

A DISSERTATION ON
Optimization and Development of Pectin Based Edible Coating
for Pomegranate Arils

SUBMITTED TO THE
DEPARTMENT OF BIOENGINEERING
FACULTY OF ENGINEERING & INFORMATION
TECHNOLOGY
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IN PARTIAL FULFILMENT
FOR THE
DEGREE OF MASTER OF TECHNOLOGY
IN FOOD TECHNOLOGY

BY
Ananya Singh
B.Tech.-M.Tech Dual Degree Food Technology (X Semester)
Roll No: 1801087006

UNDER THE SUPERVISION OF

Dr. Alvina Farooqui
Professor & Head
Department of Bioengineering

INTEGRAL UNIVERSITY, DASAULI, KURSI ROAD
LUCKNOW- 226026

DECLARATION FORM

I, **Ananya Singh**, a student of **B.Tech.-M.Tech. Dual Degree Food Technology (V Year/ X Semester)** Integral University has completed my six-month dissertation work entitled **“Optimization and Development of Pectin Based Edible Coating for Pomegranate Arils”** successfully from **Integral University, Lucknow** under the able guidance of **Dr. Alvina Farooqui**.

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

Ananya Singh

Dr. Rahul Singh
Assistant Professor
Department of Bioengineering



INTEGRAL UNIVERSITY

LUCKNOW - INDIA

Phone No.: +91(0522) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809

Kursi Road, Lucknow-226026 Uttar Pradesh (INDIA)



CERTIFICATE

Certificate that Ms **Ananya Singh** (Enrollment Number 1800102234) has carried out the research work presented in this thesis entitled “**Optimization and Development of Pectin Based Edible Coating for Pomegranate Arils**” for the award of **B.Tech.-M.Tech. Dual Degree Food Technology** from Integral University, Lucknow under my supervision. The thesis embodies results of original work and studies carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of her **B.Tech.-M.Tech. Dual Degree Food Technology** degree.

I wish her good luck and bright future.

Dr. Alvina Farooqui
(Supervisor)
Professor and Head
Department of Bioengineering
Faculty of Engineering & Information
Technology

Mrs. Poonam Sharma
(Co-Supervisor)
Assistant professor
Department of bioengineering



INTEGRAL UNIVERSITY

LUCKNOW - INDIA

Phone No.: +91(0522) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809

Kursi Road, Lucknow-226026 Uttar Pradesh (INDIA)



CERTIFICATE BY INTERNAL ADVISOR

This is to certify that **Ananya Singh**, a student of **B.Tech.-M.Tech. Dual Degree Food Technology (V Year/ X Semester)** Integral University has completed her six months dissertation work entitled **“Optimization and Development of Pectin Based Edible Coating for Pomegranate Arils”** successfully. She has completed this work from Integral University, Lucknow under the guidance of Dr. Alvina Farooqui, Professor and Head, Department of Bioengineering. The dissertation was a compulsory part of her **B.Tech.-M.Tech. Dual Degree Food Technology** degree.

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Dr. Alvina Farooqui

Professor and Head

Department of Bioengineering

Faculty of Engineering & Information Technology



INTEGRAL UNIVERSITY

LUCKNOW - INDIA

Phone No.: +91(0522)2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809

Kursi Road, Lucknow-226026 Uttar Pradesh (INDIA)



TO WHOM IT MAY CONCERN

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I wish her good luck and bright future.

Dr. Alvina Farooqui

Professor and Head

Department of Bioengineering

Faculty of Engineering & Information Technology

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Place: Lucknow

Ananya Singh

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ABSTRACT

One of the primary methods in increasing shelf life and maintaining horticulture product quality is the application of edible coatings at the product surface. This study was conducted to investigate the effect of Edible Coating (Pectin) and Chemical Dips of pomegranate arils to evaluate the change in weight loss, pH, titratable acidity, total soluble solids (TSS), moisture content, antioxidant, antibacterial, and antifungal. Pomegranate arils were immersed in pectin and some chemical dips solution with different formulations for two minutes. After applying treatments, arils were air-dried, weighed, put in petri plates, and kept at room temperature for 5 days, and in refrigerator for 12 days. Some quantitative and qualitative traits were measured and compared with the non-treated samples. Results revealed using 1% pectin had a different effect during storage time. The results also showed that the 1% pectin coating could control the reduction of fruit appearance quality. The pomegranate arils coated with 1% pectin solution could improve weight loss, pH, TSS, TA, and Titratable Acidity during storage time at room temperature, and in refrigeration. Pectin coating could improve fruit quality and extend the shelf life of pomegranate arils during the storage period and these were Ready-To-Eat products that can be consumed and also recommended to stores during their storage period.

Keywords: Pomegranate Arils, Edible Coating, Pectin, Shelf Life, Quality

CHAPTER 1

INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the Punicaceae family and is one of the oldest delicious edible fruits. It has been extensively planted in numerous tropical and subtropical countries, and recent years have seen a surge in its cultivation. Iran is one of the main producers and exporters of pomegranate in the world. India besides Spain, Israel, and the United States is one of the major cultivation areas of pomegranate (M.E. Pena-Estevez et al. 2016). Arils, comprised of seeds surrounded by pulp, are the edible part of the pomegranate fruit which forms approximately 50% of the total fruit weight (A. Tehranifar et al. 2010). The inedible part of the pomegranate is a hard outer rind and an inner soft white segment (locular septa) separating bunches of arils. Pomegranates may either be consumed as fresh fruit or as juice. Fresh fruit is eaten or processed fruit is made into juice, jam, syrup, and sauce.

Pomegranate is a rich source of various bioactive components such as organic acids, vitamins, anthocyanins, and polyphenols (A. Tehranifar et al. 2010). The main reason why pomegranates are so popular is because they play a preventive role in lowering blood pressure, inflammation, atherosclerosis, prostate cancer, heart disease, and HIV-1. Pomegranate's antioxidant action is due to phenolic acids, anthocyanin, and ascorbic acid, either individually or collectively. Recent studies have shown that extracts from the peel, juice, and seeds of pomegranate fruit have high antioxidant activity.

Several food commodities may benefit from Edible Coatings to raise their quality and lengthen their shelf lives. The application of a layer of any edible material on the product surface with the goal of giving it a modified atmosphere, delaying gas transfer, reducing moisture and aroma loss, preventing color changes, and improving the product's overall appearance through storage constitutes the use of edible coatings on fresh commodities such as fruits and vegetables, either whole or cut. Depending on the intended applications, a widely diverse spectrum of chemicals can be used to create edible coatings. The two substances that are most frequently utilized in edible coating formulations for freshly cut fruits and vegetables are hydrocolloids (polysaccharides and proteins). In comparison to lipids, which often provide hydrophobic coatings with strong water barrier qualities, polysaccharides, and proteins are effective barriers to oxygen and carbon dioxide but poor barriers to water permeability. Pomegranate seeds are

coated with various formulations made of pectin, calcium chloride, and calcium carbonate to improve their quality and shelf life after being peeled.

Pectin is a class of complex water-soluble polysaccharides used to form coatings. It is a purified carbohydrate product made from some edible plant material that has been extracted using water, commonly citrus fruits or apples. Pectin has the ability to gel under specific conditions, making it a crucial ingredient in jellies, jams, marmalades, confections, and edible coatings. Pectin is a plentiful and potentially significant dietary component that is present in large quantities in agricultural waste. Furthermore, it has nutritional advantages for human health and its medicinal properties make its usage in a range of culinary products fascinating. Pectin coatings have also been investigated for their capacity to reduce moisture loss and lipid migration, as well as to enhance food look and handling.

In the fruit and vegetable sector, Calcium Chloride (CaCl_2) has been widely utilized as a firmness-increasing agent and preservative for entire, as well as for fresh-cut, produce. By making the middle lamella more rigid and acting as a binding agent in the cell wall to form calcium pectate, calcium chloride applied to many fruits and vegetables after harvest has been found to delay softening, preserve quality, and make them firmer (Mehmet et al., 2016; Gao et al., 2020).

It has been demonstrated that several new formulations based on Calcium Carbonate have positive effects on fruit, trees, or plants, but they can also have positive effects on vegetable crops. When applied to fruit surfaces, they consist of stable fluid dispersions of finely micronized Calcium Carbonate (CaCO_3) that produce a more-or-less uniform whitish layer. Additionally, it is crucial as a fertilizer, antacid, food coloring, food firming, and culinary ingredient.

Fruits that have been freshly cut and barely processed are increasingly preferred for today's healthier diets. Fruits that require little preparation are best, especially those that are huge or challenging to peel. Due to the fast-paced lifestyle that people have chosen, the extraction and separation of the arils from the endocarp in pomegranates is a challenging and time-consuming process that may limit their usage. Instead of full pomegranate fruit, consumers would prefer to buy minimally processed or ready-to-eat pomegranate arils. The shelf life of fruits and vegetables is shortened by minimum or fresh-cut processing, which makes them more prone to deterioration than entire fruits. Pomegranate fruits are also susceptible to water loss after harvest, cold damage, physical ailments, and fungi. The use of edible coatings seems to be a

potential solution for preserving the quality and extending the shelf life of fresh-cut or barely processed vegetables. Edible-coated pomegranate arils are healthier than preserved pomegranate arils since pomegranate arils are now sold in the market with added preservatives and a significant amount of sugar, which may potentially cause obesity.

1.2 OBJECTIVES:

- Preparation of Edible Coating using Pectin, Calcium Chloride, and Calcium Carbonate.
- Optimization of various methods of coating for pomegranate arils preservation.
- Evaluation of efficacy of pectin coating in shelflife extension of peeled pomegranate arils.

CHAPTER 2

REVIEW OF LITERATURE

Fruit coating has a long history. In China around the 12th century, oranges and lemons were waxed to prevent moisture loss and enhance visual appeal. Wax was first used commercially to lessen fruit and vegetable postharvest losses, but, not until 1922. However, because these waxes have been deemed detrimental to consumers, a market for a more natural, sustainable coating application has emerged. The application of edible coating has been the subject of study for the past 20 years. Since edible coatings are made of materials that are safe for consumption, they can be safely consumed with the product. Fruit is coated with liquid substances by dipping, spraying, brushing, or dripping. By forming a semipermeable protective barrier around the fruit's surface that controls moisture, solute, and gaseous exchange between the fruit's internal environment and the external atmosphere, they reduce quality losses. Therefore, by delaying ripening, delaying physico-chemical changes, and limiting the emergence of physiological diseases, coatings may improve postharvest shelf life.

