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DECLARATION

I am Sharmin Parvin, hereby declare that this thesis paper is the result of my own works and it has not been submitted or accepted for acceptance degree in any other university.

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Sharmin Parvin

DEDICATED TO

MY BELOVED PARENTS...

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ABSTRACT

The study was designated to evaluate the effectiveness of guava leaf extract as preservative for fruits. Guava leaf extract was extracted from mature green leaves by the hot water extraction method. The 0.5% guava leaf extract was sprayed on the surface of banana, carambola and tomato. The treated fruits were stored in a safe place in the laboratory along with the controlled one. All the fruits were checked every day until the controlled fruits were damaged completely. The fruits were tested for weight loss, pH, percentage disease index (PDI), protein content, carbohydrate content and finally the moisture content. The weight loss, pH, PDI and all other properties showed better performance compared with the untreated fruits of that category. The results of this study showed that guava leaves extract have excellent potential to be used on fresh produce to maintain quality and to extend the shelf-life of different fruits.

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CHAPTER ONE: INTRODUCTION

1. Introduction

1. 1 Background of the Study

Food production and supply does not always tally with the demand or meets of the people. In some places there is surplus production of a food product, whereas in some other place there is inadequate supply (Rasooli, 2007). Even foods are perishable and semi-perishable like juicy fruits, vegetables, mangoes, tomato, papaya and many more, which very quickly gets spoilt. It is therefore important to improve and expand facilities for storage and preservation of food (Gatto, 2011). Preservation and storage problems are the two most important challenges of food products continuous supply for both on-season and off-season (Kanaani and Ginsburg, 1992). At present, almost every food products are sold far distance from its production site. So, declination of food product's shelf-life associate with large volume of food loss occurred due to microbial activity and physiological activity of the food itself (Ofor, 2011). There comes the need of the preservation process to extend the shelf-life (preservation) of food products for fulfilling the consumer demand (Jean, 1994).

Artificial preservative like sodium benzoate, potassium meta bisulphate, and citric acid are also used to preserve food. Salt, sugar, lemon juice, spice etc. is used to preserve food and known as natural preservative. Recently there has been a lot of attention focused on producing medicines and products that are natural (Rukayadi *et al.,* 2013). Modern technology has been widely introduced in the branch of food preservation like drying, freeze drying, freezing, vacuum packing, canning, microwaving or irradiating, pickling, salting, smoking, and preserved in syrup, alcohol, and sugar (Tajkarimi *et al.,* 2010). All these food preservation methods are broken down into three categories, i.e., antimicrobial – prevent the growth of yeasts, moulds, and bacteria; antioxidants - slow the oxidation of fats and lipids; and ripening inhibition - slow down the enzymatic processes of ripening after harvest (Cowan, 1999). Additives are added to the foods for most of these three types of food preservation. In developed countries, these food additives or preservatives must receive a generally recognized-as-safe (GRAS) status of the respective authority, and are not only helpful, but also healthful to people as well as to other animals.

Chemical fungicides can control postharvest pathogens to a certain limit of efficiency (Foster *et al.*, 2007). However, the situation is quite critical in developing countries like Bangladesh (Gatto *et al.*, 2011).

In Bangladesh, different techniques are practiced for food preservation including traditional as well as modern food preservation methods. However, now a day these foods are being preserved and contaminated by toxic and toxic levels of chemicals like formalin (37% formaldehyde solution), calcium carbide, sulphuric acid, industrial dyes etc. The chemical fertilizer urea is used to whiten rice; fruits, fish and vegetables in kitchen markets are sprayed and stored in formalin to keep fresh look and increase storage (Garcia, 2002). Synthetic colors and sweeteners are also injected into fruits. Use of these preservatives has been reported as critical for human health and environment. The use of these toxic preservatives not only creating major health concerns but also incurring economic loss to the producers and traders as adulterated food items are destroyed in government crackdown and unwilling consumers. Therefore, it is urgent to find eco-friendly and healthy preservatives for food items to save consumer, producers, traders, and economy. Different plant species (Bhatnagar, 1988) and marine resources (Richards *et al.*, 2005) have been studied to determine their efficacy in food preservation in other countries. Success stories of plant and marine resource based preservatives are emerging in food science and seen as revolution of green technology in the science of food preservation. Tiilikkala (2010) reported that the discovery of plant based preservatives has been found health and eco-friendly low cost technology.

Variety of different components of the plant like bark, fruit, flower, roots, leaves, etc. may provide extracts with antimicrobial activity and many of them have been enjoyed generally recognized-as-safe (GRAS) status (Negi, 2012). The adsorption of polyphenols to bacterial membrane with membrane disruption and subsequent leakage of cellular contents (Otake *et al.* 1991) with the combination of hydroperoxide generation from polyphenols (Akagawa *et al.*, 2003) are the most likely the reasons of antibacterial activity of plant extracts. Some studies have shown that plant extracts also provide antifungal properties from a wide range of fungi ((Davidson and Parish, 1989; Grange and Ahmed, 1988; Jayaprakasha *et al.*, 2001); antioxidant and antimutagenic activities (Boubaker *et al.*, 2011; Cherdshewasart *et al.*, 2009; Horn and Vargas, 2003) and inhibited lipid oxidation in food (Shan *et al.*, 2009). Although, many studies

have been done in vitro on the antimicrobial properties of plant extracts, but a very little was done on the food products especially in the underdeveloped or developing country like Bangladesh. This study was designed to extract the guava (*Psidium guajava*) leaf extract by the hot water method which was then applied to preserve the banana (*Musa acuminata*), carambola (*Averrhoa carambola*) and tomato (*Solanum lycopersicum*). It was conducted to find out the way to preserve food for a moderate time with the best return with guava leaf extract.

1.2 Objectives of the study

Foods get spoilt mainly due to the presence of microorganisms, enzymes (present in food), insects, worms and rats. As chemical preservative is health hazardous and environmentally not sound, people look for a natural one. There are bioactive components in the guava leaf that can fight against pathogens, regulate blood glucose levels, and can even aid in weight loss. The leaves of guava contain an essential oil rich in cineol, tannins, triterpenes, flavonoids, resin, eugenol, malic acid, fat, cellulose, chlorophyll, mineral salts, and a number of other fixed substances. The present study is carried out by evaluation of antifungal properties of *Psidium guajava* against fungal pathogens. Measure the availability of guava leaves and its production because this has the possibility to use as a food preservative.

Thus, this research has been conducted with the following specific objective -

- To assess the efficacy of guava (*Psidium guajava*) leaf extracted by hot water extraction method to lengthen the shelf life of banana (*Musa acuminata*), carambola (*Averrhoa carambola*) and tomato (*Solanum lycopersicum*).

CHAPTER TWO: LITERATURE REVIEW

2. Literature Review

The problem of protecting food from spoilage has been with us since prehistoric times. The solutions to this problem have changed with advances in technology and knowledge about what causes food to spoil. This project will focus on retarding microbial growth, which is only one of the causes of food spoilage.

Food preservation involves preventing the growth of bacteria, fungi (such as yeasts), or other micro-organisms (although some methods work by introducing benign bacteria or fungi to the food), as well as retarding the oxidation of fats that cause rancidity. Food preservation may also include processes that inhibit visual deterioration, such as the enzymatic browning reaction in apples after they are cut during food preparation. Antioxidant preservatives stop the chemical breakdown of food when products are exposed to the air. Unsaturated fatty acids in oils and lipids are especially susceptible to oxidation and will take on a rancid flavor and odor as a result. There are many ways that food can be spoiled. For example, oils in food can become oxidized, releasing free fatty acids that cause a bitter, rancid taste. Additionally, natural enzymes that take part in the ripening process of fruits and vegetables can remain active after harvest, causing spoilage. Different chemical preservatives have been developed to counteract each of these different mechanisms: Preservatives can be categorized into three general types: antimicrobials that inhibit growth of bacteria, yeasts, or molds; antioxidants that slow air oxidation of fats and lipids, which leads to rancidity; and a third type that blocks the natural ripening and enzymatic processes that continue to occur in foodstuffs after harvest (Dalton, 2002).

In order for an antimicrobial preservative to work, it must be used at the right concentration. Ideally, it will disrupt microbial growth while at the same time preserving most of the nutritional value of the food.

