EFFECT OF SELECTED ORGANIC ACIDS ON THE PROPERTIES OF *Borassus aethiopum* STARCH-BASED FILMS FOR FRUITS AND VEGETABLE STORAGE

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AUGUST, 2023

ABSTRACT

The increasing use of synthetic polymers to preserve foods is of more concern to the society. Edible coatings serve as the best alternative to reduce the post-harvest losses by delaying the ripening of fruits and vegetables and increasing the shelf life without affecting the quality. The study investigates the effects of formic, acetic, lactic, butyric, pentanoic and citric acids on *Borassus aethiopum* starch-based films for fruits and vegetable storage. Starch was extracted from *Borassus aethiopum* shoot using wet extraction method. Starch-based films were produce by casting method with the incorporation of six organic acids (Formic, acetic, lactic, butyric, pentanoic and citric acids) having five concentrations (0.2 %, 0.4 %, 0.6 %, 0.8 % and 1 %) each. Biodegradability test of starch-based films was carried out using soil burial test and antimicrobial activities of the starch-based films were carried out using agar diffusion method. Shelf-life determination of tomato, banana and spinach was carried out using dipping method. The result showed that *Borassus aethiopum* had (64.24%) starch content and the six organic acids incorporated had better film forming property. *Borassus aethiopum* starch-based films incorporated with organic acids had improved biodegradability and water absorption capacity. The antimicrobial inhibitory activities of the starch-based films incorporated with citric and lactic acids at 1 % concentration showed that citric acid films had highest inhibitory activity (1.85 ± 0.05) followed by lactic acid (3.97 ± 0.07) at $p \leq 0.05$ when compared with the control sample (9.57 ± 0.08) for *E. coli*). For *klebsiella pneumonia* inhibition, citric acid and lactic acid showed significant ($p \leq 0.05$) inhibitory effect at 1% concentration (1.31 ± 0.06 and 4.60 ± 0.03) respectively when compared with the control (8.66 ± 0.06). There was significant $p \leq 0.05$ improvement in the tensile strength and elongation at break of both citric acid and lactic acid films when compared with the control sample. The shelf-life of stored coated tomato, banana and spinach with citric and lactic acids at 1 % concentration was found to be extended up to 30 days for tomatoes, 10 days for banana and 4 days for spinach. The coated samples were firmer, fresh looking and less decayed when compared with the uncoated samples (control) at the end of the storage. It can be concluded from the results obtained that *Borassus aethiopum* starch could be a good source of starch for film production and citric and lactic acids could be incorporated into it to increase the shelf life of fruits and vegetables.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Most polymers used to manufacture packaging materials are petroleum-based and are therefore not biodegradable, causing a great impact on the environment when discarded (Arfat *et al*., 2017). This reality encourages the use of natural biodegradable polymers for food packaging (Priyadarshi *et al*., 2018). Starch becomes one of the best material for packaging due its biodegradability and availability. Starch is a complex carbohydrate which is insoluble in water. It is usually extracted from cereal seeds (corn, wheat, and rice), roots and tubers (potato, taro, tapioca). Starch has been widely used as a raw material in film production, because of increasing prices and decreasing availability of conventional film-forming resins. It is also useful for making films because it degrades into harmless products when placed in contact with soil microorganisms (Olusauya *et al*., 2023).

Example of starch from plants that are used for food packaging include potato, maize, corn, rice, ginger, cassava, tapioca, sago, yam, oat, wheat, baeley, banana, rye, taro, beans, quinoa, mung and pea starch. Starch has a wide range of application beyond human consumption, such as in the food, chemical, textile and packaging industries (Colussi *et al*., 2021). The main reason is because the starch has the characteristics of biodegradability and biocompatibility. Films produced from different types of starch formulations will have different characteristics (Erna *et al*., 2022). Starch from young germinating shoot of *Borassus aethiopum* (Muruchi) is underutilized, it is basically used as an important source of food for the rural people in Northern Nigeria (Muhammad *et al.*, 2019).

In this study a *borassus aethiopum* starch-based film was prepared by adding glycerol as a plasticizer and incorporated with an antimicrobial agent (organic acids) as a chelating agent. Organic acids and salts of acids have been widely used in animal feed as acidifiers to modify the intestinal environment as well as to enhance nutrient digestibility (Papatsiros and Billinis, 2012). The most commonly used acids include formic acid, citric acid, benzoic acid, carboxylic acids, and salts of short chain fatty acids (SCFAs) (Liu *et al*., 2018). Recently, combinations of organic acids and medium chain fatty acids (e.g., lauric acid) have also demonstrated synergistic benefits on animal intestinal health and performance, compared with the individual products (Zentek *et al*., 2019). In general, antimicrobial activity has been claimed or suggested as one of the primary mechanisms of action through which organic acids could enhance animal health (Long *et al*., 2018). It is theorized that organic acids in their un-dissociated and uncharged state are capable of bypassing bacterial cell membranes due to their lipophilic nature modifying the proton and associated anion concentrations in the cytoplasm (Guan and Liu, 2019). Upon entering the more alkaline interior of a bacterium, the anion and proton from organic acids may have deleterious effects on the bacterium by increasing osmotic stress and disrupting important biomolecule synthesis, which finally causes bacterial death (Hirshfield *et al*., 2018 and Salsali *et al*., 2008).

The objective of this study was to determine the effect of selected organic acids on the properties of *Borassus aethiopum* shoot (muruchi) starch-based films to be applied as coating for fruits and vegetables storage.

1.2 Statement of the Research Problem

Packagings from synthetic polymers cause serious ecological problems due to their non-biodegradability. This issue has increased environmental concern and encouraged the packaging industries to expand their research using biodegradable materials (Silva *et al*., 2019).

Organic acids are known to confer antimicrobial activities to starch-based films thus, increase the performance and extend the shelf life of the packaged food (Mazumdar, 2018). *Borassus aethiopum* starch is underutilized in Nigeria. Its food use has not gone beyond mere cooking for ultimate consumption, while it also served as a form of income generating produce for the farmers in rural areas (Akinniyi *et al*., 2010).

1.3 Aim and objectives of the study

The aim of this study was to determine the effects of selected organic acids on the properties of *Borassus aethiopum* starch-based films for fruits and vegetable storage.

The objectives of this study were to determine:

i. the effect of different organic acids on the biodegradability and water sensitivity of *Borassus aethiopum* starch-based films.

ii. the effect of different organic acids on the antimicrobial properties of *Borassus aethiopum* starch-based films.

iii. the mechanical properties of selected *Borassus aethiopum* starch-based films incorporated with citric and lactic acids that shown best antimicrobial activity

iv. the shelf-life of tomato, banana and spinach coated with *Borassus aethiopum* starch incorporated with citric and lactic acids.

1.4 Justification of Study

By using starch from underutilize source such as *Borassus aethiopum* starch to produce starch-based films and incorporating organic acids will inhibit the growth of microorganisms.

Postharvest loss usually occurred to fruits and vegetables, that is, degradation in both quantity and quality of a food product from harvest to consumption (Henz, 2017). To reduce this loss, fruits and vegetable can now be stored by coating its surface with *Borassus aethiopum* starch incorporated with organic acids to increase their shelf-life.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Polymers: Synthetic Polymer and Natural Polymers

A polymer is a large molecule (macromolecules) composed of repeating structural units. These subunits are typically connected by covalent chemical bonds. Both synthetic and natural polymers are available but the use of natural polymers for pharmaceutical applications is attractive because they are economical, readily available and non-toxic. They are capable of chemical modifications, potentially biodegradable (Satturwar *et al.*, 2019).

Synthetic polymers can be classified as addition polymers, formed from monomer units directly joined together or condensation polymers, formed from monomer units combining such that a small molecule, usually water, is produced during each reaction. Some examples of synthetic polymers are polyethylene, polyester, polyurethane, polyvinyl chloride, polyvinyl acetate, nylon, low density polyethylene and many more. These types of polymers basically cannot be degraded and thus need to be recycle in order to avoid landfills problems (Satturwar *et al*., 2019).

Synthetic polymers such as polyethylene is used in plastic bags, films, wrapa, bottles, electrical insulation and toys. Polyvinyl chloride (PVC) is used in siding, pipes, and flooring purposes. polystyrene is used in cabinets and in packaging. Polyvinyl acetate is used in adhesives and latex paints. Polypropylene is used in packaging, labelling, stationery, textile, plastics, reusable containers and laboratory equipment (Satturwar *et al*., 2019).

Natural polymers are large molecules, produced by plants and animals that carry out many life-sustaining processes in a living cell. The cell membranes of plants and the woody structure of trees are composed of cellulose, a polymeric carbohydrate. Carbohydrates, which comprise one of the three classes of foodstuffs, contain carbon, hydrogen and oxygen atoms. They can be classified as monosaccharides, disaccharides and polysaccharides (Shah *et al*., 2016).

2.2 Starch

A starch occurs in plants in the form of granules, and these are particularly abundant in seeds especially the cereal grains and tubers, where they serve as a storage form of carbohydrates (Shah *et al*., 2016). It is used in a wide range of foods for a variety of purposes including thickening, gelling, adding stability and replacing or extending food systems (Huang *et al*., 2021). Food starches are favored for their availability, comparatively low cost and unique properties; their native form is a versatile product, and the raw material for production of many modifications, sweeteners and ethanol (Evgeniy *et al*., 2022). Starch is isolated mainly from corn, potatoes, cassava, and wheat in the native and modified forms and this account for 99% of the world production (Dhull *et al*., 2021). Some other starches are also available commercially; recently, starches obtained from legumes (peas, lentils) have become more interesting because they have properties which appear to make them a suitable substitute for chemically modified starches for series of products (Kaur *et al*., 2020).

2.2.1 Structure and properties of starch granules

Starch is a mixture of two polymers: amylose and amylopectin. Natural starches consist of about 10 to 30% amylose and 70 to 90% amylopectin (Kaur *et al*., 2020). Amylose: It is a linear polysaccharide composed entirely of D-glucose units joined by the α-1,4-glycosidic bonds. Evidence indicates that amylose is not a straight chain of glucose units but coiled like a spring, with six glucose monomers per turn. When coiled in this fashion; amylose has enough room in its core to accommodate an iodine molecule. The characteristic blue-violet color that appears when starch is treated with iodine is due to the formation of the amylose-iodine complex (Kaur *et al*., 2020).

Amylopectin: It is a branched-chain polysaccharide composed of glucose units linked primarily by α-1,4- glycosidic bonds but with occasional α-1,6-glycosidic bonds, which are responsible for the branching. A molecule of amylopectin may contain many thousands of glucose units with branch points occurring about every 25 to 30 units (Kaur *et al*., 2020).

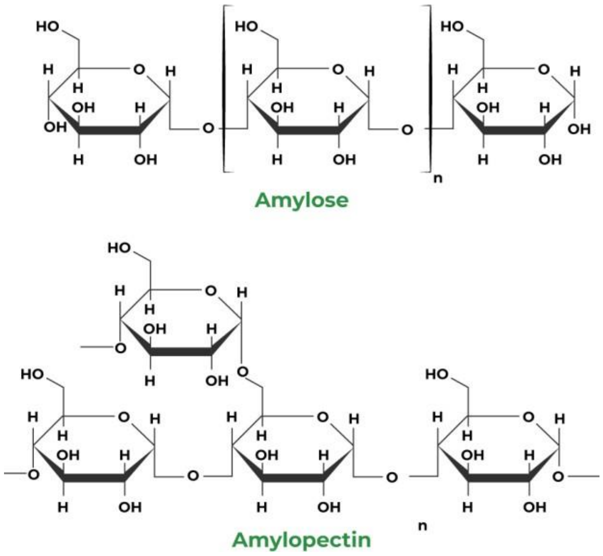


Figure 2.1: Amylose and Amylopectin molecules (Kaur *et al*., 2020).