2.1 Pomegranate Fruit Quality:

According to the variety and state of ripening, pomegranate fruits have an irregularly spherical shape with coriaceous rinds that range from bright deep red to yellow, green, or pink (Holland et al. 2009; Elyatem and Kader 1984). There are, nevertheless, some unique cultivars, such the black pomegranate. According to Holland et al. (2009), these cultivars develop black color relatively early and maintain it through the ripening process. Pomegranates feature a fleshy mesocarp and a multi-ovule chamber inside that are divided by membrane walls (the septum). Aril-like seeds are contained within the chambers. The arils, which grow from the seed's outer epidermal cells and extend greatly in a radial direction, are the succulent and edible part (Fahan 1976). While certain types are described to as seedless but really contain soft seeds, arils vary in size and hardness depending on the varieties. Depending on the type, the arils' color ranges from white to deep crimson (Holland et al. 2009). There can occasionally be many colored seeds inside a pomegranate fruit, a condition known as metaxenia (Levin 2006). Several

researchers have reported on the physicochemical characteristics of pomegranate fruit cultivars grown in various countries (Artés et al. 2000a, b; Al-Said et al. 2009; Al-Yahyai et al. 2009; Opara et al. 2009; Zarei et al. 2010). The fruit weight, entire fruit, aril color, juice volume, and juice dry matter volume are among the physical characteristics that are given. These researchers, along with others, have also demonstrated that different agroclimatic zones have different physico-chemical characteristics for pomegranate cultivars (Al-Said et al. 2009; Opara et al. 2009; Zarei et al. 2010). Furthermore, several chemical properties and phytonutrients such as vitamin C, total phenolics, total tannins and condensed tannins, total soluble solids, and anthocyanins in the peel and arils of various pomegranate cultivar have been reported (Artés et al. 2000a, b; Al-Said et al. 2009; Opara et al. 2009; D' Aquino et al. 2010; Zarei et al. 2010).

2.2 Properties of Edible Coating:

Several coating properties should be considered when applying edible coatings to any fruit. Most significantly, coatings should minimize postharvest quality losses and increase shelf life rather than reduce fruit quality overall or cause physiological problems. All coating materials used in edible coatings should be approved as food additives by the European Union or recognized by the Food and Drug Administration as "Generally Regarded as Safe" (GRAS). Additionally, all procedures and tools used in the creation of edible coatings should adhere to the same high standards as those used in the food processing industry. Fruit with coating is fit for consumption. Therefore, it's crucial that edible coverings don't in any way change how fruit feels to the touch. Therefore, while formulating a coating, consideration should be given to characteristics including coating thickness, adherence to the surface, transparency, plasticity, waxiness, taste, and odor. Furthermore, the application of a coating should regulate gaseous exchange while allowing fruit respiration to continue in some cases. Due to the formation of fermentative volatiles, the fruit could develop an unfavorable off-flavor.

For edible coatings to compete as a potential postharvest technology, coatings materials should be low-cost and highly available. Coatings should be easy to apply, with good adhesive properties and immediate uniform drying characteristics (Ncama et al., 2018). Furthermore, coating functionality and structural integrity must be maintained over extended storage periods. Coatings should have good flexibility to adapt to morphological changes that are peculiar to

the cuticle of stone fruit such as fruit shrinkage, shriveling, or mechanical damage. One of the major benefits of edible coating is to provide a glossy-finished surface to the food products along with the replacement of plastic packaging with natural and environmentally safe edible coatings which helps in retarding the waste disposal issues and thus protect the environment. Commercially, different edible coatings are utilized for different products such as coatings from oils, waxes, and resins; chocolate coatings employed for confections; corn zein coatings for candy, nutmeats, and pharmaceuticals; gelatin coatings for pharmaceuticals; collagen casings coatings for sausages and cellulose ether coatings for food ingredients. Among fruits and vegetables, whole and fresh-cut can be coated which include apple, grapefruit, passion fruit, avocado, lime, peach, lemon, orange, melons, tomato, cucumber, bell pepper, fresh-cut pear, fresh-cut apple, fresh-cut peach, fresh-cut lettuce, fresh-cut cabbage, fresh-cut potato, fresh-cut tomato, fresh-cut muskmelon, fresh-cut cantaloupe, minimally processed carrot, and minimally processed onion. Although edible coatings offer many advantages but still exert various disadvantages to the food products, especially postharvest fruits and vegetables. For example, they provide rancid flavor due to oxidation of lipids, they contain their inherent color which might be unappealing for the consumers, and they also require special compounds in order to meet the appropriate effectiveness of the bioactive ingredients used in the coating material. Therefore, in order to eradicate this problem, nanoemulsion acts as an emerging and novel technology to enhance the quality, stability, and shelf life of food products.

2.3 Types Of Edible Coating:

Proteins, polysaccharides, lipids, or a combination of these substances can all be found in edible coatings, which are made of composite components. All varieties of fruit are commonly coated with polysaccharides (gums, starches, pectin, and cellulose derivatives), with pectin, alginate, aloe, chitosan, gum Arabic, and methylcellulose receiving particular attention. Four broad categories of edible films and coatings include:

2.3.1 Polysaccharide-based edible films and coatings:

Edible films and coatings are created using polysaccharides such as starch, pectin, cellulose, exudate gums, seaweed extracts, etc. The physical, mechanical, and functional qualities of edible films and coatings are taken into consideration when choosing these constituents. Although polysaccharide-based films and coatings have poor moisture barrier qualities, they are only slightly less permeable to oxygen and selectively permeable to oxygen and carbon dioxide (CO₂). Because they can alter the atmosphere inside the product, they are useful for the preservation of fruits and vegetables where they can lower the respiration rate. Pea starch-based edible films improved food and non-food uses by combining guar gum, glycerol, and other ingredients.

2.3.2 Protein-based edible films and coatings:

Protein alterations provide films and coatings with improved functional and technical qualities. Scientists and producers are becoming increasingly interested in this aspect of protein-related films and coatings. For the creation of edible films and coatings, proteins from both plants and animals, such as gelatin and soy protein, can be used. For instance, the protein from sesame seeds was successfully used to create edible film.

2.3.3 Lipid-based edible films and coatings:

Lipids have been used as edible coatings for food preservation for a very long time. Citrus fruits were preserved in ancient China using wax coats. Later, larding (using lards or fats) was used to extend the shelf life of beef products in England. The following list includes some of the lipids, waxes, and resins used to create edible films and coatings:

- Lipids, such as cocoa butter, coconut oil, palm oil, and sunflower oil.
- Wax types: Candelilla wax, beeswax, carnauba wax, jojoba oil, etc.
- Resins: Tragacanth gum, Arabic gum, mesquite gum, etc.

2.3.4 Composite edible films and coatings:

The development of composite edible films and coatings involves using more than one of the aforementioned ingredients. Utilizing many ingredients allows you to benefit from their synergistic interactions. The physical and barrier qualities of tragacanth-locust gum bean blend films were said to be superior to those of their individual counterparts.

2.4 Pectin:

The principal cell walls of plants and fruits receive structure from pectin, a high-molecular-weight water-soluble polysaccharide that is primarily made up of (1 4) linked -d-galacturonic acid esterified units. The pectin polymer chains create a flexible network that solidifies into a robust gel in the presence of calcium or other multivalent metal cations. To improve food items' viscosity and gel strength, pectin is derived from a variety of fruit by-products. As amply demonstrated in other investigations, the calcium treatment required to cause pectin gelation (often 1% CaCl₂) assists in retaining fruit firmness throughout storage. However, consumers may occasionally taste bitterness because of the remaining calcium chloride on the fruit's surface. It is also fascinating to employ in a variety of food products due to its medicinal activity and nutritional advantages for human health. Pectin coatings have also been investigated for their capacity to reduce moisture loss and lipid migration, as well as to enhance food look and handling.

2.5 Methods of edible coating:

The effectiveness of applied coating materials on food products such as fruits, vegetables, meat, etc., is influenced by the various applied techniques such as dipping, spraying, fluidized-bed, and panning (Yanyun, 2011; Raghav et al., 2016). These deposition methods of coating applications on food products are dependent on the nature of food that should be coated, the surface attributes, and the primary objective of the coating. The applying procedure of edible coating solution on food products is accompanied by the adhesion procedure which involves

diffusion between the coating solution and the surface area of the food product (Parreidt et al., 2018). The wetting stage of the coating application procedure is vital because when the coating affinity for the product is effective, the time required for such an operation will be minimal letting the coating solution to be applied spontaneously (Park & Seo, 2011). Before the application of edible coating on food products, two product ripening patterns such as climacteric and non-climacteric should be considered (Flores-López et al., 2016). The coating application methods on food products have demonstrated numerous merits/demerits and effectiveness. These methods are dependent on the properties of food products and physical characterizations such as surface tension, density, and viscosity of coating materials (Andrade et al., 2012). The physical parameters of food products such as shape and size, desired coating thickness influence the selection of the coating system and device (Debeaufort & Voilley, 2009). The coating 14 application methods are used for the deposition and adhesion on the surface of the food products and casting of the coating materials in the form of a liquid solution, suspension, emulsions, and powder. The adjustments of covering layers of coating materials on the surface of food products were conversed by using drying, warming, cooling, and coagulation process.

