peeled and blended into homogeneous solutions using an electric blender. The blended solutions were then analyzed for physico-chemical parameters including weight change, ascorbic acid, total soluble solids, titratable acidity, and pH changes, as affected by the various treatments. Initial determinations were done on day zero (0) and thereafter at three (3) days intervals until the mangos got spoilt.

3.13.2 Weight Loss

Three fruits from each batch were randomly marked and individually weighed before storage commenced. The fruits were subsequently weighed at each sampling interval date to track their weight changes for the entire experiment. Weight measurements was taken by weighing balance (Mettler-Toledo) and their averages determined in order to compute

the overall weight changes (Daisy *et al*., 2020). Percentage weight change/loss was calculated using (Equation 3.6).

$$(\%)\mathrm{Wl} = \frac{(\mathrm{wi}-\mathrm{wf})}{\mathrm{wi}} \times 100 \quad \text{(Equation 3.6)}$$

Where;

Wl was the weight loss (%)

Wi (g) was the initial weight

Wf (g) was the final weights.

3.13.3 Determination of Ascorbic Acid Content of Mango Fruits

Fifty (50) mL of the sample solution were pipetted into a 250 mL volumetric flask. Two (2) g of potassium iodide (KI) was added. Ten (10) mL of both 0.5 M H_2SO_4 and 0.01 M potassium iodate solutions was then added into the flask simultaneously. The solution was then titrated immediately with 0.07 M sodium thiosulphate until the entire color got almost pale yellow. Two (2) mL of starch indicator was then added and the titration process completed. The procedure was replicated twice for all the samples. The titer values were obtained, and their means calculated to get the amount of Vitamin C concentration in the samples (Dioha *et al*., 2012).

3.13.4 Determination of Total Soluble Solids (ºBrix)

This was determined by use of a digital bench refractometer (range 0–32%). Before taking readings, it was standardized with distilled water and adjusted to reading 0 ˚brix. Pulp tissue (10 g) was homogenized in 50 mL distilled water in the blender jug, and filtered with cotton wool. Then, two drops of filtrate were placed on the glass prism of the

refractometer and the reading was recorded. This was done on three replicated sample solutions and the refractometer was then calibrated afresh using distilled water prior to use for the next sample (Majidi *et al*., 2011).

3.13.5 Determination of Titratable Acidity (TA)

Ten (10) mL of the various prepared mango sample solutions were transferred into a 250 mL conical flask by a pipette. An equal amount of distilled water was added into the flask. Phenolphthalein indicator (3–4 drops) was then added and the solution stirred. The contents was rapidly titrated with 0.1 N NaOH solution and end point determined when there was a definite color change from colorless to pink (Majidi *et al*., 2011). The final burette reading was then noted, and the calculation of titratable acidity was done using Equation 3.7.

$$\% \text{ Acid} = \frac{(\text{mls NaOH used} \times 0.064)}{\text{grams of sampe}} \times 100 \quad \text{(Equation 3.7)}$$

3.13.6 Determination of pH

The pH of the sample solutions was measured using an electric pH meter (Sr. No 1630). Ten (10) mL of freshly prepared samples were placed in a beaker. The electrode end of the pH meter was used to agitate the solution until a stable reading was obtained. This was done on the six batch samples of the same solutions respectively. Between readings the electrode was rinsed with distilled water to eliminate cross-contamination (Majidi *et al*., 2011).

3.14 Data Analysis

GC-MS data files were analyzed with reference to NIST 14 library, web-based resources and published research articles. FT-IR data was analyzed using Origin pro software and

FT-IR Explorer. All experiments were done in triplicates and the data obtained from the experiments above were expressed as means ± standard deviations and subjected to statistical analysis using Microsoft excel (Microsoft corporation, USA) as shown in (Appendix VII). Means were analyzed for significance difference by one-way analysis of variance (ANOVA) at $P < 0.05$ with turkey post hoc test.

CHAPTER FOUR: RESULTS AND DISCUSSION

This chapter presents results on percentage yield of *O. gratissimum* leaves and *M. esculenta* tubers crude extracts, percentage yield of *O. gratissimum* leaves essential oils, phytochemical screening of *O. gratissimum* leaves and *M. esculenta* tubers; total phenolic content and total flavonoid content of modified *M. esculenta* coating; characterization of *O. gratissimum* EOs by FT-IR and GC-MS, characterization of unmodified and modified *M. esculenta* coating using UV-Vis and FT-IR. Results for antioxidant and antibacterial properties of the modified *M. esculenta* edible coating and evaluated physico-chemical parameters on mango fruits coated with unmodified and modified *M. esculenta* edible coating at low temperature (4 ± 2 ℃) and room temperature (23 ± 2 ℃) are also presented.

4.1 Percentage Yield of *O. gratissimum* leave and *M. esculenta* tubers of Crude Extracts

The percentage yield for *O. gratissimum* leaves and *M. esculenta* tubers crude extracts was determined and the results are tabulated as (Table 4.1).

Table 4.1: *Ocimum gratissimum* leaves and *M. esculenta* tubers percentage yields of Methanol, Ethyl acetate, acetone and hexane extracts

Plant used	**Hexane**	**Ethylacetate**	**Acetone**	**Methanol**
Ocimum gratissimum leaves	0.66 ± 0.02	1.52 ± 0.02	1.92 ± 0.01	2.76 ± 0.01
M. esculenta tubers	0.51 ± 0.03	1.34 ± 0.02	1.71 ± 0.01	2.52 ± 0.01

Results obtained demonstrated significant differences ($P<0.05$) in yield of crude extracts of methanol, acetone, ethyl acetate and hexane of *O. gratissimum* leaves and *M. esculenta* tubers as shown in (Table 4.1). These results are comparable with that of (Nassazi *et al*.,

2020). The author reported that, the methanol extracts exhibited the highest yield for *O. gratissimum* leaves 54.14 ± 0.01 and *R. officinalis* 27.66 ± 0.01 while n-hexane extracts showed the lowest (*O. gratissimum* 3.19 ± 0.03; *R. officinalis* 21.35 ± 0.02).

These results demonstrate that methanol is a good solvent for extraction of plant parts as compared to hexane, ethyl acetate and acetone probably indicating that most compounds in the leave parts of *O. gratissimum* plant and *M. esculenta* tubers extracted could be polar, hence were able to dissolve in the more polar methanol than other non-polar solvents (hexane) and mid-polar solvents (ethyl acetate and acetone) used.

Differences in solvent polarities used for extraction is known to play a key role in increasing the solubility of phytochemical constituents (Altemimi *et al*., 2017). Moreover, differences in the structure of phytochemical constituents similarly determine their solubility in solvents of different polarities (Ingle & Kapgatte, 2018). As a result, the current study's findings validated the impact of solvent extraction and on yield as well as the existence of polar and non-polar components in the extracts.

4.1.1 Percentage Yield of *O. gratissimum* Leaves Essential Oil

The hydro-distillation extraction of *O. gratissimum* leaves essential oil yielded pale yellow aromatic oil. The yield was obtained using (Equation 3.1).

$$\% \text{ yield } = \frac{1.6\text{g}}{200\text{g}} \text{ X } 100$$

The approximate yield of oil that was obtained for every run of 2 hours was 0.80 ± 0.03% w/w. Time affects extraction yield in that, the extraction is rapid at the beginning of experiment but get slow gradually by time. This is an indication that, when the plant material is exposed to the heat, the plant cells begins to disintegrate resulting to release and evaporation of volatile component in the oil (Nour *et al*., 2013).

4.2 Phytochemical Screening of *O. gratissimum* Leaves and *M. esculenta* Tubers

The Phytochemical screening of *O. gratissimum* leaves crude extracts revealed the presence of important phytogenic compounds such as alkaloids, flavonoids, terpenoids, steroids, glycosides, phenols and tannins. Alkaloids, terpenoids, steroids, glycosides and phenols were in abundance in methanol extracts while saponins were not detected all (Table 4.2).

Table 4.2: Phytochemicals screened for their presence in *O. gratissimum* leave extracts

Phytochemical	**Hexane**	**Ethyl acetate**	**Acetone**	**Methanol**
Alkaloids	+	+	+	+++
Flavonoids	+	+	+	++
Terpenoids	++	++	++	+++
Glycosides	+	+	+	+++
Steroids	++	++	+	+++
Phenols	++	++	+	+++
Saponins	-	-	-	-
Tannins	+	+	+	+

Key; +++ = abundant ++ =moderate += traces - = not detected

This result agreed with those of other researchers who indicated the presence of alkaloids, flavonoids, tannins, terpenoids, glycosides, saponins and steroids with aqueous, ethanolic, methanolic, chloroform, hexane crude extracts (Pandey, 2017; Airaodion *et a*l., 2019; Ae *et al*, 2018; Tella & Oseni, 2019). The only difference was the absence of saponins which could be attributed by difference in geographical region, plant physiological aspects and maturity (Mann, 2012). Additionally, environmental stressors

such as, microbial attack, UV radiation and nutrient deficiency may affect the synthesis of secondary metabolites (Kiazolu *et al*., 2016).

The phytochemical screening of *M. esculenta* tubers indicated presence of alkaloids, flavonoids, tannins, glycoside, steroids and phenols in hexane, ethyl acetate, acetone and methanoic crude extract. Alkaloids, flavonoids, steroids, glycosides and phenols were in abundance in methanol while terpenoids and saponins were not detected (Table 4.3).

Table 4.3: Phytochemicals screened for their presence in *Manihot esculenta* tubers crude extracts

Phytochemical	**Hexane**	**Ethyl acetate**	**Acetone**	**Methanol**
Alkaloid	++	+	+	+++
Flavonoids	+	++	+	+++
Terpenoids	-	-	-	-
Saponins	-	-	-	-
Steroids	+	+	+	+++
Glycosides	++	++	++	+++
phenols	+	+	+	+++
Tannins	+	++	+	+++

Key; +++ = abundant ++ = moderate += traces - = not detected

Similar work by Thiyagarajan, (2010) showed presence of alkaloids, tannins, saponins, flavonoids, glycosides and phenols in methanolic and aqueous crude extract. Previous reports indicated presence of this compounds in other parts of the plants such as the leaves (Quartey *et al*., 2016; Ehiobu *et al*.,2018).

Phytochemicals are non-nutritive plants bioactive compounds which are polar and non-polar in nature (Aparadh *et al*., 2014). This study showed that most compounds were dissolved in methanol solvent as shown in Table 4.2 and 4.3.

This finding indicates that methanol is a good solvent for extraction of plant parts as compared to hexane, ethyl acetate and acetone. Probably indicating that, most compounds in the leave parts of *O. gratissimum* plant and *M. esculenta* tubers extracted could be polar, hence were able to dissolve in the more polar methanol than other non-polar solvents (hexane) and mid-polar solvents (ethyl acetate and acetone) used. Differences in solvent polarities used for extraction is known to play a key role in increasing the solubility of phytochemical compounds (Altemimi *et al*., 2017).

4.2 Total Phenolic Content and Total Flavonoid Content of Modified *M. esculenta* Coating Results

4.2.1 Total Phenolic Content (TPC)

Results for the calculation of Total phenolic content are presented in (Figure 4.1). The correlation coefficient of the graph (R^2=0.9993) was obtained for the standard Gallic acid curve. The total phenol contents (TPC) in modified *M. esculenta* coating was found to be 18.106 ± 0.5 mg GAE/mL. Similar to that work, are findings of Dávila-Aviña *et al*., (2014) who studied the effects of edible coatings on bioactive compounds and antioxidant capacity of tomatoes at different maturity stages. They found the TPC range between 20.22, 18.22, and 18.74 (mg GAE/100 g), these results were consistence with the ones reported in this study. Additionally, study work by Oriani *et al*., (2014) reported the TPC range from 6.10 to 11.55 mg GAE/mL on cassava starch edible coating incorporated with

fennel and cinnamon essential oils. These results were slightly lower than the results recorded in this study.

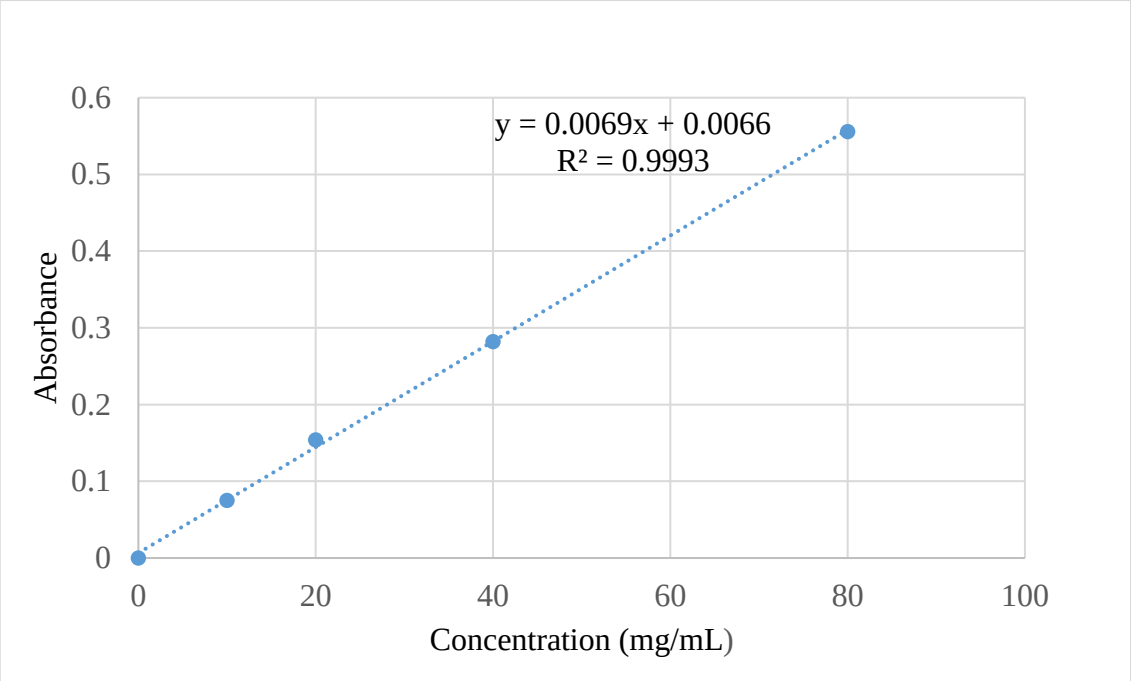


Figure 4.1. Calibration curve for gallic acid standard of the modified coating.

A different result from the above was reported by (Piñeros *et al*., 2017). Their TPC of cassava starch film blended with rosemary extracts was 5.04 ± 2.3 mg GAE/mL which was three times lower than the TPC reported in this study. The variations in these findings could be attributed to the differences in active composition in the formulation of edible coating, and the time of analysis (Doi & Enstit, 2019). Differences in ecological factors for instance, soil composition concentration, rainfall patterns, light intensities, ultraviolet radiation affects the concentration of phenolic constituents in plants (Nawaz *et al*., 2020). According to Chavan *et al*., (2013) variation in phenolic concentration could be attributed to severe climactic conditions, such as low humidity which leads to increase of total phenolic in plants. Moreover, the type of solvent used for extraction, methodology, type of plants used contributes greatly in dissolving phytochemical compounds (Doi & Enstit,

2019). As such, phenolic compounds contain hydroxyl groups which makes them to dissolve in more polar solvents (Sobiesiak, 2017).

4.2.2 Total Flavonoid Content (TFC)

Results for the calculation of Total Flavonoid content are presented in (Figure 4.2). The correlation coefficient of the graph (R^2=0.998) was obtained for the standard gallic acid curve.

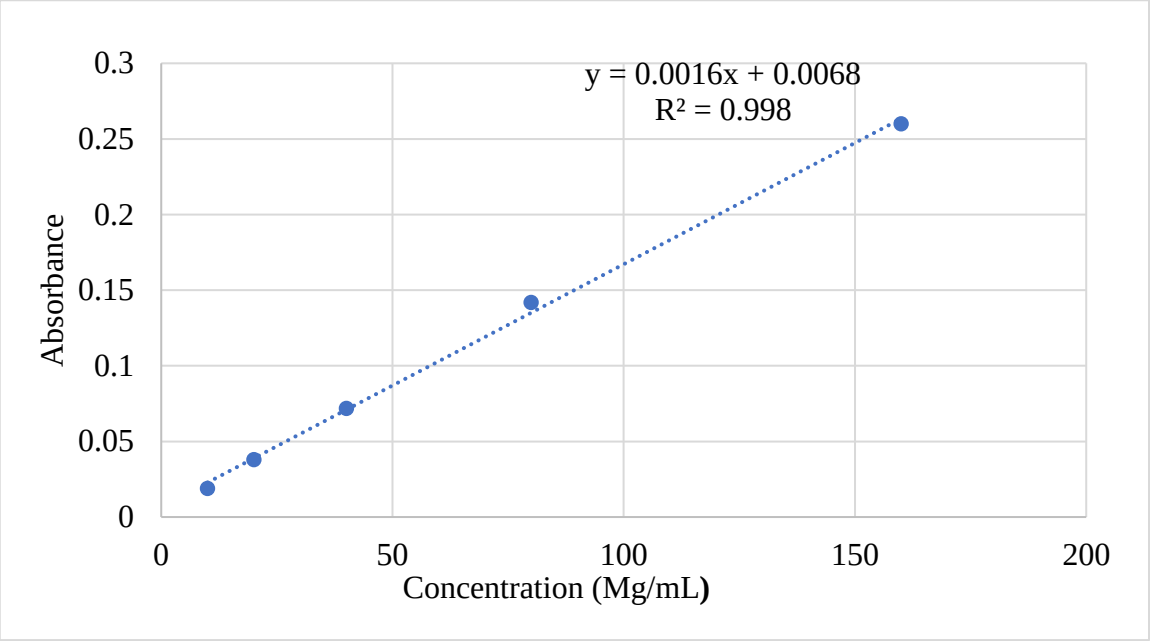


Figure 4.2. Quercetin calibration curve of the modified coating.