2.1 Methods of Preservation

2.1.1 Traditional Method

There are several traditional methods of food preservation used at the household level that can be classed as chemical methods (Ofor, 2011). Substances such a sugar, salt, vinegar, spices and

wood-smoke are generally regarded as safe and natural preservatives. Salting, sugaring and smoking are all methods of curing foods. Curing is a general term that covers all these types of food preservation.

a) Salting

Salt draws water out of cells via the process of osmosis. Essentially, water moves across a cell membrane to try to equalize the salinity or concentration of salt on both sides of the membrane. If you add enough salt, too much water will be removed from a cell for it to stay alive or reproduce. Organisms that decay food and cause disease are killed by a high concentration of salt. A concentration of 20% salt will kill bacteria. Lower concentrations inhibit microbial growth, until you get down to the salinity of the cells, which may have the opposite and undesirable effect of providing ideal growing conditions.

b) Sugaring

Sugaring refers to the action of sugar in food preservation. It is similar to the action of salt in that it depends on the removal of water. In concentrations of at least 65%, sugar solution is widely used as a sweetening and preserving agent. However, care is needed because at low concentrations, sugar solution can support the growth of microorganisms. It has been found that microorganisms rarely survive in solutions above 20–25% sugar concentration.(Hamdi, 2007).

c) Smoking

Smoking is one of the oldest methods used to improve the quality of food and is commonly used to preserve meat and fish. The smoking process involves exposing food to smoke from burning or smoldering wood or other plant material. It partially preserves the food by surface drying, i.e. removing moisture from the surface of the food, but it is not a reliable method of preservation unless combined with some other method such as salting or drying.

d) Spices

Spices also have some uses in food preservation because they tend to inhibit the growth of staphylococci and other bacteria. However, they have a very limited application because they often get contaminated themselves by a number of bacteria.

2.1.2 Industrial Method

a) Pasteurization

Pasteurization is a process for preservation of liquid food. It was originally applied to combat the souring of young local wines. Today, the process is mainly applied to dairy products. In this method, milk is heated at about 70 °C for 15 to 30 seconds to kill the bacteria present in it and cooling it quickly to 10 °C to prevent the remaining bacteria from growing. The milk is then stored in sterilized bottles or pouches in cold places. This method was invented by Louis Pasteur, a French chemist, in 1862.

Advantages

- It does not produce an unpleasant cooked flavor.
- Shelf life of milk is increased due to a marked decrease in the total bacterial count.
- Harmful pathogens, especially TB bacteria are destroyed.
- It inactivates enzymes such as phosphates and lipase in milk, which adversely affect the quality of milk.

Disadvantages

- Proteins are denatured only slightly and minerals are not appreciably precipitated.

b) Vacuum packing

Vacuum-packing stores food in a vacuum environment, usually in an airtight bag or bottle. The vacuum environment strips bacteria of oxygen needed for survival. Vacuum-packing is commonly used for storing nuts to reduce loss of flavor from oxidization. A major drawback to vacuum packaging, at the consumer level, is that vacuum sealing can deform contents and rob certain foods, such as cheese, of its flavor (Cowan, 1999).

c) Artificial food additives

Preservative food additives can be *antimicrobial*, which inhibit the growth of bacteria or fungi, including mold, or *antioxidant*, such as oxygen absorbers, which inhibit the oxidation of food constituents. Common antimicrobial preservatives include calcium propionate, sodium nitrate, sodium nitrite, sulfites (sulfur dioxide, sodium bisulfate, potassium hydrogen sulfite, etc.) and

disodium EDTA. Other preservatives include formaldehyde (usually in solution), glutaraldehyde (kills insects), ethanol, and methyl chloro isothiazolinone.

d) Irradiation

Irradiation of food is the exposure of food to ionizing radiation. The two types of ionizing radiation used are beta particles (high-energy electrons) and gamma rays (emitted from radioactive sources as cobalt-60 or cesium-137). Treatment effects include killing bacteria, moluds, and insect pests, reducing the ripening and spoiling of fruits, and at higher doses inducing sterility. The technology may be compared to pasteurization; it is sometimes called "cold pasteurization", as the product is not heated. Approximately 500,000 tons of food items are irradiated per year worldwide in over 40 countries.

Advantages

- Prevention of post-harvest losses by destruction of insects in stored cereals, fresh and dried fruits, nuts, oilseeds and pulses, or phytosanitary (quarantine) treatment for insect pests infesting fresh fruits and vegetables.
- It causes inactivation/destruction of various food-borne parasites.
- It shortens drying and cooking times of vegetables and fruits.

e) Hurdle technology

Hurdle technology has been defined by Leistner (2000) as an intelligent combination of hurdles that secures the microbial safety and stability as well as the organoleptic and nutritional quality and the economic viability of food products.

f) Modified atmosphere

Modifying atmosphere is a way to preserve food by operating in the atmosphere around it. Salad crops that are notoriously difficult to preserve are now being packaged in sealed bags with an atmosphere modified to reduce the oxygen concentration and increase the carbon dioxide concentration. There is concern that, although salad vegetables retain their appearance and texture in such conditions, this method of preservation may not retain nutrients, especially vitamins. There are two methods for preserving grains with carbon dioxide. One method is placing a block of dry ice in the bottom and filling the can with the grain. Another method is

purging the container from the bottom by gaseous carbon dioxide from a cylinder or bulk supply vessel.

g) Non-thermal plasma

This process subjects the surface of food to a "flame" of ionized gas molecules, such as helium or nitrogen. This causes micro-organisms to die off on the surfaces.

h) High-pressure food preservation

High-pressure food preservation or pascalization refers to the use of a food preservation technique that makes use of high pressure. "Pressed inside a vessel exerting 70,000 pounds per square inch (480 MPa) or more, food can be processed so that it retains its fresh appearance, flavor, texture and nutrients while disabling harmful microorganisms and slowing spoilage.

2.2 Types of Preservative

2.2.1 Artificial preservatives

Artificial preservatives are chemical compounds synthesized for use in food to prolong its shelf life. Chemical compounds added into food to enhance color, retard spoilage, preserve texture and increase shelf life. The primary purpose of artificial preservatives is to enable bakery products to remain fresh and at high quality during transport and delivery to consumers. Artificial preservatives prevent spoilage by two ways: 1) microbial contamination or 2) oxidation leading to rancidity (Negi, 2012).

The three major categories of artificial preservatives are:

a. antimicrobials, (microbial contamination is stopped or delayed by antimicrobials),
b. antioxidants, (antioxidants inhibit or delay oxidation in oils as well as fatty foods, and
c. chelating agents (a chelating agent prevents spoilage by binding aforementioned metal ions, copper or iron, slowing the act of oxidation).

a. Antimicrobials

Sorbets : Sorbet acid combined with mineral salts such as calcium sorbate, potassium sorbate, and sodium sorbate. Easily dissolves in water. Prevents bacteria, mould, and fungi growth in food products.

Potassium sorbate: the product of the reaction of potassium hydroxide and sorbic acid, is utilized in baking to inhibit mould growth.

Benzoates: Salts of benzoic acid utilized to inhibit growth of mould and fungi in acidic liquids such as vinegar, fruit juices, and soft drinks.

Propionates: Created from the salts of propionic acid such as calcium, sodium, or potassium propionate. Utilized in the baking industry to prevent molud growth in baked items such as breads, muffins, pastries, and cakes and subsequently increasing product shelf life.

Nitrates: Salts of nitric acid. Utilized in the food industry mainly to prevent the growth of *Clostridium botulinum* in meat items, as well as enhance color (Lee, 2011).

b. Antioxidants

Sulfites: Sodium sulfite, sodium bisulfite, sodium metabisulfite, potassium bisulfite, and potassium metabisulfite are artificial preservatives used to preserve wine or beer, fruits, meats, and dried potato products. In the banking industry, sulfites are found in potato chips and snack items to prolong freshness.

Ascorbic Acid: Also known as vitamin C, occurs naturally in many fruits as well as vegetables. When utilized as an artificial preservative, ascorbic acid increases longevity and freshness of fresh cut fruits or vegetables or juices.

Butylated Hydroxyanisole (BHA): A waxy, yellow solid used to prevent oxidation in baked items, as well as many items across the board in the food industry.

Butylated Hydroxytoluene (BHT): A white powdery substance added to packaging and fats or oils. Of course, in baking, BHT is found in shortening which is very similar to BHA.

c. Chelating Agents

Disodium Ethylenediaminetetraacetic acid (EDTA): EDTA is used in food processing to bind manganese, cobalt, iron, or copper ions in order to retard oxidation.