2.3 *Borassus aethiopum* (Muruchi)

The plant *Borassus aethiopum* (Figure 2.2) has been described as a palm tree with huge fan shaped leaves. In Nigeria, the hausa people call it Giginya, the Fulanis call it Dubbe, the yorubas call it Agbonolodu and the igbos call it Ubiri while the Kanuris call Kemelutu. The plant is a dioecious plant and can reach up to 20m high on average and 1m in diameter (Ahmed *et al.,* 2010).

The shoot of *Borassus aethiopum* (Figure 2.3) is obtained by burying the matured seeds of the plant in pit and allowed to germinate. The young germinating shoot or hypocotyls known as Muruchi or Gazari, is usually harvested after 7 to 8 weeks of planting (Ahmed *et al.,* 2010). Muruchi is an important source of food for the rural people in Northern Nigeria. The people consume it either raw or boiled and claimed that it enhances libido in women and aphrodiasiac in men (Akinniyi *et al*., 2010). The shoots are potential source of starch in Cote d'Ivoire which is an important raw material in industry (Mazumdar, 2018). Muhammad *et al*., (2019) reported high concentration of carbydrate (83.00%) and crude fibre (3.96%) and low fat (1.49%) in the shoot of *Borassus aethiopum* plant on dry weight basis. Similarly, the shoots contained an appreciable amount of both macro and micro mineral elements.

The sap is usually boiled immediately after extraction to make sugar or fermented to produce an alcoholic beverage. Also, evidence suggests its folkloric anti-inflammation activity (Sakande *et al*., 2019). Other studies have revealed that the young shoot of *Borassus aethiopum* extract has an anabolic effect of androgens; thus, supporting its local use as an aphrodisiac (Akinniyi *et al*., 2010).

The methanolic seed coat of *Borassus aethiopum* has been shown to possess free radical scavenging action and its leaves have an effective anthelmintic activity against Indian adult earth warms (Sastry *et al*., 2018). The anti-fungal and anti-bacterial activities of a 50:50 dichloromethane methanol extract of the male inflorescences of *Borassuss aethiopum* in mice have been found (Sakande *et al*., 2019).

Figure 2.2: *Borassus aethiopum* tree Salako *et al*. (2019).

Figure 2.3: *Borassus aethiopum* shoot Sirajo *et al*. (2019)

2.4 Organic Acids

Organic acids have long been used in their natural forms. These are low-molecular weight compounds that contain one or more carboxyl groups and are observed in almost every microorganism. They have numerous applications in industries related to bio-commodities such as food, cosmetics, surfactants, and textile industries (Panda *et al*., 2016). Production of organic acids for commercial exploitation is carried out either by chemical synthesis or fermentation, with preference for fermentation because of its high yield in the microbiological processes. The initial production practices of acetic acid date back to 1823 and citric acid to 1913. But commercial fermentation of citric acid production via microbe-assisted processing started around 1920 (Sneh *et al*., 2022). Because of their functional groups such as keto and hydroxyl groups, organic acids are enormously valuable as initial substances for chemical industries. Besides their traditional usage in food, feed, and pharmaceuticals, production of organic acids as monomers with bi-functional characteristic has been triggered due to their surgence of bioplastics. Indulgence of recombinant DNA technology and metabolic engineering techniques have efficiently engineered organic acid-producing microorganisms to enhance and speed up process developments for preferred bio-products at high concentration, quality, and productivity (Li and Borodina, 2015; Cho *et al*., 2015). The organic acid with highest annual production is citric acid at 1.6 million tons, followed by acetic and lactic acid with 0.19 and 0.15 million tons, respectively (Vandenberghe *et al*., 2018).

2.4.1 Types of organic acid

Organic acids are segregated into various clusters. Common organic acids constitute the carboxylic acids, containing one or more carboxyl groups (–COOH). As most of the organic acids are weak acids, in aqueous solutions they work as a buffer, and this property is used in the production of food and feed (Vandenberghe *et al*., 2018).

Table 2.1: Common names of some basic organic acids, natural sources and their chemical formula

S/N	Common name	Natural sources	Chemical formula
1	Formic acid	Ants (Formica)	$HCOOH$
2	Acetic acid	Vinegar	CH_3COOH
3	Lactic acid	Sour milk	$CH_3CHOHCOOH$
4	Butyric acid	Rancid butter	$CH_3(CH_2)_2COOH$
5	Pentanoic acid	Seefood	$CH_3(CH_2)_3COOH$
6	Citric acid	Lemons	$C_6H_8O_7$

2.4.1.1 *Formic acid*

Formic acid is a colorless liquid with a pungent odor, which is completely miscible with water and many polar solvents but only partially miscible with hydrocarbons. Formic acid derived its name from the red ant, Formica rufa, in which it was discovered around 1670. Formic acid has been detected in the poison or defense systems of ants, bees, and other insects and also of cnidarians. Formic acid is used primarily in dyeing, in the textile and leather industries; in rubber production; and as an intermediate in the chemical and pharmaceutical industries (Banerjee and Arora, 2023).

2.4.1.2 *Acetic acid*

Acetic acid is also known as ethanoic acid, ethylic acid, vinegar acid, and methane carboxylic acid; it has the chemical formula of CH3COOH. Acetic acid is a byproduct of fermentation, and gives vinegar its characteristic odor. Vinegar is about 4-6% acetic acid in water. More concentrated solutions can be found in laboratory use, and pure acetic acid containing only traces of water is known as glacial acetic acid (Banerjee and Arora, 2023). Acetic acid is the 33rd highest volume chemical produced in the United States. Acetic acid is used in the manufacture of acetic anhydride, cellulose acetate, vinyl acetate monomer, acetic esters, chloracetic acid, plastics, dyes, insecticides, photographic chemicals, and rubber. Other commercial uses include the manufacture of vitamins, antibiotics, hormones, and organic chemicals, and as a food additive. Typical concentrations of acetic acid occurring naturally in foods are 700 to 1,200 milligrams/kilogram (mg/kg) in wines, up to 860 mg/kg in aged cheeses, and 2.8 mg/kg in fresh orange juice (Banerjee and Arora, 2023).

2.4.1.3 *Lactic acid*

Lactic acid (LA) is a three-carbon (2-hydroxypropionic acid) carboxylic acid present in plants and microorganisms. It is widely available organic acid and is generated by lactic acid bacteria, which have a long history in the food industry and microbial processes over the last century (Magnuson and Lasure, 2018). Worldwide production of lactic acid is mostly contributed by bacterial fermentation, which is more economic friendly with superior yields, comparatively fast, and produces one of the two stereo isomers of lactic acid as well as their racemic mixture (Magnuson and Lasure, 2018).

Lactic acid is safe for human consumption and as a food additive. It has been utilized for the manufacture of biocompatible polylactate polymers such as polylactic acid (Lee *et al*., 2015) that has boosted the commercial exploration of this acid.

Lactic acid is used in the food, pharmaceutical, cosmetic, and textile industries. The free acid is used as an acidulant and preservative in several food products, such as cheese, meat, beer, and jellies. Lactic acid has applications for the production of dairy products, such as yogurt, buttermilk, acidophilus milk, cottage cheese, cream cheese, and fermented cheese etc (Suyama *et al*., 2017).

2.4.1.4 *Butyric acid*

Butyric acid can be directly produced by fermentation and used in the manufacturing of pharmaceuticals, perfumes, plastics, plasticizers, and fuels, as well as in the making of butyrate esters, such as cellulose butyrate (Qureshi, 2017). It can be converted into important fine chemicals and biofuels such as butanol and butyl– butyrate (Sjöblom *et al*., 2016). Butyric acid can be produced from numerous sources of sugars including glucose derived from corn, sugarcane molasses, beet molasses, paper mill sludges, sweet sorghum stalks and juice, and hydrolysates of cellulosic residues including straws (wheat, barley, and rice), reed canary grass, and switch grass (Xiao *et al*., 2018).

2.4.1.5 *Citric acid*

Citric acid Citric acid ($C_6H_8O_7$, 2-hydroxy-1,2,3-propane tricarboxylic acid), a natural constituent and common metabolite of plants and animals, is the most versatile and widely used organic acid in the fields of food (60%) and pharmaceuticals (10%). It also has several other applications in various other fields. Citric acid is a tricarboxylic acid with a molecular weight of 210.14 g /mol and contains three carboxylic functional groups with three different pKa values (3.1, 4.7, and 6.4). Citric acid is a primary metabolic product that is formed in the tricarboxylic acid (Krebs) cycle and is found in small quantities in virtually all plants and animals. Citric acid was first isolated by Karls Scheels in 1874 in England from lemon juice imported from Italy. The main application of citric acid (70%) is in the food and beverage industry, as the most versatile and widely used acidulant. Its pleasant taste, high water solubility, and property of enhancing flavors have affirmed its application in this market, which has been largely responsible for the increases in demand for citric acid. A buffering capacity and the ability to form complexes with heavy metals have driven the application of citric acid and several of its salts in the pharmaceutical and chemical-processing industries as sequestering agents (Xiao *et al*., 2018).

2.5 Tomatoes

Tomato (Solanum Lycopersicum L. (or) Lycopersicon esculentum Mill.) is considered as one of the most important and known vegetables in the world. By weight, tomatoes rank second only to potatoes in global production of all horticultural produce of high yielding, better adaptability and multipurpose uses for instance, it can be eaten in various ways and in a countless number of dishes and also eaten raw in salads or as an extract or sauce in many dishes and in drinks. Globally, tomato production accounts 162 million tons from about 4.8 million hectares of land area (Rosalina *et al*., 2020).

This fruit is one of food sources rich in micronutrients that are necessary for health benefits include reduced risks of cancer, skin health and cardiovascular disease. Therefore, tomato is known for its outstanding nutritive and medicinal values and therefore grouped under protective foods (Ayari *et al*., 2020).

Despite numerous nutritious and health benefits of tomatoes, storage life has been limited by several factors including physiological losses and senescence, biological factor, physical or mechanical injuries, and environmental condition. Uneven handling of tomatoes can also result in the damage of the fruit cell wall leading to softening, and increased respiration which results in faster fruit ripening and deterioration of fruit quality and reduced marketability of the product (Mai *et al*., 2021).

Several postharvest interventions have been introduced to help maintain quality and extend storage life. These interventions include low temperature storage, edible coating, and modified atmosphere packaging. The combinations of treatments such as low temperature, coatings, low oxygen and high carbon dioxide storage and ethylene inhibitor such as calcium chloride treatment have been reported to have the potential to extend the storage life of fresh produce such as tomatoes (Genanew, 2019).

CHAPTER THREE

3.0 RESEARCH METHODOLOGY

3.1 Materials

Borassus aethiopum shoot (muruchi) starch was used in this study. Fresh muruchi (*Borassus aethiopum*) tubers were purchased from Kasuwan Gwarri market in Minna, Niger state, Nigeria.

3.1.1 Chemicals

All chemicals used in this study were of analytical grade, products of Sigma Aldrich. The chemicals used include formic acid, acetic acid, lactic acid, butyric acid, pentanoic acid, citric acid, glycerol among others.

3.1.2 Equipments

The equipments used in this study were volumetric flask, conical flask, measuring cylinder, funnel, beaker, pipette, glass rod, dry air oven, water bath, filter paper, test tube, refrigerator, weighing balance, pH meter, refractometer, petri dish among others.