The total flavonoid content of the modified edible coating blended with essential oils was found to be 1.778 ±0.1 mg/g expressed in quercetin equivalents (QE) per gram of sample in dry weight (mg/g). Similar to that work, are findings of Dávila-Aviña *et al.*, (2014) who studied the effects of edible coatings on bioactive compounds and antioxidant capacity of tomatoes at different maturity stages. They found the TFC range between 0.0622, 0.0597, 0.0583 (mg RE/g) which was two times lower than the TFC reported in this study. The variations in these findings could be differences in formulation processes,

standards used and the time of analysis (Doi & Enstit, 2019). According to Sobiesiak, (2017) variation in soil condition such as lack of nutrients and enough water can lead to differences an amount of flavonoid present in plants. Also ecological factors including harsh or change on climactic conditions such as, rainfall patterns, ultraviolet radiations, drought, variation in temperature and humidity may affect presence of flavonoid contents in plants (Pramila *et al*., 2012).

4.3 Ultraviolet-Visible Spectra of the Modified and Unmodified coatings

The results of UV-Vis analysis for the modified and unmodified cassava starch coating film are shown in (Appendix VI). The UV-Vis spectra for modified and unmodified coatings showed absorbance in the spectral range from 300 to 600 nm. Simona *et al*., (2021) reported an absorbance in the spectral range from 356 to 600 nm, in the study based on carrageenan starch edible film enriched orange essential oils. Regarding on the high absorbance in the UV region, the coating films have excellent potential barrier properties against UV radiation (Shankar & Rhim, 2017).

The UV-Vis spectra of the coating film blended with 0.5%, 1% and 1.5% essential oil were shifted to higher absorbance values due to the impact of essential oil composition. On the other hand, the unmodified coating resulted to the lowest absorbance. A higher absorbance of the coating film with *O. gratissimum* essential oil can be as a result of the existence of lipid droplets in coting films that have a different refractive index, inducing light scattering meaning that there is a synergistic effect between coating film and the EOs (Zang *et al*., 2017).

Generally, analysis of packaging material requires low transmittance of UV radiation as much as it lengthens the lifespan of the packaged food. Also, the packaging film should

have high transparency in the visible region for consumer's visual regulatory foodstuff condition (Jancikova *et al*., 2020). Additionally, the UV protection of natural extracts such essential oils is due to the presence of active aromatic compounds (Christaki *et al*., 2012). These types of edible coating films combined with natural preservatives such as essential oils can be applied as a UV protector, since a lot of undesirable reactions occurring in foodstuffs are caused by UV radiation (Jancikova *et al*., 2020).

4.4 Fourier Transform Infrared Analysis of *O. gratissimum* EOs

FT-IR spectroscopy was used to identify the functional groups in *O. gratissimum* EOs, unmodified and modified coatings.

Table 4.4: Summary of the functional groups of *O. gratissimum* EOs by the FT-IR spectrum

Wave numbers (cm^{-1})	**Assignments**
3400 cm^{-1}	-OH stretch
2929 cm^{-1}	C-H stretch
1586 cm^{-1}	C=C stretch
1409 cm^{-1}	C-O-H bending
1150 cm^{-1}	C-O stretch
1076 cm^{-1}	C-O stretch vibration
1025 cm^{-1}	C-O stretch
745 cm^{-1}	=CH benzene ring
594 cm^{-1}	aromatic ring

Figures 4.3, 4.4, 4.5, 4.6, 4.7 and 4.8 show the FT-IR spectra for *O. gratissimum* essential oils, unmodified *M. esculenta*, 0.5%, 1% and 1.5% modified *M. esculenta* edible coating.

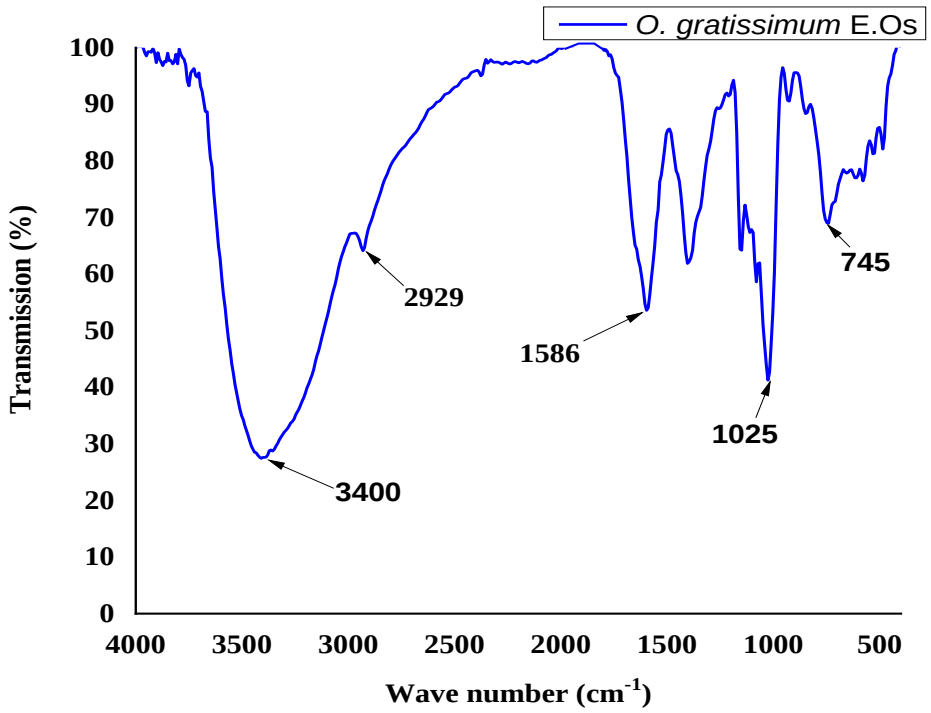


Figure 4.3. FT-IR spectrum of essential oil from the leaves *O. gratissimum*.

Figure 4.3 was used to identify the functional groups of the active components of *O. gratissimum* essential oil based on the peak value in the region of infrared radiation and finger print region. The intense absorption at 3400 cm^{-1} corresponds to the stretching of hydroxyl groups from phenolic compounds present in the extract (Jain *et al*., 2016). The peak at 2929 cm^{-1} is ascribed to aromatic (C-H stretching) bond. The absorption at 1586 cm^{-1} is attributed to the aromatic ring C=C skeleton vibration of an aromatic constituent.

The peak at 1409 cm^{-1} is characteristic for an alcohol C-O-H within the bending vibration absorption. The three peaks at 1150 cm^{-1}, 1076 cm^{-1} and 1025 cm^{-1} is assigned to C-O stretching vibrations from secondary and primary alcohols respectively. The peak at 748 cm^{-1} is assigned to benzene rings CH vibration absorption, and the absorption at 594 cm^{-1} represents the aromatic H out of plane bending (Shigwenya *et al*., 2012).

4.5. GC-MS Analysis of *O. gratissimum* essential oil

The hydro-distillation of O. *gratissimum* essential oil from the leaf extract yielded pale yellow aromatic oil. A Shimadzu QP 2010-SE GC-MS was used to analyze he pale-yellow EOs. The total run time was 44 minutes. Several compounds were detected, and their fragmentation patterns and retention indexes compared to NIST 2014, MS library. Table 4.5 shows the compounds identified in *O. gratissimum* leaves essential oils by GC-MS and (Figure 4.4) shows the GC chromatogram for *O. gratissimum* leaves essential oil.

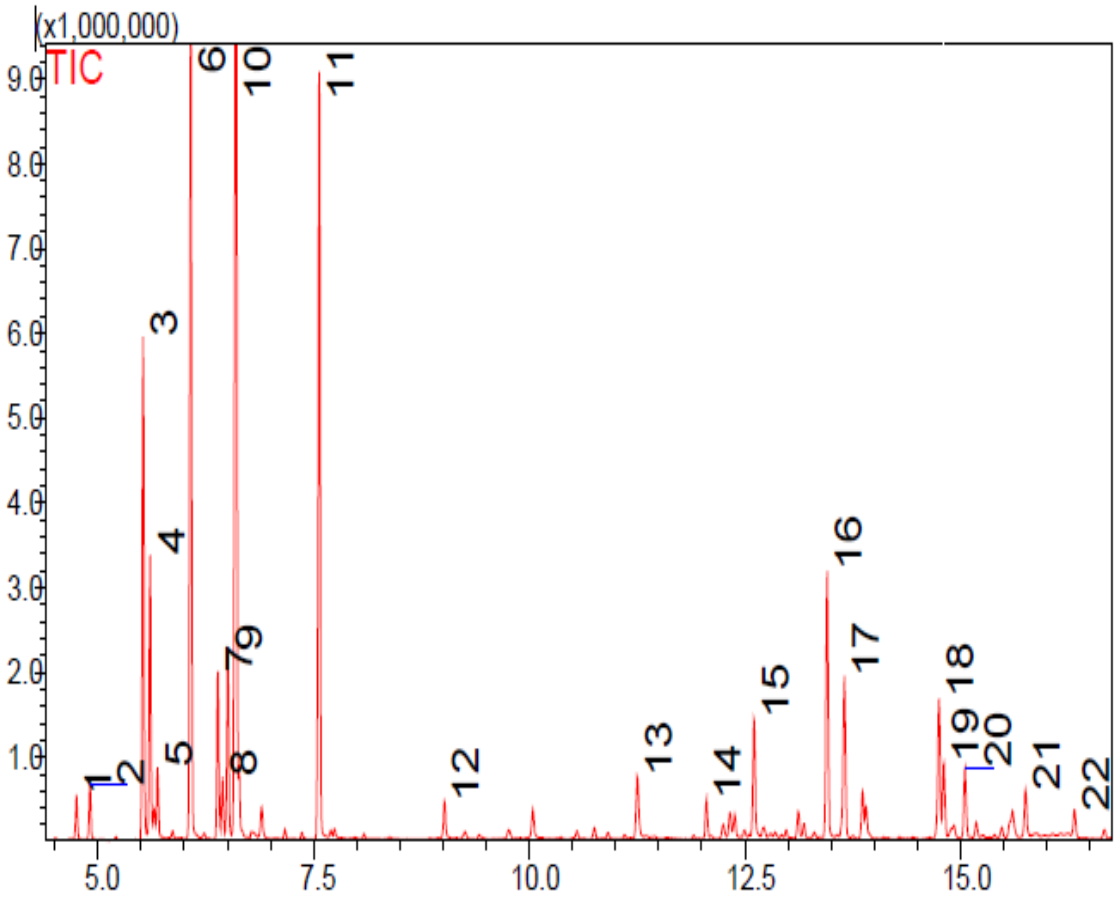


Figure 4.4. GC chromatogram for *O. gratissimum* leaves essential oil.

Table 4.5: Compounds identified in *O. gratissimum* leaves essential oils by GC-MS

Peak No.#	Compound	MF	RI	RT	MW	Area%
1	α-Thujene	$C_{10}H_{16}$	902	4.751	136	0.64
2	α-Pinene	$C_{10}H_{16}$	948	4.907	136	0.90
3	4(10)-Thujene	$C_{10}H_{16}$	897	5.524	136	9.14
4	1-Octen-3-ol	$C_8H_{16}O$	969	5.603	128	4.07
5	β.-Myrcene	$C_{10}H_{16}$	958	5.691	136	0.88
6	γ-Terpinene	$C_{10}H_{16}$	998	6.075	136	16.14
7	β.-Cymene	$C_{10}H_{14}$	1042	6.390	134	2.68
8	D-Limonene	$C_{10}H_{16}$	1018	6.444	136	0.69
9	β.-Phellandrene	$C_{10}H_{16}$	964	6.505	136	2.40
10	2-Octen-1-ol, (Z)	$C_8H_{16}O$	1067	6.600	128	26.66
11	1-Octen-3-yl-acetate	$C_{10}H_{18}O_2$	1109	7.566	170	15.00
12	cis-. β.-Terpineol	$C_{10}H_{18}O$	1137	9.018	154	0.60
13	Terpinen-4-ol	$C_{10}H_{18}O_2$	1276	11.253	170	1.32
14	β.-Bourbonene	$C_{15}H_{24}$	1339	12.055	204	0.65
15	Caryophyllene	$C_{15}H_{24}$	1494	12.606	204	2.24
16	Germacrene D	$C_{15}H_{24}$	1515	13.454	204	5.80
17	γ-Elemene	$C_{15}H_{24}$	1431	13.654	204	3.13
18	α- Bourbonene	$C_{15}H_{26}O$	1344	14.751	222	2.84
19	(-)-Spathulenol	$C_{15}H_{24}O$	1536	14.807	220	1.14
20	Epiglobulol	$C_{15}H_{26}O$	1530	15.052	222	1.44

21	α.-Cadinol	$C_{15}H_{26}O$	1580	15.754	222	0.97
22	Shyobunol	$C_{15}H_{26}O$	0	16.322	222	0.65
Monoterpene hydrocarbons						33.47%
Sesquiterpene hydrocarbons						9.88%
Oxygenated monoterpenes						47.65%
Oxygenated sesquiterpenes						7.03%
Total						97.97%

Key; RI= Retention index, RT= Retention time, MW= Molecular weight, MF= Molecular weight.

A total of twenty-two compounds were identified by the GC-MS analysis according to their mass spectra and their relative retention indices comprising 97.97% of the total oil constituents as shown in (Table 4.5). Each of the identified compounds and structures are shown in (Appendix VII). The detailed chromatogram and spectra, are attached in (Appendix VII). The major compounds were 2-octen-1-ol, (Z) (26.66%), α-thujene (16.14%), 1-octen-3-yl-acetate (15.00%), 4(10)-thujene (9.14%), germacrene D (5.80%), 1-Octen-3-ol (4.07%), γ-elemene (3.13%), α-bourbonene (2.84%), β.-cymene (2.68%), β.-phellandrene (2.40%), caryophyllene (2.24%), epiglobulol (1.44%), terpinen-4-ol (1.32%), and (-)-spathulenol (1.14%). Other compounds were present in trace amounts. The oil was found to be rich in oxygenated monoterpene (47.65%), followed by monoterpene hydrocarbon 33.74(%), sesquiterpene hydrocarbons (9.88%) and oxygenated sesquiterpenes (7.03%) as shown in (Figure 4.4) and (Table 4.5).

The compounds α-thujene, α-pinene, 4(10)-thujene, γ-terpinene, β.-myrcene and β.-cymene are natural organic compound classified as monoterpene hydrocarbons (Joshi, 2017). These compounds have been previously detected by GC-MS in aerial parts of *O. gratissimum* (Joshi, 2017), *Ocimum basilicum* L, *O. gratissimum* L. and *O. americanum* L (TA, 2017). D-limonene and β.-phellandrene are also monortepene hydrocarbons which have been previously identified in *Ocimum lamiifolium* and *Ocimum kilimandscharicum* (G *et al.*, 2019). These compounds have been previously reported to have strong antimicrobial and antioxidant activities (Joshi, 2017; Tangpao *et al*., 2018).

The compounds 2-octen-1-ol, (Z), 1-octen-3-ol, 1-octen-3-yl-acetate, cis-.β.-terpineol, and terpinen-4-ol are oxygenated monoterpenes which occur naturally in aromatic plants (Joshi, 2017). The constituents 1-octen-3-ol and 1-octen-3-yl-acetate have been previously identified from *Ocimum lamiifolium* species and have been reported to possess remarkable antimicrobial and antioxidant activities (G *et al*., 2019). Additionally, 1-octen-3-ol, was previously identified by GC-MS in *Ocimum forskolei* and reported as a mosquito repellent (Dekker *et al*., 2011). Cis-. β.-terpineol and terpinen-4-ol has been previously identified from *O. gratissimum* aerial parts and flowering parts and reported to have antimicrobial, antioxidant and anti-flammatory properties (Dung *et al*., 2021). They have also been reported in other *ocimum* species for instance *Ocimum basilicum* L, and *Ocimum americanum* (TA, 2017).

The compounds caryophyllene, germacrene D, β.-bourbonene and γ-elemene belong to a class of sesquiterpene hydrocarbons (John Mary *et al.*, 2020). These compounds have been previously identified in *O. gratissimum* aerial parts and flowers (Saliu, 2011; Joshi, 2017; Silva *et al*., 2016). They have also been reported in other *ocimum* species such as *Ocimum lamiifolium, Ocimum kilimandscharicum, Ocimum americanum, Ocimum kenyense* and

ocimum basillum (TA, 2017; G *et al*., 2019). These compounds have also been reported to have strong antimicrobial and antioxidant properties. Caryophyllene and its derivative oxide have severe woody aroma and are applied in food manufacturing (Sarpietro *et al*., 2015). Most of the essential oil constituents reported in this study, have been reported elsewhere as food flavors which are being utilized by Food and Drug Administration (FDA) and by the European Safety Authority (EFSA) due to their low water solubility and low toxicity (Al-Hashimi *et al*., 2020). Essential oils are a complex mixture of constituents and the antimicrobial and antioxidant properties relies on the active principle of synergism and antagonism among compounds (Petretto *et al*., 2013).

4.5.1 Correlation Between FT-IR and GC-MS Analysis of *O. gratissimum* leaves EOs

The compounds identified in this analysis were the complex mixtures of class monoterpenes and sesquiterpenoids. This was confirmed by the Fourier Transform Infrared Spectroscopy (FT-IR) analysis of EOs, which confirmed presence of functional groups such as –OH stretch which could correspond to aliphatic alcohols such as; 2-octen-1-ol (Z) (26.6%), 1-octen-3-yl-acetate (15.00%) and 1-octen-3-ol (4.07%). Moreover, the Monoterpene alcohols such as; terpinen-4-ol (1.32%) and cis-. β.-terpineol (0.60%) (Moghaddam & Mehdizadeh, 2017). The C=C stretch could correspond to monoterpene hydrocarbon (alkenes) such as; γ-terpinene (16.14%), 4(10)-thujene (9.14%), β.-phellandrene (2.40%), α-pinene (0.90%), β.-myrcene (0.88%), D-limonene (0.69%) and α-thujene (0.64%). A benzene ring =CH that was observed at this region 745.24 cm^{1} and an aromatic ring around 594.79 cm^{-1} could correspond to β.-cymene (Tisserand & Young, 2013). Figure 4.5 shows structures of some of the compounds identified by the GC-MS.