Polyphosphates: Inhibits or slows the act of oxidation, preventing browning in food products such as fruits or vegetables.

Citric Acid: Present naturally in many foods; in addition to utilization as a chelating agent, citric acid prolongs shelf life and adds flavor to food items (Hirasawa, 1991).

2.2.2 Natural Preservative

Nature gave us real food to preserve other foods the simple way, in a much healthier fashion. Preserving our food helps keep left over fresh, keeps items from spoiling more quickly, and many can prevent bacteria from developing, even if they just sit a day in the fridge.

Here are five options

a. Lemons

Lemons are a natural source of citric acid, a fantastic preservative found in their peel and flesh, but not the kind you find in store-bought items that is derived from yeast. Lemons are a fantastic anti-bacterial food too, but need to be sure the lemons you buy are fresh and not fixing to spoil.

b. Garlic

Garlic is a potent anti-viral food that's incredibly well at fighting bacteria—both in body and in food. Using a whole clove or minced garlic in a soup, dressing, an entree, a dip, or anything else will help ward off harmful bacteria to prevent it spoiling quicker. As an added bonus, it's a cheap instant upgrade to make anything taste better too (Kumar, 2012).

c. Pink Unprocessed Sea Salt (Himalayan Rock Salt)

This salt is a special one; it is considered a raw salt because it's produced through a mill and doesn't undergo the harsh refinement of regular sea salts find at the store. Because it is still pink when purchase it (often in a grinder), it's also mineral-rich and a fantastic alkalizing food, yet actually improves stomach acid levels needed for digestion. Using just a tiny grind or pinch (very small) will help preserve food in a much healthier way.

d. Fermented Foods

Fermented foods are nature's most miraculous preservatives. They're rich in probiotics and aid in gut health, but also help fight bad bacteria and can help your foods keep longer.

e. Cayenne, Hot Sauce, and Mustard

Possibly the most surprising of all, are spicy foods. Cayenne, hot sauce and mustard are three of the best natural foods that will help us to keep foods longer. Mustard and hot sauce have the added benefits of containing vinegar, another natural preservative, but cayenne is also effective. Spicy foods have been shown to fight bacteria, which may be one reason why they're so good for us outside of the metabolism-boosting benefits.

2.3 Common fruit preservatives

- Formaldehyde
- Soaking with NaCl
- Unhairing/liming with KOH, Na_2S0^3/bi Sulphide
- Deliming/bating with Na_2SO^3, NH_4C1, Na2SO4
- Picling with H_2SO_4, H-COOH, NaCl
- Chrome Tanning
- Sammying, splitting with dyes, fixing, agent, Condensation of urea
- Buffing with Liquid pigment, polymer, fixative, preservatives and aromatic ingredients
- Shaving, dyeing

2.4 Bio preservation

Bio preservation is the use of natural or controlled micro biota or antimicrobials as a way of preserving food and extending its shelf life.Beneficial bacteria or the fermentation products produced by these bacteria are used in bio preservation to control spoilage and render pathogens inactive in food. It is a benign ecological approach which is gaining increasing attention.Of special interest is lactic acid bacteria (LAB). Lactic acid bacteria have antagonistic properties that make them particularly useful as bio preservatives .These days, LAB bacteriocins are used as an integral part of hurdle technology (Ibrahim, 2013).

Advantages

- It is environmentally sound and healthy.
- It is free from toxic chemicals.
- It can be found from natural sources.

2.5 Use of Plant Products as Antimicrobials

Since ancient times, Essential oils also called volatile or ethereal oils were the active principle of many important herbal remedies (Guenther, 1948; Hammer *et al.*, 1999). This has also been applied to food preservation where the presence of naturally occurring antimicrobial agents in plants have been used against the spoilage microorganisms found in food in order to increase the storage properties of food (Magiatis *et al.*, 2002; Burt, 2004) . The suitability of a plant as an antimicrobial agent is dependent largely on the active components present in the plant part being used as an antimicrobial. The presence of an active component at a particular time is determined by factors such as environmental conditions, the period during which the plant part was collected, method of drying the plant part, storage condition and isolation methods (Magiatis *et al.*, 2002).

2.6 Guava leaves show phyto chemicals activity as antimicrobial

The antimicrobial activity of guava leaves is an outcome of certain compounds regarded as active compounds. These substances are naturally produced in plants as defense mechanisms against pathogenic microorganisms and insect pests. Its photochemical are classified broadly as terpenoids, phenolic and alkaloids (Croteau *et al.*, 2000). The antifungal compound mainly found in *Psidium guajava* were tannins, phlobatannins, saponins, terpenoids, alkaloids and poly phenols. The leaves of guava were rich in flavonoids in particular quercetin, saponins, tannins, alkaloids anthraquinones, phlobatannins and cardiac glycosides (Biswas *et al.*, 2002). Much of the guava therapeutic activity was attributed to these flavonoids. The flavonoids had demonstrated antibacterial activity. Guava also had antioxidant properties which were attributed to the poly phenols found in the leaves. Guava leaves were often boiled into a tea to treat diarrhea on many pacific islands. In many of the developing countries the used of the plant drugs was increasing because modern life saving drugs and people spend 40-50% income in drugs for health care. Among ancient civilization, India had been known to be rich repository of medicinal plants (Rukayadi *et al.*, 2013).A wide spectrum of activities against a variety of human ailments found in guava leaf extract. The presence of ascorbic acid and other phytonutrients such as

carotinoids, is flavonoids and polyphenols (quercetin in particular) in guava leaves has led to it being effective antioxidant (Hartwell, 2012).

a) Phenols

Phenols and their derivatives which possess oxygen molecules are secondary metabolites. They generally include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (Nohyneke*t al.* 2006). Phenols and phenolic acids are bioactive photochemical consisting of a single substituted phenolic ring. They contain varying number of hydroxyl groups and this determines the level of toxicity to microorganisms. Flavones, flavonoids and flavonols have the phenolic structure with one carbonyl group. They are synthesized by plants in response to microbial infection and have been found to be effective in vitro as antimicrobial substance against a wide array of microorganisms (Bennett and Wallsgrove, 1994). Due to their ability to form complexes with nucleophilic amino acids in proteins and bacterial cell wall,lead to enzyme inactivation (Masson and Wasserman, 1987). Tannins are polymeric phenolic substances possessing the astringent property. Tannins succeed in their antimicrobial activity by making substrates required for growth unavailable, directly inhibiting oxidative phosphorylation and in some cases inhibition of extracellular enzymes in microorganisms (Scalbert, 1991). Coumarins are phenolic substances made of fused benzene and pyrone rings (Kennedy and Thornes, 1997). They have a characteristic odor and several of them have antimicrobial properties.

b) Terpenes

Essential oils contain many substances including isoprene structure based substances called terpenes and terpenoids. Terpenes are known to disrupt membranes in microorganisms, alter their permeability and affect their ability to effectively carry out osmoregulation.The 1, 8 cineole is a terpenoid with the ability to reduce growth, inhibit the spore production in fungi and germination of wide range of microbes (Magiatis *et al.* 2002). Since fungi absorb nutrients from their environment, they have been found to absorb terpenoids which lead to hyphae malformations, disorganization of cell wall, and leakage of cytoplasmatic material. (Tang and Cronin, 2007)

c) Alkaloids

The diversity of alkaloids is an indication of their efficiency in antimicrobial activities ofguava extracts. These compounds occur in unstable concentrations in different plants and plant parts as well as having derivatives themselves, are all efficient against microbes (Gatto, 2011). Some of these substances are lipohilic and hydrophobic in nature thereby altering the integrity of the cell wall and mitochondria while affecting the transport system and causing cell content leakages (Kanaani and Ginsburg, 1992). They have also been found to be able to intercalate with DNA (Phillipon and Neill, 1987).

Food preservation involves preventing the growth of bacteria, fungi (such as yeasts), or other micro-organisms (although some methods work by introducing benign bacteria or fungi to the food), as well as retarding the oxidation of fats that cause rancidity (Magiatis *et al.* 2002). Biopreservation offers the potential to extend the storage life and food safety. Biopreservation may be effectively used in combination with other preservative factors (called hurdles) to inhibit microbial growth and achieve food safety (Scalbert, 1991).

There has been increased interest in subjects related to preservation. It is common to think that we ought to preserve valuable things. In fact, preservation has a specific purpose, and when that purpose is frustrated, the importance of preservation fades away (Bennett and Wallsgrove, 1994).