3.2 Methodology

3.2.1 Determination of the effect of different organic acids on biodegradability and water absorption capacities of *Borassus aethiopum* starch-based films

3.2.1.1 *Extraction of Borassus aethiopum starch*

Starch was extracted by the method of Honda *et al*. (2019) with slight modification. The freshly bought raw Muruchi tubers were washed thoroughly with tap water, peeled, rewashed, cut into pieces and milled into pulp. The pulp was suspended in excess distilled water and homogenized in a blender at a medium speed for 5 minutes at room temperature (30^o C). The homogenized slurry was filtered through a muslin cloth and the waste residue in the cloth was washed four times with 20 ml distilled water until cleared. The resulting milky filtrate was allowed to settle for 5 hours and the water decanted off. The whole starch sediment was washed with 10 ml of distilled water to remove adhering protein layer. The wet cakes of the starch were sun dried. Finally, the isolated starch was pulverized into powder, weighed for starch yield calculation, packed in transparent sample bottles and stored at room temperature.

3.2.1.2 *Production Borassus aethiopum starch-based films incorporated with organic acids*

A film forming dispersion was prepared using a method of Ahmadi *et al*. (2019) with some modification. About 1 g of starch was weighed and 10 ml of distilled water was added and stirred for 60 minutes and then dispersion was gelatinized in a shaker water bath at 78 – 80°C for 10 minutes. Glycerol was added as plasticizer to reach a concentration of about 0.3 g glycerol/g starch. Organic acids were incorporated at different concentration (0.2, 0.4, 0.6, 0.8 and 1 percent). Then 5 ml of starch solution while still hot was transferred into petri dishes of 100 mm diameter and 15 mm deep. The dishes were placed for 24 hours in an oven and the films were peeled off.

3.2.1.3 *Biodegradability test of Borassus aethiopum starch-based films incorporated with organic acids using soil burial testE*

Biodegradability test was carried out using Soil Burial Test (SBT) following the method of Thakore *et al*. (2022) with some modification. Starch film samples (1x1 cm) were buried in soil at 7.5 cm depth, then incubated at room temperature for 5 days with sampling time every 24 hours. The buried samples were then cleaned from the soil and weighed. The weight loss of the films was calculated using the following equation:

$$\% \text{ Weight loss} = \frac{W_0 - W_f}{W_0} \times 100 \tag{3.1}$$

Where, W_0 is the weight of the original films before SBT and W_f is the weight of residual films after SBT and subsequent drying till constant weight for different times.

3.2.1.4 *Water absorption test of Borassus aethiopum starch-based films incorporated with organic acids*

Water absorption tests were performed on the samples following the method of Thakore *et al*. (2022) with modification. The starch-based film sample samples were immersed in distilled water at room temperature for 5 days. The samples were taken out every 24 hours from the water and were weighed immediately after wiping out the water on the surface of the sample. The amount of water absorbed was determined by calculating the weight gain after immersion

% Water absorption = $\frac{W_f - W_i}{W_i} \times 100$ (3.2)

Where, W_f = Final weight and W_i = Initial weight

3.2.2 Determination of the effect of organic acids on the antimicrobial properties of *Borassus aethiopum* starch-based films

Antimicrobial activity test on films was carried out using the agar diffusion method of Daoud *et al*. (2015), and the antimicrobial effects of films containing organic acids against *Escherichia coli*, *Klebsiella pneumonia and Pseudomonas aeruginosa* were carried out using plate count assay on solid media. The starch based films were cut into a circle form with 7.1 mm diameter and placed on the surface of the solid media which had been inoculated with 0.1 ml culture, approximately containing 106 to 107 cfu / ml. The plates were then incubated at 37°C for 24 h, and after incubation time, the inhibitory zone by organic acid films was counted on the film discs. The entire test was conducted in triplicate and the mean values were expressed in colony forming units per gram (CFU/g). The starch films incorporated with organic acids showing the best antimicrobial properties were selected for further studies.

3.2.3 Determination of mechanical properties of *Borassus aethiopum* starch-based films incorporated with citric and lactic acids

Mechanical test was performed using Universal Testing Machine. Film samples were cut into rectangular strips (100 mm x 15 mm) and the tensile strength (TS), strain, modulus of elasticity (ME) and the elongation at break (EAB) were measured in accordance to standard method ASTM D882-02 (ASTM, 2020).

The film thickness was measured using a hand-held micrometer with an accuracy of 0.01 mm at five random points and the average value was determined.

3.2.4 Determination of shelf-life of tomato, banana and spinach coated with *borassus aethiopum* starch-based films incorporated with citric acid and lactic acid

Coatings were produced according to Valencia *et al*. (2016), with slight modifications. Ten grams of *Borassus aethiopum* starch was added to 100 ml of distilled water incorporated with 1 % citric acid (for citric acid coatings) and 1 % lactic acid (for lactic acid coatings). This solution was heated at 70 °C for 20 minutes on a hot plate with the addition of 5 g glycerol (0.5 g glycerol/g starch) under stirring until complete starch gelatinization. Three types of edible coatings were developed (citric acid, lactic acid and control coatings). Tomatoes, banana and spinach were washed with water, sanitized with sodium hypochlorite solution at 100 ppm for 10 minutes, rinsed in chlorinated water at 3 ppm, and placed in plastic trays to dry at room temperature (26 °C). The samples were immersed for 1 minute in the coatings solution and returned to the trays to dry at room temperature (26 °C) for 1 hour. Subsequently, the coated fruits were stored in a Biochemical Oxygen Demand (BOD) refrigerator at 25 °C for up to 30 days, 10 days and 4 days for tomatoes, banana and spinach respectively.

3.2.4.1 ***Determinaton of physico-chemical properties of stored coated tomatoes with Borassus aethiopum starch-based films incorporated with 1 % citric and 1 % lactic acids after 30 days***

3.2.4.2 ***Percentage weight loss of citric and lactic acid coated tomatoes***

The weight loss of tomato fruit was determined by using the methods of AOAC (2019). The percentage weight loss was calculated for each sample by taking mass on laboratory weight balance using the following equation:

$$\% \text{ weight loss} = \frac{W_i - W_f}{W_i} \times 100 \quad (3.3)$$

Where, W_i = initial weight, W_f = final weight.

3.2.4.3 ***pH determination of coated tomatoes with 1 % citric and lactic acids***

Tomatoes samples were cut into small pieces and homogenized in a semi-industrial blender. Ten grams of the sample was suspended in 100 ml of distilled water and then filtered. The filtered sample was used for assessment of pH using pH meter (AOAC, 2019).

3.2.4.4 ***Total soluble solids of coated tomatoes with 1 % citric and lactic acids***

Tomato samples was ground in an electric juice extractor for freshly prepared juice. Total soluble solids content was measured using digital hand held pocket Refractometer (% Brix). The range of the refractometer is 0 to 85 % (AOAC, 2019).

3.2.4.5 ***Titratable acidity of coated tomatoes with 1 % citric and lactic acids***

Titratable acidity was determined by AOAC (2019). Five grams of tomato juice was diluted in 25 ml of distilled water, two drops of phenolphthalein indicator was added and titrated against 0.1 N sodium hydroxide (NaOH). The titratable acidity was expressed as g citric acid /kg tomato in following equation.

$$\text{Titratable acidity} = \frac{v \times 0.1 \times 1000 \times 0.064}{m} \quad (3.4)$$

Where, v is the volume of NaOH required (ml), 0.1 is the normality of NaOH (N), 0.064 is the conversion factor for citric acid and m is the mass of tomato juice sample used (g).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Effect of different organic acids on biodegradability and water absorption capacities of *Borassus aethiopum* starch-based films

4.1.1.1 *Biodegradability of Borassus aethiopum starch-based films incorporated with six organic acids*

The percentage degradability of *Borassus aethiopum* starch films in soil after 5 days is shown in Table 4.1. The result indicate that the films were degradable in soil. The obtained result showed that starch-based films incorporated with organic acids had high degradation (75.5 %) for pentanoic acid at 0.4 % concentration and low degradation (51.0 %) for acetic acid at 1 % concentration compared with the control (59.0 %).

Table 4.1: Biodegradability of *Borassus aethiopum* Starch-Based Films in Soil After 5 Days of Burial

	% weight loss after 5days				
Acids (%)	0.2	0.4	0.6	0.8	1.0
Citric acid	64.50 ± 0.50 ^{d}	65.00 ± 0.01^{b}	61.00 ± 0.01^{c}	58.00 ± 1.00 ^{b}	66.00 ± 1.00 ^{c}
Butyric acid	62.50 ± 0.50 ^{c}	66.00 ± 0.01^{c}	57.00 ± 1.00^{ab}	54.00 ± 0.01^{a}	53.00 ± 1.00 ^{ab}
Pentanoic acid	60.00 ± 0.01 ^{b}	75.50 ± 0.01^{e}	70.00 ± 0.01^{d}	65.00 ± 1.00 ^{c}	53.00 ± 1.00 ^{a}
Lactic acid	53.50 ± 0.50 ^{a}	62.00 ± 0.01^{a}	60.00 ± 0.01^{c}	71.00 ± 1.00 ^{d}	52.00 ± 0.01 ^{a}
Formic acid	63.00 ± 1.00 ^{cd}	70.00 ± 0.01^{d}	61.00 ± 1.00 ^{c}	69.00 ± 1.00 ^{d}	55.00 ± 1.00 ^{b}
Acetic acid	64.00 ± 0.01 ^{cd}	62.00 ± 0.01^{a}	56.50 ± 0.50 ^{a}	54.50 ± 0.50 ^{a}	52.00 ± 0.01 ^{a}
Control			59.00 ± 1.00		

Values are mean ± S.E. (S.E = Standard error of Mean), n = 3
Values between experimental treatments Within the same column bearing the same superscript are not significantly different at the 5 % level (P ≥ 0.05).

4.1.1.2 *Water Absorption of Borassus aethiopum starch-based films incorporated with six organic acids*

The water absorption capacity of *Borassus aethiopum* starch films with the incorporation of organic acids are shown in Table 4.2. From the result obtained, starch film incorporated with 1 % citric acid had lowest water absorption capacity (20.0 %) after 5 days and starch film incorporated with 0.2 % formic acid film had highest water absorption (56.0 %) compared with the control sample (44.0 %).

Table 4.2: Water Absorption Capacities of *Borassus aethiopum* Starch-based Films Incorporated with Organic Acids After 5 Days

	% water absorption after 5days				
Acids	0.2	0.4	0.6	0.8	1.0
Citric acid	43.50 ± 1.50^{c}	52.00 ± 0.01^{e}	44.00 ± 0.01^{d}	36.50 ± 1.50^{b}	20.00 ± 0.01^{a}
Butyric acid	45.00 ± 0.01^{c}	30.50 ± 0.50^{c}	29.00 ± 0.01^{b}	52.00 ± 2.00^{e}	41.00 ± 0.01^{c}
Pentanoic acid	$35.00 \pm 0.\ 01^{b}$	51.00 ± 0.01^{e}	30.00 ± 0.01^{b}	45.00 ± 1.00^{d}	43.00 ± 0.01^{d}
Lactic acid	44.50 ± 1.50^{c}	26.00 ± 1.00^{b}	45.00 ± 0.01^{c}	45.50 ± 1.00^{c}	36.00 ± 0.01^{b}
Formic acid	56.00 ± 1.00^{d}	21.00 ± 0.01^{a}	39.50 ± 1.50^{c}	42.50 ± 1.50^{cd}	50.00 ± 0.01^{e}
Acetic acid	24.00 ± 1.00^{a}	35.00 ± 1.00^{d}	45.00 ± 1.00^{c}	40.00 ± 0.01^{b}	44.00 ± 0.01^{d}
Control			64.00 ± 1.00		

Values are mean ± S.E. (S.E = Standard error of Mean), n = 3
Values between experimental treatments Within the same column bearing the same superscript are not significantly different at the 5 % level ($P \geq 0.05$).