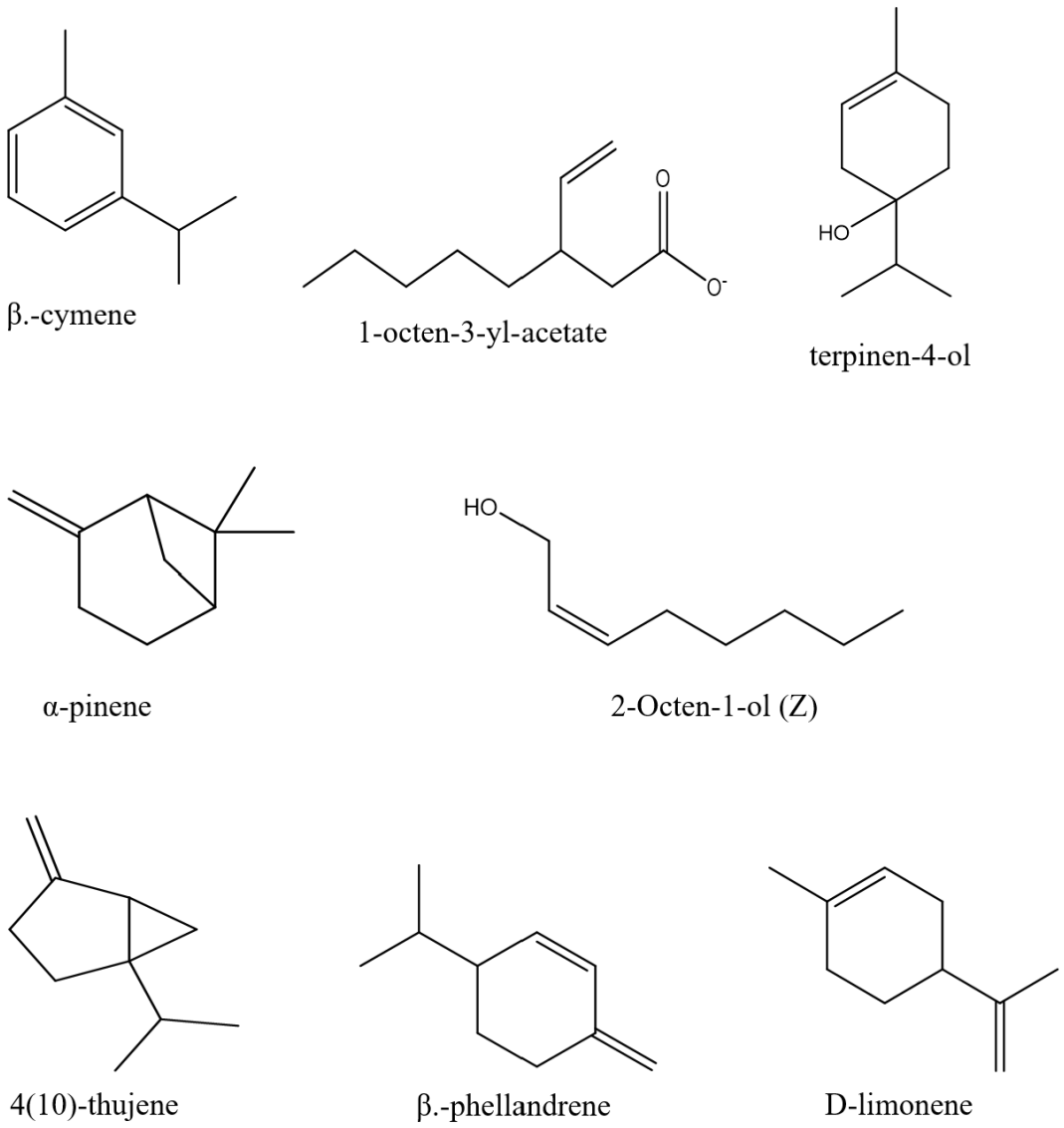


Figure 4.5. Structures of some of the compounds identified by GC-MS from *O. gratissimum* leaves EOs.

4.6 Fourier Transform Infrared Analysis of Unmodified *M. esculenta* Coating

Table 4.6: Summary of the functional groups of unmodified (*M. esculenta*) coating by the FT-IR spectrum

Wave numbers (cm^{-1})	**Assignments**
3300-3379	-OH stretch
2937	C-H
1608	C=O
1372	C-O-H
1112	C-O

The spectrum of the unmodified *M. esulenta* (CS) coating film showed characteristic bands comprising of starch and glycerol as the plasticizer (Figure 4.6).

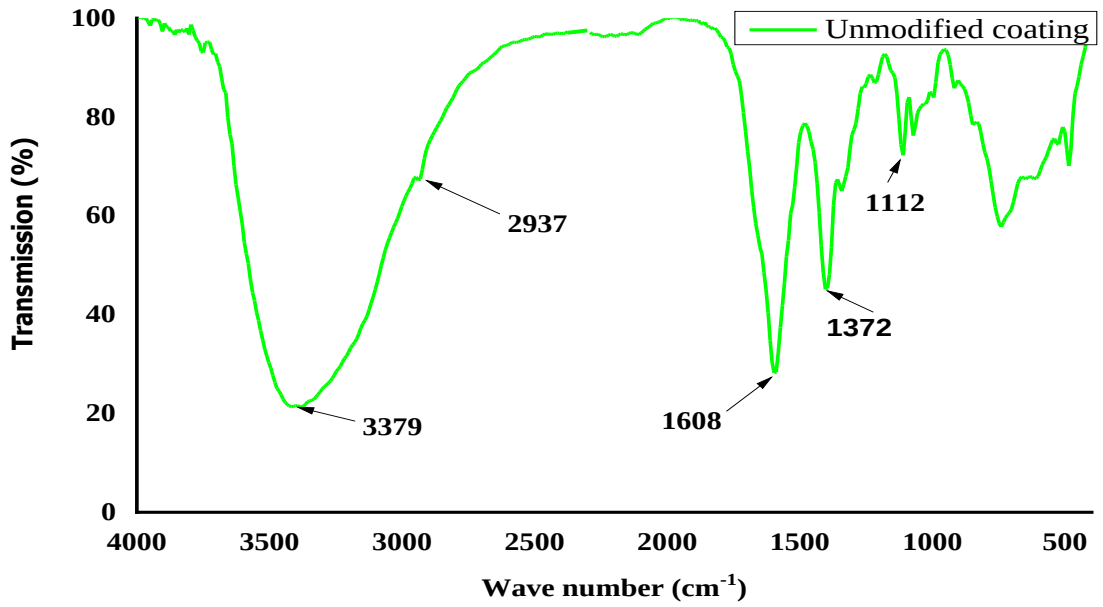


Figure 4.6. FT-IR spectrum of unmodified *M. esculenta* (CS) coating.

The broad absorption and centered in the range of 3300 -3379 cm^{-1} is as a result of O-H stretching which is found in both the starch coating components as well in glycerol plasticizer. The concentration of glycerol as plasticizer affects the water content value of cassava coating, the higher the concentration of glycerol, the higher the water content value. The concentration of glycerol makes the adsorption of water molecules larger because glycerol is hydrophilic. Glycerol is also able to hold water in the film matrix and form hydrogen bonds (O-H) (Cerqueira *et al*., 2012).

The peak at region 2937 cm^{-1} could be attributed to the symmetric C-H bond stretching of aliphatic compounds. The sharp absorption peak at 1608 cm^{-1} is attributed to C=O stretching, the peaks at 1372 and 1112 cm^{-1} are due to C-O-H bonding vibrations and C–O stretching respectively, this are vibrations of glycoside bonds associated to ordered structure in the starch branch, which are constituted of internal long chains of amylose

and amylopectin (Khatoon *et al*., 2009). All the spectra below 800 cm^{-1} shows complex vibrational modes as a result of the skeletal mode of vibrations of the pyranose ring in the glucose unit (Ayu *et al*., 2018).

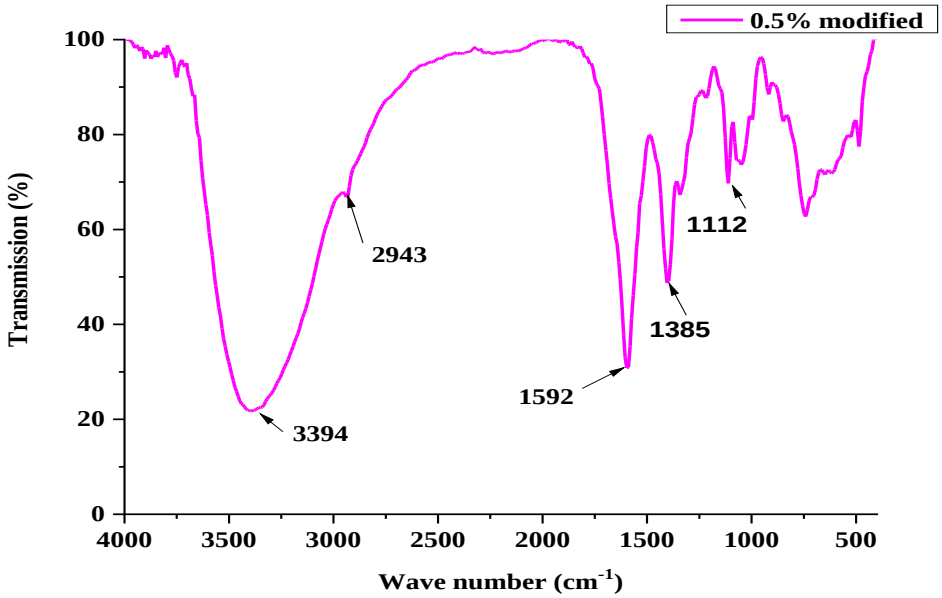


Figure 4.7. FT-IR spectrum of *M. esculenta* (CS) modified with 0.5% of *O. gratissimum* EOs.

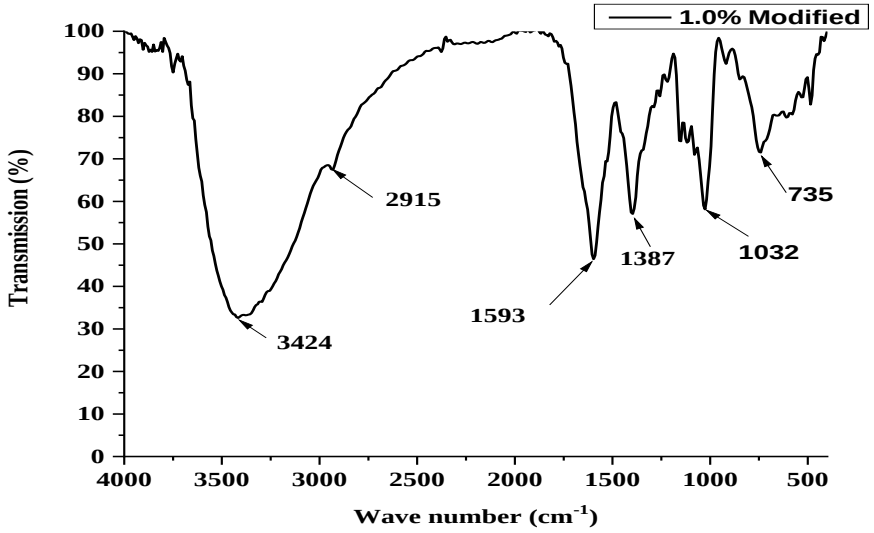


Figure 4.8. FT-IR spectrum of *M. esculenta* (CS) modified with 1% of *O. gratissimum* EOs.

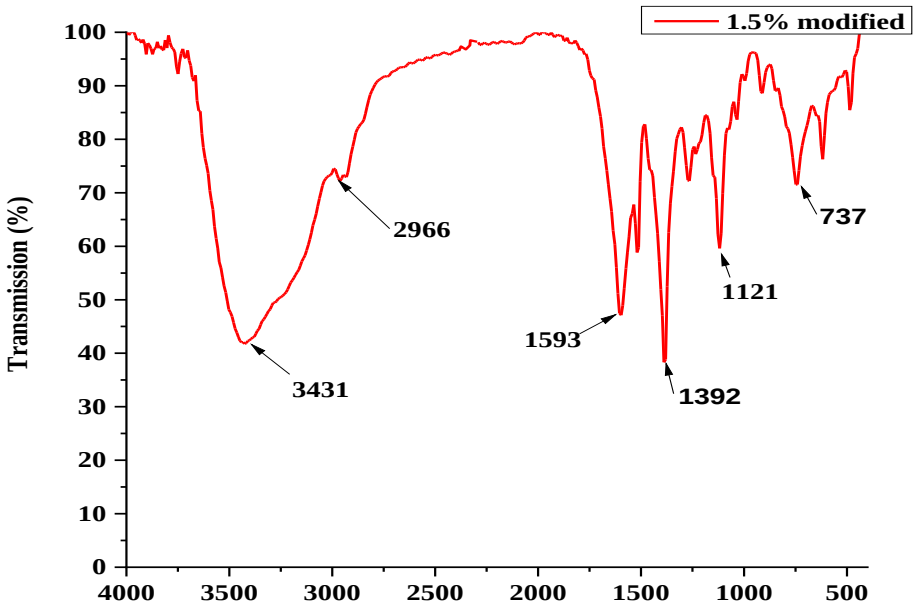


Figure 4.9. FT-IR spectrum of *M. esculenta* (CS) modified with 1.5% of *O. gratissimum* EOs**.**

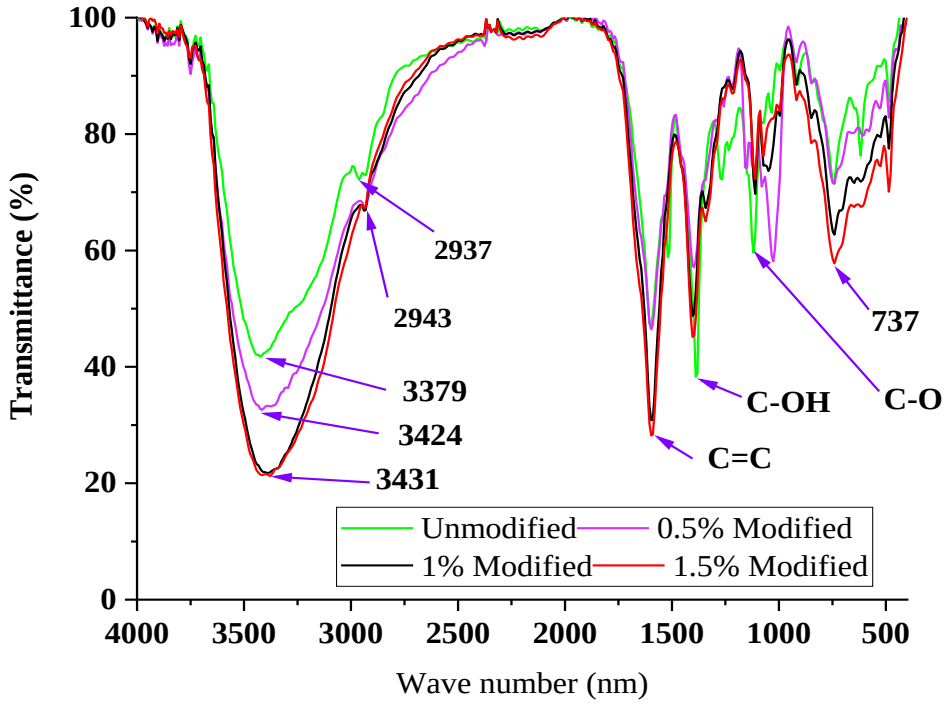


Figure 4.10. FT-IR spectra showing shift in absorbance of unmodified and modified coatings.

The modification of *M. esculenta* coating with 0.5%, 1% and 1.5% of *O. gratissimum* essential oil resulted in the shifting of peak absorbances. The unmodified *M. esculenta* coating showed a broad absorption band in the range of 3300-3379 cm^{-1} which corresponds to O-H stretching (Figure 4.6). By incorporating cassava starch with 0.5%, 1% and 1.5% *O. gratissimum* essential oil, the O-H band of the coating films shifted to 3394 cm^{-1}, 3424 cm^{-1} and 3431 cm^{-1} respectively. The shifting of the peaks at these regions indicates that, the hydrogen bonding between molecules was partially destroyed (Syafiq *et al*., 2020). Also, the differences in absorption peaks for the coating incorporated with *O. gratissimum* EOs demonstrates that the molecular structure was altered (Liu *et al*., 2020). This implies that the modified coating with 1.5% EOs is much better compared to the ones modified with 1% and 0.5% (Figure 4.10).

The peak of C-H stretching of unmodified coating film that appeared at 2937 cm^{-1} shifted to 2915 cm^{-1}, 2943 cm^{-1} and 2966 cm^{-1} respectively, see Figures 4.7, 4.8, and 4.9. The water absorption peak of unmodified cassava starch at 1608 cm^{-1} shifted to C=C aromatic ring at peak regions of 1592 cm^{-1}, 1593 cm^{-1} and 1593 cm^{-1} respectively, upon addition of increasing concentration of essential oil. The C-OH bending of unmodified cassava starch coating film that appeared at 1372 cm^{-1} was shifted to 1385 cm^{-1}, 1387 cm^{-1} and 1392 cm^{1} respectively. The modified cassava starch composite showed an increase in intensity as the concentration of essential oil was increased forming sharper peaks than the unmodified cassava starch. The shifting of the peaks from lower to higher wavenumbers was largely contributed by different conformations of molecular structures induced by the addition of *O. gratissimum* EOs (Ilyas *et al*., 2018).

The C-O stretch of the unmodified cassava starch that appeared at 1112 cm^{-1} remained unaffected 0.5% and 1.0 % essential oil but got shifted with 1.5% modification. The 1.5%

modified cassava starch exhibited increased intensities which resulted to sharper peaks, besides that more absorption peaks were observed at 1527 cm^{-1} corresponding to aromatic ring C=C skeleton vibration of an aromatic compounds from essential oils, 1261 and 1121 cm^{-1} attributed to C-O stretching vibrations (Figure 4.9). The combination of starch coating and essential oil resulted to physical blends against chemical reactions which caused shifting in the spectral peaks (Syafiq *et al*., 2020).

Other minor peaks were observed in the fingerprint region that is 900 cm^{-1} below corresponding to complex vibrational modes as a result of the skeletal mode of vibrations of the of the pyranose ring in the glucose unit (Ayu *et al*., 2018). These results indicated that, the increase in concentration of essential oils in the coating film resulted to different bands shift. Jaiswal *et al*., (2021) reported that this impact is as a result of altered intermolecular interaction among essential oils and coating film matrix activated by the increase in concentration of essential oils. These findings also corresponded with those of (Tongdeesoontorn *et al*., 2011) on the effects of different constituents blended with cassava starch.