Modern technologies in food processing and microbiological food safety standards have reduced but not eliminated the likelihood of food related illness and product spoilage in industrialized countries. Food spoilage refers to the damage of the original nutritional value, texture, flavour of the food that eventually render food harmful to people and unsuitable to eat (Moteriya *et al.*, 2015)

2.7 Extraction Method of Guava Leaves

The guava tree is an evergreen small tree. The guava leaves are 2 to 6 inches long and 1 to 2 inches wide, aromatic when crushed, and appear dull-green with stiff but coriaceous with pronounced veins. The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, soxhletm extraction, aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction, supercritical fluid extraction, and phytonic extraction. Maceration extraction is crude extraction;

solvents diffuse into the solid plant material and solubilize compounds with similar polarity. Effect of plant material depends on its origin, variations in the extraction technique, the time, temperature of extraction, solvent concentration and polarity, quantity, and secondary metabolite composition of an extract .Variations in extraction methods are usually found in the length of the extraction period, the solvent used pH, temperature, particle size, and the solvent-to-sample ratio.To the family without refrigerator and also the people who cannot afford commercial preservative, this study is an effective opening for them to preserve fruit or vegetable for a certain time. This study was designed to use the preservative for fruits locally available in Bangladesh (Malik, 1990).

CHAPTER THREE: MATERIALS AND METHODS

3. Materials and Methods

3.1 Collection of Fruits

Banana (*Musa acuminata*), carambola (*Averrhoa carambola*) and tomato (*Solanum lycopersicum*) were collected from agricultural fields and home garden around the Khulna city. (Fig: 3.1). Around the same sized fruits were selected for this study. Fruits were separated into two groups and each group contained at least ten fruits. Finally the fruits were washed and dried at room temperature properly before preservative treatment.

Figure.3.1. Different types of fruits (banana, carambola, tomato)

3.2 Preparation of Guava Extract

Mature green guava leaves were collected from Khulna University campus. The leaves were washed properly with distilled water. Around ¼ kg guava leaf were taken into 2 kg of water. It was then boiled at 180°C for 30 minutes. After that, the solution was screened to remove the bigger particles. The solution was then slowly evaporated with the temperature of 70°C for a concentration of 0.5%. (Fig: 3.2). This concentration of preservative was applied to the selected fruits.

Figure.3.2. Guava leaf extract

3.3 Application of extracts for food preservation

By using a sprayer, guava leaf extract was sprayed to the group of bananas, carambola and tomato. The treated samples were placed in the room free from insects along with the controlled samples. The samples were observed daily at the same time until the controlled samples were damaged completely.

3.4 Data collection

3.4.1 Weight Loss

Samples were weighed using a digital electronic balance with an accuracy of 0.0001 g after every 4 days. Calculation of weight of fruits was done using Equation 1.

$$\text{Weight Loss}(\%) = \frac{\text{Weight}_{\text{initial}} - \text{Weight}_{\text{final}}}{\text{Weight}_{\text{initial}}} \times 100 \quad \text{---------- Eq.1}$$

`Where,

$\text{Weight}_{\text{initial}}$ = Initial weight of a fruit (g),

$\text{Weight}_{\text{final}}$ = Weight after treatment (g).

3.4.2 p^H of Sample juice

After the treatment period, samples were peeled and blended with a vita-mix digital blender (Vita-Prep 3®, England) having the 37000 rpm to prepare juice. The pH of that juice was measured by a pH meter (pH-009).

3.4.3 Percentage disease index (PDI)

Disease index was assessed only for banana by a scanner (Canon DR M140, New Zealand) and Adobe Photoshop (Version CS6, USA) software. The banana peel was scanned and then analyzed for yellow and black portion percentage.

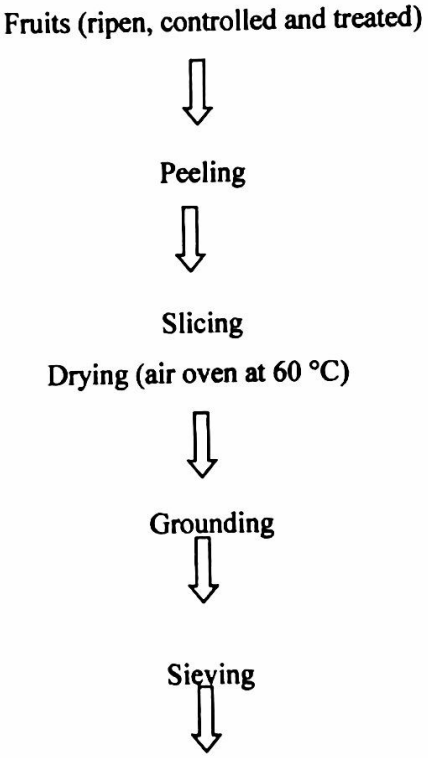
PDI = no. of infection categories/no. of infected fruit falling to this category×100/maximum no. of infection categories ---------- Eq.2

3.4.4 Peel color, flavor and firmness

Peel color, flavor, firmness and overall acceptability of the fruits were assessed by a panel of 10 members among the students of Khulna University selected randomly. They were asked to fill a questionnaire comparing with the original color and flavor.

3.4.5 Determination of nutritional content

The ripe fruits were bought from a garden around the Khulna city. They were well peeled (banana), (carambola) and (tomato) cut into small sizes and dried in an air at 60°C. The dried fruits were grinded using grinder mill into flour. The flours were then subjected to proximate analysis. A determination was carried out in duplicate for each sample.



3.4.5.1 Protein Content

The protein content in the fruits was estimated by Lowry's method using a standard curve of Bovine Serum Albumin (BSA) solution (20-100 Mg/ml) and absorbance at wavelength of 660 nm using double beam UV-Visible spectrophotometer. The percentage concentrations of protein was estimated using standard graph.

3.4.5.2 Total Carbohydrates Determination

The Lane-Eynon method was used to determine the total carbohydrates. A burette was used to add the carbohydrate solution being analyzed to a flask containing a known amount of boiling copper sulfate solution and a methylene blue indicator. The carbohydrate solution reacts with the copper sulfate present in the flask. Once all the copper sulfate in solution is reacted, any further addition of reducing sugars causes the indicator to change from blue to white. The volume of sugar solution required to reach the end point is recorded and total carbohydrate was determined.

3.4.6 Moisture Content Determination

Aluminum dishes were washed and dried in an oven. The dishes were weighed and recorded. Five gram of the samples was weighed into the aluminum dish. The dishes were placed in an oven regulated at 105°C for 3 hours during which the weight was checked at specific time interval. This was done until constant weight was observed. This made it possible to obtain the moisture content of the original weight of the sample.

3.5 Statistical Analysis

The result was evaluated by using statistical analysis (One way ANOVA) for its application. Treatment means will be separated by comparing the means at $p \leq 0.05$ using Microsoft Office Excel 2007 and SAS (Statistical Analysis System) (Version 6.12) software to visualize the LSD (Least Significance Difference) test.

CHAPTER FOUR: RESULTS AND DISCUSSIONS

4. Result and Discussion

4.1 Weight Loss Percentage (WLP)

Control fruits of banana had significantly ($p \leq 0.05$) higher WLP (25%) whereas lower WLP (5%) was found in guava extract coated fruits after 12 days storage. (Fig: 4.1) shows that for sprayed fruits WLP decreased on the 8th day compared to the 4thday.

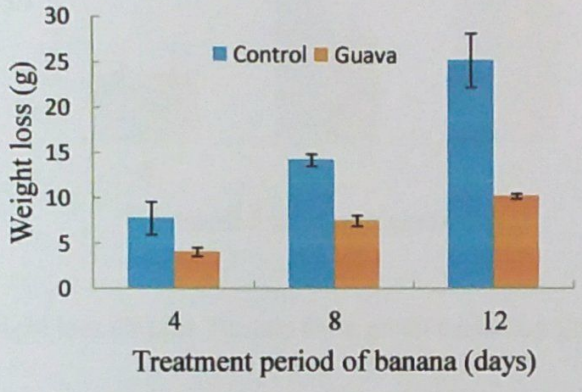


Figure.4.1. Weight loss (%) for banana for a given period by guava leaf extracts treatment

For Carambola, control fruits had significantly ($p \leq 0.05$) higher WLP (15%) whereas lower WLP (8%) was found in guava extract sprayed fruits after 12 days storage. (Fig: 4.2) shows that for sprayed fruits WLP decreased on the 8th day compared to the 4th day.