4.1.2 Antimicrobial activity of Borassus aethiopum starch-based films incorporated with organic acids

The antimicrobial load (cfu / g × 10^4) in starch- based films incorporated with organic acids cultured plate samples on selected microorganisms base on their frequency of occurrence in fruits and vegetables is shown in Tables 4.3; 4.4 and 4.5. The result obtained indicates that starch-based film incorporated with Citric acid had lowest microbial load. That is, highest inhibitory activity on *Escherichia coli* (1.85 ± 0.05), *Klebsiella pneumoniae* (1.31 ± 0.06) and *Pseudomonas aeruginosa* (1.65 ± 0.05) at 1 % concentration. Lactic acid films had second highest inhibitory activity at 1 % concentration when compared with other organic acid films and the control sample.

Table 4.3: *Escherichia coli* Count (cfu / g x 10^4) in *Borassus aethiopum* Starch-Based Films Incorporated with Organic Acids

Acids	0.2(%)	0.4(%)	0.6(%)	0.8(%)	1.0(%)
Citric acid	5.70 ± 0.01^a	6.42 ± 0.02^b	5.12 ± 0.02^a	3.25 ± 0.05^a	1.85 ± 0.05^a
Butyric acid	8.07 ± 0.07^d	8.04 ± 0.04^e	7.07 ± 0.07^c	8.06 ± 0.06^f	4.13 ± 0.03^b
Pentanoic acid	7.58 ± 0.02^c	5.25 ± 0.05^a	7.79 ± 0.03^e	7.36 ± 0.16^e	5.88 ± 0.08^e
Lactic acid	6.45 ± 0.05^b	8.31 ± 0.01^f	7.65 ± 0.03^d	6.45 ± 0.05^c	3.97 ± 0.07^b
Formic acid	8.38 ± 0.03^e	7.71 ± 0.01^d	8.17 ± 0.07^f	6.89 ± 0.02^d	4.66 ± 0.06^c
3Acetic acid	8.15 ± 0.02^d	7.22 ± 0.01^c	6.17 ± 0.07^b	6.19 ± 0.02^b	5.66 ± 0.06^d
Control			9.57 ± 0.08		

Values are mean ± S.E. (S.E = Standard error of Mean), n = 3
Values between experimental treatments Within the same column bearing the same superscript are not significantly different at the 5 % level (P ≥ 0.05).

Table 4.4: ***Klebsiella pneumoniae*** **Count (cfu / g x 10^4) in** ***Borassus aethiopum*** **Starch-Based Films Incorporated with Organic Acids**

Acids	0.2(%)	0.4(%)	0.6(%)	0.8(%)	1.0(%)
Citric acid	7.25 ± 0.05^{cd}	6.15 ± 0.05^{a}	4.60 ± 0.10^{a}	3.86 ± 0.06^{a}	1.31 ± 0.06^{a}
Butyric acid	6.15 ± 0.05^{b}	7.25 ± 0.05^{cd}	6.40 ± 0.01^{c}	6.01 ± 0.10^{d}	5.26 ± 0.10^{d}
Pentanoic acid	7.38 ± 0.03^{d}	7.12 ± 0.05^{c}	6.46 ± 0.04^{c}	6.90 ± 0.01^{f}	6.12 ± 0.01^{b}
Lactic acid	5.26 ± 0.05^{a}	6.50 ± 0.01^{b}	5.95 ± 0.05^{b}	5.17 ± 0.03^{b}	4.20 ± 0.03^{c}
Formic acid	7.17 ± 0.07^{c}	8.25 ± 0.05^{e}	7.72 ± 0.02^{d}	6.31 ± 0.01^{e}	6.17 ± 0.01^{e}
Acetic acid	8.30 ± 1.00^{e}	7.28 ± 0.08^{d}	6.46 ± 0.04^{c}	5.79 ± 0.01^{c}	4.61 ± 0.01^{e}
Control			8.66 ± 0.06		

Values are mean ± S.E. (S.E = Standard error of Mean), n = 3
Values between experimental treatments Within the same column bearing the same superscript are not significantly different at the 5 % level ($P \geq 0.05$).

Table 4.5: *Pseudomonas aeruginosa* Count (cfu / g x 10^4) in *Borassus aethiopum* Starch-Based Films Incorporated with Organic Acids

Acids	0.2(%)	0.4(%)	0.6(%)	0.8(%)	1.0(%)
Citric acid	5.15 ± 0.05^a	6.26 ± 0.05^a	4.15 ± 0.05^a	3.69 ± 0.01^a	1.65 ± 0.05^a
Butyric acid	8.57 ± 0.03^f	7.25 ± 0.15^c	7.21 ± 0.11^f	5.55 ± 0.05^d	4.35 ± 0.25^c
Pentanoic acid	7.65 ± 0.05^e	7.25 ± 0.05^c	6.46 ± 0.06^d	4.75 ± 0.05^c	3.15 ± 0.05^b
Lactic acid	6.45 ± 0.05^c	6.65 ± 0.05^b	4.86 ± 0.06^b	4.28 ± 0.03^b	2.74 ± 0.05^b
Formic acid	7.28 ± 0.03^d	7.16 ± 0.04^c	6.89 ± 0.02^e	5.69 ± 0.02^e	4.45 ± 0.05^c
Acetic acid	6.22 ± 0.01^b	6.09 ± 0.02^a	5.25 ± 0.05^c	3.66 ± 0.01^a	2.85 ± 0.15^b
Control			8.36 ± 0.06		

Values are mean ± S.E. (S.E = Standard error of Mean), n = 3
Values between experimental treatments Within the same column bearing the same superscript are not significantly different at the 5 % level ($P \geq 0.05$).

The *Borassus aethiopum* starch-based films incorporated with citric and lactic acids that confer best antimicrobial activities are shown below:

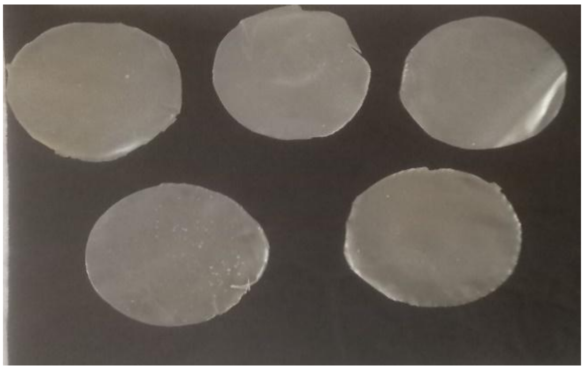


Plate 1: *Borassus aethiopum* starch-based films incorporated with Citric acid

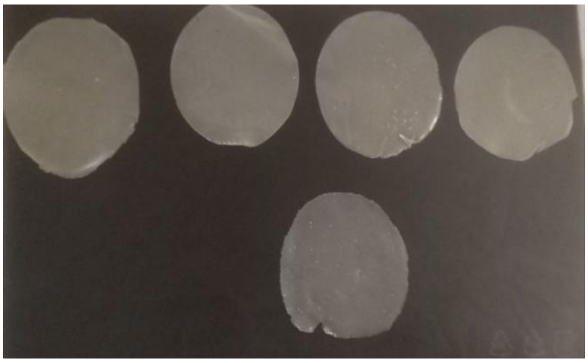


Plate 2: *Borassus aethiopum* starch-based films incorporated with Lactic

4.1.3 Mechanical properties of *borassus aethiopum* starch-based films incorporated with 1 % citric and 1 % lactic acids

The mechanical properties of the selected *Borassus aethiopum* starch-based films incorporated with citric acid and lactic acid at 1 % concentrations is shown in Table 4.6. That is, citric and lactic acid films that confer best antimicrobial activity against the three microorganisms tested at 1 % concentration. From the result obtained, it was observed that lactic acid film tensile strength was significantly higher (1.40 ± 0.12) compared to citric acid film (0.58 ± 0.06). The percentage elongation at break of lactic acid films was significantly ($p \leq 0.05$) higher (51.00 ± 2.00) compared to citric acid film (42.00 ± 2.12) and the control sample (26.00 ± 2.40).

Table 4.6: Mechanical Properties of *Borassus aethiopum* Starch-Based Films Incorporated with 1 % Citric and 1 % Lactic Acids

Film type	Thickness (mm)	Tensile Strength (MPa)	Strain	Modulus of Elasticity (MPa)	Percentage Elongation at Break
Control	0.10 ± 0.02^{b}	0.52 ± 0.03^{b}	0.26 ± 0.14^{b}	1.40 ± 0.16^{c}	26.00 ± 2.40^{c}
Citric acid (1%)	0.43 ± 0.01^{a}	0.58 ± 0.06^{b}	0.42 ± 0.07^{a}	2.00 ± 0.14^{b}	42.00 ± 2.12^{b}
Lactic acid (1%)	0.39 ± 0.04^{a}	1.40 ± 0.12^{a}	0.50 ± 0.04^{a}	2.80 ± 0.13^{a}	51.00 ± 2.00^{a}

Values are mean ± standard error of mean. Values within the same column bearing the same superscript are not significantly different at the 5 % level ($P \geq 0.05$).

4.1.4 Coating of fruits and vegetable with *Borassus aethiopum* starch-based films incorporated with citric and lactic acids

The *Borassus aethiopum* starch-based films that confer best antimicrobial activity (citric acid and lactic acid films) at 1 % concentration were selected for the coating of tomatoes, banana and spinach. The coated tomatoes were observed for a period of 30 days at ambient temperature and was still looking fresh, less decayed when compared with uncoated (control) sample that started decaying after 10 days. The shelf life of coated bananas was found to be extended up to 10 days and had less shrinkage and less decayed when compared with the control sample that shrinked and dried completely after 5 days. The coated spinach was observed for a period of 4 days and had less decay when compared with the uncoated sample that dried completely on the second day of storage.