2.5.1 Dipping method:

Dipping is the most common method of coating on the food product. It is the immersion of a food sample in the coating-forming dispersion (dipping process) (Senturk et al., 2018). The dipping method for applying edible coating on food products consists of three steps: i) immersion & dwelling, ii) deposition and iii) evaporation of solvents (Andrade et al., 2012; Brinker, 2013; Costa et al., 2014; Tavassoli-Kafrani et al., 2016). In the first step, the substrate is immersed in the coating emulsion/solution at a constant speed, the dwelling ensures enough quantity of solution for wetting substrate and complete interaction between both substrate and coating matrix (Valdés et al., 2017). The deposition process is used to develop thin layers of the precursor emulsion on the surface of food products. The excess surface liquid drains and removes by deposition (Costa et al., 2014). During evaporation step, the solvent and excess liquid are evaporated from the surface of food products by using heating and drying procedure. The product will be dried either at room temperature or with the help of a dryer when the surplus coating is drained away (Andrade et al., 2012). Thickness of liquid coated

films has been shown to rely on the characteristics of a coating solution such as density, viscosity, surface tension as well as surface withdrawal rate (Cisneros-Zevallos & Krochta, 2003).

2.5.2 Spraying method:

Spraying is the most common method used in the application for coating on food products (Debeaufort & Voilley, 2009; Parreidt et al., 2018b). It increases the liquid surface through forming droplets and dispenses them across the food surface with a set of nozzles. Three types of 16 spraying techniques have been used in the industries for applying an edible coating on food products.

- i) Air spray atomization: In this method of spraying the high velocity stream of air is surrounding fluid flowing from a tube at a low speed. Fluid-air friction speeds up and disrupts the liquid fluid flow and induces atomization. It is also known as cost effective spraying application method used on food products to apply edible coating (Valdés et al., 2017). In this technique, air is used for fine spraying of the droplet on food products. The air jet nozzle jounces are used to break water jet (deflector) cylindrical into fine droplets for spraying of coating on food product.
- ii) Air assisted airless atomization: Air-assisted airless spray guns first partially atomize the fluid with a special fluid nozzle tip similar to a standard airless tip. Second, they complete the atomization with small amounts of compressed air from the face and/or the horns of the air nozzle that they use. The result is a finely atomized spray pattern closely resembling that of a compressed air system. Air-assisted airless atomization solves many problems with the use of high-viscosity and high-solids coatings, and issues associated with heating and using higher fluid pressures for the atomization of viscous materials. This method offers high production levels and a high-quality finish (Peretto et al., 2017).
- iii) Pressure atomization: In this technique, the edible coating is applied to food products by using pressure. Therefore, the air is not used in this technique so it is also known as air less atomization technique. The small size nozzles are used to pass high pressure energy to provide surface tension and high viscosity of coating solution for applying on food products. During the processing, the pressure

maintains below 3.5 bars to avert the annihilation of the film forming system (Andrade et al., 2012).

In this method, after the application of the initial coating and drying processes to the top, the bottom surface of the product may also be covered separately. The spray-coating process has been reported to form a thin film on food surfaces and can be well controlled despite the need to carefully modify the viscosity of the coating solutions for application with a specific spray-gun application (Andrade et al., 2012). Spraying enables thin or thick layers of suspensions of aqueous solution and molten lipids or chocolate to be deposited (Debeaufort & Voilley, 2009).

2.5.3 Fluidized-bed processing method:

The fluidized-bed method of edible coating is frequently utilized in applications for preparing food and conducting research. It is used to coat dry particles that are extremely small and/or low in density with thin coatings of material (Solis-Morales et al., 2009). In a fluidized coating process, the coating solution and suspension are sprayed onto the fluidized powder surface via a number of nozzles to form a shell-like structure. When a liquid flow rises through a bed of particles at a fast enough rate to assist the particles without pulling them into the liquid stream, fluidization takes place. At this moment, the bubbling fluid's characteristics referred to as fluidization are accepted by the particle bed. The three types of fluidized-bed process methods are top spray, bottom spray, and rotary-fluidized bed. However, in the food industry, top spraying is still the most efficient technique (Jacquot & Perneti, 2004). Because the powders in the conventional bed do not fluidize steadily or form excessive agglomerations in the smaller sizes, the size of the particulate matter in the fluidized bed should be more than 100 μ m (Guignon et al., 2002; Chen et al., 2009). The fluidized bed coating technique's particle aggregation fosters the coating material's solubility and dispersion. In addition, a liquid binder is utilized to increase the material's stability; this process is completed under heat treatment before being sprinkled on meals. This process results in the adhesion, aggregation, and drying of the particles. It applies to both continuous and batch processes (Debeaufort & Voilley,

2009). The commonly used nozzles in the fluidized-bed coating are pneumatic or binary: the fluid is delivered at low pressure and the air is sheared into the nozzles.

2.5.4 Panning method:

Greek Arabian society invented the panning coating technique, which was applied to the coating of drugs. The food and other products that need to be coated are put in a big rotating bowl called a pan using the panning technique. In order to evenly distribute the coating solution across the surface of the food product, the layering solution is then drizzled or dusted into a bowl that is spinning. Using forced air and temperatures of room temperature or greater, coating layers are cured (Pandey et al., 2007; Dangaran et al., 2009). Heat is produced by friction with the cold air during the panning method of coating food goods.

2.6 Functional properties of edible coating:

In the food processing industry, edible coating/film offers a stable quality for food products with market safety, nutritional value, and an affordable cost of manufacturing (Bhardwaj et al., 2019). Due to its promising barrier properties against moisture and gas transmission, lipid oxidation, and control of enzymatic activities and microbial spoilage, it preserves the quality parameters, appearance, and increases the shelf life of food products (fruits, vegetables, meat, fish, bakery, dairy products, etc.) (Rooney, 2005; Parreidt et al., 2018b). To prevent the oxidation of high-fat foods and to boost the oil resistance of fried food products, natural antioxidant and antibacterial compounds have also been added to edible films (Daraei et al., 2012; Ganiari et al., 2017). Edible material provides barrier properties against moisture and gases of fresh produce during storage to retard the enzymatic oxidation, which provide protection of the food from browning discoloration and texture softening. It also has the ability to prevent the loss of natural volatile flavour compounds and color components (Sapper & Chiraly, 2018).

2.7 Applications of edible films and coatings:

Some of the common applications of edible films and coatings are in the preservation of fruits and vegetables, meat and poultry, bakery and confectionery products, breakfast cereals, dry fruits like raisins and nuts, etc. Some of these applications have been discussed below in detail.

2.7.1 Preservation of fruits and vegetables:

Due to their high respiration rates and significant free moisture content, fruits and vegetables are regarded as perishable commodities. By lowering respiration, edible films and coatings sometimes serve as protective coverings surrounding the surface of fruits and vegetables, postponing ripening. Applications that are often used include:

- a) Applications for edible coatings to regulate mass transfer in fruits and vegetables.
- b) Edible coverings applied to fresh and cooked fruits and vegetables to lessen oxidative responses.
- c) Edible coatings for enhancing the gloss and shine on the surfaces of fruits and vegetables.
- d) Edible coatings to enhance the textural qualities.

2.7.2 Meat, fish and poultry:

The following table summarizes the use of edible films and coatings in the preservation of meat, poultry, and fish products:

Table 2.1: Application of edible films and coatings in meat preservation

Type	Applications
Starch	Beef, pork
Carageenam	Beef, fish, pork
Chitosan	Beef, fish, pork, and poultry
Collagen	Beef, pork

Gelatin	Beef, pork, fish
---------	------------------

2.8 Limitations related to edible films and coatings:

Edible films and coatings have various benefits, including low cost, simple application, use of natural components, etc. However, there are a few trouble spots that need research and development. However, many of these issues can be resolved by creating composite edible films, in which the limiting qualities are improved by synergistic reactions between ingredients. Below are some disadvantages associated with various edible films and coatings:

Table 2.2: Limitations of edible films and coatings

Type of edible film/ coating	Limitations
Protein based	Poor physical properties like tensile strength, elongation at break, puncture strength, etc.
Polysaccharide based	Poor moisture barrier properties due to the hydrophilic nature of constituents.
Lipid-based	a) Poor oxygen barrier property which makes the food prone to oxidative spoilage. b) Negatively affect the sensory properties of food.

The aim of this study was to extend the shelf life and preserve the quality of fresh pomegranate seeds during storage by using pectin-based edible coatings in combination with chemical dips containing calcium chloride and calcium carbonate.

CHAPTER 3

MATERIAL AND METHODS

3.1 Materials Required:

A nearby grocery store sold us pomegranates that were free of any physical flaws, such as bruising. Pomegranates are in good health and are of uniform size. The arils were cleaned and stored in the refrigerator until processing. Online pectin was introduced. In the lab, calcium carbonate and calcium chloride are available.

Laboratory Glassware: Burette, conical flask, beaker, petri plates, pipette, measuring cylinder, glass rod stirrer, funnel, cuvettes.

3.2 Equipment and Instruments:

The equipment and instruments that were used are as follows:

3.2.1 Autoclave:

An Autoclave is a device that uses highly pressurized saturated steam to efficiently kill microorganisms to sterilize medical equipment, glassware, and laboratory equipment. An autoclave is a sealed vessel that sterilizes materials by operating at high pressure and temperature. An autoclave machine also neutralizes biological risks. The physical process of disinfection and sterilizing is provided by autoclaves. They combine steam, pressure, and time to operate. In medical, dental, and laboratory settings, autoclaves are used to sterilize tools and equipment by the use of high temperature steam.



Figure 3.1: Autoclave

3.2.2 Hot Air Oven:

Dry heat sterilization is a technique used in Hot Air Ovens. Microorganisms and bacterial spores are destroyed in hot air ovens using extremely high temperatures maintained for several hours. By heating the outside surfaces of the item and allowing them to absorb the heat, the ovens use conduction to sterilize the item's center. One needs to be methodical in deciding what will be sterilized when, as hot air ovens sanitize equipment over a long period of time. This is dependent upon when the equipment must be made available once more.



Figure 3.2: Hot Air Oven

3.2.3 Electronic Analytical Balance:

The device used to monitor sudden weight fluctuations. The fundamental component is an analytical balance with a magnet and solenoid so that weight fluctuations can be electrically balanced. By including a photoelectric sensor device to track the motion of the balance beam and control the solenoid current, automatic balancing is made possible. According to a preliminary experiment, assuming the magnet's location in relation to the solenoid does not fluctuate noticeably, the current needed to maintain balance is proportional to the weight.