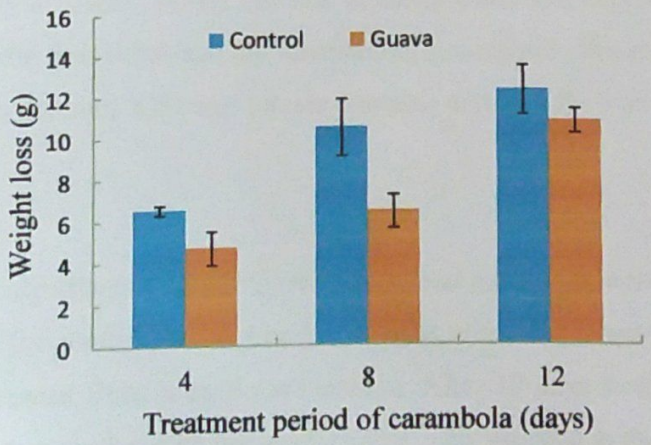


Figure.4.2. Weight loss (%) for carambola for a given period by guava leaf extracts treatment

In the same way, For Tomato higher WLP (22%) was found in control and lower WLP (7%) found on the 12th day in guava extract treatment compared to 8th day.

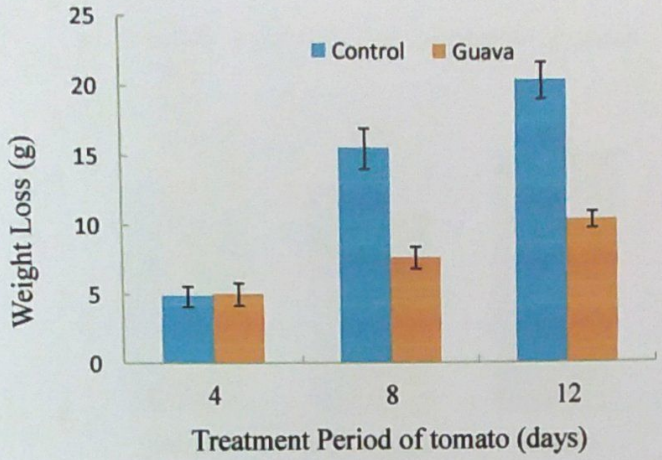


Figure.4.3. Weight loss (%) for Tomato for a given period by guava extracts treatment

The water loss percentage increased with increasing time (Fig: 4.3). However, this increase was higher for the first four days and then gradually decreases. Controlled samples had higher WLP compared to the treated samples. Controlled samples had highest WLP (25.56) whereas the maximum WLP of treated fruits was 10.39% only.

Weight loss mainly occurs due to water loss by transpiration and loss of carbon reserves due to respiration (Zagory & Kader, 1988). The rate at which water lost depends on the water pressure gradient between the fruit tissue and the surrounding atmosphere. Banana contains 74% water by weight, carambola contains 82% and tomato contains almost 94% water by weight (Jayathunge *et al.*, 2011).

4.2 p^H Value

The pH increased significantly ($P \leq 0.05$) with increased storage time in uncoated (Fig: 4.4). The mean pH value of the control banana was 4.5 whereas minimal change was noticed in pH values of guava extract coated fruits after 8 days storage. After 12 days storage the pH values of the coated fruits increased further with the lowest value. This was due to the semi-permeable coating on the fruit surface which modified the internal atmosphere, i.e., the endogenous carbon-di-

oxide and oxygen concentration of the fruit. The increase in pH during storage was due to the metabolic processes of the fruit that resulted in a decrease of the organic acids Coseteng and Lee (1987).

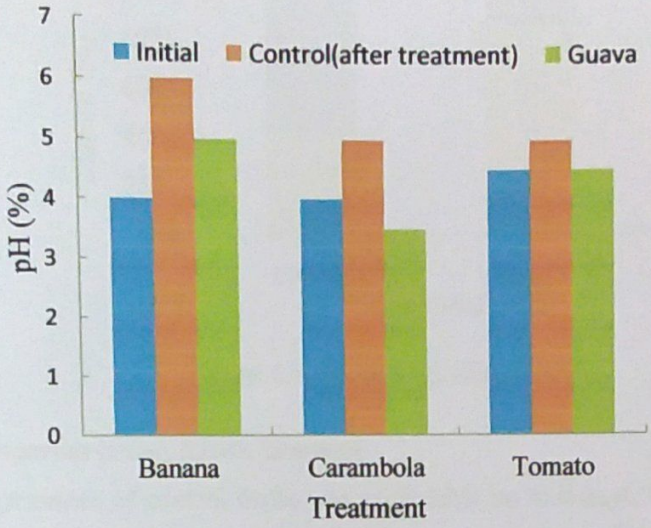


Figure.4.4. pH Percentage

4.3. Percentage Disease Index (PDI)

PDI was used to observe the effectiveness of coated material on fruit in retarding fruit disease. No disease signs were observed in treated fruits until 1 week after the beginning of the storage period. This was due to the anti-microbial potentiality of sprayed materials.

Figure.4.5. Scanning the outer layer of banana for calculation of PDI

At 12 days storage around 80% disease incidences was observed in controls, whereas for guava treated fruits it shows the antimicrobial activity against disease (Fig: 4.5).

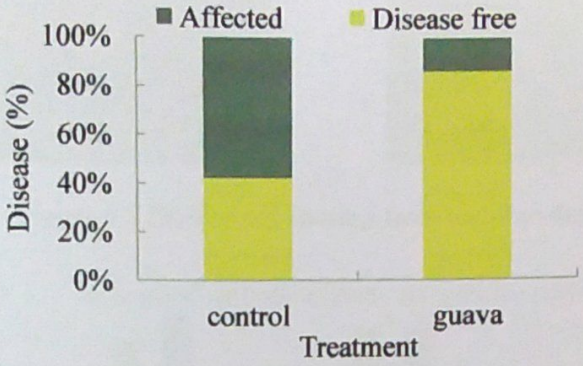


Figure.4.6. Percentage disease index

4.4. Sensory properties (color, flavor, firmness)

The overall appearance of control fruits was acceptable up to 4 days, but it became inferior from the 6th day onward. The flavor of fruits was found to be satisfactory and firmness was slightly soft.

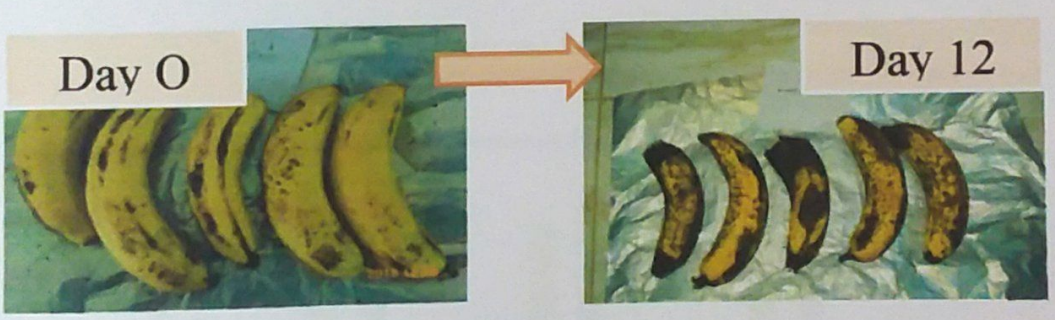


Figure 4.7 (a) Treated Banana from day 0 to day 12

However, after the 6th day the treated fruits started to deteriorate as they ripened fully and peel color changed to a lower amount. At 8th day their firmness became very soft and flavor was completely unsatisfactory. In the case of guava-coated fruits, the color was bright yellow until the 4th day of storage, but from the 6th day they started to show a slight color combination.