Plate 3: Spinach Coated with 1 % Citric acid and 1 % Lactic Acid Films

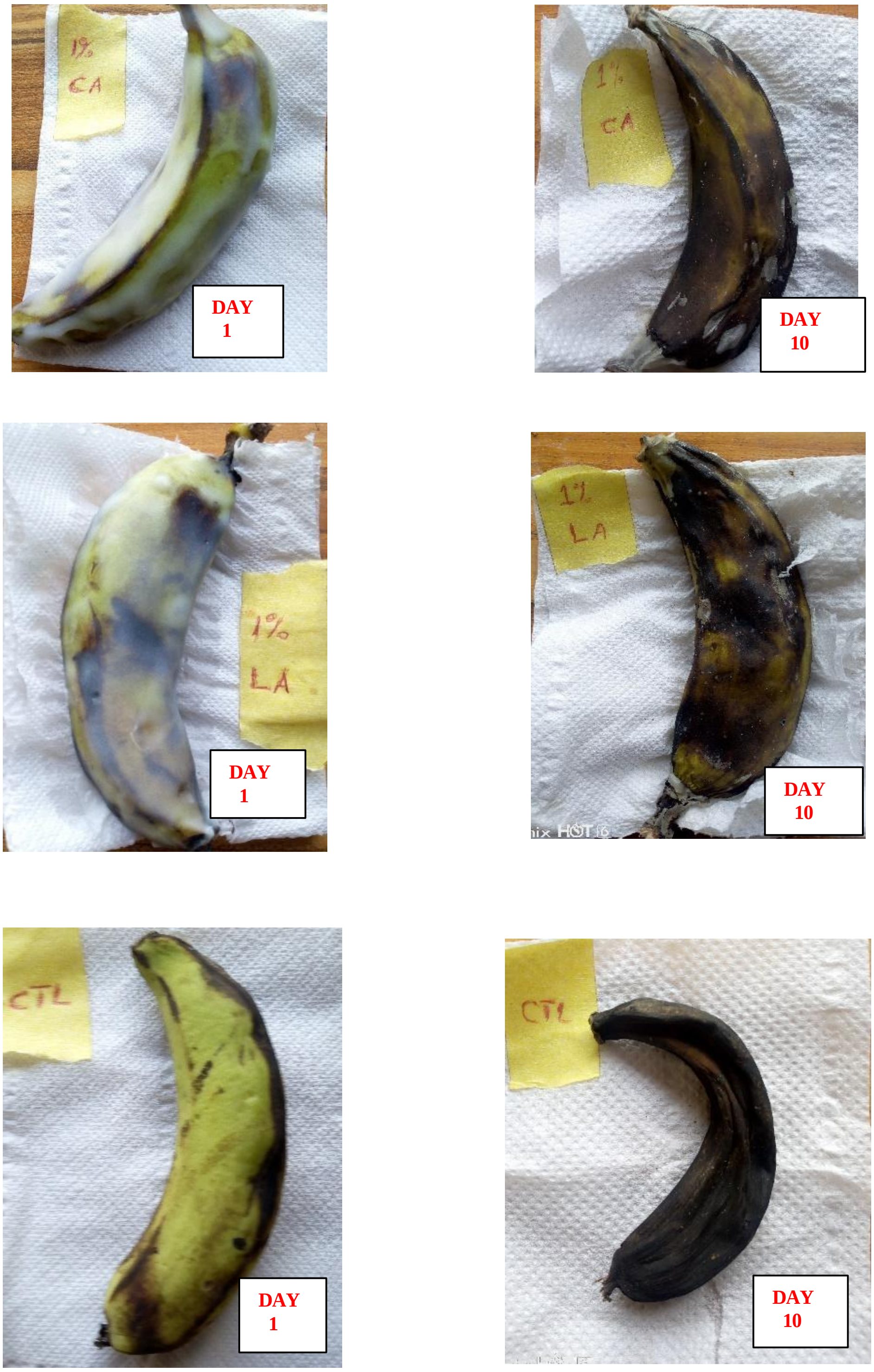


Plate 4: Bananas Coated with 1 % Citric acid and 1 % Lactic Acid Films

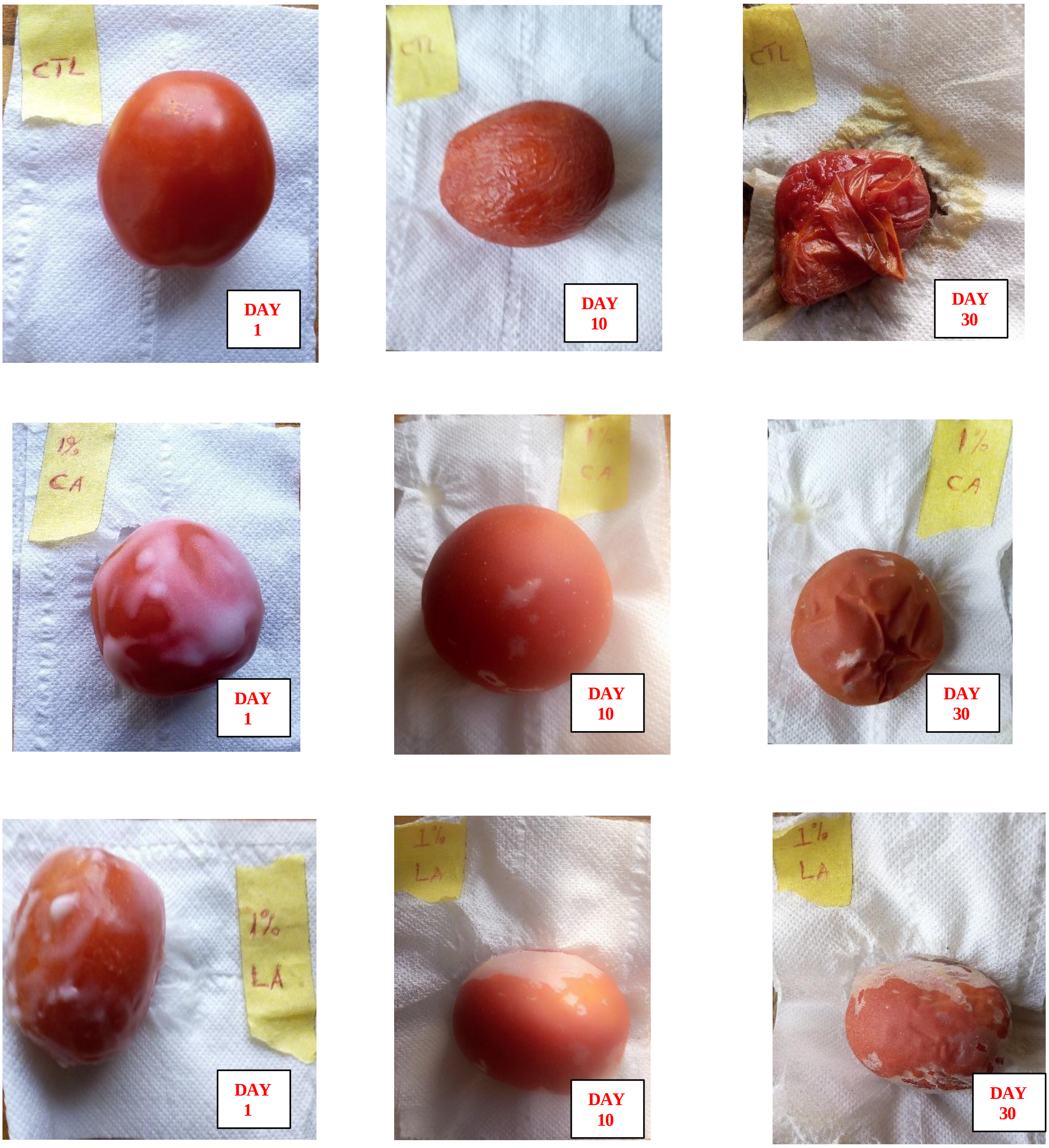


Plate 5: Tomatoes Coated with 1 % Citric acid and 1 % Lactic Acid Films

4.1.5 Physico-chemical parameters of stored coated tomatoes coated with *borassus aethiopum* starch-based films incorporated with 1 % citric acid and 1 % lactic acid after 30 days

The Physico-chemical parameters of stored tomatoes with citric and lactic acids films after 30 days of storage is shown in table 4.7. The parameters determine include percentage shrinkage, Total Soluble Solids (% Brix), pH value and Titratable acidity. The result obtained shows that lactic acid film had less shrinkage (70 %) compared to the citric acid film (72 %) and the control sample (82 %). The total soluble solids in lactic acid film was significantly ($p \leq 0.05$) lower (4.94) compared to the citric acid film (5.00) and the control sample (6.00). The values of total soluble solids in stored tomatoes coated with citric, lactic acid and control films were significantly lower compared to the fresh tomato (3.40).

Table 4.7 Physico-chemical Properties of Stored Coated Tomatoes with *Borassus aethiopum* Starch-Based Films Incorporated with 1 % Citric and 1 % Lactic acids

Sample	% Shrinkage	Total soluble solids (%Brix)	pH	Titratable acidity
Citric acid (1%)	72.00 ± 1.00^a	5.00 ± 0.05^b	4.50 ± 0.14^b	4.56 ± 0.17^b
Lactic acid (1%)	70.00 ± 1.04^a	4.94 ± 0.04^b	4.52 ± 0.12^b	4.74 ± 0.19^b
Control	82.00 ± 1.32^b	6.00 ± 0.24^c	4.82 ± 0.18^c	4.35 ± 0.23^a
Fresh tomato (no film, no acid)	0.00	3.40 ± 0.02^a	4.34 ± 0.10^a	4.92 ± 0.27^c

Values are mean ± standard error of mean. Values within the same column bearing the same superscript are not significantly different at the 5 % level ($P \geq 0.05$).

4.2 Discussion

4.2.1 Biodegradability of *Borassus aethiopum* starch films incorporated with organic acids

The biodegradability of starch based films incorporated with six organic acids after five days are shown in Table 4.1. From the result obtained, starch films incorporated with citric acid (CA) had highest degradation at 1% concentration (66.0 %). Starch films incorporated with butyric acid (BA), pentanoic acid (PA) and formic acid (FA) had their highest degradation at 0.4 % concentration (66.0 %, 75.5 % and 70.0 % respectively). Also, lactic acid (LA) film had high degradation at 0.8 % concentration (71.0 %) and acetic acid (AA) film at 0.2 % concentration (64.0 %). There was a significant ($p \leq 0.05$) difference in the values between the organic acid films used.

The degradation pattern is in the order: PA > LA > FA > CA > BA ≥ AA

Borassus aethiopum starch-based films incorporated with organic acids showed appreciable degradation at certain concentration compared with the control sample (59.0 %). It would have been expected that the organic acids will influence the film according to their chain length but that was not observed. Therefore, the degradation pattern may be by other mechanisms. Form the pattern of degradation, *Borassus aethoiopum* starch films incorporated with pentanoic acid will degrade faster and will not be good enough for storage of food products. Starch-based films incorporated with butyric and acetic acids will degrade slower and will be good for food storage. This result is similar to Lin (2010) who reported that the degradation capacity of native corn starch is faster than that of the modified corn starches. However, the decrease in the degradation of starch films incorporated with organic acids at some percentage concentrations means that it could be a good alternative to synthetic packaging materials used in most developing world.

4.2.2 Water absorption capacities of *Borassus aethiopum* starch films incorporated with organic acids

Water absorption of *Borassus aethiopum* starch-based films incorporated with organic acids was represented in Table 4. From the result obtained, it is shown that *Borassus aethiopum* starch-based film without organic acid (control had the highest water absorption capacity of 64.0 %). However, *Borassus aethiopum* starch-based films incorporated with organic acids clearly caused the decrease in water absorption (Table 4.2). This was due to esterification reaction which caused less free hydroxyl groups, resulting in more hydrophobicity and lower water absorption. The lowest percentage water absorption was observed with *Borassus aethiopum* starch-based film incorporated with citric acid at 1 % concentration (20.0 %), suggesting the highest degree of esterification and cross-linking. The pattern of water absorption of organic acid incorporated starch films in the order: PA > BA > LA > AA > FA > CA

From the pattern of water absorption, it would have been expected that the organic acids will influence the films according to their chain length but that was not observed. Therefore, it may also be by other mechanisms. Starch-based film incorporated with citric acid will form better film quality for food storage compared to the pentanoic acid incorporated films that shown high percentage water absorption. The result obtained was in line with the reports from Olsson *et al*. (2016) that the potato starch film modified with citric acid noticeably showed the decrease in water absorption and water vapor permeability.