Figure 3.3: Electronic Analytical Balance

3.2.4 Laminar Air Flow:

In microbiology labs, equipment with a Laminar Air Flow is widely used. Laminar airflow (LAF) equipment is frequently used in aseptic processing as an engineering control to ensure that the production environment is free of airborne and surface contamination by microorganisms, pyrogenic and drug residues, and other materials that pose a risk of intravascular infection, pyrogenic response, or occlusion of the peripheral vasculature. It consists of a chamber with a connected air blower that directs air in straight, parallel lines at a consistent speed. The purpose of a laminar flow cabinet/primary hood is to maintain a hygienic workplace. It uses a filter pad and a specialized filter system known as a HEPA filter, which can remove airborne impurity particles as fine as 0.3 micrometers, or an air laminar.



Figure 3.4: Laminar Air Flow

3.2.5 Magnetic Stirrer:

A spinning magnet or a stationary electromagnet that generates a rotating magnetic field make up a Magnetic Stirrer, a common laboratory tool. This tool can be used, for instance, to quickly spin, stir, or mix a solution. It can also be used to create a stir bar. A linked heating system for the liquid is typically part of a magnetic stirring system.



Figure 3.5: Magnetic Stirrer

3.2.6 Spectrophotometer:

The relationship between values of the same photometric magnitude is measured using wavelengths by a Spectrophotometer, a device for chemical analysis. In other words, it calculates the amount of light that is absorbed after striking a sample solution. As a result, it relies on the following factors: transmittance, which describes the connection between input and output light beam intensities for a given wave frequency; absorbance, which describes how much light intensity is absorbed by the sample; and solution concentration.



Figure 3.6: Spectrophotometer

3.2.7 Refractometer:

Through refraction, the sucrose content of the sample is determined using Brix Refractometers. The amount of sucrose in a solution of pure water is measured in Brix as a percentage of weight. The liquid sample is put on a prismatic element in refractometers. The output of these devices is determined by the intensity of a light beam at the liquid-prism interface. In the most basic refractometers, the index is assessed visually using a linseed viewing tube and a graduated scale, where the output beam intersects. To calculate the Total Soluble Solids of the supplied sample, a Brix Refractometer was utilized.

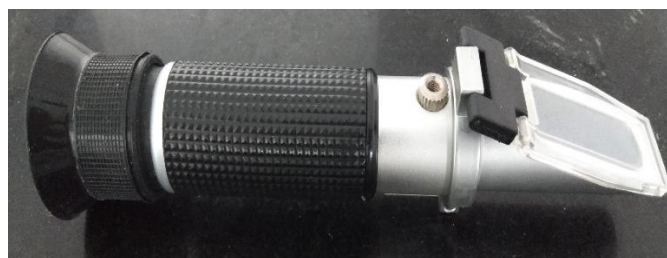


Figure 3.7: Refractometer

3.2.8 Incubator:

By controlling factors like humidity, carbon dioxide, and temperature, microbiological Incubators typically provide a contaminant-free environment for safe, controlled, and dependable work with cultures. In general, bacterial cultures are grown and stored in microbiological incubators during the storage period.



Figure 3.8: Incubator

3.2.9 pH Meter:

The acidity and alkalinity of liquids or semi-solid samples are measured using a pH meter. A probe makes up this three-in-one device. It has a temperature probe, a reference electrode, and a hydrogen ion-sensitive electrode. To ensure that any temperature difference is automatically adjusted, a temperature probe is employed. On a scale of 1 to 14, the pH of a solution is measured. A pH of 7 denotes a neutral solution. 1 is a highly acidic pH value, whereas 14 is a highly alkaline pH value.



Figure 3.9: pH Meter

3.3 Methodology:

3.3.1 Preparation of Coating Solutions:

For the preparation of pectin edible coating, 50ml of distilled water were used to dissolve 1% (w/v) and 2% (w/v) of pectin powder. The mixture was then homogenized by magnetic stirring until it became clear. The mixture was homogenized by magnetic stirring until it became clear after being dissolved in 50ml of distilled water with 1% (w/v) each of calcium chloride and calcium carbonate. The subsequent coating solution preparation involved dissolving 2% (w/v) pectin and 1% (w/v) calcium chloride in a (4:1) and (4:2) ratio before homogenizing the liquid with magnetic stirring until it was transparent. Before applying the coating solution to the fruit, it was chilled. In Table 3.1, the therapy formulations were displayed.

Table 3.1 Treatment Formulations-

Treatments	Components
T0	Control
T1	Pectin (1%)
T2	Pectin (2%)
T3	CaCl ₂ (1%)
T4	CaCO ₃ (1%)
T5	Pectin (2%) + CaCl ₂ (1%) (4:1)
T6	Pectin (2%) + CaCl ₂ (1%) (4:2)

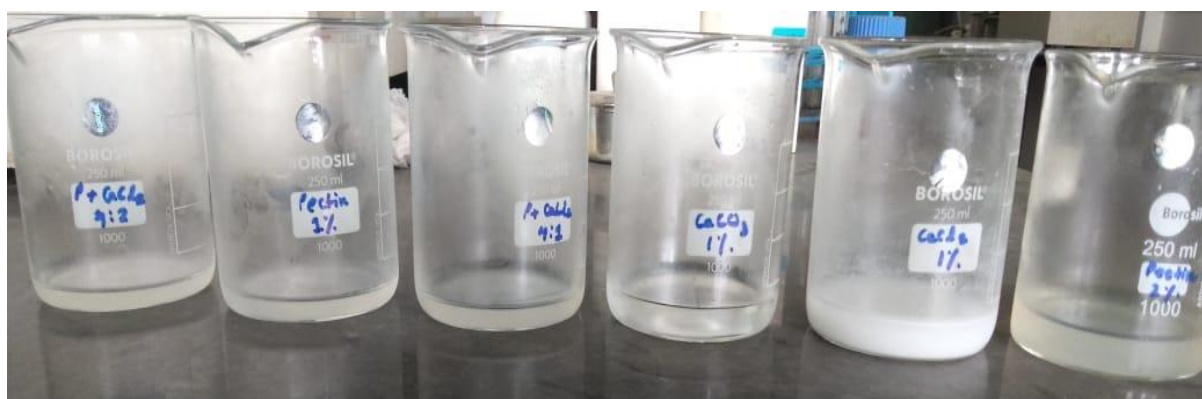
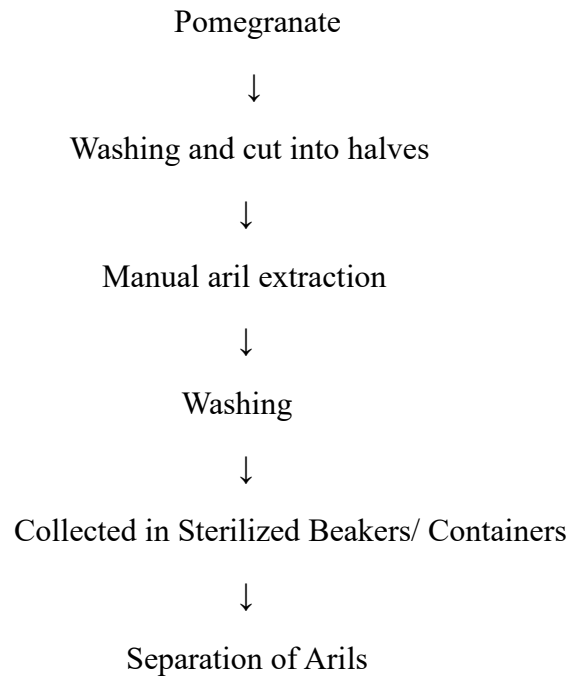


Figure 3.10 Prepared Coating Samples

3.3.2 Pomegranate Aril Processing:

Pomegranate were carefully preserved by carefully removing the exterior hard peel with sharp blades. The arils were then meticulously and physically removed, and collected in sterilized beakers or containers. For various treatments, the arils were separated. Hand gloves were worn at every stage of the pomegranate aril production procedure to prevent contamination.



Pomegranate



Pomegranate Arils

3.3.3 Fruit Preparation and Treatment:

Pomegranate Arils were submerged for a short time in distilled water, and then air-dried at room temperature. For two minutes, arils were submerged in the chemical solution. After being allowed to drip off the pomegranate arils for 5 minutes on the tissue paper, then the arils was kept on petri plates for 5 days at room temperature and 12 days in the refrigerator. Slices were submerged in distilled water as a control.

Arils were submerged in Distilled Water



Air Dried



Arils were submerged in Chemical Solutions



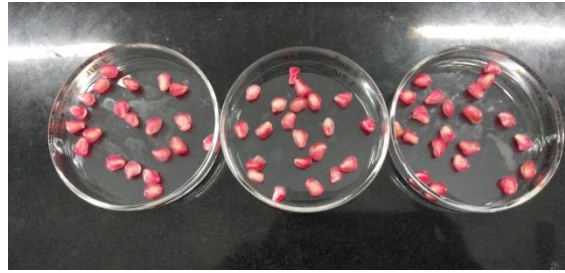
Drip off the Arils for 5 minutes on the tissue paper



Arils was kept on petri plates for 5 days at room temperature and 12 days in the refrigerator



Treated Pomegranate Arils



Treated Pomegranate Arils

3.4 Quality Evaluation:

3.4.1 Weight Loss:

Using an electronic analytical balance, the weight of the samples was measured on the day of sample preparation, followed by the specific analysis days, which were for 5 days (0, 3, and 5) at ambient temperature and for 12 days (0, 3, 6, 9, and 12), when the samples were stored in the refrigerator. The percentage of weight loss was calculated using the formula below:

Formula Used: $\text{Weight loss (\%)} = [(W_0 - W_S) / (W_0)] * 100$

where, W_0 is the initial weight (g) on day 0 and W_S is the measured weight (g) of each sample on a particular day of analysis during storage.