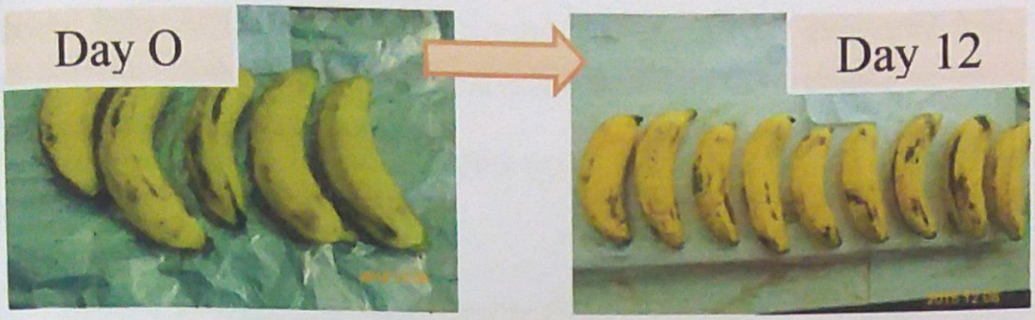


Figure 4.7 (b) Treated Banana from day 0 to days 12

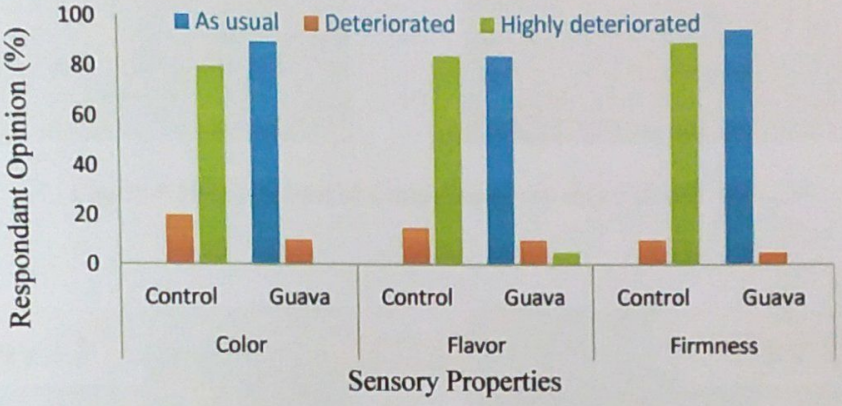


Fig.4.8 Color, flavor and firmness for banana

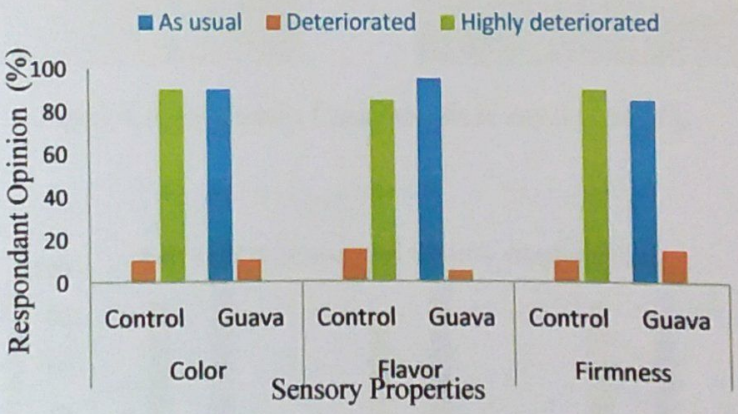


Fig.4.9. Color, flavor and firmness for carambola

The initial firmness values were similar for both fresh (control) and sprayed fruits ($p \leq 0.05$). After 8 days of storage, the control fruits began to show a gradual loss of firmness compared to

the sprayed fruits. At 12 days of storage, control fruits decayed and the treated fruits were slightly soft. This indicated that the ripening of treated fruits was delayed by delaying softening.

According to questionnaire's people's perception (appendix 1) shows the differences of the result of the treatments.

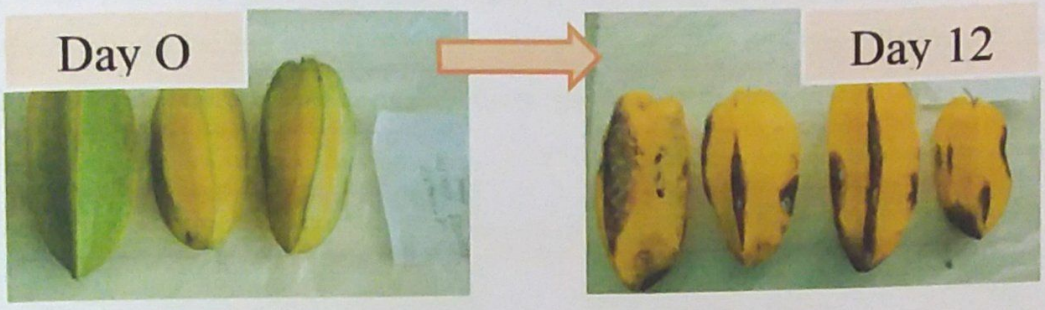


Figure 4.10 (a) Untreated Carambola from day 0 to day 12.

Figure 4.10 (b) Treated Carambola from day 0 to day 12.

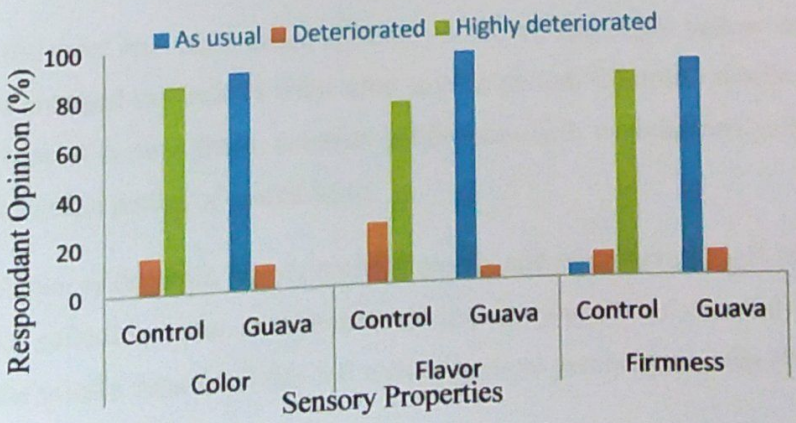


Figure.4.11. Color, flavor and firmness for tomato

Visual assessment is the first impression and a key feature in the choice of the fruit. Surface color of fruit sample is one of the most important criteria in determining ripening of fruit. Color retention of coated fruits was due to the delay in ripening of coated fruits. The modified atmosphere created by the edible coating material retarded the ethylene production rate. At 8th day their firmness became slightly soft and even at 12th day they were also slightly soft. During storage intervals (0, 4, 8 and 12 days) their flavor was found to be satisfactory.

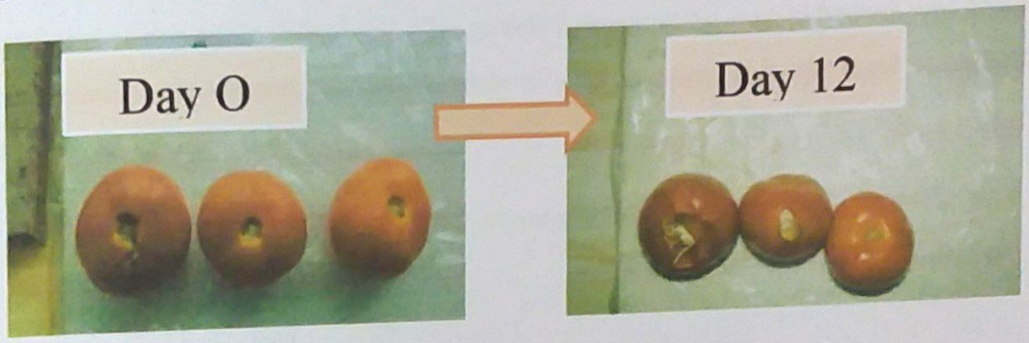


Figure.4.12 (a) Untreated Tomato from day 0 to day 12.

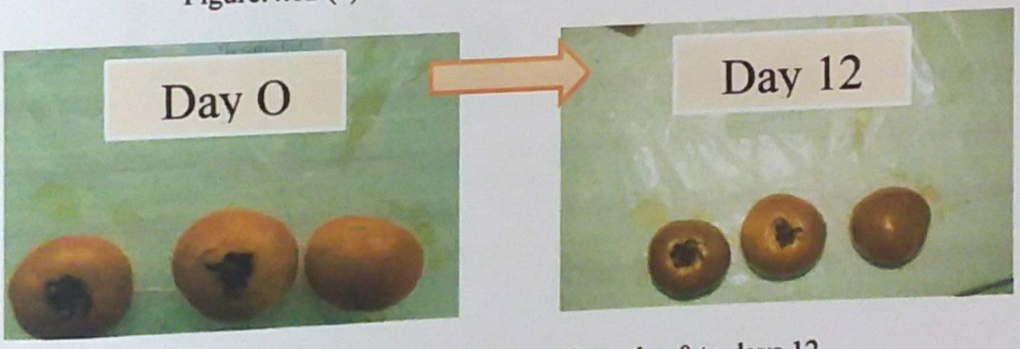


Figure.4.12 (b) Treated Tomato from day 0 to days 12.