4.7 Antioxidant and Antibacterial Activity of Modified *M. esculenta* Edible Coating

4.7.1 Antioxidant Activity

The DPPH scavenging assay was carried out to elucidate the antioxidant activity of the modified *M. esculenta* edible coating of different concentrations as shown in (Figure 4.11). The DPPH % inhibition of this study indicated 24.73 ± 1.2%, 39.74 ± 0.40% and 51.72 ± 0.8% for coating sample blended with 0.5%, 1% and 1.5% EOs respectively. As the concentration of essential oil increased, the DPPH scavenging activity of the modified coating was significantly increased. These results were comparable ($P < 0.05$) to that of positive control ascorbic acid which showed DPPH % inhibition of 27.55 ± 0.70%, 42.73 ± 0.46% and 57.01 ± 0.44% for the abovementioned concentrations.

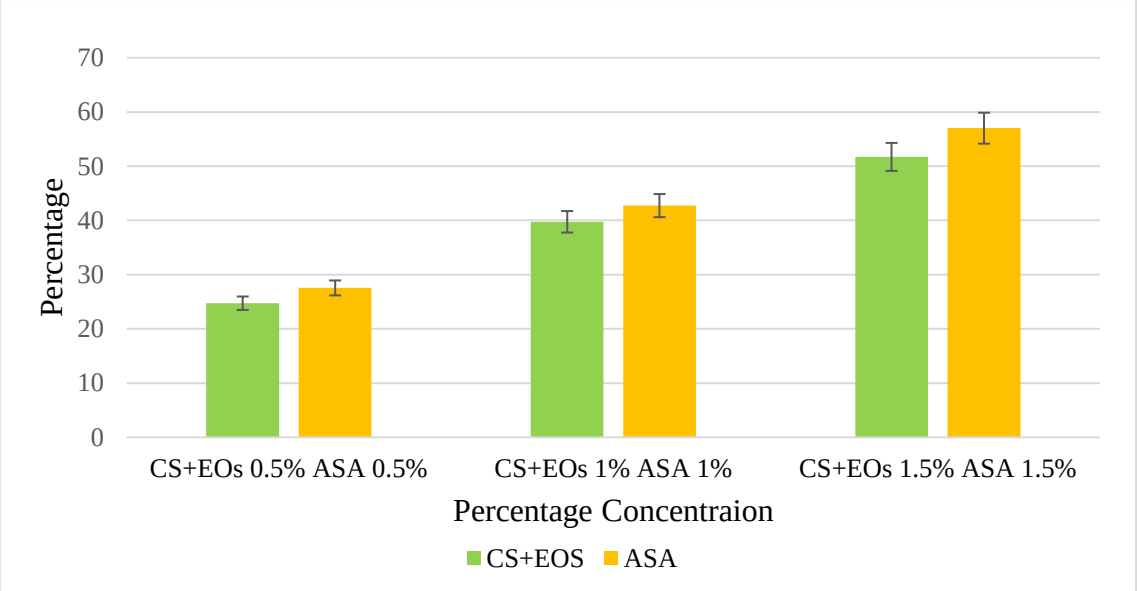


Figure 4.11. Antioxidant activity percentage inhibition of modified coating.

Key; CS= cassava starch, EOs= essential oils, ASA= ascorbic acid

The findings in this study were in agreement with Piñeros *et al.*, (2017) who reported an increasing trend of DPPH inhibition activity of 28.6 ± 0.3, 54.4± 1.6 and 81.9 ± 1.7 % for cassava starch edible films carrying rosemary extracts of increasing concentrations

(TPS-RE5%, TPS-RE10% or TPS-RE20%). Similarly, Al-Hashimi *et al*., (2020) indicated an increase of antioxidant activity which ranged from 36.57 to 85.96% of millet starch film encapsulated with clove essential oil of an increasing activity of (1%, 2% and 3%). Several other researchers such (Dávila-Aviña *et al*., 2014; Ruiz *et al*., 2013; Chiumarelli *et al*., 2014) reported the increasing activity of edible films and coating enriched with essential oils in increasing concentration.

Generally, food deterioration occurs as a result of oxidation process which affects foodstuffs during harvesting, transportation, processing and storage. Lipid oxidation causes perishability in fresh produces as a result of rapid discoloration, change in rancid flavor, odor and texture.

The use of edible coating with essential oils is utilized to maintain the quality of fresh produce and protect them against oxidation processes (Wang *et al*., 2019). Moreover, essential oils are commonly utilized in formulation of edible coatings and films because they are rich in antioxidants compounds. The antioxidant property of essential oils can be determined by their capability to act as oxygen scavengers and allow the diffusion of active agents into coated food products (Fernández-López & Viuda-Martos, 2018). This effect could be attributed to the presence of active phytochemical compounds contained in essential oils blended with the edible coating.

The GC-MS analysis identified some of the compounds such as; β.-cymene, D-limonene, caryophyllene, γ-terpinene, β.-phellandrene among others. Most of these compounds are phenolic or aromatic which have been previously reported to have antioxidant activities. A study by Miguel, (2010) reported that phenolic compounds are responsible for antioxidant activity in essential oils. These was supported by (de Oliveira *et al*., 2012)

who revealed that phenolic compounds are receptors for free radicals by breaking chain oxidation reactions which can be an indicator of the antioxidant activity of essential oils.

4.7.2 Antibacterial Activity

The *M. esculenta* (cassava starch) edible coating containing increasing concentrations of *O. gratissimum* oil (0.5%, 1% and 1.5%) showed promising results of antibacterial activity against two standard bacteria strains which are widely studied for food contamination, Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*. The antibacterial results are shown in (Table 4.7) and (Figure 4.12).

The *M. esculenta* modified with 0.5%, 1%, and 1.5% exhibited inhibitory zones of 10.35 ± 0.30mm, 12.37 ± 0.32mm and 17.53 ± 0.03mm against *E. coli* and 12.30 ± 0.32mm, 16.76 ± 0.08mm and 22.22 ± 0.28mm against *S. aureus* respectively. *Ocimum gratissimum* EOs showed inhibitory zones of 9.32 ± 0.08mm, 11.27 ± 0.25mm, 17.53mm against *E. coli* and 9.40 ± 0.36mm, 15.43 ± 0.40mm and 19.20 ± 0.17mm against *S. aureus* respectively for the abovementioned concentrations. The results of the modified coatings were significantly comparable ($P < 0.05$) to ciprofloxacin (positive control), which showed inhibition zones of 20.21 ± 0.04mm, 21.54 ± 0.10mm and 24.74mm against *E. coli* and 20.49 ± 0.02mm, 23.42 ± 0.03mm and 26.46 ± 0.03mm against *S. aureus* for the abovementioned concentrations. The negative control that is unmodified edible coating *M. esculenta* (CS) coating showed no effect against the tested microorganisms as shown in (Table 4.7).

Table 4.7: Antibacterial activity of modified coating and *O. gratissimum* EOs

		E. coli	***S. aureus***
CS (2%w/v) +	EOs (% v/v)	**Inhibitory zone (*mm*)**	**Inhibitory zone (*mm*)**
	0.5	10.35 ± 0.30	12 .30± 0.35
	1.0	12.37 ± 0.32	16.76 ± 0.08
	1.5	17.53 ± 0.03	22.22 ± 0.28
Essential oils (EOs)			
	0.5	9.32 ± 0.08	9.40 ± 0.36
	1.0	11.27 ± 0.25	15.43 ± 0.40
	1.5	17.53 ± 0.03	19.20 ± 0.17
(Ciprofloxacin)(+ve) control			
	0.5	20.21 ± 0.04	20.49 ± 0.02
	1.0	21.54 ± 0.10	23.42 ± 0.03
	1.5	24.74 ± 0.04	26.46 ± 0.03
CS (negative control)		0.00 ± 0.00	0.00 ± 0.00

This results corresponded with those of literature work by Iamareerat *et al*., (2018) who reinforced cassava starch edible film with cinnamon essential oil in an increasing concentration of 1.5%, 2% and 2.5% against *E.coli* and *S. aureus*. The modified coating showed inhibition zones of 8.33 ± 0.29mm, 12.17 ± 0.29mm and 13.00 ± 1.32mm against *E. coli* and 10.00 ± 1.00mm, 11.17 ± 0.29mm and 11.67 ± 1.15mm against *S. aureus* for the aforementioned concentration respectively. Another study on millet starch modified

with 1%, 2% and 3% clove oil by; Al-Hashimi *et al*., (2020) indicated inhibition concentration of 16 ± 0.13mm, 18 ± 0.10mm and 23 ± 1.00mm against *S. aureus* and 10 ± 0.18mm, 11 ± 0.52mm and 18.95mm against *E.coli* respectively. The inhibitory effect of the modified edible coating increased significantly with the increase of essential oil concentrations (Chiumarelli *et al*.,2014).

Comparing the two strains, the positive strain (*S. aureus*) showed sensitivity slightly higher than negative strain (*E. coli*) towards the modified coating and the essential oil (Figure 4.12).

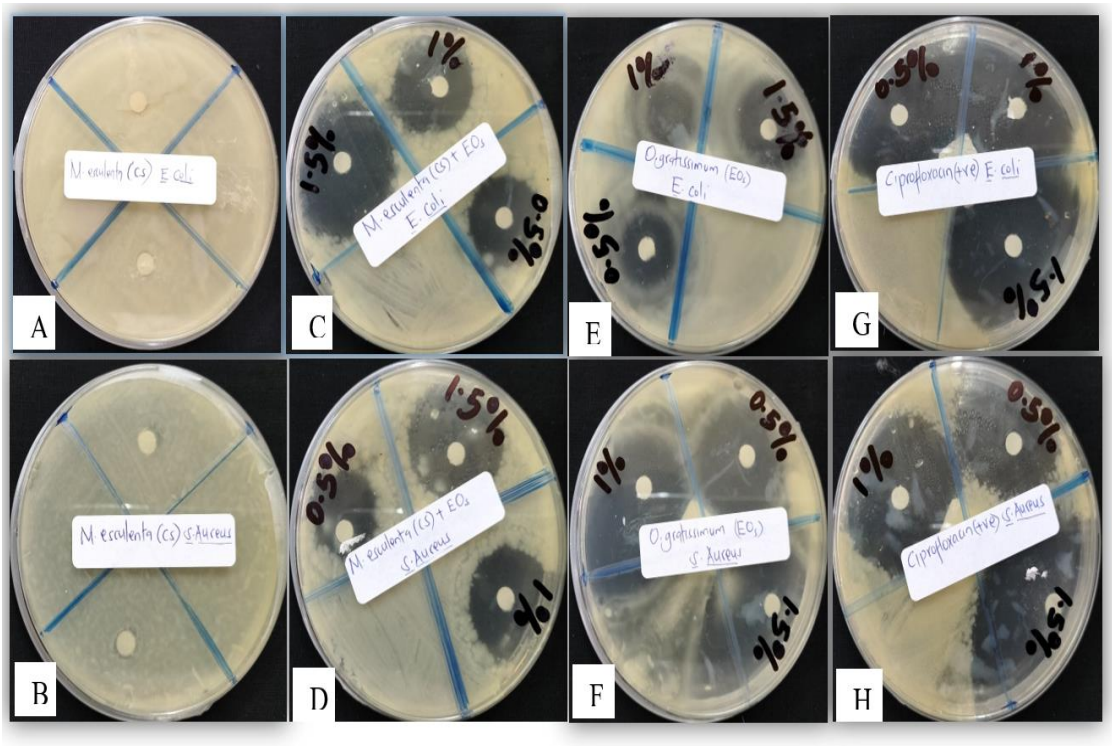


Figure 4.12. Inhibition zones for (A)-*M. esculenta* (*E. coli*), (B)-*M. esculenta* (*S. aureus*), (C)- *M. esculenta* + EOs (*E. coli*), (D)- *M. esculenta* + Eos (*S. aureus*), (E)- *O. gratissimum* EOs (*E. coli*), (F)- *O. gratissimum* EOs (*S. aureus*), (G)- Ciprofloxacin (*E. coli*) and (H)- Ciprofloxacin (*S. aureus*).

This demonstrates that Gram-positive bacteria are more sensitive to EOs or the modified edible coating in contrast with Gram-negative ones (Nazzaro *et al*., 2013). Moreover,

some factors such as the type of microorganism, and structure of the cell wall affect the antimicrobial property of EOs (Pietrysiak *et al*., 2019). The gram -positive bacteria cell wall is made up of thick layer peptidoglycans, proteins and teichoic acid. Due to the lipophilic property of major constituents of EOs such as, 2-octen-1-ol, (Z), eugenol, thujene among others they can easily penetrate through the cell wall. Conversely, gram-negative bacteria have a more complex monolayer peptidoglycan entangled by an outer layer of lipopolysaccharides (LPS) and proteins. This charged hydrophilic membrane limits the diffusion hydrophobic constituents through the LPS (Vergis *et al*., 2015).

These finding demonstrates that starch-based edible coating incorporated with different essential oils have inhibitory efficacy against both positive and negative bacteria. According to Ju *et al*., (2019) the blend of polysaccharide coating and EOs produces a conjugate which has strong antibacterial and antioxidant impact, which is better able to preserve the original quality and prolong the shelf life of food. Additionally, edible coatings have high potential for carrying active constituents, for instance; anti-browning, antimicrobial antioxidant and colorants agents. Essential oil-edible coatings are favorable to the utilization of EOs in various food by increasing their distribution in the food area where pathogens grow and proliferate (Donsì & Ferrari, 2016).

Moreover, the inhibitory activity of *O. gratissimum* EOs is as a result of presence of several compounds such α-thujene, germacrene D and β- caryophyllene among others. The GC-MS analysis identified presence of such compounds such as; 2-octen-1-ol, (Z) (26.66%), α-thujene (16.14%), 1-octen-3-yl-acetate (15.00%), 4(10)-thujene (9.14%) and germacrene D (5.80%) as shown in (Table 4.6). These compounds have been previously reported to possess strong antimicrobial activities (Petretto *et al*., 2013).

These components can change protein structure and the phospholipids of cell membranes by affecting their permeability (Devi *et al*., 2010). The hydrophobic criteria of essential oils interact with the lipid structure, for instance gram-negative bacteria cell membrane, mitochondria, and most intracellular component, which results to disparaging the cell structure, leaking, ion exchange, breathing inhibition, and finally causing cell death (Atanasova-Pancevska *et al*., 2017). In many cases these activities are due to the synergistic effects of the several combined active phyto-chemical compounds (Petretto *et al*., 2013).

4.8 Effect of *M. esculenta* Edible Coating and Storage Period on the Physico Chemical Parameters of Mango Fruits

4.8.1 Weight Loss

The *M. esculenta* (cassava starch) coating treatments, significantly ($P < 0.05$) affected weight loss of mango fruits subjected to room temperature (23 ± 2 °C) and low temperature (4 ± 2 °C) conditions. In this study, weight loss of all fruit samples increased progressively with the advancement of storage period. The control (T_0) fruits at room temperature condition demonstrated higher weight loss compared to the coated fruits, unmodified (T_1) and modified (T_2), see (Figure 4.13). The mango fruits stored at low temperature (4 ± 2 °C) condition, the control (L_0), unmodified coating (L_1) and modified coating (L_2) showed weight loss which was relatively lower than those of room condition (23 ± 2 °C) as shown in (Figure 4.14).

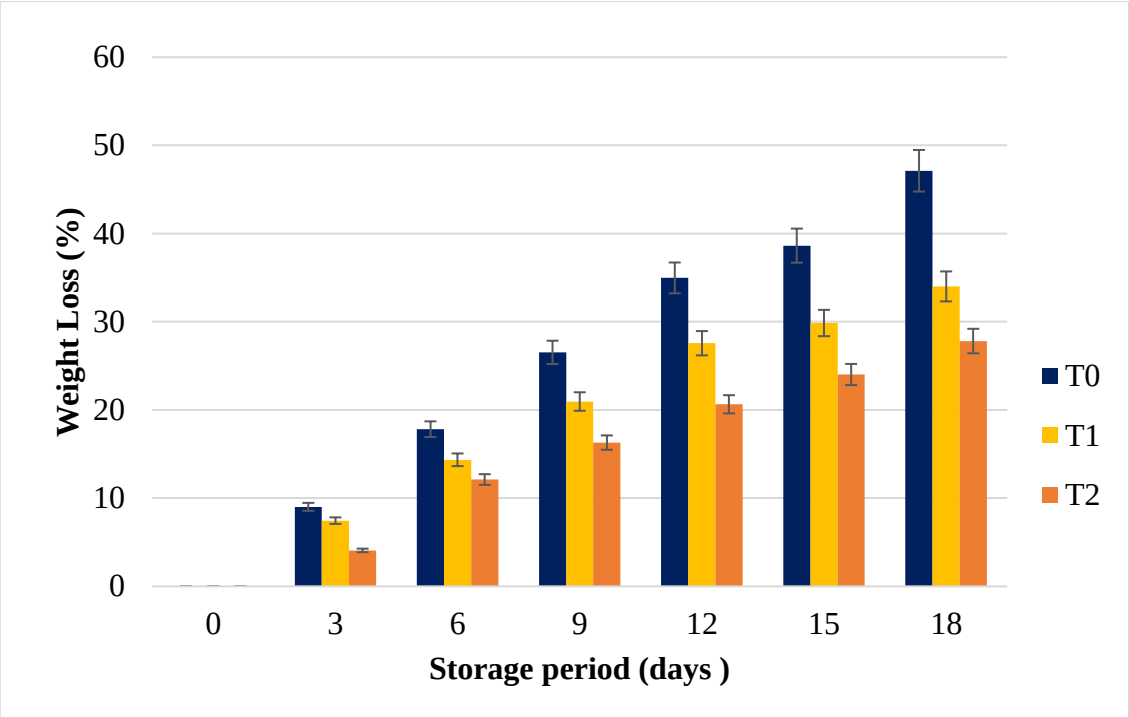


Figure 4.13. Effect of different *M. esculenta* (CS) coating treatments on the weight loss of mango fruits during storage at room temperature (23 ± 2 °C).