3.4.2 TSS (° Brix):

The amount of total soluble solids (TSS) in fruit is one of the key elements affecting how tasty it is to consume. Given that starch is hydrolyzed into simple sugars by catabolic

processes like respiration, such as ripening, TSS serves as a measure of fruit sweetness and is known to rise during ripening. Although increases in TSS brought on by the concentration of sugars within the fruit may also be attributed to postharvest moisture loss, starch breakdown frequently has a bigger impact on changes in TSS.

A digital refractometer was used to measure the amount of total soluble solids, and the results were represented as a percentage. Prior to taking readings, the refractometer was calibrated with distilled water.

3.4.3 Moisture Content:

Make a note of the empty petri plate's weight and then fill a petri plate with 2 g of the sample (pomegranate arils). Record the weight of the sample then insert the Petri dish into the hot air oven. After that set the thermostat to 135 °C and let the sample air dry for two hours. Put the petri plate in the desiccator to cool it. Make a note of the total weight. Carry out the steps again for each coated sample.

Formula Used: $\text{Moisture Content (\%)} = \frac{WS - (W2 - W1)}{WS} * 100$

Where, W1 is the weight of empty petri plate, W2 is the weight of petri plate after drying, and WS is the weight of sample.

3.4.4 Titratable Acidity:

After treatment and during the storage period, titratable acidity was assessed. Pomegranate arils' titratable acidity (TA) was measured using a titration technique and 0.1N NaOH Solution.

In 2 g of pomegranate homogenate, made up to 10 g with distilled water, and add 2-3 drops of phenolphthalein as an indicator for a color shift to light pink, the titratable acidity was calculated then make a note of the initial reading. Pomegranate arils that had been homogenized were titrated with 0.1N NaOH Solution until the color changed to bright pink. Make a note of the last reading.

Formula Used: Titratable Acidity (%) = Titre Value * 0.1N NaOH * Equivalent Weight / Weight of Sample * 100

Where, Equivalent weight of citric acid is 70mg/ m Eq

3.4.5 pH:

The covered pomegranate arils were crushed to determine the pH of the fruit and fill a beaker with 2g of the crushed sample (coated pomegranate arils) then fill the beaker with 10g of water. Use distilled water that has been immersed in the electrode soak solution to rinse and clean the pH electrode. Submerge the electrode completely. Press the run/enter key and make a note of the reading.

3.4.6 DPPH Free-Radical-Scavenging Activity:

To make a DPPH solution, combine 0.002 grams of DPPH powder with 50 ml of methanol in a beaker. Cover the beaker with foil and store in a dark area. Adding 2 mg of crushed sample (coated pomegranate arils) to 1ml of methanol will create the extract. Then add 3ml of DPPH solution, 3ml of methanol, and 1ml of the extract to the test tube. A blank sample was also made by combining 3ml of DPPH solution, 3ml of methanol, and 1ml of distilled water and cover it with foil and let it in the dark for 30 minutes. A spectrophotometer was used to measure the absorbance at 517 nm. Then make a note of the reading. For each covered pomegranate aril, repeat the process.

Formula Used: DPPH scavenging activity (%) = $[1 - \text{absorbance of sample} / \text{absorbance of blank}] \times 100$

3.4.7 Antibacterial Activity:

For the purpose of analyzing the antibacterial activity toward the test organisms, the agar well diffusion method was used.

Gram-positive (*Bacillus Pumilus*) and gram-negative (*E-Coli*) bacteria are spread out over the surface of a nutrient agar medium plate in an amount of 100 µl each. Using a sterile borer, wells were made in the agar, and coating solutions (200 µl) were poured into them. The inoculation plates were then incubated for 24 hours at 37°C. By measuring the diameter of the inhibitory zone (DIZ) of the investigated bacteria, antibacterial activity was assessed.

3.4.8 Antifungal Activity:

In order to evaluate the antifungal activity against the test species, the agar well diffusion method was used.

Take a black fungal Mold that was growing on pomegranate arils and place it on a PDA medium plate to grow. After that, let the plate sit at 25 °C for 3 to 4 days. Inoculate this into the PDB/NB medium after three to five days; a few days later, green and white Mold will appear in the media. Spread across the surface of the Nutrient Agar Medium Plate are 100 µl each of Green Mold and White Mold. Using a sterile borer, wells were made in the agar, and coating solutions (200 µl) were poured into them. The inoculation plates were then incubated for 24 hours at 28°C. By measuring the diameter of the inhibitory zone (DIZ) of the investigated bacteria, antibacterial activity was assessed.

CHAPTER 4

RESULTS AND DISCUSSION

In the current study, the pomegranate arils were coated with pectin and some other chemicals with different treatment formulations under two different temperature conditions (at room temperature and in refrigerator). The goal was to maintain the quality and extend the shelf life of pomegranate arils, and the use of edible coatings appears to be a promising option. The coated and uncoated pomegranate arils weight loss, pH, TSS, Titratable Acidity, Moisture Content, Antioxidant, Antibacterial, Antifungal, and sensory ratings were all analyzed in the study. Throughout the storage period of time (days), samples of pomegranate arils were examined every 3 days. The results are described below:

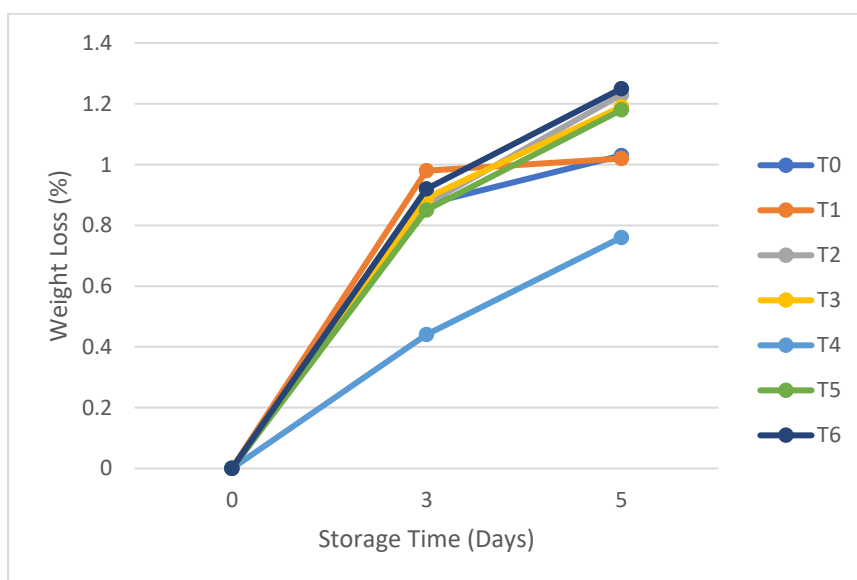
4.1 Effect of weight loss on storage time:

As moisture loss is linked to a decline in the turgescence and crispness of fresh fruit, it is a sign of freshness. The primary factor in weight loss in fresh fruits during storage is moisture evaporation through the fruit's surface. Due to the way edible coatings behave as a semipermeable barrier against moisture, it is thought that they can help fresh-cut fruits retain more water.

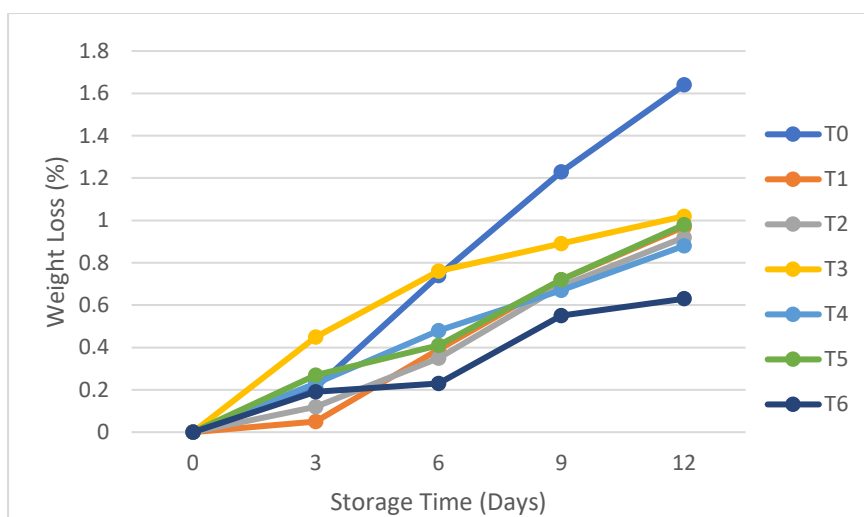
Throughout the storage period, the weight loss % increased. In comparison to other coated samples, weight loss was avoided in pomegranate arils by the use of a pectin coating cover. Consequently, the amount of weight loss in fruits with 1% coating Pectin levels were substantially lower than in the control and other coated treatment samples. A hydrophobic ingredient must be included in the coatings in order to prevent water evaporation, while hydrophilic compounds that are effective gas barriers do not affect moisture outflow. Citric acid in these coatings has caused the loss of aril water during the storage period of pomegranate arils, which is why the weight loss of arils coated with 1% pectin solution was greater than that of other treatments. Pomegranate arils' weight loss % at ambient temperature is higher than that of the sample kept in the refrigerator.

Treatments	Room Temperature			Refrigerated				
Days	0	3	5	0	3	6	9	12
T0	0	0.87	1.03	0	0.21	0.74	1.23	1.64
T1	0	0.98	1.02	0	0.05	0.39	0.72	0.97
T2	0	0.87	1.23	0	0.12	0.35	0.69	0.92
T3	0	0.89	1.19	0	0.45	0.76	0.89	1.02
T4	0	0.44	0.76	0	0.23	0.48	0.67	0.88
T5	0	0.85	1.18	0	0.27	0.41	0.72	0.98
T6	0	0.92	1.25	0	0.19	0.23	0.55	0.63

Table 4.1: Effect of weight loss on control and treated pomegranate arils stored for 5 days at room temperature and stored for 12 days in a refrigerator.



(a)



(b)

Figure 4.1: Effect of weight loss on control and treated pomegranate arils (a) Stored for 5 days at room temperature (b) Stored for 12 days in a refrigerator.