Color is one of the most important visual attributes of fruits. The bright yellow color of control and coated fruits changed to blackish color after storage period. Complete blackness was found after 8 days storage of control fruits, whereas yellow skin with well-defined yellow stripe was found at 12 days storage period of coated fruits.

Loss of texture is one of the main factors limiting quality and post-harvest shelf life of fruits and vegetables. Fruits softening occur considerably during ripening which is mainly as a result of degradation of the middle lamella of the cell wall of cortical parenchyma cells (Perkins-Veazie,

2010). Changes in cell wall structure and in their composition are mainly due to the combined action of enzymes.

4.5. Protein content

The protein content increased significantly ($P \leq 0.05$) with increased storage time both in uncoated and coated fruits (Fig: 4.13). The protein content of the control banana was around 5% whereas minimal change was noticed in values of guava extract. This statement is supported by the study of Jiang *et al.*, (2000).

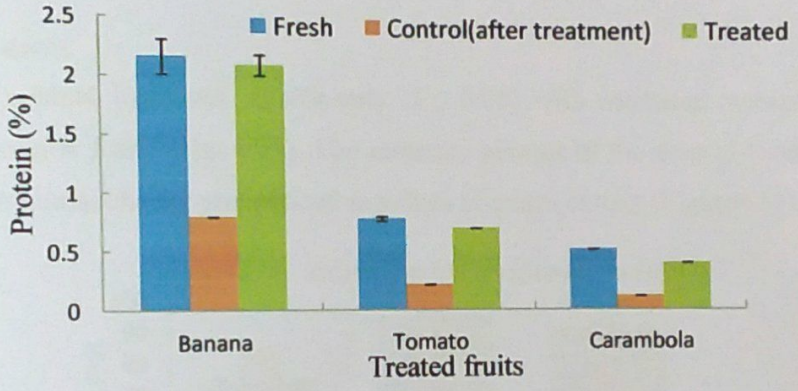


Figure4.13. Protein content of different fruit sample

4.6 Carbohydrate Content

The carbohydrate content increased significantly ($P \leq 0.05$) with increased storage time in guava treated fruits (Fig: 4.15).

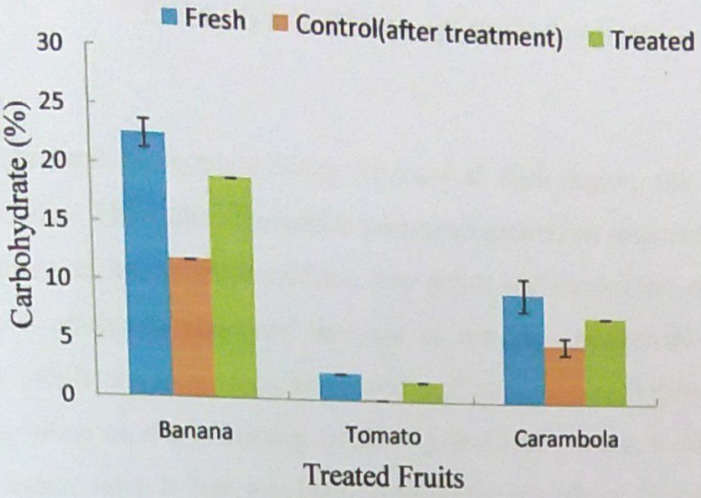


Figure.4.14. Carbohydrate content of different fruit sample

4.7 Moisture content

The moisture content increased significantly ($P \leq 0.05$) with increased storage time both in uncoated and coated fruits (Fig: 4.14). The moisture content of the control banana was around 84% whereas minimal change was noticed in values of guava extract (Figure4.14).

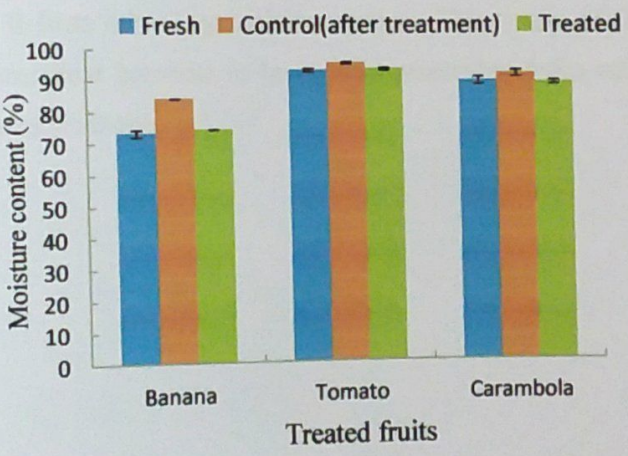


Figure4.15. Moisture content for different fruit sample

CHAPTER FIVE: CONCLUSION

5. Conclusion

With the improvement of people's living standard at each region, the fruit consumption has increased day by day. The fruit preservation problem becomes an important research direction as chemical preservatives are health hazardous. The guava leaf extract has no toxic and side effect, the raw material source is rich and the cost is low, the application process for the fruit preservation is simple and easy to be developed and used. Although there are many preliminary researches have done on the efficiency of the guava leaf extract, it still lacks the systematic experimental data and it has not been completely elucidated theoretically. This research selected the guava leaf extract as the protective agent to conduct the fruit preservation experiment on various fresh fruits. The experimental result proved that after spraying the guava leaf extract as protective agent, the fruit's weight loss rate and decay index declined and the decrease speed of the hardness slowed down. This study discovers the efficiency of guava leaves that act strongly against quick food spoilage. The application of guava leaf extract delayed the acceleration of the deterioration of organic fruits. Its unique properties and good film-forming properties separated it from other degradable polymers. The results of this study showed that guava extract have excellent potential to be used in preserving fruits with the maintenance of quality and extension of shelf-life.

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Appendices

Appendix-1

Table 1: Effect guava leaf extract of treatment on physical and chemical properties of banana

Treatment	Physical properties						Chemical properties		
	pH	PDI (%)	WLP (%)	Sensory properties (%)			Protein (%)	Carbohydrate (%)	Moisture content (%)
				Color	Flavor	Firmness			
Fresh	4	–	–	–	–	–	2.16^{A} (.25)	22.67^{A} (1.7)	73.51^{B} (1.87)
Control	6	42.41^{B}	25.56^{A} (5.20)	–	–	–	$.65^{B}$ (.14)	11.27^{C} (.72)	85.7^{A} (1.72)
Treatment	5	86.51^{A}	10.39^{B} (.45)	–	–	–	2.03^{A} .08	19.67^{B} (.49)	74.4^{B} (1.01)

Values in parenthesis are standard deviation.

Values within the same line column by different letters are significant difference at α=0.05.

Table 2: Effect guava leaf extract of treatment on physical and chemical properties of carambola

Treatment	Physical properties						Chemical properties		
	pH	PDI %	WLP (%)	Sensory properties (%)			Protein (%)	Carbohydrate (%)	Moisture content%
				Color	Flavor	Firmness			
Fresh	4	--	--	--	--	--	$.53^{A}$ (.01)	9.75^{A} (2.54)	89.89^{B} (1.16)
Control	--5	--	12.35^{A} (2.07)	--	--	--	$.16^{C}$ (.03)	5.23^{C} (1.56)	92.18^{A} (1.04)
Treatment	3.5	--	10.85^{A} (1.01)	--	--	--	$.41^{B}$ (.004)	7.70^{B} (.12)	89.33^{B} (.60)

Values in parenthesis are standard deviation.

Values within the same line column by different letters are significant difference at α=0.05.

Table 3: Effect of guava leaf extract treatment on physical and chemical properties of tomato

Treatment	Physical properties						Chemical properties		
	pH	PDI %	WLP (%)	Sensory properties (%)			Protein%	Carbohydrate %	Moisture content%
				Color	Flavor	Firmness			
Fresh	4.5	--	--	--	--	--	$.79^{A}$ (.03)	2.4^{A} (.15)	93.34^{B} (.82)
Control	5	--	20.70 (2.3)	--	--	--	$.22^{C}$ (.008)	$.15^{C}$ (.01)	95.58^{A} (.49)
Treatment	4.5	--	10.54 (1.9)	--	--	--	$.71^{B}$ (.004)	1.8^{B} (.14)	93.7^{B} (.56)

Values in parenthesis are standard deviation.