On the 9th day of storage, control (T_0), unmodified (T_1) and modified (T_2) recorded 26.53 ± 0.02 %, 20.97 ± 0.02% and 16.30 ± 0.02% weight loss respectively with a subsequent increase up to 18th days of storage, see (Appendix V). On the 18th day of storage control (T_0), unmodified (T_1) and modified (T_2) recorded 47.12 ± 0.02%, 34.01 ± 0.01% and 27.81 ± 0.02 % respectively. Similarly, the control (L_0), unmodified (L_1) and modified (L_2) respectively recorded 11.72 ± 0.02%, 8.05 ± 0.02% and 4.93 ±0.01% weight loss and the trend increased steadily up to the 27th day of storage. On the 27th day control (L_0), unmodified (L_1) and modified (L_2) respectively recorded 24.21 ± 0.02%, 19.82 ± 0.02% and 14.70 ± 0.01% as shown in (Figure 4.14).

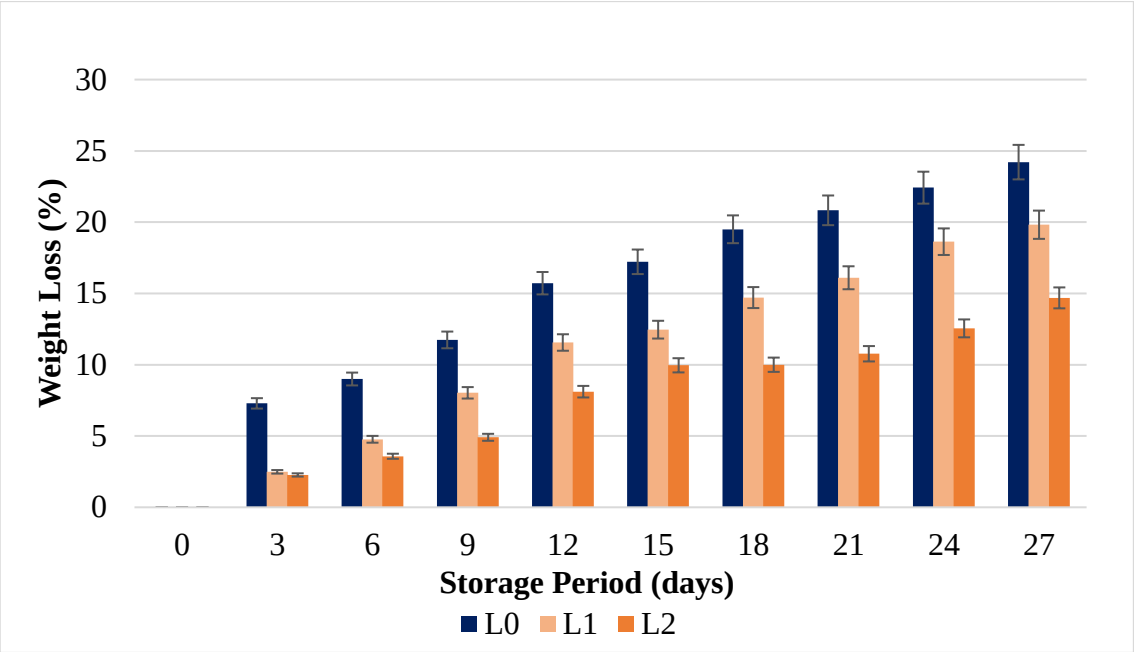


Figure 4.14. Effect of different *M. esculenta* (CS) coating treatments on the weight loss of mango fruits during storage at low temperature (4 ± 2 °C).

These results were comparable with literature work by Mandal *et al*., (2018), who reported an increasing weight loss from 0 day to a maximum of 12 days. The weight loss for the control fruits indicated a maximum of 26.41% weight loss on the 12th day, compared to mangoes coated with olive 17.95% and chitosan 1% 7.53%. Another study by Daisy *et al*., (2020) reported that, weight loss increased with the advancement of storage period and reached the maximum on the 10th day for the control and the 15th day for the treatments, after which the mangoes were spoilt, respectively. Also, the study work by Ebrahimi & Rastegar, (2020), on guar-based edible coatings blended with *Spirulina platensis* and *Aloe vera* extract on mango recorded a highest 14.33% weight loss at the end of storage period compared to 6.8% the coated sample.

Manihot esculenta edible coating significantly affected weight loss hence the shelf life of the coated mangoes was prolonged compared to the control. The progressive loss of water through evaporation from the fruit surface occurred as a result of normal metabolic

processes such transpiration owing to the water vapor pressure difference between the atmosphere and the fruit surface which leads in shrinking and deterioration of the fruits (Khaliq *et al*., 2015). However, the percentage weight loss was more intense in the control fruits compared to the coated fruits. This is as a result of higher moisture loss and increased respiration through uninterrupted atmospheric column and lower relative humidity compared to the coated ones (Guerra *et al*., 2015).

The reduced weight loss among coated fruits occurred as a result of the creation of a semi-permeable barrier which blocks the stem end scar from losing moisture, oxygen, and carbon dioxide subsequently reducing respiration and transpiration processes (Ali *et al*., 2010). Moreover, the modification of edible coating with essential oils increased their functionality by creating a more complex matrix with antioxidant, and antibacterial properties (Ambarsari *et al*., 2018).

4.8.2 Total Soluble Solids (TSS)

The *M. esculenta* (cassava) starch coating treatment significantly ($P < 0.05$) influenced the pulp TSS of mango fruits subjected to room temperature (23 ± 2 °C) and low temperature (4 ± 2 °C) conditions. The amount of the pulp TSS increased in all fruit samples during the 18^{th} and 27^{th} days of storage at room temperature (23 ± 2 °C) and at low temperature (4 ± 2 °C) respectively. The TSS for the control (T_0) increased drastically from initial day 5.81 ± 0.03% and was highest on the 6^{th} day of storage 20.20 ± 0.02% after which it dropped steadily from the 9^{th} day 18.63 ± 0.02% up to 18^{th} day 17.32 ± 0.01%.

The unmodified (T_1) treatment recorded 5.76 ± 0.01%, 7.19 ± 0.02%, 9.92 ± 0.01%, 12.22 0.03% 15.53 ± 0.02%, 19.21 ± 0.01% and 18.61 ± 0.02% from the initial day, 3^{rd}, 6^{th},9^{th},

12th, 15th, and the 18th day respectively. The unmodified treatment increasingly produced TSS from the initial day up to the maximum of 15th day and then dropped afterward up to 18th day. The modified (T_2) recorded 5.73 ± 0.02%, 6.60 ± 0.01%, 7.50 ± 0.01%, 9.12 ± 0.02%, 10.63 ± 0.02%, 13.67 ± 0.01% and 17.31 ± 0.02% from the initial day, 3rd, 6th, 9th, 12th, 15th and the 18th day respectively. The modified (T_2) mango fruits showed an increasing trend of TSS but in slower motion compared to unmodified (T_1) and modified (T_0) as shown in (Figure 4.15).

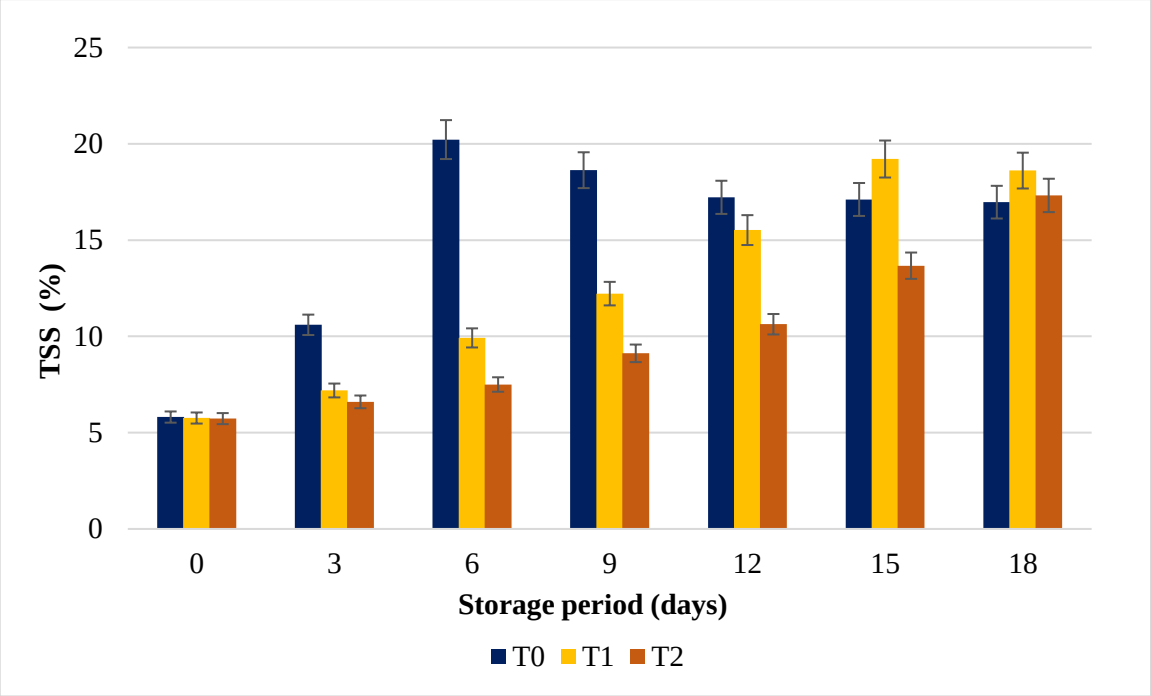


Figure 4.15. Effect of different *M. esculenta* (CS) coating treatments on the pulp TSS of mangoes during storage at room temperature (23 ± 2 °C).

The pulp TSS for the low temperature (4 ± 2 °C) fruits, the control (L_0) recorded 5.65 ± 0.01%, 7.06 ± 0.02%, 9.15 ± 0.02%, 14.21 ± 0.02% and 17.41 ± 0.01% from the initial day, 6th, 15th, 24th and the 27th day respectively. The unmodified treatment (L_1) recorded 5.60 ± 0.02%, 6.67 ± 0.02%, 8.23 ± 0.02%, 13.60 ± 0.01% and 16.24 ± 0.01% respectively for the aforementioned days. The modified (L_2) recorded 5.63 ± 0.01%, 6.13

± 0.02%, 8.15 ± 0.01%, 12.30 ± 0.01% and 14.62 ± 0.01% respectively for the aforementioned days as shown in (Figure 4.16).

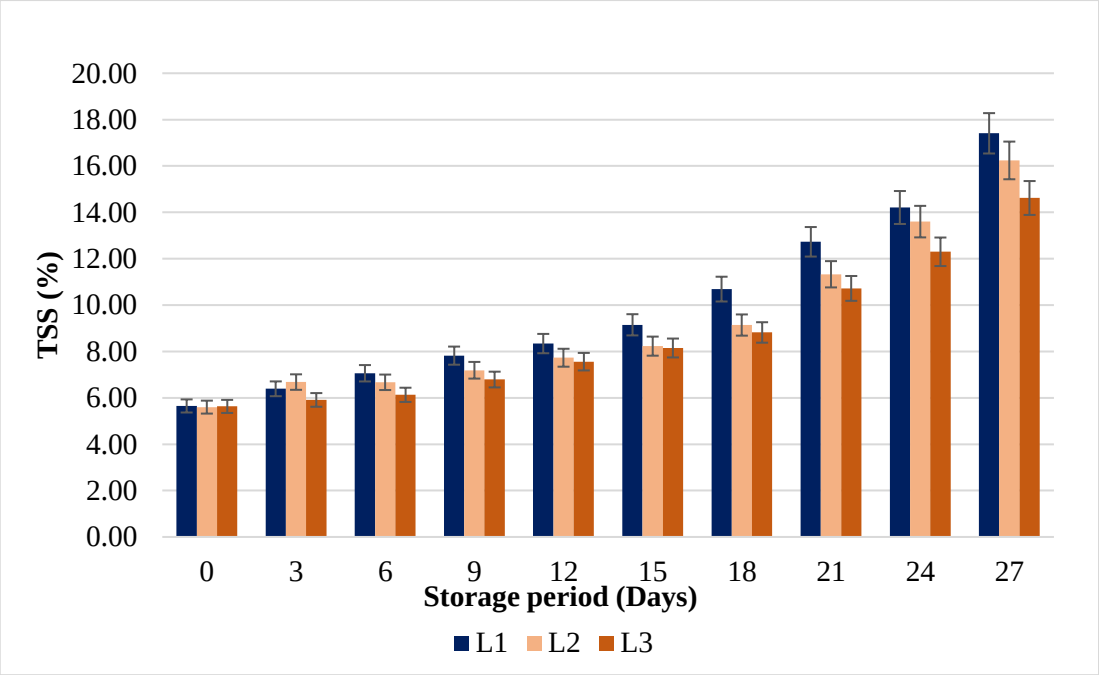


Figure 4.16. Effect of different *M. esculenta* (CS) coating treatments on the pulp TSS of mangoes during storage at low temperature (4 ± 2 °C).

The pulp TSS for the low temperature (4 ± 2 °C) fruits, the control (L_0), unmodified (L_1), and modified (L_2) samples increased gradually but much more delayed compared to those of room temperature conditions (23 ± 2 °C). This findings were comparable with literature work by Md Khairul *et al*., (2013), who reported increasing pulp TSS for the control mango fruits of 5.82%, 12.13%, 20.22%, 18.75% and 17.25% from the initial day, 3rd, 6th, 9th, and the 12th respectively. The coated mango fruits indicated 5.74%, 8.74%, 13.21%, 17.72% and 20.22% for the aforementioned respectively.

Total soluble solid is an important parameter which determines the sweetness of a particular fruit (Hosseini *et al*., 2019). The increase of the pulp TSS content throughout the storage period corresponded with the progress of the ripening process, in which the

highest total soluble solids content being those of control, followed by those of unmodified and modified fruit samples. The increasing trend of pulp TSS in the control could be attributed to the of loss of water from the fruit and the ripening process that results in the hydrolysis of complex carbohydrates into simple sugars and glucose (Thompson, 2008), see (Equation 4.1).

$$nC_6H_{10}O_5 + nH_2O \longrightarrow nC_6H_{12}O_6 \quad \text{..... Equation 4.1}$$

The low temperature (4 ± 2 °C) mango fruits presented a slow motion towards the increase of TSS as compared to room temperature mangoes (23 ± 2 °C), but much slower in the modified samples (L_2). Low temperature (4 ± 2 °C) suppressed respiration rates which resulted in lower soluble solid concentration and a minimized conversion of carbohydrates to simple sugars (Khaliq *et al*., 2015). Modified *M. esulenta* edible coating created a semipermeable barrier around the fruit and modified the internal atmosphere by reducing oxygen and increasing carbon dioxide production (Bartolomeu *et al*., 2012).

4.8.3 Titratable Acidity (TA)

Titratable acidity of the mango pulp was significantly ($P < 0.05$) affected by the *M. esculenta* edible coating and the storage duration. In this study, titratable acidity of all fruit samples decreased as the storage period progressed. The decreasing trend for control (T_0) was rapid from the initial day to the 6th day and thereafter declined steadily to the 18th day of storage. For the room temperature (23 ± 2 °C) fruits, the control (T_0) recorded a decreasing trend of 3.53 ± 0.02%, 0.74 ± 0.01%, 0.47 ± 0.01%, 0.23 ± 0.01%, 0.18 ± 0.01%, 0.15 ± 0.01% and 0.11 ± 0.02% from the initial day, 3rd, 6th,9th, 12th, 15th, and the 18th day respectively. The unmodified (T_1), 3.64 ± 0.01%,1.94 ± 0.02%, 0.82 ± 0.01%, 0.51 ± 0.01%, 0.40 ± 0.01%, 0.37 ± 0.01% and 0.31 ± 0.02% for the aforementioned days

respectively. The modified (T_2) indicated a similar trend of 3.66 ± 0.02%, 2.60 ± 0.01%, 1.43 ± 0.01%, 0.94 ± 0.02%, 0.83 ± 0.02%, 0.71 ± 0.01% and 0.66 ± 0.02% for the abovementioned days respectively. The decreasing trend of the modified (T_2) fruit samples was much lower compared to unmodified (T_1) and the control (T_0) as shown in (Figure 4.17).

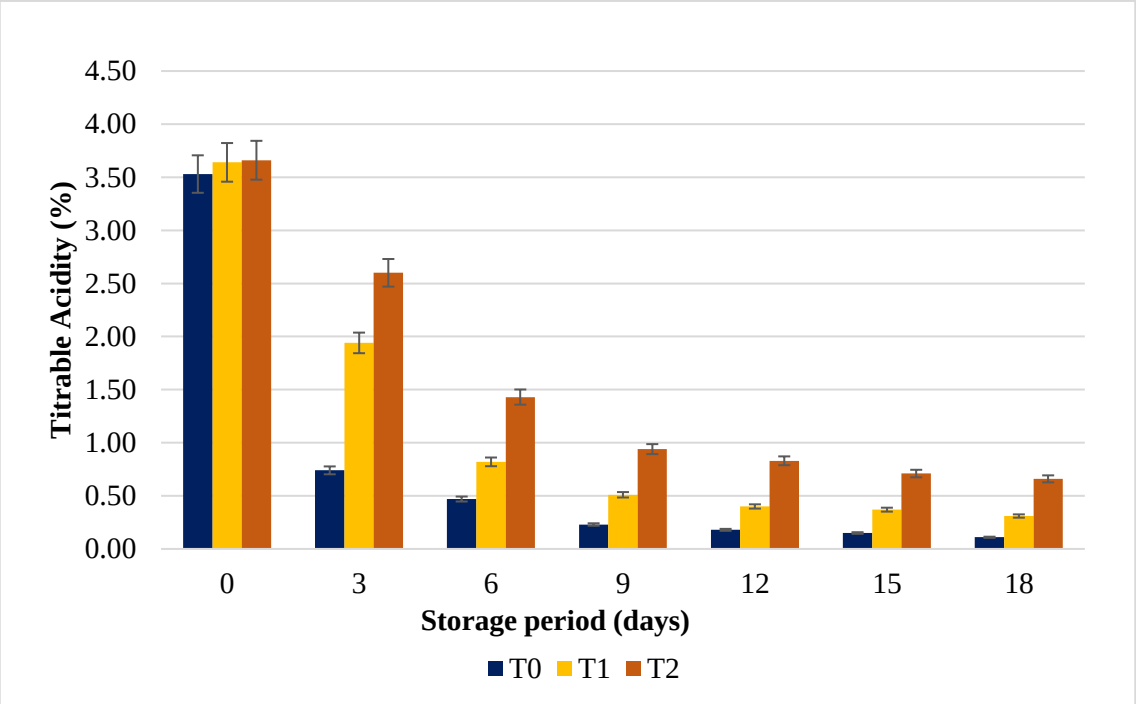


Figure 4.17. Effect of different *M. esculenta* (CS) coating treatments on the pulp TA of mangoes during storage at room temperature (23 ± 2 °C).