4.2.3 Antimicrobial activities of *Borassus aethiopum* starch-based films incorporated with organic acids

It was found that there was significantly ($P \leq 0.05$) inhibitory activity of starch-based film containing organic acids against the three microorganisms tested. A significant difference

($P \leq 0.05$) was also observed among the different kind of organic acids used on the inhibitory activity against those microorganisms. The inhibitory activity of *Borassus aethiopum* starch-based films incorporated with organic acids against *Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa* is shown in Tables 4.3, 4.4 and 4,5. The *Borassus aethiopum* starch-based film incorporated with citric acid had significantly ($P \leq 0.05$) lowest microbial counts at 1 % concentration 1.85 ± 0.05 for *E. coli,* 1.31 ± 0.06 for *Klebsiella pneumoniae* and 1.65 ± 0.05 for *Pseudomonas aeruginosa* count when compared with other organic acids films and the control sample. This means *Borassus aethiopum* starch film containing citric acid had the highest inhibitory activity against the three microorganisms tested. Lactic acid films had the second highest inhibitory activity against the three microorganisms tested at 1 % concentration when compared with the control sample and other organic acids films (Tables 4.3, 4.4 and 4,5). This condition could be as a result of the difference of organic acids effectiveness against the microorganisms tested. The inhibition trend of the six organic acids incorporated into *Borassus aethiopum* starch-based films is in the order:

CA ≥ LA ≥ BA ≥ FA ≥ AA ≥ PA for *Escherichia coli* count (cfu/g) $x10^4$

CA ≥ LA ≥ AA ≥ BA ≥ PA ≥ FA for *Klebsiella pneumoniae* count (cfu/g) $x10^4$

CA ≥ LA ≥ AA ≥ PA ≥ BA ≥ FA for *Pseudomonas aeruginosa* count (cfu/g) $x10^4$

The above trend of inhibition indicated that carbon chain length of organic acid could influence the inhibition properties of organic acids. Citric acid incorporated film could be the best film for the storage of food products. Data in Tables 4.3, 4.4 and 4.5 showed that *E. coli* was the most sensitive bacteria against the starch based films containing organic acids, especially the one containing formic, acetic, butyric and pentanoic acids. This condition indicated that there was an adaptation effect of *E. coli* against the organic acids when added to the starch based films. The result was in agreement with the report of

Mallick *et al*. (2020) who noted that *E. coli* basically had an adaptation capacity against the negative effect of organic acids. The *Borassus aethiopum* starch-based films incorporated with citric and lactic acid films that confer best antimicrobial activity was selected for further studies (mechanical properties determination and shelf-life determination of fruits and vegetable).

4.2.4 Mechanical properties of *Borassus aethiopum* starch-based films incorporated with citric and lactic acid at 1 % concentration

The mechanical properties of *Borassus aethiopum* starch-based films incorporated with citric and lactic acid are shown in Table 4.6. There was a significant ($p \leq 0.05$) increase in the thickness of citric and lactic acid films (0.43 mm and 0.39 mm) respectively when compared with the control sample (0.10 mm). The tensile strength of the starch films with incorporated citric and lactic acids was slightly higher (0.58 MPa and 1.40 MPa) respectively compared with the control (pure starch film) (0.52 MPa). These results showed that the addition of the organic acids improved the mechanical properties of the starch-based films. When the organic acids were added into the starch solution, there was a gradual increase in both the tensile strength, modulus of elasticity and percentage elongation (Table 4.6). The significant increment in the tensile strength of the film with the incorporated organic acids indicated the presence of intermolecular interactions in the film.

Consequently, there was a significant ($p \leq 0.05$) increase in modulus of elasticity (2.80 MPa for lactic acid; 2.00 MPa for citric acid film) and percentage elongation when compared with the control sample (1.40 MPa). The increase in percentage elongation of citric acid and lactic acid films (42.00 ± 2.12 and 51.00 ± 2.00) respectively, were the measure of flexibility of the starch based films. An increase in the percentage elongation with the incorporation of organic acids is due to the intermolecular cross-linking and increase in the inter-molecular interactions. The results obtained is in line with the report of Olsson *et al*. (2016). The results of this study shows that the incorporation of citric acid and lactic acid into the starch-based film not only improved the strength of starch-based films but also reduced their brittleness with lactic acid films appearing to be the best. However, starch-based films incorporated with lactic acid at 1 % concentration could be the best film for storage of food products.

4.2.5 Determination of shelf-life of tomato, banana and spinach coated with *Borassus aethiopum* starch-based films incorporated with citric and lactic acids

Borassus aethiopum Starch-Based films that confer best antimicrobial activity (citric acid and lactic acid films) at 1 % concentration were selected for the coating of tomatoes, banana and spinach. The coated tomatoes were observed for a period of 30 days at ambient temperature and was still looking fresh, less decayed when compared with the uncoated (control) sample that started decaying after 10 days. The shelf life of coated bananas was found to be extended up to 10 days and had less shrinkage and less decayed when compared with the control sample that shrink and dried completely after 5 days. The coated spinach was observed for a period of 4 days and showed less decay when compared with the uncoated sample that dried completely on the second day of storage. From the results obtained, starch-based film coatings incorporated with lactic and citric acids was efficient for prolonging the useful life of the fruits and vegetables.

4.2.6 Physico-chemical parameters of tomatoes coated with citric and lactic acid and stored for 30 days

The physico-chemical properties of coated and stored tomato is shown in Table 4.7. From the result obtained, the percentage shrinkage of coated and stored tomatoes with starch-based films incorporated with citric and lactic acids were significantly ($p \leq 0.05$) lower

(72 % and 70 %) respectively compared with the control (82 %). This means starch films incorporated with citric and lactic acid was able to protect the firmness of tomatoes after 30 days of storage.

Total Soluble Solid for both tomatoes coated with citric and lactic acid films decreased with the increase in the storage period (Table 4.7). The tomatoes coated with pure starch film (control) was found to be significantly ($p \leq 0.05$) higher than citric and lactic coated tomatoes. The result suggested that pure starch film (control) could not extend the shelf-life of the tomato fruit when compared with those of citric and lactic acid films coatings. For the coated samples, storage period was found to have a significant effect on the total soluble solid content. The total soluble solids increased during storage (Riveria, 2005). The increase in total soluble solids could be attributed to the breakdown of starch (Ze *et al*., 2022) into sugars (Crouch, 2003) or the hydrolysis of cell wall polysaccharides (Ben and Gaweda, 2018).

The titratable acidity of the stored tomato fruits was also significantly affected by coating materials and storage durations. The control sample showed lower titratable acidity values than those of the citric and lactic acid films coated fruits. This can be explained by the fact that ripening process is faster and consequent conversion of acid into sugar and other substance is greater than citric and lactic acids coated fruits in which respiration and other biochemical activities were slowed down. From the above result, citric and lactic films were able to preserve the tomato compared with the control. The decreasing trend of titratable acidity during storage period was reported by Raleng *et al*. (2018). This finding is in agreement with the report of Formiga *et al*. (2019) who stated that titratable acidity (TA) values decreased with storage time in both the coated and uncoated fruit.

There was a significant increase in pH during storage (Table 4.7). The pH of the stored tomato coated with pure starch film (control) was found to be higher compared to those of citric and lactic acid coated tomato fruits. This might be due to decrease in titrable acidity, as acidity and pH are inversely proportional to each other (Bhardwaj *et al*., 2005). However, during the processing, a large part of the quality characteristics of the fresh fruits undergo remarkable changes which could reduce the nutritional value of the products.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The results from this study confirmed that *Borassus aethiopum* starch-based films incorporated with organic acids (Citric acid and Lactic acid) improved the qualities of the starch-based films. The starch-based films incorporated with citric and lactic acids also showed higher inhibitory activities against *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* at 1 % concentration.

Tomatoes, banana and spinach coated with *Borassus aethiopum* starch-based films incorporated with citric and lactic acids at 1 % concentrations had extended shelf-life up to 30 days for tomato, 10 days for banana and 4 days for spinach.

5.2 Recommendations

- Further study is recommended on purely starch-based films with other blends like protein for improved film properties.
- The combination of citric and lactic acid films for the storage of other fruits and vegetables is also recommended.

5.3 Contribution of Research to Knowledge

The result obtained for *Borassus aethiopum* starch-based films incorporated with citric and lactic acids at one percent concentrations caused remarkable inhibition of spoilage microorganisms in fruits and vegetable. Citric and lactic acid films can be used as a surface coating on fruits and vegetable to extend their shelf-life.

REFERENCES

Ahmadi, R., Ghanbarzadeh, B., & Ayaseh, A. (2019). The antimicrobial bio-nanocomposite containing non-hydrolysed cellulose nanofiber (CNF) and miswak extract. *Journal of Carbohydrate Polymer*, 214, 15-25.

Ahmed, A., Djibrilla, A., & Clerge, T.S. (2010). Physico-chemical properties of palmyrah palm (*Borassus aethiopum*) fruits from Northern Cameroon. *African Journal of Food Science*, 4(3), 115 – 119.

Akinniyi, J.A., Waziri, M., & Usman, H. S. (2010). Assessment of the anabolic effect of androgens of the edible portion of the shoot of giginya plant (*Borassus aethiopum* mart). *Journal of Scientific Research*, 2(2), 362-368.

American Society for Testing Materials - ASTM. (2020). *Standard test method tensile properties of thin plastic sheeting* (D882-02). Pennsylvania: ASTM International.

Arfat, Y. A., Ejaz, M., Jacob, H., & Ahmed, J. (2017). Deciphering the potential of guar gum/Ag-Cu nanocomposite films as an active food packaging material. *Carbohydrate Polymers*, 157, 65-71.

Association of Official Analytical Chemists – AOAC. (2019). *Official methods of analysis of AOAC international* (19th ed.). Arlington: AOAC.

Ayari, A., Achir, N., Servent, A., Ricci, J., & Brat, P. (2020). Development of a nutritional profile predicting tool for fresh and processed tomato-based products. *International Journal of Food Science and Technology*, 50, 1598–1606

Banerjee, S., & Arora, A. (2023). Microbial processing for valorization of horticultural wastes. *Journal of Evironmental Microbial and Biotechnology*, 28(2), 203–221.

Ben, J., & Gaweda, M. (2018). Changes of pectic compounds in jonathan apples under various storage conditions. *Journal of Acta Physiologiae Plantarum*, 7, 45–54.

Bhardwaj, R.L., Sen, N.L., & Mukherjee, S. (2005). Effect of benzyl adenine on physico-chemical characteristics and shelf-life of Mandarin Nagpur santra. *Indian Jornal of Horticulture*, 62(2), 181–183.

Cho, C., Choi, S.Y., Luo, Z.W., & Lee, S.Y. (2015). Recent advances in microbial production of fuels and chemicals using tools and strategies of systems metabolic engineering. *Journal of Biotechnology Advances*, 33(7), 1455–1466.

Colussi, R., Avila L., & Singh, L. (2021). Potential use of starch from different sources in the preparation of mucoadhesive films. *International Journal of Biological Macromolecule, 13, 1-9.*

Crouch, I. (2003). 1–Methylcyclopropene as an alternative to modified atmosphere and controlled atmosphere storage of apples and pears. *Journal of Acta Horticulture*, 600, 433–436.

Daoud, A., Maliki, D., Bakari, S., Hfaiedh, N., Mnafgui, K., & Kadri, A. (2015). Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of date palm pollen (DPP) from two Tunusian cultivars. *Arabian Journal of Chemistry*, 8, 10-16.

Dhull, S.B., Malik, T., & Kaur, R. (2021). Banana starch: Properties illustration and food applications. *Starch Starke Review*, 73, 1-2.

Erna, K.H., Felix, W.L., Rovina, K., Vonnie, J.M., & Huda, N. (2022). Development of curcumin/rice starch films for sensitive detection of hypoxanthine in chicken and fish meat. *Carbohydrate Polymer Technology Application*, 3, 367-385.

Evgeniy, V.P., & Christoph, H.B. (2022). Protein chemistry combined with mass spectrometry for protein structure determination. *Journal of Chemistry Reserve*, 122(8), 7488-7499.

Formiga, A. S., Pinsetta Junior, J. S., Pereira, E. M., Cordeiro, I. N., & Mattiuz, B. H. (2019). Use of edible coatings based on hydroxypropyl methylcellulose and beeswax in the conservation of red guava. *Journal of Food Chemistry*, 290, 144-151.

Genanew, T. (2019). Effect of postharvest treatments on storage behavior and quality of tomato fruits. *World Journal of Agricultural Sciences*, 9, 29-37.

Guan, N., & Liu, L. (2019). Microbial response to acid stress: Mechanisms and applications. *Journal of Applied Microbiology and Biotechnology*, 104, 51-65.