Values within the same line column by different letters are significant difference at α=0.05.

Appendix-2

QUESTIONNAIRE FOR TESTING OF PHYSICAL PROPERTIES

Name of the fruit: Condition of the fruit: Treated/ Controlled

Name of preservative: **Guava extract**

Date: 17/01/2016

Name and address of the respondent:

.....

.....

.....

Preservative percentage: 0.5%

Color:	As usual	☐	Deteriorated	☐	Highly deteriorated	☐
Flavor:	As usual	☐	Deteriorated	☐	Highly deteriorated	☐
Firmness:	As usual	☐	Deteriorated	☐	Highly deteriorated	☐
Overall:	As usual	☐	Deteriorated	☐	Highly deteriorated	☐

Overall comments: Good ☐ Moderate ☐ Bad ☐ Very bad ☐

Appendix-3

Analysis of Variance: Protein (banana)

Class	Levels	Values
Treatment	3	T1 T2 T3

Number of observations in data set = 9

Dependent Variable: Protein

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	3.56222222	62.25	0.0001
Error	6	0.17166667		
Corrected Total	8	3.73388889		

T tests (LSD) for variable: Protein (banana)

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	2.1667	3	T1
A			
A	2.1000	3	T3
B	0.8000	3	T2

Analysis of Variance: Protein (Carambola)

Class	Levels	Values
Treatment	3	T1 T2 T3

Number of observations in data set = 9

Dependent Variable: Protein

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	0.26660000	99999.99	0.0001
Error	6	0.00000000		
Corrected Total	8	0.26660000		

T tests (LSD) for variable: Protein

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	0.5300	3	T1
B	0.4100	3	T3
C	0.1200	3	T2

Analysis of Variance: Protein (Tomato)

Class	Levels	Values
Treatment	3	T1 T2 T3

Number of observations in data set = 9

Dependent Variable: Protein

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	0.57140000	659.31	0.0001
Error	6	0.00260000		
Corrected Total	8	0.57400000		

T tests (LSD) for variable: Protein

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	0.79000	3	T1
B	0.71000	3	T3
C	0.22000	3	T2

Analysis of Variance: Carbohydrate (banana)

Class	Levels	Values
Treatment	3	T1 T2 T3

Number of observations in data set = 9

Dependent Variable: Carbohydrate

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	176.90888889	61.24	0.0001
Error	6	8.66666667		
Corrected Total	8	185.57555556		

T tests (LSD) for variable: Carbohydrate

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	22.6667	3	T1
B	19.1000	3	T3
C	12.0000	3	T2

Analysis of Variance: Carbohydrate (carambola)

Class	Levels	Values
Treatment	3	T1 T2 T3

Number of observations in data set = 9

Dependent Variable: Carbohydrate

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	30.73380000	99999.99	0.0001
Error	6	0.00000000		
Corrected Total	8	30.73380000		

T tests (LSD) for variable: Carbohydrate

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	9.750	3	T1
B	7.700	3	T3
C	5.230	3	T2

Analysis of Variance: Carbohydrate (Tomato)

Class	Levels	Values
Treatment	3	T1 T2 T3

Number of observations in data set = 9

Dependent Variable: Carbohydrate

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	8.14500000	99999.99	0.0001
Error	6	0.00000000		
Corrected Total	8	8.14500000		

T tests (LSD) for variable: Carbohydrate

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	2.400	3	T1
B	1.800	3	T3
C	0.150	3	T2

Analysis of Variance: Moisture Content (banana)

Class	Levels	Values
Treatment	3	T1 T2 T3

Number of observations in data set = 9

Dependent Variable: Moisture Content (mc)

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	214.16820000	91.52	0.0001
Error	6	7.02060000		
Corrected Total	8	221.18880000		

T tests (LSD) for variable: MC

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	84.3600	3	T2
B	74.6000	3	T3
B			
B	73.5100	3	T1

Analysis of Variance: Moisture Content (carambola)

Class	Levels	Values
Treatment	3	T1 T2 T3

Number of observations in data set = 9

Dependent Variable: Moisture Content

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	13.71448889	7.33	0.0245
Error	6	5.61140000		
Corrected Total	8	19.32588889		

T tests (LSD) for variable: MC

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	92.1867	3	T2
B	89.8933	3	T1
B			
B	89.3333	3	T3

Analysis of Variance: Moisture Content (tomato)

Class	Levels	Values
Treatment	3	T1 T2 T3

Number of observations in data set = 9

Dependent Variable: Moisture Content

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	8.61962222	10.51	0.0110
Error	6	2.46093333		
Corrected Total	8	11.08055556		

T tests (LSD) for variable: Moisture Content

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	95.5767	3	T2
B	93.7000	3	T3
B			
B	93.3467	3	T1

Analysis of Variance: Weight Loss (banana)

Class	Levels	Values
Treatment	2	T1 T2

Number of observations in data set = 6

Dependent Variable: Weight Loss

Source	DF	Sum of Squares	F Value	Pr > F
Model	1	345.04166667	25.29	0.0073
Error	4	54.57613333		
Corrected Total	5	399.61780000		

T tests (LSD) for variable: Weight Loss

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	25.563	3	T1
B	10.397	3	T2

Analysis of Variance: Weight Loss (carambola)

Class	Levels	Values
Treatment	2	T1 T2

Number of observations in data set = 6

Dependent Variable: Weight Loss

Source	DF	Sum of Squares	F Value	Pr > F
Model	1	3.40506667	1.28	0.3217
Error	4	10.66966667		
Corrected Total	5	14.07473333		

T tests (LSD) for variable: Weight Loss

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	12.357	3	T1
A			
A	10.850	3	T2

Analysis of Variance: Weight Loss (carambola)

Class	Levels	Values
Treatment	2	T1 T2

Number of observations in data set = 6

Dependent Variable: Weight Loss

Source	DF	Sum of Squares	F Value	Pr > F
Model	1	154.94001667	34.35	0.0042
Error	4	18.04326667		
Corrected Total	5	172.98328333		

T tests (LSD) for variable: Weight Loss

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	20.703	3	T1
B	10.540	3	T2

Analysis of Variance: Percentage of Disease Index (PDI) (banana)

Class	Levels	Values
Treatment	2	T1 T2

Number of observations in data set = 6

Dependent Variable: PDI

Source	DF	Sum of Squares	F Value	Pr > F
Model	1	2916.33306667	54.41	0.0018
Error	4	214.40213333		
Corrected Total	5	3130.73520000		

T tests (LSD) for variable: PDI

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	86.507	3	T2
B	42.413	3	T1

Analysis of Variance: p^H (banana)

Class	Levels	Values
Treatment	2	T1 T2

Number of observations in data set = 6

Dependent Variable: pH

Source	DF	Sum of Squares	F Value	Pr > F
Model	1	2.16000000	216.00	0.0001
Error	4	0.04000000		
Corrected Total	5	2.20000000		

T tests (LSD) for variable: pH

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	6.10000	3	T1
B	4.90000	3	T2

Analysis of Variance: p^H (banana)

Class	Levels	Values
Treatment	2	T1 T2

Number of observations in data set = 6

Dependent Variable: pH

Source	DF	Sum of Squares	F Value	Pr > F
Model	1	2.16000000	216.00	0.0001
Error	4	0.04000000		
Corrected Total	5	2.20000000		

T tests (LSD) for variable: pH

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	6.10000	3	T1
B	4.90000	3	T2

Analysis of Variance: pH (carambola)

Class	Levels	Values
Treatment	2	T1 T2

Number of observations in data set = 6

Dependent Variable: pH

Source	DF	Sum of Squares	F Value	Pr > F
Model	1	1.92666667	289.00	0.0001
Error	4	0.02666667		
Corrected Total	5	1.95333333		

T tests (LSD) for variable: pH

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	5.10000	3	T1
B	3.96667	3	T2

Analysis of Variance: pH (tomato)

Class	Levels	Values
Treatment	2	T1 T2

Number of observations in data set = 6

Dependent Variable: pH

Source	DF	Sum of Squares	F Value	Pr > F
Model	1	0.54000000	162.00	0.0002
Error	4	0.01333333		
Corrected Total	5	0.55333333		

T tests (LSD) for variable: pH

Means with the same letter are not significantly different.

T Grouping	Mean	N	Treatment
A	5.03333	3	T1
B	4.43333	3	T2