The low temperature (4 ± 2 °C) fruits indicated abated trend which was much slower compared to that of at room temperature (23 ± 2 °C) conditions. The control (L_0) fruits recorded TA of 3.58 ± 0.01%, 1.54 ± 0.01%, 0.52 ± 0.02%, 0.33 ± 0.01%, 0.30 ± 0.01% and 0.23 ± 0.02% from initial day, 6^{th}, 15^{th}, 18^{th}, 24^{th} and the 27^{th} day respectively. Unmodified (L_1) recorded 3.70 ± 0.02%, 1.63 ± 0.02%, 0.71 ± 0.02%, 0.54 ± 0.02% and 0.49 ± 0.01% respective days. The modified (L_2) fruits recorded 3.65 ± 0.01%, 1.79 ± 0.01%, 1.30 ± 0.01%, 1.18 ± 0.01%, 1.01 ± 0.01% and 0.87 ± 0.02% for the

aforementioned days respectively. The modified (L_2) fruits retained the highest amount of TA compared to unmodified (L_1) and the control (L_0) fruits shown in (Figure 4.18).

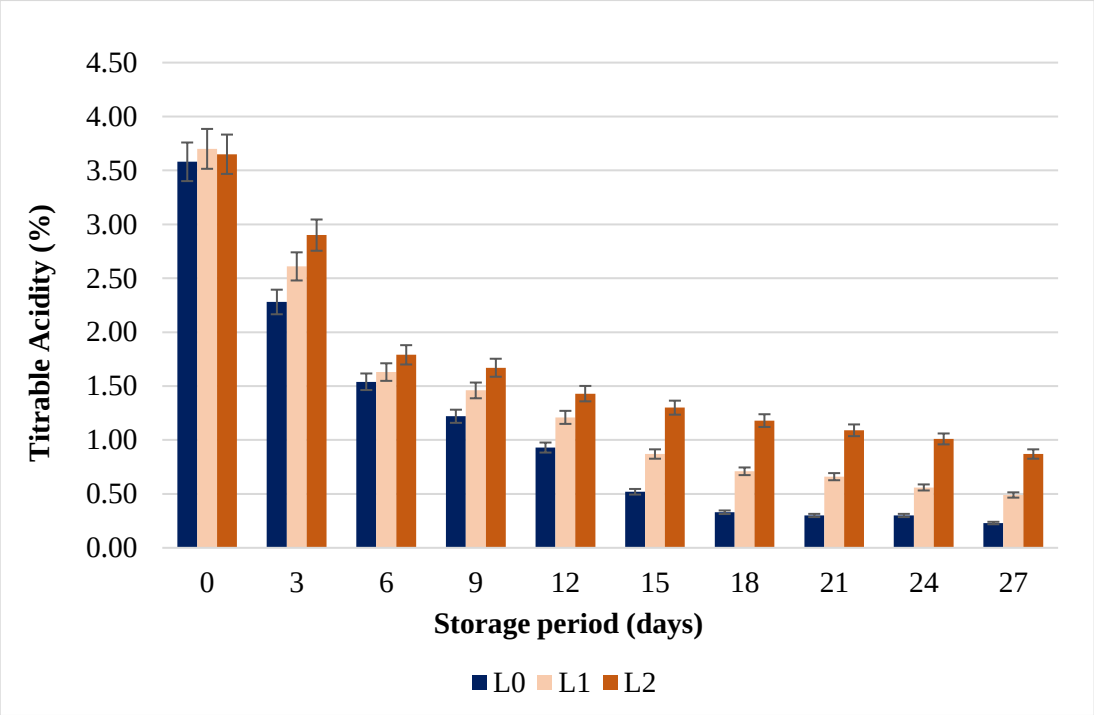


Figure 4.18. Effect of different *M. esculenta* (CS) coating treatments on the pulp TA of mangoes during storage at low temperature (4 ± 2 °C).

This result corresponded to literature survey of mango fruits by (Islam *et al.*, 2016). The author reported a decreasing trend of TA as 4.25%, 1.50%, 0.60%, 0.35% and 0.18% from the initial day, 3rd, 6th, 9th and 12th day respectively for the control fruits. The fruits coated with paraffin coating recorded 4.30%, 2.45%, 1,20%, 0.80% and 0.65% for the respective days. Similarly, the low temperature coated fruits showed the highest amount TA at the end of storage period. They indicated TA amount of 4.42%, 2.83%, 1.60%, 1.35% and 1,10% for above-mentioned days respectively.

Titratable acidity (TA) is an important factor which determines maturity and the taste quality of the fruit (Khorram *et al.*, 2017). The control (L_0) showed a greater decline of titratable acidity and this might be attributed by increased respiration rates that resulted

into disintegration of organic acids that were used up for enzymatic reactions (Khaliq *et al*., 2015). Also, the decreased amount of TA in the control fruits could be attributed to the increase in ethylene production and respiration rate during the advent of ripening process (Oz & Ulukanli, 2012). In this study, mango fruits treated with Modified coating showed higher retention of TA, which indicated that the treatment might have preserved the organic acids by inhibiting oxidation and respiration rates (Chiumarelli *et al*., 2010).

4.8.4 pH Changes

The *M. esculenta* (cassava starch) edible coating and storage time significantly ($P < 0.05$) impacted the pulp pH of the mango fruits subjected to room temperature (23 ± 2 °C) and low temperature (4 ± 2 °C) conditions. The pulp pH of mango fruits was increased during storage period, but it was greater in the control samples (T_0, L_0), as compared to the coated samples. The control(T_0) fruits for the room temperature (23 ± 2 °C) condition, recorded a rapid increasing trend of 3.62 ± 0.02, 4.63 ± 0.03, 5.79 ± 0.01, 6.60 ± 0.02, 7.19 ± 0.02, 7.51 ± 0.01 and 8.21 ± 0.02 from the initial day, 3rd, 6th,9th, 12th, 15th and the 18th day of storage respectively. The unmodified (T_1) recorded a trend of 3.61 ± 0.02, 3.91 ±0.01, 4.22 ± 0.02, 4.6 ±0.02, 5.39 ± 0.01, 5.92 ±0.02 and 6.30 ±0.01 for the abovementioned days respectively. The modified (T_2) fruit samples recorded a pulp pH of 3.64 ± 0.01, 3.73 ± 0.03, 3.81 ± 0.01, 4.01 ± 0.02, 4.40 ± 0.01, 4.80 ± 0.01 and 5.20 ± 0.02 for the respective days as shown in (Figure 4.19).

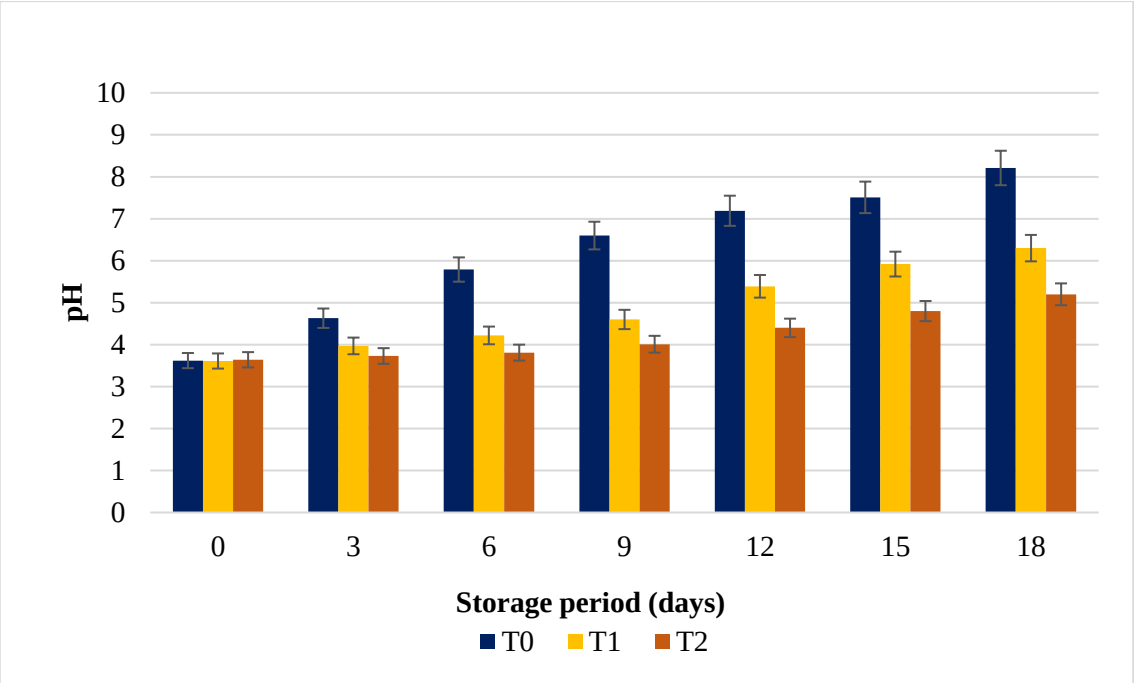


Figure 4.19. Effect of different *M. esculenta* (CS) coating treatments on the pulp pH of mangoes during storage at room temperature (23 ± 2 °C).

The control (L_0) mango fruits for low temperature (4 ± 2 °C) condition, recorded 3.59 ± 0.02, 3.81 ± 0.02, 3.90 ± 0.02, 4.08 ±0.01, 4.39 ±0.02, 4.80 ±0.01, 5.18 ±0.01, 5.39 ±0.01, 5.79 ±0.02 and 6.02 ±0.01 from the initial day, 3rd, 6th, 9th,12th, 15th, 18th, 21st, 24th and the 27th day respectively. The unmodified (L_1) fruit samples recorded 3.66 ± 0.01, 3.77 ± 0.01, 3.87 ±0.01, 3.93 ± 0.02, 4.19 ± 0.01, 4.60 ± 0.02, 4.81 ± 0.01, 4.88 ± 0.01, 5.11 ± 0.01 and 5.21 ± 0.02 for the aforementioned days respectively. The modified (L_2) samples recorded 3.62 ± 0.02, 3.71 ± 0.02, 3.79 ± 0.01, 3.89 ± 0.01, 4.09 ± 0.02, 4.31 ± 0.01, 4.49 ± 0.02, 4.61 ± 0.01, 4.79 ± 0.01 and 4.93 ± 0.02 for the aforementioned days respectively as shown (Figure 4.20).

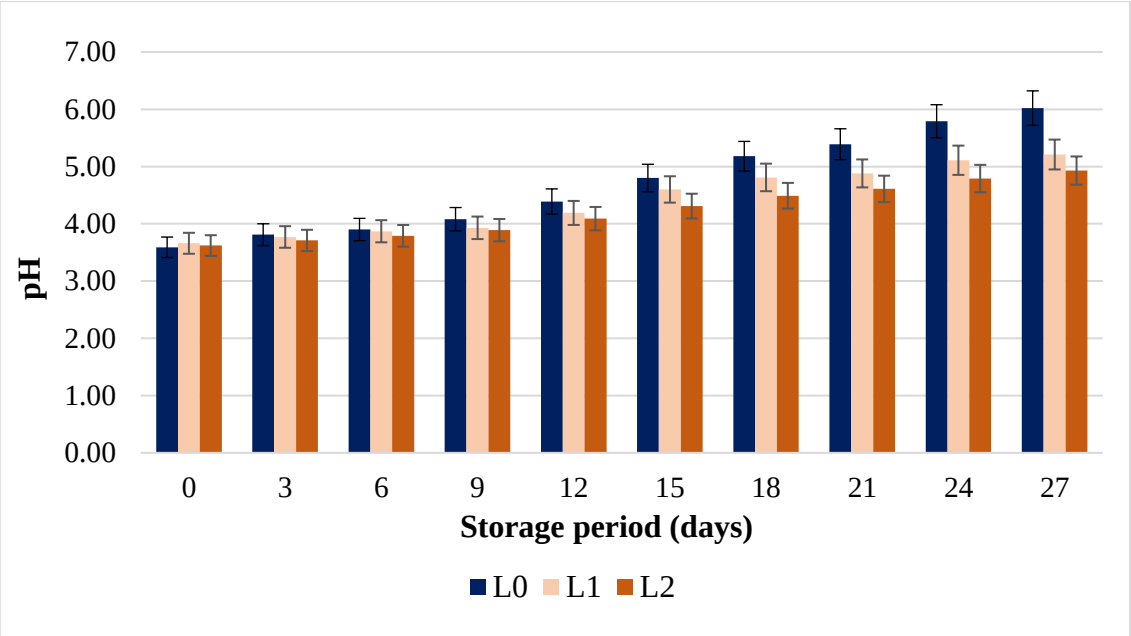


Figure 4.20. Effect of different *M. esculenta* (CS) coating treatments on the pulp pH of mangoes during storage at low temperature (4 ± 2 °C).

Results of this study were comparable to those by (Islam *et al*., 2013). The author reported a rapid increasing trend of pulp pH in control mango fruits as 3.70, 4.80, 5.70, 6.80 and 6.90 for a period of 12 days of storage. The coated fruits indicated a pulp pH of 3.50, 3.60, 3.80, 4.00 and 4.40 for the aforementioned 12 days of storage. The increasing trend for the coated mango fruits was relatively lower compared to that of the control.

The increasing trend of pH for the control samples, corresponded with the decreasing amount of titratable acidity, due increased rate of respiration and oxidation of organic acids such as citric acid to simple sugars over the storage time (Mannozzi *et al*., 2017). On the hand, for the treatment at low temperature (4 ± 2 °C) samples, the pH increment was much delayed which corresponded with the slight changes in titratable acidity. Low temperature (4 ± 2 °C) condition suppressed the samples' reaction processes, while the *Manihot esculenta* coating modified the atmosphere around the treated fruits by creating a semi-permeable film that inhibited the adsorption of gases across the coat (Cortés Rodríguez *et al*., 2020). This significantly barred the development of acids leading to

lower pH values in coated samples unlike the uncoated or control fruits (Cortés Rodríguez *et al*., 2020).

4.8.5 Vitamin C Concentration (mg/100g)

The amount of pulp vitamin C concentration in the mango fruits was significantly ($P < 0.05$) influenced by *M. esculenta* coating on the mango fruits subjected to room temperature (23 ± 2 °C) and low temperature (4 ± 2 °C) conditions. The amount of vitamin C concentration for the pulp of fruits decreased throughout the storage period. The control (T_0) fruits for the room temperature (23 ± 2 °C) condition, displayed a greater decreasing amount of vitamin C concentration of 86.23 ± 0.01 mg/100g, 55.43 ± 0.02 mg/100g, 27.30 ± 0.01 mg/100g, 15.64 ± 0.02 mg/100g, 7.34 ± 0.01 mg/100g, 5.25 ± 0.01 mg/100g and 3.74 ± 0.01 mg/100g from the initial day up to 18 day of storage at an interval evaluation of 3 days.

The unmodified (T_1) fruits indicated 83.43 ± 0.02mg/100g, 70.65 ± 0.01 mg/100g, 38.21 ± 0.01 mg/100g, 22.61 ± 0.01 mg/100g, 11.78 ± 0.01 mg/100g, 8.19 ± 0.01 mg/100g and 5.97 ± 0.01 mg/100g for the aforementioned days respectively. The modified (T_2) mango fruit samples indicated 85.23 ± 0.02 mg/100g, 78.73 ± 0.02 mg/100g, 49.5 ± 0.01 mg/100g, 28.41 ± 0.01 mg/100g, 16.84 ± 0.01 mg/100g, 11.63 ± 0.02 mg/100g and 8.13 ± 0.02 mg/100g for the respective days as shown in (Figure 4.21).

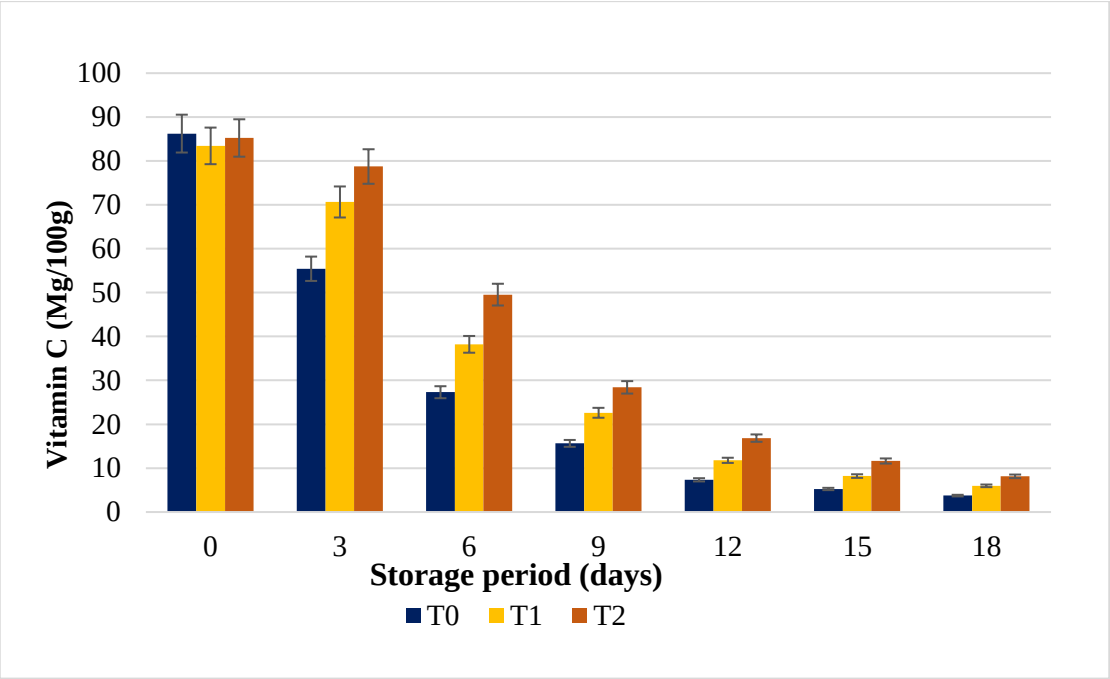


Figure 4.21. Effect of different *M. esculenta (CS*) coating treatments on the vitamin C of mangoes during storage at room temperature (23 ± 2 °C).