T0



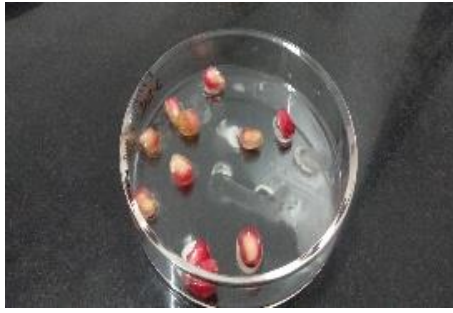
T1



T2



T3



T4



T5



T6

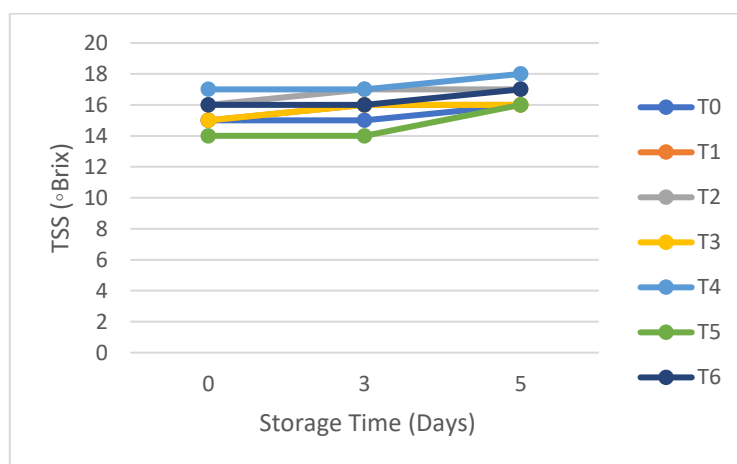
Figure 4.2: Weight loss of control and treated pomegranate arils (T0 – T6) during storage time (days).

4.2 Effect of TSS(°Brix) on storage time:

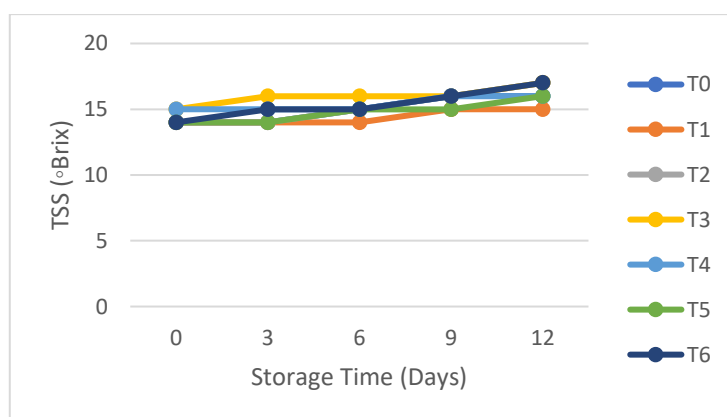
The pomegranate arils' total soluble solid content revealed noticeably higher TSS values during storage. When compared to other coated samples, pectin-coated samples that had been dipped had the lowest TSS values. When compared to samples stored in the refrigerator, TSS grew more gradually throughout the first three days in the majority of ambient temperature samples. TSS content has risen in the majority of Control samples over the past three days of storage. The ongoing acid metabolism, which transforms starch and acid into sugar during post-harvest storage, has been blamed for the rise in TSS. This could be explained by the fact that these edible coatings, when compared to uncoated samples, might slow down the metabolic processes that lead to degradation.

Treatments	Room Temperature				Refrigerated			
Days	0	3	5	0	3	6	9	12
T0	15	15	16	14	14	15	16	16
T1	15	16	16	14	14	14	15	15
T2	16	17	17	14	14	15	16	17
T3	15	16	16	15	16	16	16	17
T4	17	17	18	15	15	15	16	16
T5	14	14	16	14	14	15	15	16
T6	16	16	17	14	15	15	16	17

Table 4.2: Effect of TSS ($^{\circ}$ Brix) on control and treated pomegranate arils stored for 5 days at room temperature and stored for 12 days in a refrigerator.



(a)



(b)

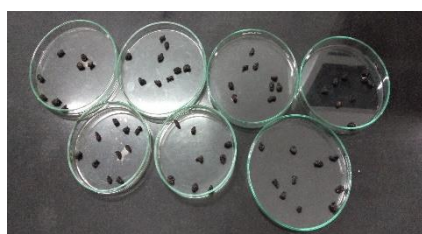
Figure 4.3: Effect of TSS ($^{\circ}$ Brix) on control and treated pomegranate arils (a) Stored for 5 days at room temperature (b) Stored for 12 days in a refrigerator.

4.3 Effect of Moisture Content on storage time:

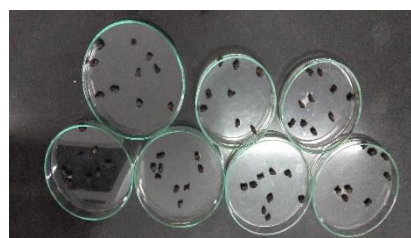
Pomegranate arils' moisture content decreased over the course of storage at both temperatures. The moisture content of coated and uncoated pomegranate arils should differ somewhat at both temperatures. Pomegranate arils typically have an initial moisture content of 81%, and the outcome of coated pomegranate arils demonstrates that moisture content is closer to that level.

Treatments	Room Temperature			Refrigerated				
Days	0	3	5	0	3	6	9	12
T0	80.12	77.14	75.23	81.37	77.45	76.77	75.49	75.32
T1	79.14	78.54	76.24	78.81	77.72	77.54	76.31	75.86
T2	81.38	79.48	75.67	80.82	79.24	78.77	77.12	76.14
T3	80.54	78.24	75.86	90.59	78.66	77.81	76.50	75.76
T4	83.14	80.18	78.24	83.33	81.37	79.95	76.07	75.23
T5	80.78	78.37	76.18	81.24	79.24	77.38	76.29	75.34
T6	81.98	79.14	78.39	80.18	78.12	77.68	76.34	75.77

Table 4.3: Effect of moisture content on control and treated pomegranate arils stored for 5 days at room temperature and stored for 12 days in a refrigerator.

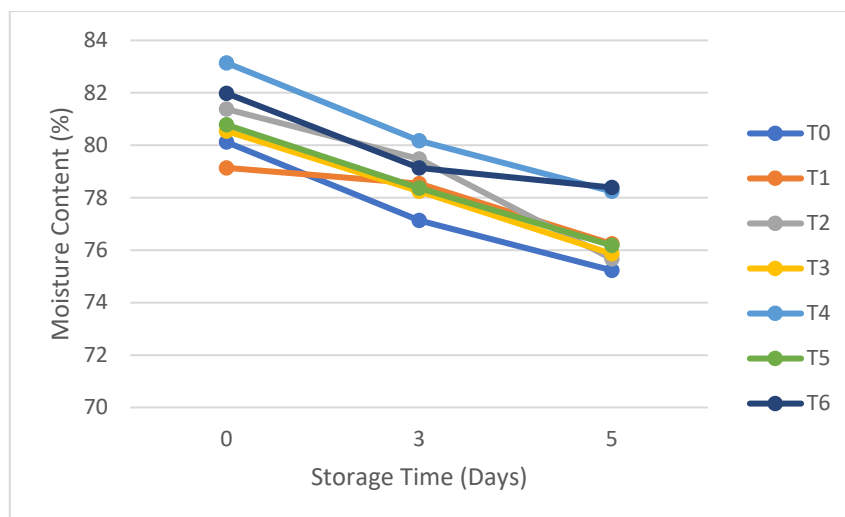


(a)

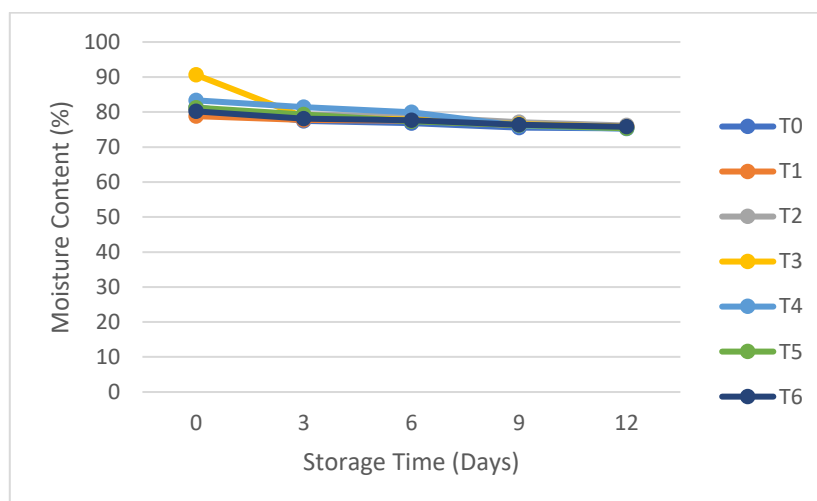


(b)

Figure 4.4: Endpoint observation of moisture content (a) At Room Temperature (b) In Refrigerator.



(a)



(b)

Figure 4.5: Effect of moisture content on control and treated pomegranate arils (a) Stored for 5 days at room temperature (b) Stored for 12 days in a refrigerator.

4.4 Effect of Titratable Acidity on storage time:

All samples' titratable acidity generally increased throughout the course of storage days, most likely as a result of organic acids' conversion to sugars and subsequent usage as respiratory metabolism substrates. It was interesting to see that treatment T5 and T6 did not exhibit an increase in titratable acidity after 3 days of storage at room temperature, and treatment T2 and T5 did not exhibit an increase in titratable acidity after 9 days of storage in refrigerator samples. This was in contrast to the other samples, where titratable acidity increased, likely because

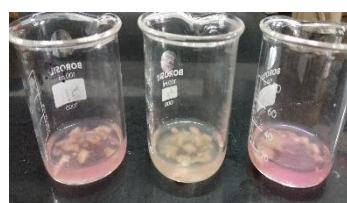
fermentation had begun in these samples. This might mean that the treatments created a gas barrier on the fruit's surface or reduced surface-gas permeability, which caused the respiration rate to slow down.