Henz, G. P. (2017). Postharvest losses of perishable fruits in brazil. *Horticultura Brasileira*, 35(1), 6-13.

Hirshfield, I.N., Terzulli, S. and Byrne, C. (2018). Weak organic acids: A panoply of effects on bacteria. *Journal of Science Progeny*, 86, 245–269.

Honda, Y., Yamazaki, T., Katsumi, N., Fujita, N., Matsumota, K., Okazaki, M., & Miwa, S. (2019). Physicochemical properties of starches from *Lotus rhizomes* harvested in different months. *Journal of Applied Glycoscience*, 66(2), 51-57.

Huang, L., Tan, H., Zhang, C., Li, Q., & Liu, Q. (2021). Starch biosynthesis in cereals endosperms. *Journal of Plant Biology*, 2(5), 100-117.

Kaur, L., Dhull, S.B., & Kumar, P. (2020). Banana starch: Properties, description, and modified variations: A review. *International Journal of Biological Macromolecule*, 165, 2096-2098.

Lee, J.Y., Kang, C.D., & Lee, S.H. (2015). Engineering cellular redox balance in saccharomyces cerevisiae for improved production of L-lactic acid. *Journal of Biotechnology and Bioengineering*, 112, 751–758.

Li, M., & Borodina, I. (2015). Application of synthetic biology for production of chemicals in yeast *Saccharomyces cerevisiae*. *Journal of Microbiology and Biotechnology*, 15(1), 1–12.

Lin, S. (2010). Chemical modification of starch and preparation of starch-based nanocomposites. *A dissertation presented to the faculty of the University of Akron*, 72-75.

Liu, Y., Espinosa, C.D., Abelilla, J.J., Casas, G.A., Lagos, L.V., Lee, S.A., Kwon, W.B., Mathai, J.K., Navarro, D.M., & Jaworski, N.W. (2018). Non-antibiotic feed additives in diets for pigs: A review. Journal of *Animal Nutrition*, 4, 113–125.

Long, S.F., Xu, Y.T., Pan L., Wang, Q.Q., Wang, C.L., Wu, Y.Y., Han, Y.M., Yun, C.H., & Piao, X.S. (2018). Mixed organic acids as antibiotic substituents improve performance, serum immunity, intestinal morphology and microbiota for weaned piglets. *Journal of Animal Feed Science Technology*, 235, 23-32.

Magnuson, J.K., & Lasure, L.L. (2018). Organic acid production by flamentous fungi. *Advances in Fungal Biotechnology for Industry, Agriculture, and Medicine*, 8, 307–340.

Mai, A., Pankaj, B.P., & Adil, A. (2021). Effect of storage conditions on postharvest quality of tomatoes. *Journal of Agricultural and Marine Science,* 26(1), 13-20.

Mallick, N., Pal, D., Soni, B.A., Jhariya, D., & Singh, D. (2020). Starch-based antimicrobial food packaging film towards a sustainable environment. *Journal of Earth and Environmental Science*, 597(1), 1315-1755.

Mazumdar, B.C. (2018). Palmyrah the widely grown palm of India of versatile use. *Journal of World Crops*, 7(2) 20 -25.

Muhammad, S.J., Umar, K.J., & Hassan, S.W. (2019). Proximate value and mineral content of the shoots of *Borassus aethiopum* mart (Giginya). *Journal of Chemical Society of Nigeria*, 36(1), 10 – 14.

Olsson, E., Hedenqvist, M.S., Johansson C., & Jarnstrom, L. (2016). Influence of citric and curing on water sorption, diffusion and permeability of starch films. *Journal of Carbohydrate Polymers*, 94(2),765-772.

Olusauya, J.O., Mohan, T.P., & Kanny, K. (2023). Effect of nano-clay incorporation method on mechanical and water vapor barrier properties of starch-based films. *Journal of industrial crops and products*, 33, 605-610.

Panda, S.K., Mishra, S.S., Kayitesi, E., & Ray, R.C. (2016). Microbial-processing of fruit and vegetable wastes for production of vital enzymes and organic acids. *Journal of biotechnology and Environmental Resources*, 146,161–172.

Papatsiros, V.G., & Billinis, C. (2012). The prophylactic use of acidifiers as antibacterial agents in swine. *Journal of Industrial* Microbiology, 9, 295–310.

Priyadarshi, R., Sauraj, K. B., & Negi, Y. S. (2018). Chitosan film incorporated with citric acid and glycerol as an active packaging material for extension of green chilli shelf life. *Carbohydrate Polymers*, 195, 329-338.

Qureshi, N. (2017). Solvent (Acetone-Butanol) production. *Reference Module in Life Sciences*, 3, 1–20.

Raleng, A., Manikantan, M.R., & Mishra, A.A. (2018). Effect of packaging materials on storage stability of protein rich flour from deoiled sesame cake. *International Journal of Advanced Research*, 2(12), 248-254.

Riveria, J. (2005). Cutting shape and storage temperature affect overall quality of fresh cut papaya. *Maradology Journal of Food Science*, 70(7), 488-489.

Rosalina, Y., Endang, K., & Sri, P. (2020). Characterization of lactic acid bacteria and antimicrobial activity in Sui wu'u. *Asian Journal of Pharmaceutical and Clinical Research*, 13(1), 36-76.

Sakande, J., Nikiema, A., Kabre, E., Lompo, M., & Nikiema, J.B. (2019). In vitro assay of potential antifungal and antibacterial activities of extracts of *Borassus aethiopum* mart. *International Journal of Nigerian Society*, 51, 280-282.

Salako, V. K., Vihotogbé, R., Houéhanou, T., Sodé, I. A., & Glèlè Kakaï, R. (2019). Predicting the potential impact of climate change on the declining agroforestry species Borassus aethiopum Mart. in Benin: a mixture of geostatistical and SDM approach. Agroforestry Systems, 93, 1513-1530.

Salsali, H.R., Parker, W.J., & Sattar, S.A. (2008). The effect of volatile fatty acids on the inactivation of *Clostridium perfingens* in anaerobic digestion. *World Journal of Microbiology and Biotechnology*, 24, 659-665.

Sastry, N.Y., Padmaja, I.J., Rao, R.P., Kirani, K.R., & Kaladhar, D. (2018). In vitro dose dependant study on anti-human pathogenic bacterial and free radical scavenging activities of methanolic seed coat extract of *Borassus aethiopum. Asian Journal of Phamacology and Clinical Research*, 5, 83-86.

Satturwa, P.M., Fulzels, S.V., & Dorle, A.K. (2019). Biodegradation and in vivo biocompatibility of rosin: A natural film -forming polymer. *Journal of Pharmaceutical Science and Technology*, 4, 1-6.

Shah, U., Naqash, F., Gani, A., & Masoodi, F. A. (2016). Art and science behind modified starch edible films and coatings: A Review. *Comprehensive Reviews in Food Science and Food Safety*, 15(3), 568-580.

Silva, O. A., Pellá, M. G., Caetano, J., Simões, M. R., Bittencourt, P. R. S., Dragunski, D. C. (2019). Synthesis and characterization of a low solubility edible film based on native cassava starch. *International Journal of Biological Macromolecules*, 128, 290-296.

Sirajo, M., Umar, K. J., & Hassan, S. W. (2019). Toxicity studies of African Palmyrah palm (Borassus Aethiopum) shoots. African Journal of Pure and Applied Chemistry, 13(2), 27-33.

Sjöblom, M., Matsakas, L., Christakopoulos, P., & Rova, U. (2016). Catalytic upgrading of butyric acid towards fine chemicals and biofuels. *Journal of Microbiology*, 363, 1–7.

Sneh, P.B., Shweta, S., Monica, T., & Fatih, O. (2022). Organic acid production from lactic acid bacteria: A preservation approach. *Journal of Food Sciences*, 46(1), 101-115.

Suyama, A., Higuchi, Y., & Urushihara, M. (2017). Production of 3-hydroxypropionic acid viathemalonyl-CoA pathway using recombinant fission yeast strains. *Journal of Bioscience and Bioengineering*, 124(4), 392–399.

Tan, H.L., Thomas-Ahner, J.M., Grainger, E.M., Wan, L., & Francis, D.M. (2021). Tomato-based food products for prostate cancer prevention. *Journal of Cancer Metastasis Reverve*, 29, 553–568.

Thakore, I.M., Desai, S., Sarawade, B.D., & Davi, S. (2022). Studies on biodegradability, morphology and thermomechanical properties of LDPE/modified starch blends. *Journal of European Polymer*, 37(1), 151-160.

Valencia, G. A., Lourenco, R. V., Bittante, A. M., & Amaral-Sobral, P. J. (2016). Physical and morphological properties of nanocomposite films based on gelatin and laponite. *Journal of Applied Clay Science*, 124, 260-266.

Vandenberghe, L.P.S., Karp, S.G., & Oliveira, P.Z. (2018). Solid-state fermentation for the production of organic acids. *Current Development in Biotechnology and Bioengineering*, 13, 415–434.

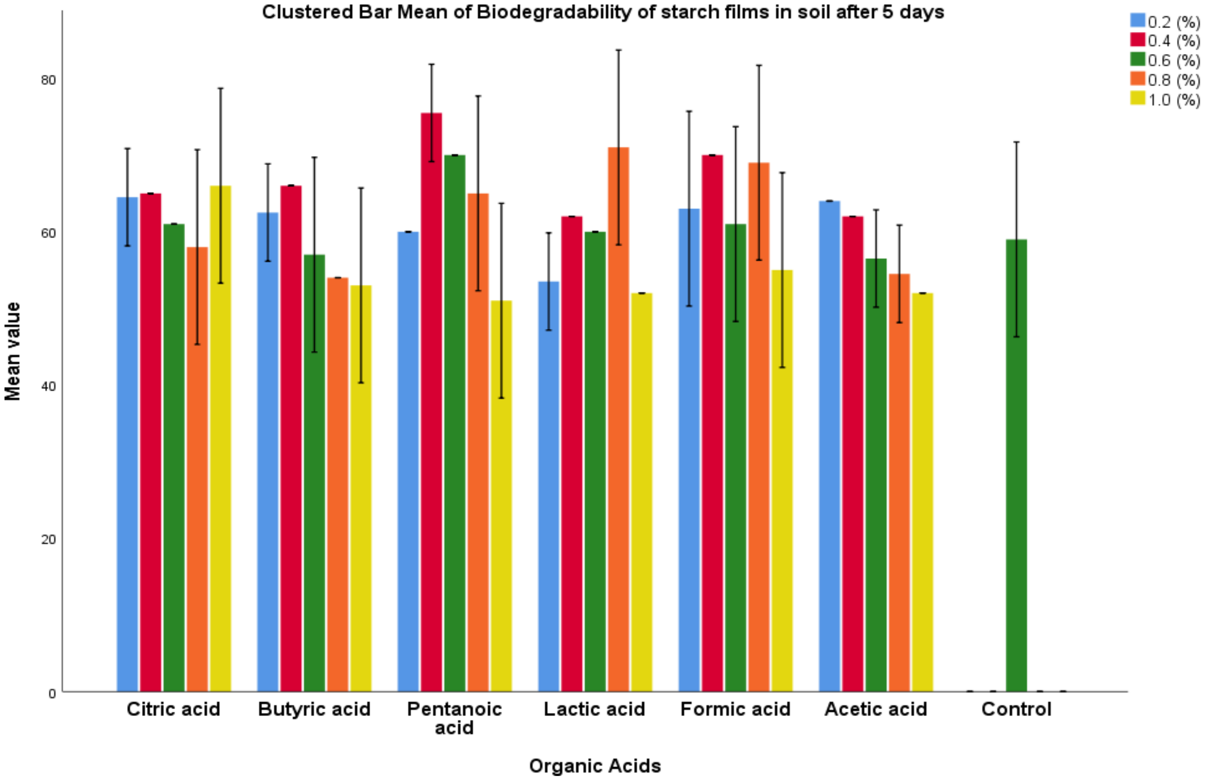
Xiao, Z., Cheng, C., Bao, T., Liu, L., Wang, B., Tao, W., Pei, X., Yang, S.T., & Wang, M. (2018). Production of butyric acid from acid hydrolysate of corn huskin fermentation by *Clostridium tyrobutyricum*: Kinetics and economic analysis. *Journal of Biotechnology and Biofuels*, 11, 164-166