The control (L_0) mango fruits for the low temperature (4 ± 2 °C) conditions, recorded 88.13 ± 0.02 mg/100g, 78.33 ± 0.02 mg/100g, 71.49 ± 0.02 mg/100g, 59.60 ± 0.01 mg/100g, 47.30 ± 0.03 mg/100g, 35.71 ± 0.03 mg/100g, 31.30 ± 0.01 mg/100g, 12.20 ± 0.02 mg/100g and 12.11 ± 0.02 mg/100g from the initial day up to 27 days at storage interval evaluation of 3 days. The unmodified (L_1) fruit 84.24 ± 0.02 mg/100g, 79.87 ± 0.01 mg/100g, 75.40 ± 0.01 mg/100g, 61.74 ± 0.01 mg/100g, 49.24 ± 0.01 mg/100g, 37.54 ± 0.01 mg/100g, 34.81 ± 0.02 mg/100g, 17.31 ± 0.02 mg/100g and 14.4 ± 0.02 mg/100g for the aforementioned days. The modified (L_2) mango fruits indicated 87.45 ± 0.01 mg/100g, 81.77 ± 0.02 mg/100g, 77.34 ± 0.01 mg/100g, 65.11 ± 0.01 mg/100g, 57.24 ± 0.01 mg/100g, 40.27 ± 0.01 mg/100g, 38.39 ± 0.02 mg/100g, 19.72 ± 0.01 mg/100g and 17.84 ± 0.03 mg/100g for the respective days as given in (Figure 4.22).

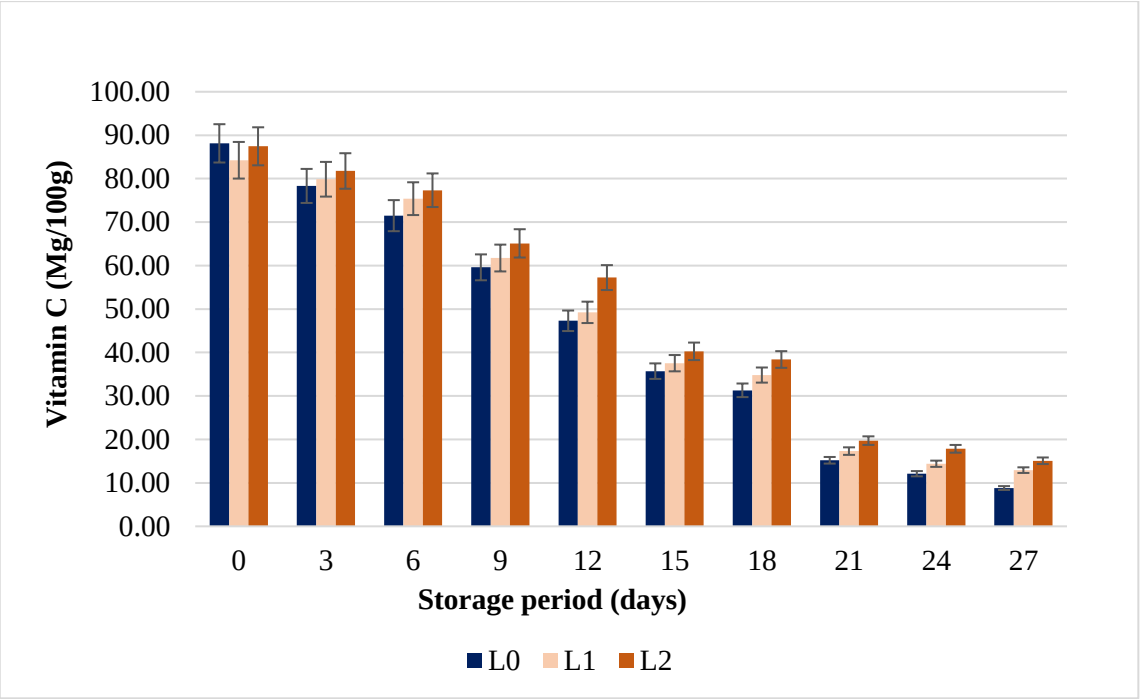


Figure 4.22. Effect of different *M. esculenta* (CS) coating treatments on the vitamin C concentration of mangoes during storage at low temperature (4 ± 2 °C).

The findings of this study were comparable to that of literature work on the study of coated mangoes by (Khairul *et al*., 2013). The uncoated mango fruits indicated a decreasing amount of vitamin C content of 150.06 mg/100g, 110.70 mg/100g, 45.60 mg/100g, 24.80 mg/100g and 15.60 mg/100g for 12 days' storage at 3 days' evaluation period. The coated mangoes indicated 155.30 mg/100g, 125.80 mg/100g, 95.60 mg/100g, 58.20 mg/100g, 35.80 mg/100g for 12 days' storage. The coated mango fruits indicated lower decrease of vitamin C concentration as compared to the uncoated ones.

The decreasing trend in vitamin C concentration in the mango pulps at different storage intervals might be due to oxidation during respiration processes and low temperature could be possibly delayed the oxygen dependent processes. *Manihot esculenta* coating modified the atmosphere around the treated fruits by creating a semi-permeable film that delayed oxidation and respiration processes during storage period (Mandal *et al*., 2018).

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Phytochemical screening indicated presence of alkaloids, flavonoids, terpenoids, steroids, glycosides, phenols and tannins with abundance of alkaloids, glycosides, terpenoids, steroids and phenols in methanol extracts compared to ethyl acetate, acetone, and hexane. Time affects extraction yield of *O. gratissimum* essential oil in that, the extraction is rapid at the beginning of experiment but get slow gradually by time.

Cassava (*M. esculenta*) starch formed a clear transparent gel after homogenization which when modified with *O. gratissimum* essential oils formed barrier properties on mango fruits against oxygen dependent processes extending the shelf life of mango fruits. This was also established by the antioxidant and antibacterial properties of *O. gratissimum* essential oils and the modified cassava starch coating.

Ocimum gratissimum leaves EOs contains significant compounds such as 2-octen-1-ol, (Z), α-thujene, 1-octen-3-yl-acetate, 4(10)-thujene, germacrene, 1-octen-3-ol, among others. These compounds possess strong antimicrobial activity which when incorporated with *M. esculenta* coating makes it useful.

Ocimum gratissimum essential oils and modified edible coating had significant antibacterial and antioxidant activities.

M. esculenta edible coating incorporated with *O. gratissimum* EOs (1.5%) extended the shelf-life of mango fruits by up to 18 and 27 days of storage at room temperature (25 ± 2 °C) and low temperature (4 ± 2 °C) conditions respectively. Control samples got fully ripened at the 9^{th} day and 15^{th} day of storage at room temperature (25 ± 2 °C) and low temperature (4 ± 2 °C) conditions respectively.

5.2 Recommendations

i. Further studies on various concentrations of *O. gratissimum* EOs to be incorporated for modification of *M. esculenta* edible coating need to be done to establish the best composite for coating.

ii. Further characterization of modified coating and *O. gratissimum* EOs needs to be done to determine the synergistic effect of the coating composite and the essential oils.

iii. Mechanism of interaction of *O. gratissimum* essential oil and the *M. esculenta* edible coating is recommended.

iv. Studies on EOs of other plants from the *Ocimum* family is recommended to compare their biological and chemical activities.

v. Modified *M. esculenta* edible coating is recommended for preserving fresh produce while maintaining their physico-chemical qualities during storage.

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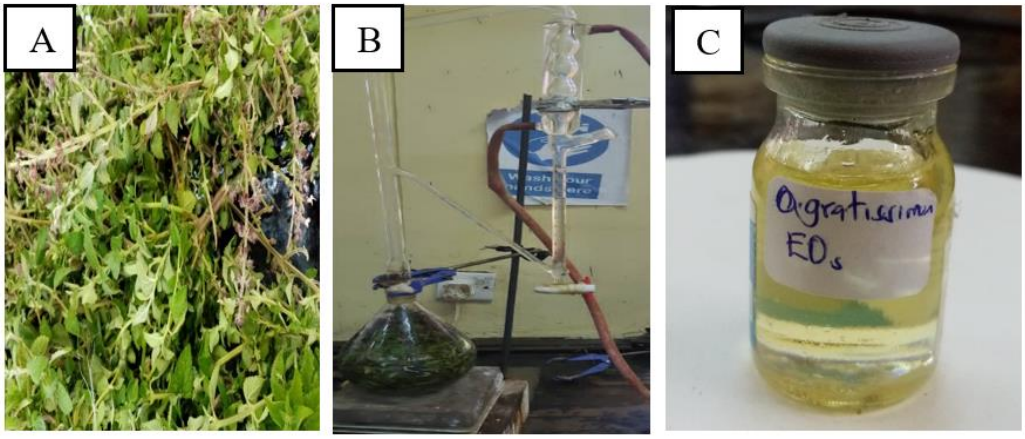
APPENDICES

Appendix I: Sample Preparation and extraction for phytochemical screening

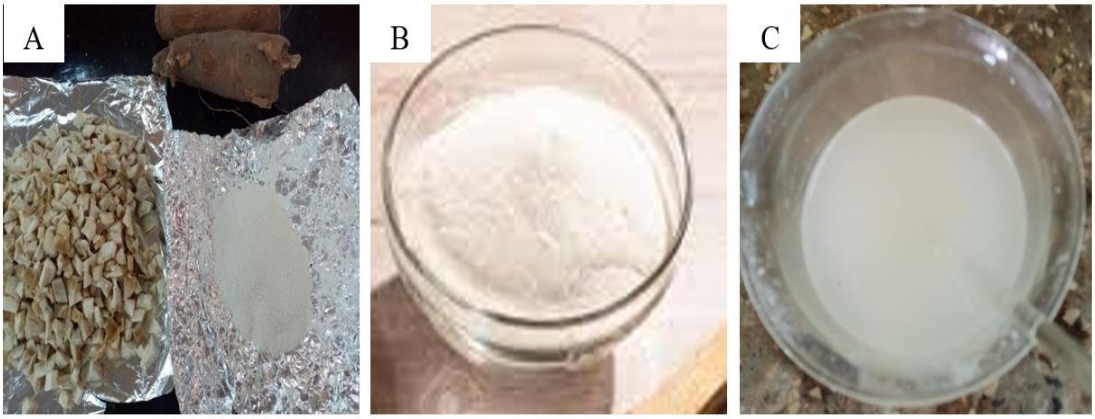
Dry ground powders of (A) *O. gratissimum* leaves and (B) *M. esculenta* tubers

Appendix II: The *O. gratissimum* leaves, hydro-distillation process and EOs



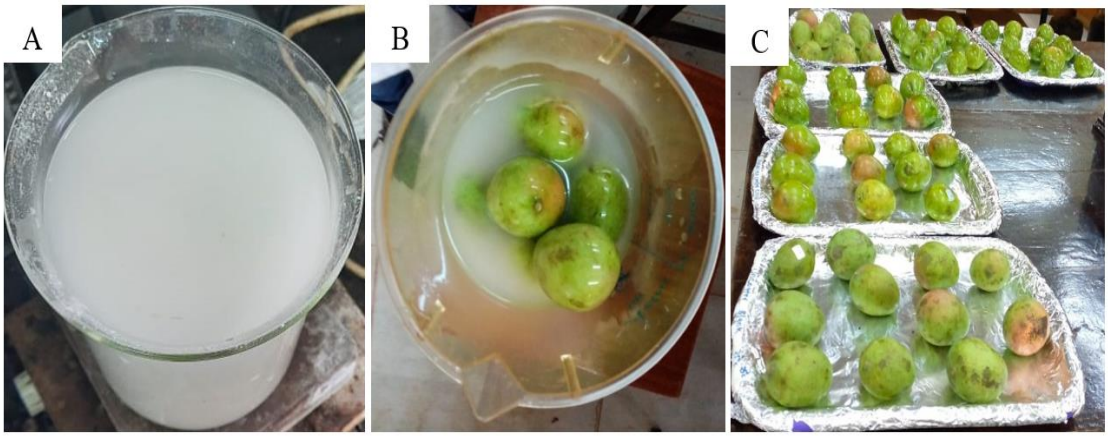
Ocimum gratissimum leaves (A), hydro-distillation extraction (B) and EOs (C)

Appendix III: Preparation of cassava starch and edible coating



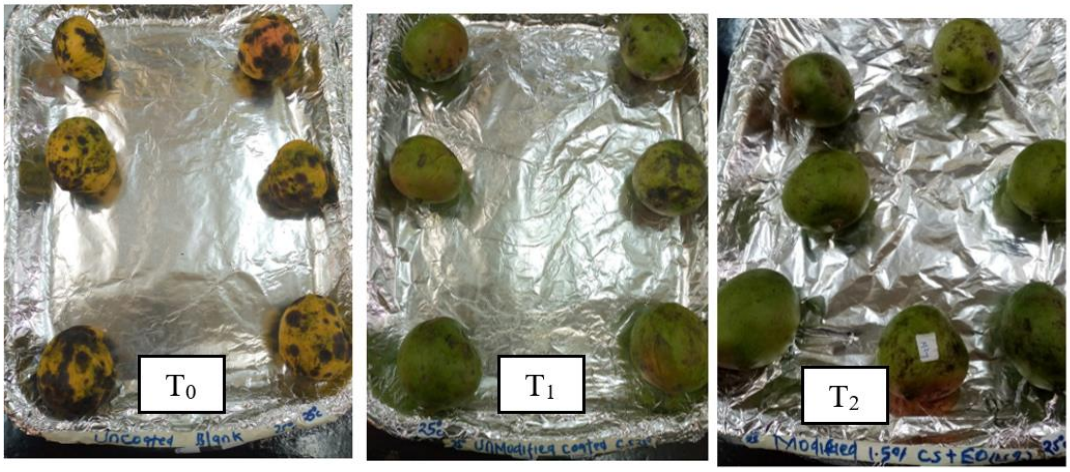
Grinded *M. esculenta* tubers (A), Dried and sieved starch (B), and edible coating (C).

Appendix IV: Fruit coating process

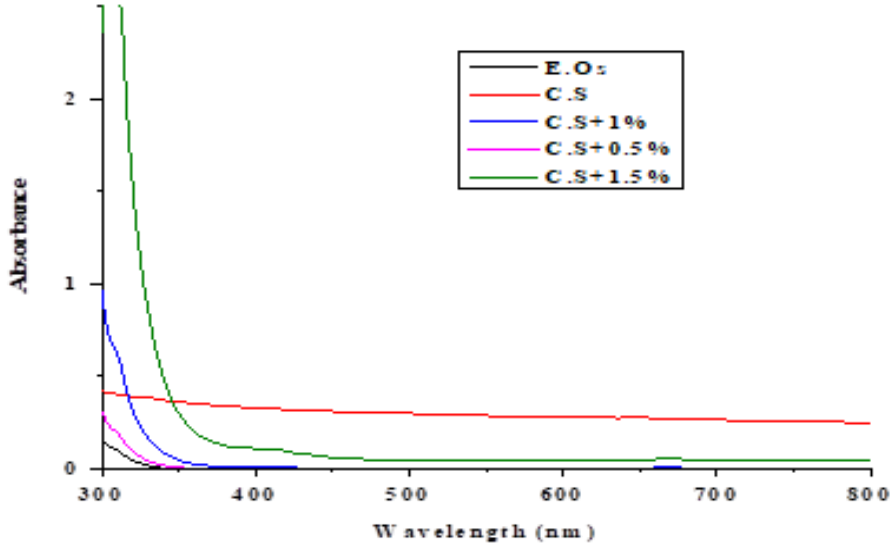


Prepared edible coating (A), Dipping process (B), Coated fruits (C).

Appendix V: Shelf-life of mango fruits on the 9th day at room temperature condition (25 ± 2 °C)

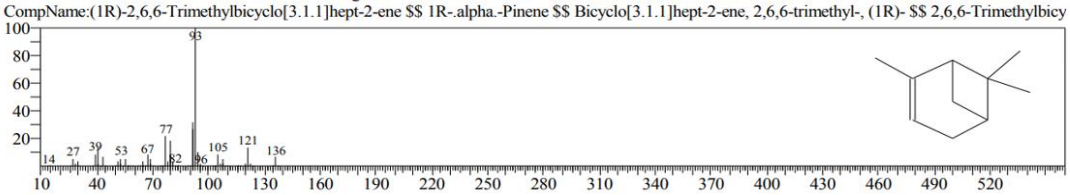


Appendix VI: UV-Vis spectra of modified, unmodified coating and the EOs.