Treatments	Room Temperature			Refrigerated				
Days	0	3	5	0	3	6	9	12
T0	0.14	0.14	0.17	0.14	0.14	0.17	0.17	0.24
T1	0.17	0.17	0.21	0.14	0.14	0.14	0.14	0.21
T2	0.17	0.21	0.21	0.14	0.17	0.17	0.21	0.21
T3	0.17	0.17	0.21	0.17	0.17	0.17	0.17	0.24
T4	0.17	0.21	0.21	0.14	0.17	0.17	0.17	0.21
T5	0.14	0.17	0.17	0.14	0.17	0.17	0.21	0.21
T6	0.14	0.17	0.17	0.14	0.14	0.14	0.17	0.21

Table 4.4: Effect of titratable acidity on control and treated pomegranate arils stored for 5 days at room temperature and stored for 12 days in a refrigerator.

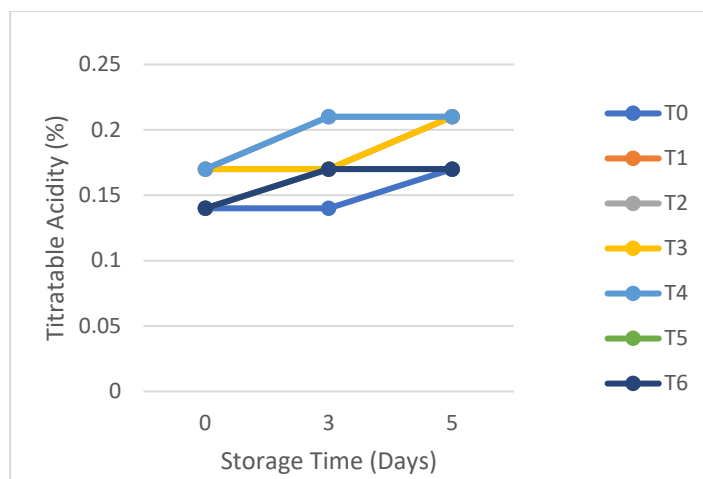


(a)

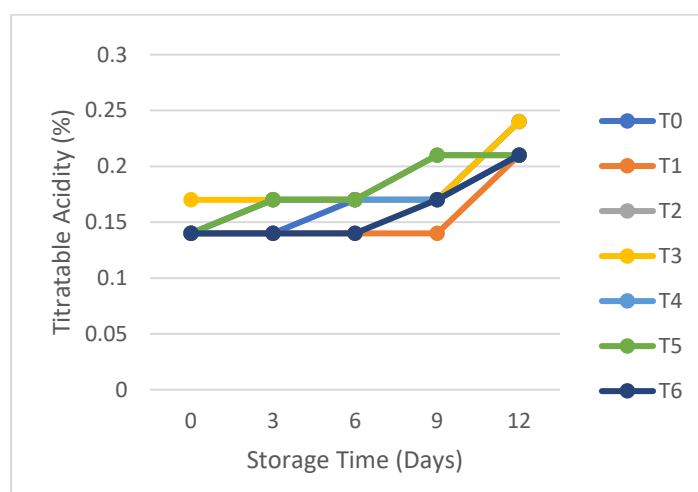


(b)

Figure 4.6: Endpoint observation of titratable acidity (a) At Room Temperature (b) In Refrigerator



(a)



(b)

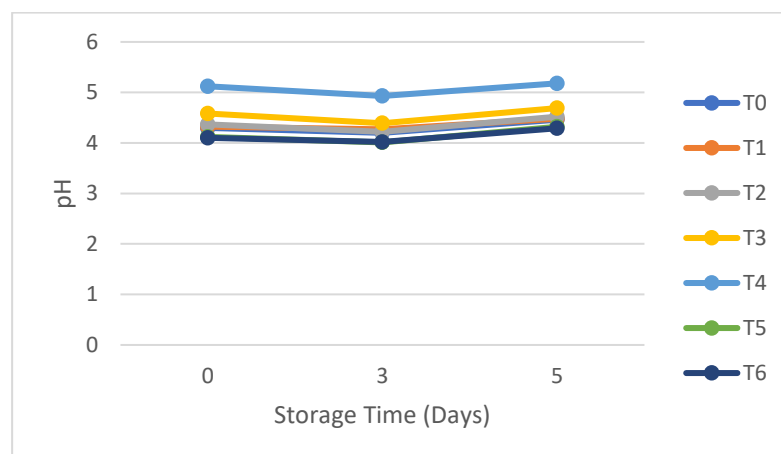
Figure 4.7: Effect of titratable acidity on control and treated pomegranate arils (a) Stored for 5 days at room temperature (b) Stored for 12 days in a refrigerator.

4.5 Effect of pH on storage time:

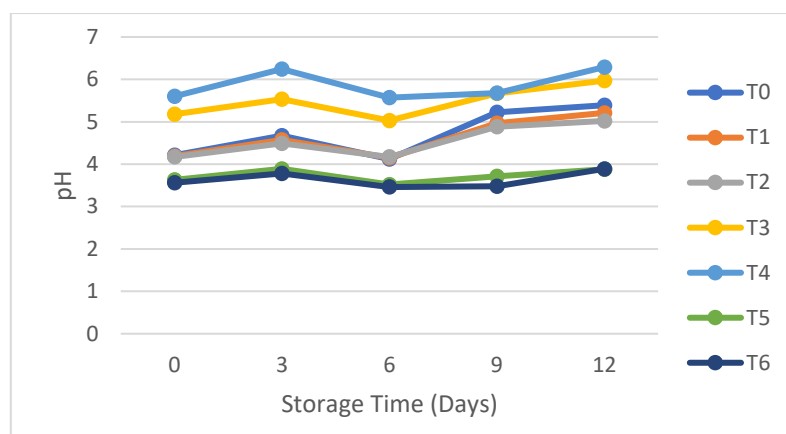
After a few days of storage under both conditions, the pH of all coated and uncoated samples decreased for the first time. The pH of the samples stored at room temperature initially increased after 0 days and then reduced after 3 days, but the pH of the samples stored in the refrigerator first increased but after 0 days and then decreased after 3 days. The pH of the chilled sample rose once more after six days of storage. The pH of the chilled sample during treatments T5 and T6 is less pronounced than under the other treatments.

Treatments	Room Temperature			Refrigerated				
Days	0	3	5	0	3	6	9	12
T0	4.30	4.20	4.46	4.21	4.67	4.12	5.22	5.39
T1	4.32	4.27	4.48	4.19	4.57	4.15	4.97	5.21
T2	4.37	4.22	4.52	4.17	4.49	4.17	4.88	5.02
T3	4.58	4.39	4.69	5.18	5.53	5.03	5.67	5.97
T4	5.12	4.93	5.18	5.60	6.24	5.57	5.68	6.29
T5	4.12	4.01	4.32	3.63	3.89	3.52	3.71	3.88
T6	4.10	4.02	4.29	3.56	3.78	3.46	3.48	3.89

Table 4.5: Effect of pH on control and treated pomegranate arils stored for 5 days at room temperature and stored for 12 days in a refrigerator.



(a)



(b)

Figure 4.8: Effect of pH on control and treated pomegranate arils (a) Stored for 5 days at room temperature (b) Stored for 12 days in a refrigerator.

4.6 Effect of DPPH Free-Radical-Scavenging Activity on storage time:

Pomegranate arils' DPPH free-radical-scavenging activity as influenced by pectin coating and other chemical treatments. The antioxidant activity of pomegranate arils was not considerably impacted by pectin coating or other treatments. The DPPH Free-Radical-Scavenging Activity under both circumstances decreased with storage time. On both circumstances with storage periods, treatment samples T0 and T4 had considerably higher DPPH antioxidant activity than other treatment samples. Antioxidant substances like ascorbic acid and polyphenols, which are rapidly decreased in the presence of oxygen via enzyme-mediated processes, may be to blame for the oxidation of antioxidant activity values. When compared to other treatments in both settings and for the duration of storage, treatments T5 and T6 exhibit slightly different antioxidant activity.

Treatments	Room Temperature			Refrigerated				
Days	0	3	5	0	3	6	9	12
T0	48.29	42.24	38.23	45.96	42.27	40.03	38.29	35.92
T1	28.87	24.89	21.27	23.57	20.22	18.16	16.24	13.12
T2	25.17	22.72	20.46	24.14	22.89	20.18	18.72	16.82
T3	35.18	31.87	28.89	35.09	33.29	30.98	28.24	25.24
T4	40.14	36.14	33.14	38.94	35.29	32.18	30.98	29.87
T5	34.42	31.29	28.42	32.29	30.29	28.87	26.72	24.78
T6	36.12	32.93	29.72	34.18	32.29	30.89	28.24	25.19

Table 4.6: Effect of DPPH Antioxidant Activity on control and treated pomegranate arils stored for 5 days at room temperature and stored for 12 days in a refrigerator.