Ze, Y., Huijun, G., Xi, C., Xuewu, D., & Yueming, J. (2022). The role of hydrogen water in delaying ripening of banana fruit during postharvest storage. *Journal of Food Chemistry*, 373(1), 131-590

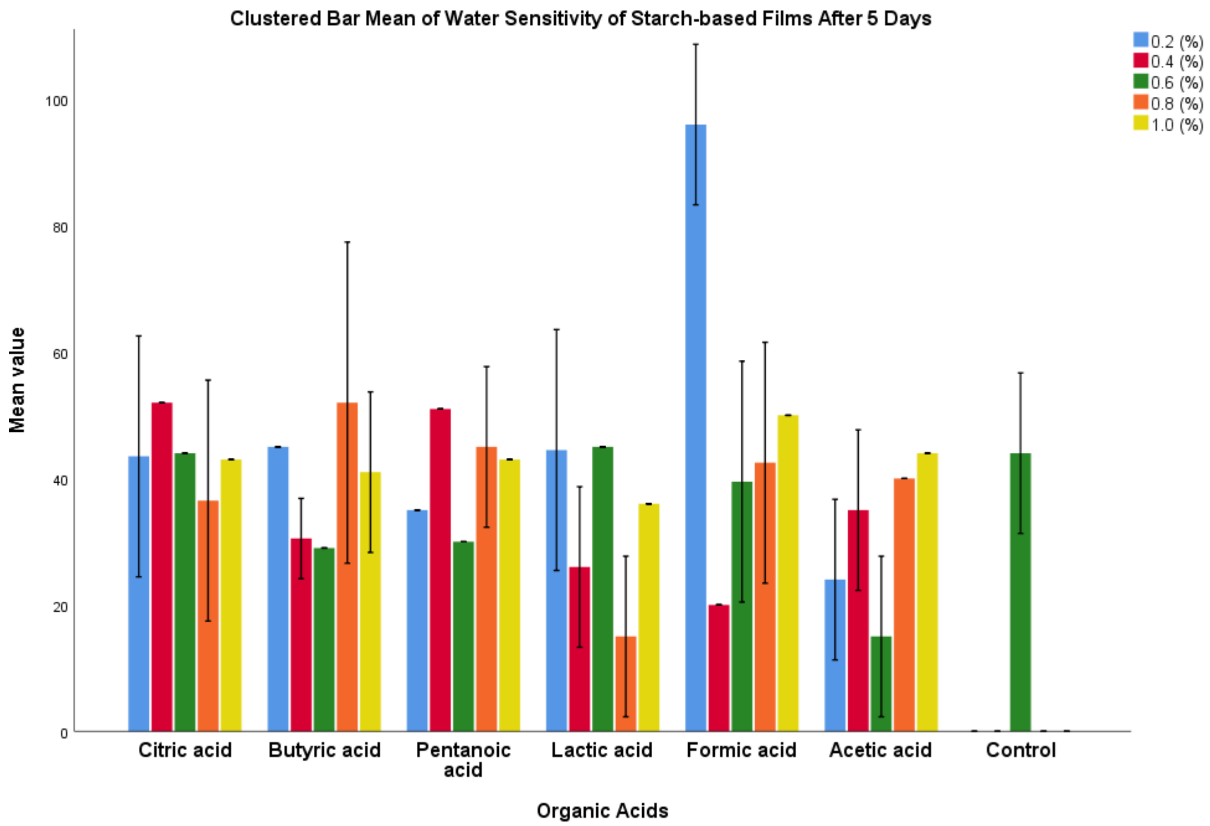
Zentek, J., Ferrara, F., Pieper, R., Tedin, L., Meyer, W., & Vahjen, W. (2019). Effects of dietary combinations of organic acids and medium chain fatty acids on the gastrointestinal microbial ecology and bacterial metabolites in the digestive tract of weaning piglets. *Journal of Animal Sciience*, 91, 3200–3210.

APPENDICES

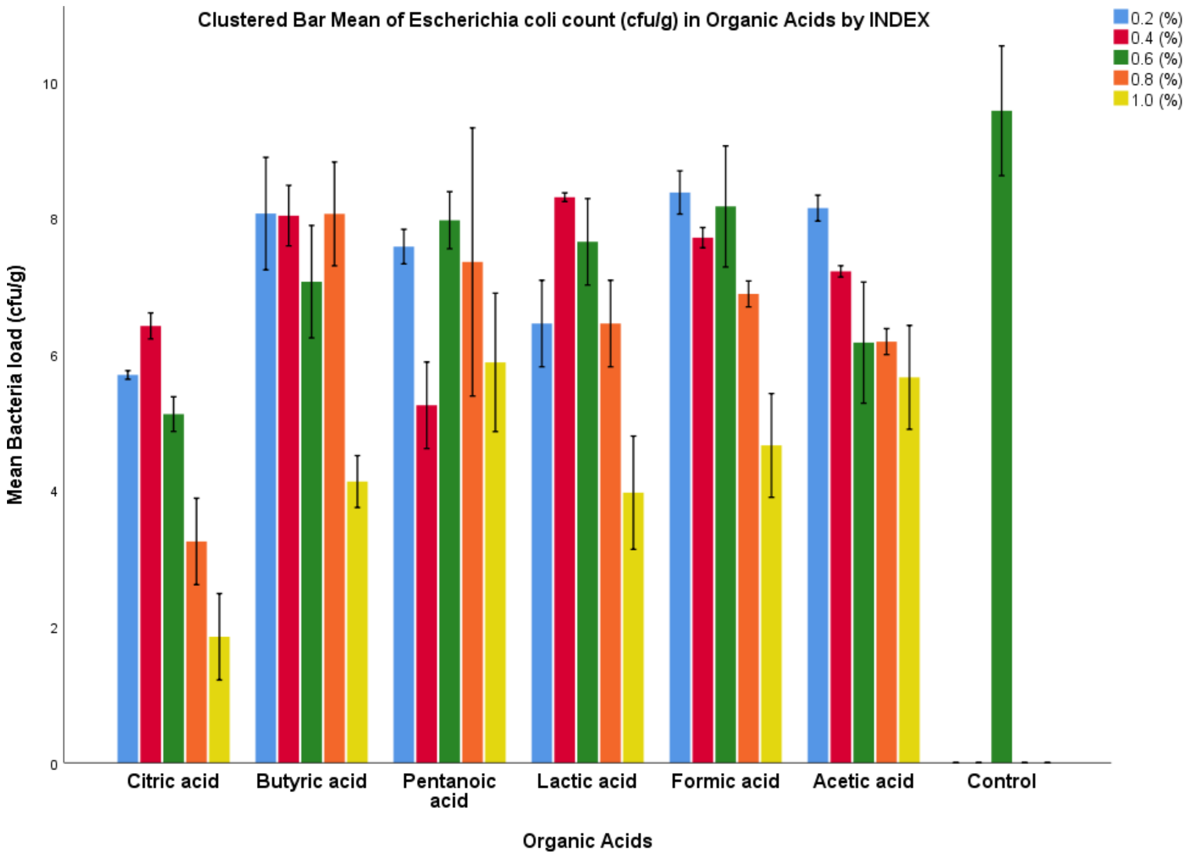
APPENDIX I: Percentage Degradation of *Borassus aethiopum* Starch Films in Soil after Five Days



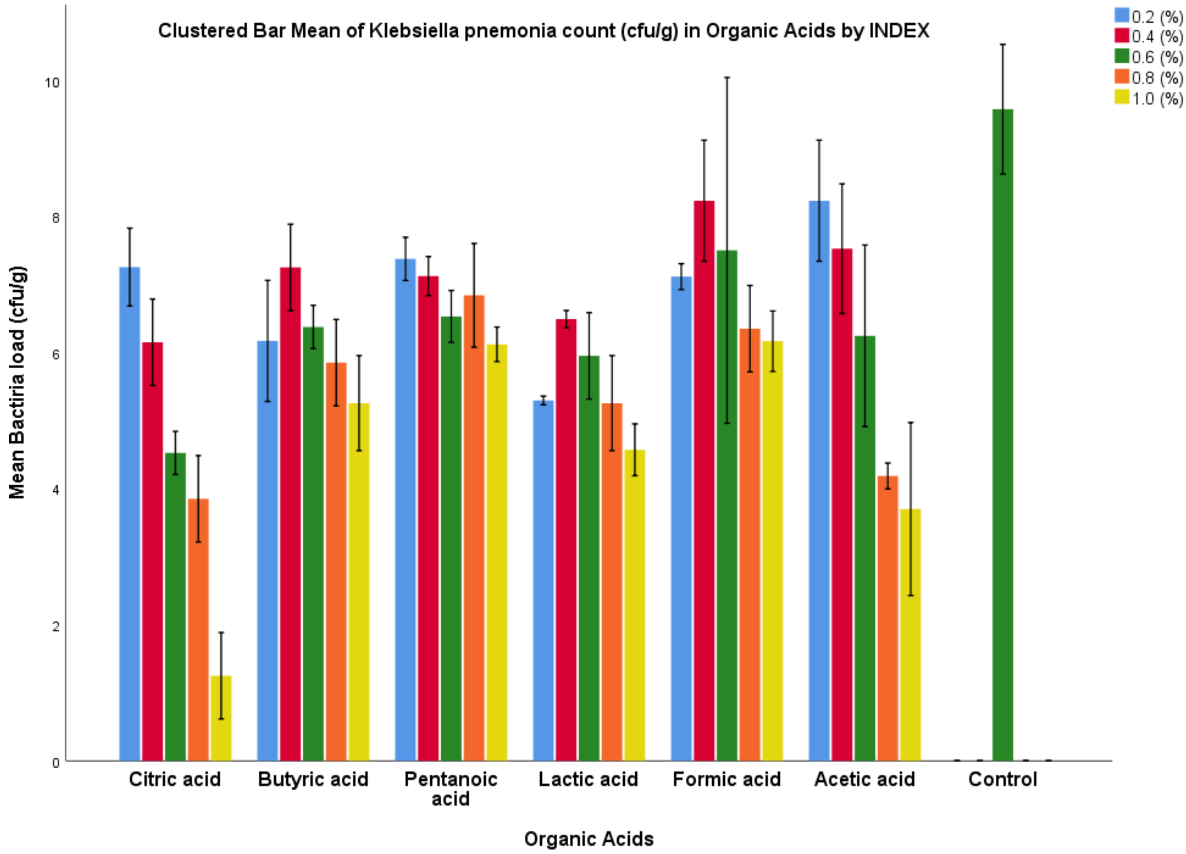
APPENDIX II: Percentage Water Absorption of *Borassus aethiopum* Starch films after Five Days



APPENDIX III: Inhibitory Effect of *Borassus aethiopum* Starch Films Incorporated with Organic Acids Against *Escherichia coli*



APPENDIX IV: Inhibitory Effect of *Borassus aethiopum* Starch Films Incorporated with Organic Acids Against *Klebsiella pneumonia*



APPENDIX V: Inhibitory Effect of *Borassus aethiopum* Starch Films Incorporated with Organic Acids Against *Pseudomonas aeruginosa*