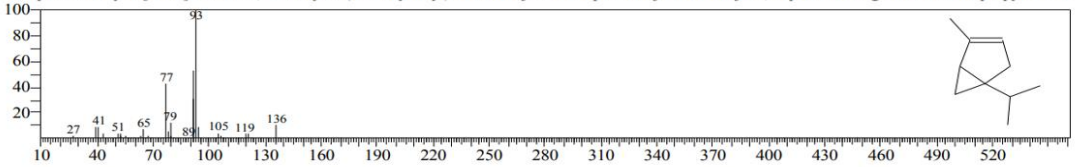


Appendix VII: Mass spectra of compounds in *O. gratissimum* EOs

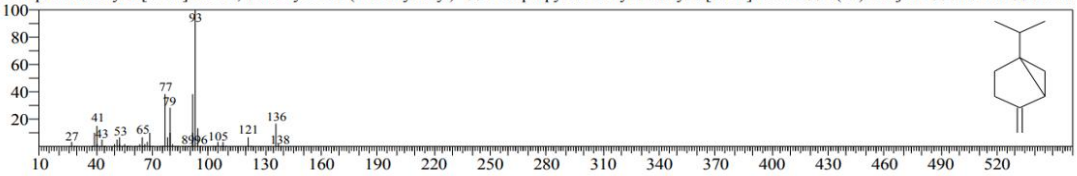
Hit#:1 Entry:10022 Library:NIST14.lib
SI:95 Formula:C10H16 CAS:7785-70-8 MolWeight:136 RetIndex:948
CompName:(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene $$ 1R-.alpha.-Pinene $$ Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (1R)- $$ 2,6,6-Trimethylbicy



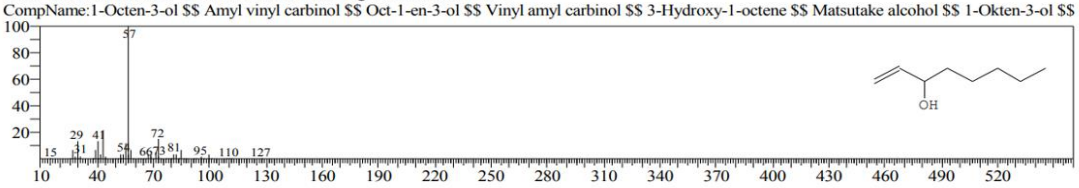
Hit#:1 Entry:10012 Library:NIST14.lib
SI:98 Formula:C10H16 CAS:2867-05-2 MolWeight:136 RetIndex:902
CompName:Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)- $$ 3-Thujene $$ .alpha.-Thujene $$ Thujene, .alpha.- $$ Origanene $$ 5-Isopropyl-2-met



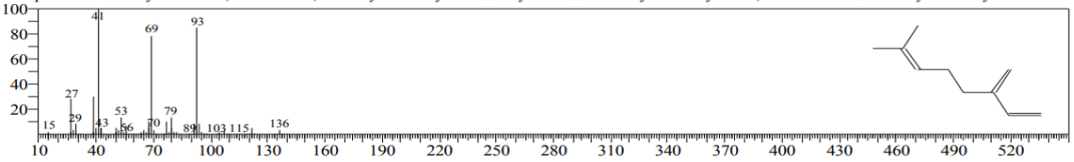
Hit#:1 Entry:10011 Library:NIST14.lib
SI:98 Formula:C10H16 CAS:3387-41-5 MolWeight:136 RetIndex:897
CompName:Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- $$ 1-Isopropyl-4-methylenebicyclo[3.1.0]hexane $$ 4(10)-Thujene $$ Sabinen $$ Sabin



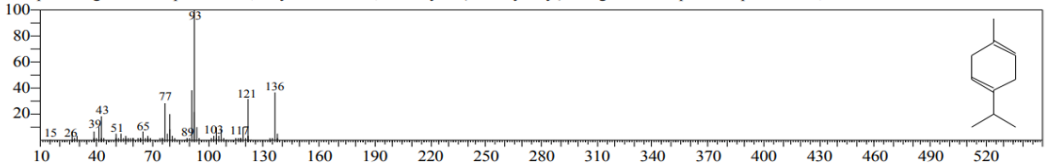
Hit#:1 Entry:7486 Library:NIST14.lib
SI:99 Formula:C8H16O CAS:3391-86-4 MolWeight:128 RetIndex:969
CompName:1-Octen-3-ol $$ Amyl vinyl carbinol $$ Oct-1-en-3-ol $$ Vinyl amyl carbinol $$ 3-Hydroxy-1-octene $$ Matsutake alcohol $$ 1-Okten-3-ol $$



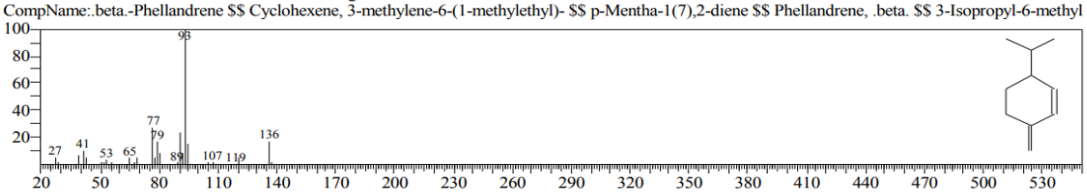
Hit#:1 Entry:9926 Library:NIST14.lib
SI:94 Formula:C10H16 CAS:123-35-3 MolWeight:136 RetIndex:958
CompName:.beta.-Myrcene $$ 1,6-Octadiene, 7-methyl-3-methylene- $$ Myrcene $$ 7-Methyl-3-methylene-1,6-octadiene $$ 7-Methyl-3-methyleneoctadie



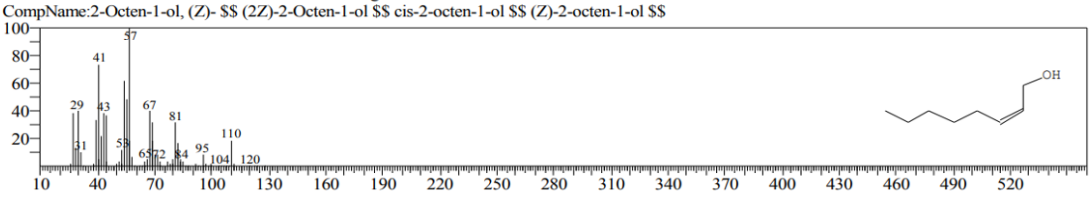
Hit#:4 Entry:10019 Library:NIST14.lib
SI:89 Formula:C10H16 CAS:99-85-4 MolWeight:136 RetIndex:998
CompName:.gamma.-Terpinene $$ 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- $$ .gamma.-Terpinen $$ p-Mentha-1,4-diene $$ Crithmene $$ Moslen



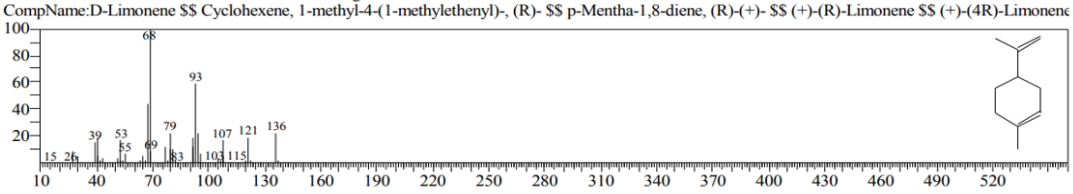
Hit#:2 Entry:9997 Library:NIST14.lib
SI:95 Formula:C10H16 CAS:555-10-2 MolWeight:136 RetIndex:964
CompName:.beta.-Phellandrene $$ Cyclohexene, 3-methylene-6-(1-methylethyl)- $$ p-Mentha-1(7),2-diene $$ Phellandrene, .beta. $$ 3-Isopropyl-6-methyl



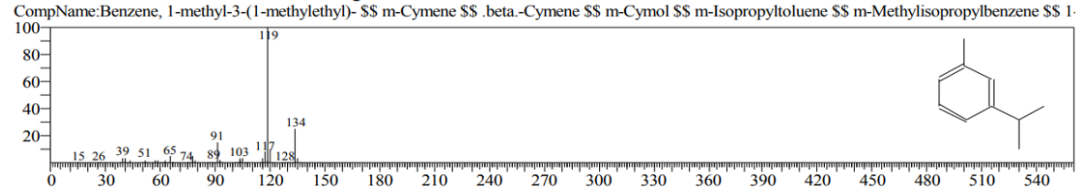
Hit#:1 Entry:7473 Library:NIST14.lib
SI:91 Formula:C8H16O CAS:26001-58-1 MolWeight:128 RetIndex:1067
CompName:2-Octen-1-ol, (Z)- $$ (2Z)-2-Octen-1-ol $$ cis-2-octen-1-ol $$ (Z)-2-octen-1-ol $$



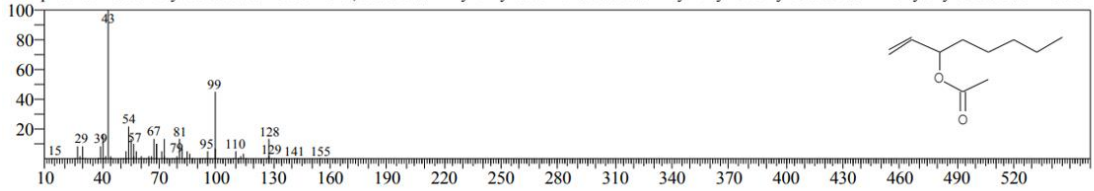
Hit#:2 Entry:9955 Library:NIST14.lib
SI:91 Formula:C10H16 CAS:5989-27-5 MolWeight:136 RetIndex:1018
CompName:D-Limonene $$ Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (R)- $$ p-Mentha-1,8-diene, (R)-(+)- $$ (+)-(R)-Limonene $$ (+)-(4R)-Limonene



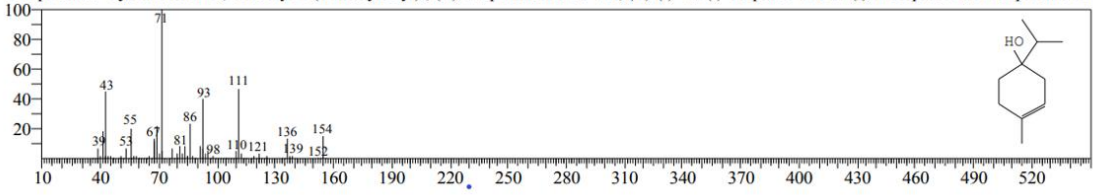
Hit#:1 Entry:9321 Library:NIST14.lib
SI:95 Formula:C10H14 CAS:535-77-3 MolWeight:134 RetIndex:1042
CompName:Benzene, 1-methyl-3-(1-methylethyl)- $$ m-Cymene $$ .beta.-Cymene $$ m-Cymol $$ m-Isopropyltoluene $$ m-Methylisopropylbenzene $$ 1-



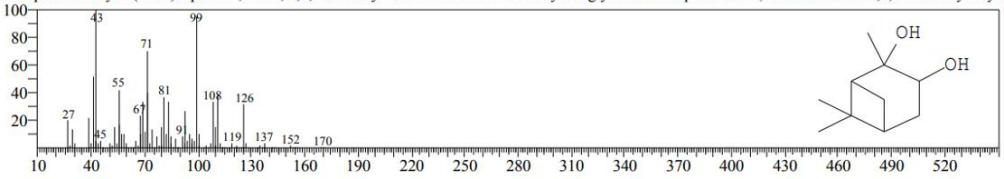
Hit#:1 Entry:26928 Library:NIST14.lib
SI:95 Formula:C10H18O2 CAS:2442-10-6 MolWeight:170 RetIndex:1109
CompName:1-Octen-3-yl-acetate $$ 1-Octen-3-ol, acetate $$ Amyl vinyl carbinol acetate $$ Amyl vinyl carbinyl acetate $$ 1-Pentylallyl acetate $$ 1-Octen



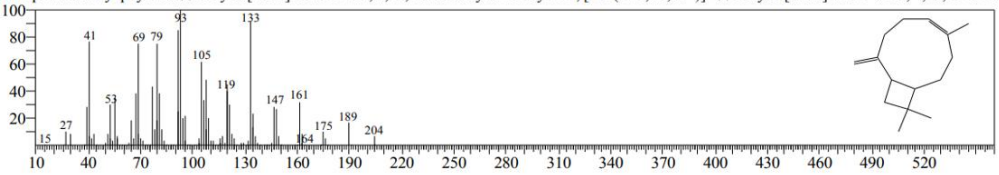
Hit#:1 Entry:18072 Library:NIST14.lib
SI:95 Formula:C10H18O CAS:20126-76-5 MolWeight:154 RetIndex:1137
CompName:3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)- $$ p-Menth-1-en-4-ol, (R)-(-)- $$ (-)-Terpinen-4-ol $$ (-)-4-Terpineol $$ L-terpinen-4-ol



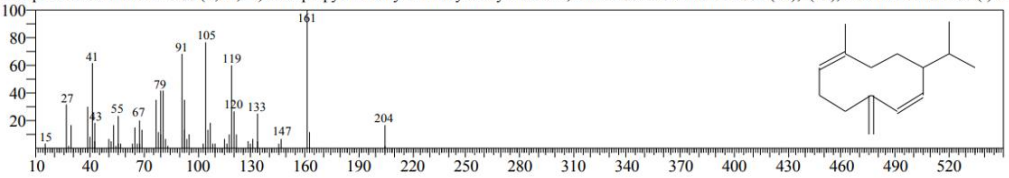
Hit#:1 Entry:26930 Library:NIST14.lib
SI:83 Formula:C10H18O2 CAS:53404-49-2 MolWeight:170 RetIndex:1276
CompName:Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl- $$ DHS activator $$ Ethylene glycol ether of pinene $$ 2,3-Pinanediol $$ 2,6,6-Trimethylbicyc



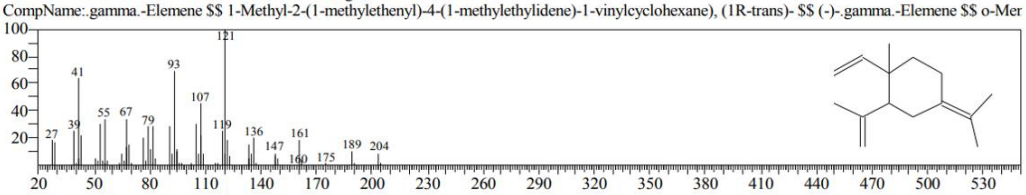
Hit#:1 Entry:49890 Library:NIST14.lib
SI:97 Formula:C15H24 CAS:87-44-5 MolWeight:204 RetIndex:1494
CompName:Caryophyllene $$ Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4E,9S*)]- $$ Bicyclo[7.2.0]undec-4-ene, 4,11,11-trim



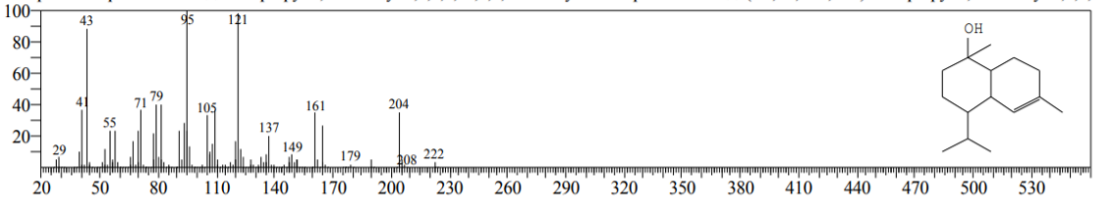
Hit#:1 Entry:50023 Library:NIST14.lib
SI:95 Formula:C15H24 CAS:23986-74-5 MolWeight:204 RetIndex:1515
CompName:Germacrene D $$ (S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene $$ D-Germacrene $$ 1(10),4(14),5-Germacratriene $$ (-)-G



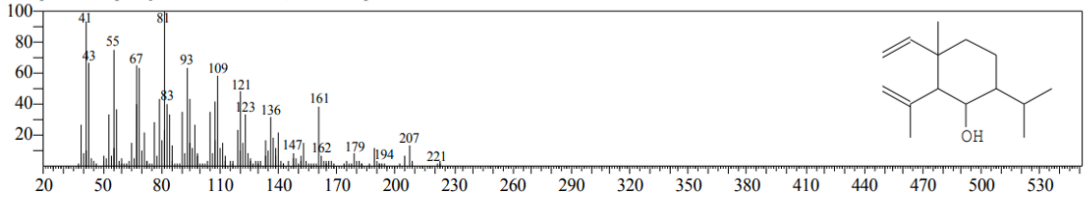
Hit#:2 Entry:49977 Library:NIST14.lib
SI:93 Formula:C15H24 CAS:29873-99-2 MolWeight:204 RetIndex:1431
CompName:.gamma.-Elemene $$ 1-Methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-1-vinylcyclohexane), (1R-trans)- $$ (-)-.gamma.-Elemene $$ o-Mer



Hit#:1 Entry:64366 Library:NIST14.lib
SI:96 Formula:C15H26O CAS:481-34-5 MolWeight:222 RetIndex:1580
CompName:.alpha.-Cadinol $$ 4-Isopropyl-1,6-dimethyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenol # $$ (1R,4S,4aR,8aR)-4-Isopropyl-1,6-dimethyl-1,2,3,



Hit#:2 Entry:64350 Library:NIST14.lib
SI:85 Formula:C15H26O CAS:0-00-0 MolWeight:222 RetIndex:1555
CompName:6-epi-shyobunol $$ elema-1,3-dien-6.alpha.-ol $$



Appendix VIII: Analysis of Variance for the total weight loss, TSS, TA, pH and Vitamin C concentration

Weight loss (25 ± 2 °C)

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	296.45265	3	98.81755	1.87461022	0.03513354	2.86626555
Within Groups	1897.6915	36	52.7136517			
Total	2194.1441	39				

Weight loss (4 ± 2 °C)

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	942.57494	3	314.191648	2.13666045	0.02200931	3.00878657
Within Groups	3529.152	24	147.048001			
Total	4471.727	27				

Total soluble solids (25 ± 2 °C)

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	92.52174	2	46.26087	1.922704	0.017507	3.554557
Within Groups	433.0858	18	24.06032			
Total	525.6075	20				

Total soluble solids (4 ± 2 °C)

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	8.24408	2	4.12204	0.351299	0.040694	3.354131
Within Groups	316.8098	27	11.73369			
Total	325.0538	29				

Titratable acidity (25 ± 2 °C)

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.099924	2	1.049962	0.717406	0.011452	3.554557
Within Groups	26.34397	18	1.463554			
Total	28.4439	20				

Titratable acidity (4 ± 2 °C)

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.603487	2	0.801743	0.781051	0.0368	3.354131
Within Groups	27.7153	27	1.026493			
Total	29.31879	29				

pH (25 ± 2 °C)

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	14.54427	2	7.272133	5.31893	0.01531	3.554557
Within Groups	24.60991	18	1.367217			
Total	39.15418	20				

pH (4 ± 2 °C)

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.134827	2	0.567413	1.290307	0.029162	3.354131
Within Groups	11.87327	27	0.439751			
Total	13.0081	29				

Vitamin C (25 ± 2 °C)

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	430.0235	2	215.0117	0.217037	0.020698	3.554557
Within Groups	17832.02	18	990.6675			
Total	18262.04	20				

Vitamin C (4 ± 2 °C)

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	139.3725067	2	69.68625	0.089078	0.015042	3.354131
Within Groups	21122.27648	27	782.3065			
Total	21261.64899	29				