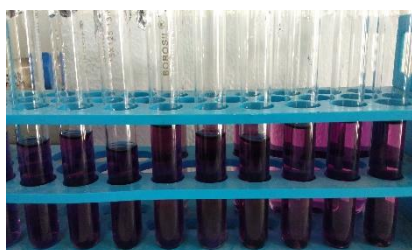
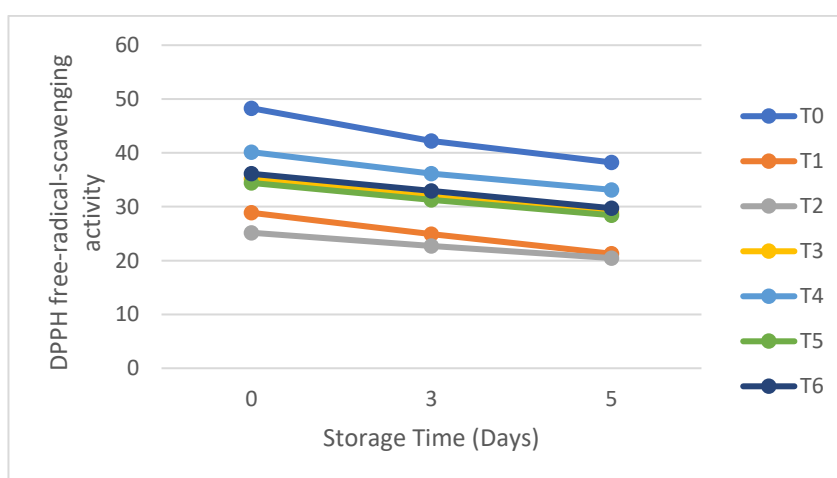


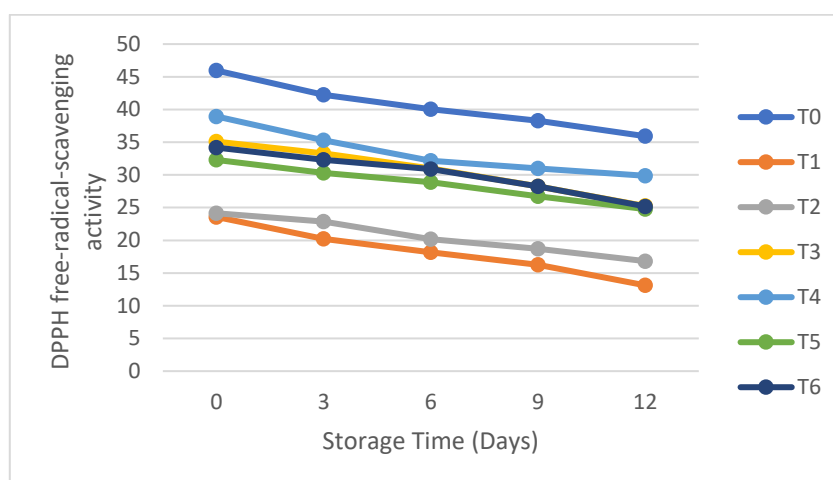
Figure 4.9 DPPH Antioxidant Activity



Figure 4.10 Spectrophotometer



(a)



(b)

Figure 4.11: Effect of DPPH Antioxidant Activity on control and treated pomegranate arils (a) Stored for 5 days at room temperature (b) Stored for 12 days in a refrigerator.

4.7Antibacterial Activity of prepared coatings against Gram Positive and Gram Negative Bacteria:

Gram-positive *Bacillus Pumilus* is one of two bacteria employed, and Gram-negative *E-Coli* is the other bacteria.

Gram positive and Gram negative bacteria in the treatment groups (T0-T6) did not exhibit any zone of inhibition. Only antibiotics exhibit a zone of inhibition for both bacteria and under both conditions, whereas distillate water did not exhibit a zone of inhibition as indicated on the table.

Organisms	Diameter of zone of inhibition					
	Room Temperature			Refrigerated		
	Antibiotic	DW	(T0-T6)	Antibiotic	DW	(T0-T6)
Bacillus Pumilus	0.7	0	0	0.6	0	0
E-Coli	0.8	0	0	0.8	0	0

Table 4.7: Antibacterial Activity of prepared coatings

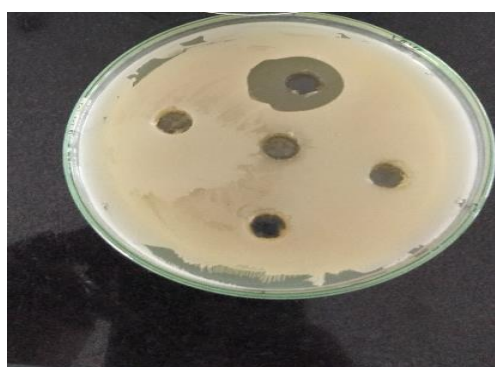
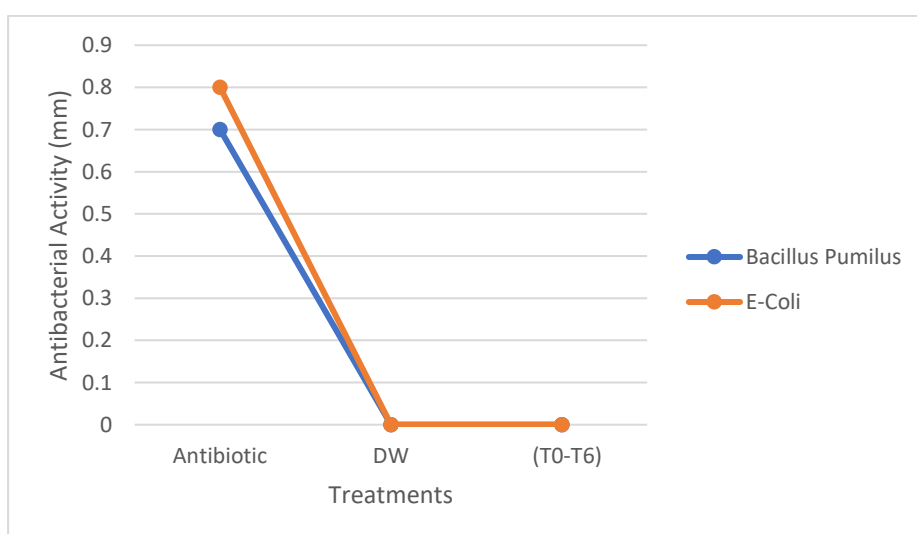
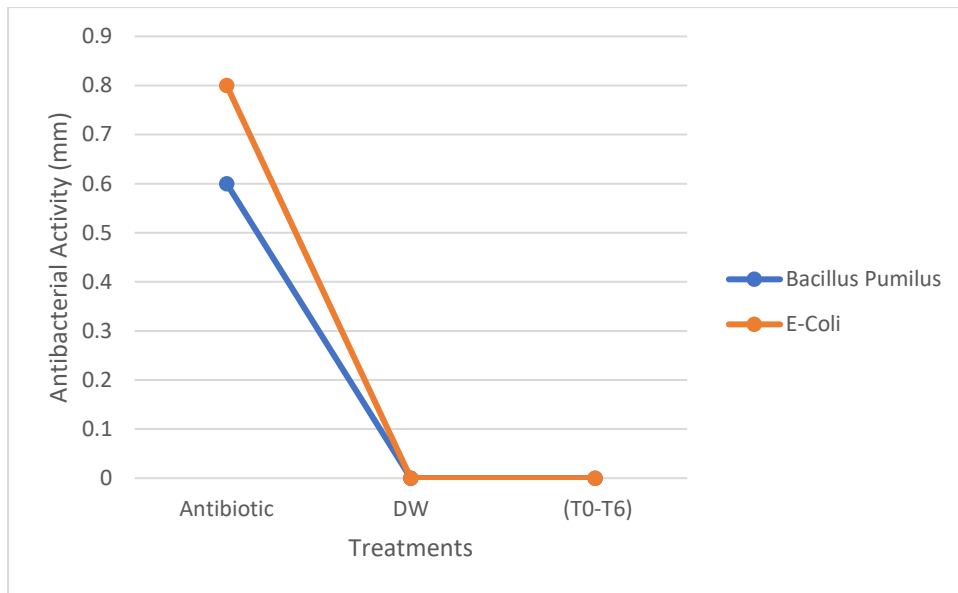


Figure 4.12: End Point Observation of Antibacterial Activity



(a)



(b)

Figure 4.13: Antibacterial Activity of prepared coatings (a) At Room Temperature (b) In Refrigerator

4.8 Antifungal Activity of prepared coatings against Green Mold and White Mold:

Green Mold and White Mold are the two types of Mold that are employed.

Treatments (T0-T6) failed to demonstrate a zone of inhibition for either White Mold or Green Mold. In contrast to Distilled Water, which did not exhibit any zone of inhibition as indicated in the table, only Antibiotic exhibits the zone of inhibition of both Mold and on both conditions.

Organisms	Diameter of zone of inhibition					
	Room Temperature			Refrigerated		
	Antibiotic	DW	(T0-T6)	Antibiotic	DW	(T0-T6)
Green Mold	0.6	0	0	0.7	0	0
White Mold	0.7	0	0	0.8	0	0

Table 4.8: Antifungal Activity of prepared coatings

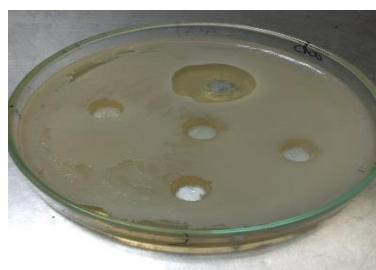
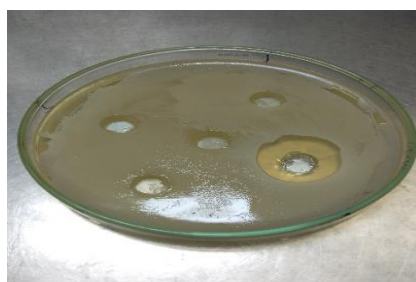
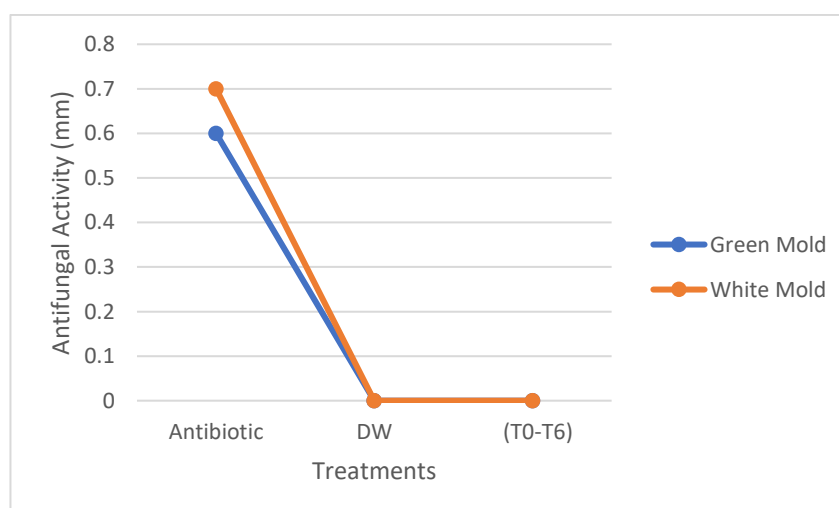
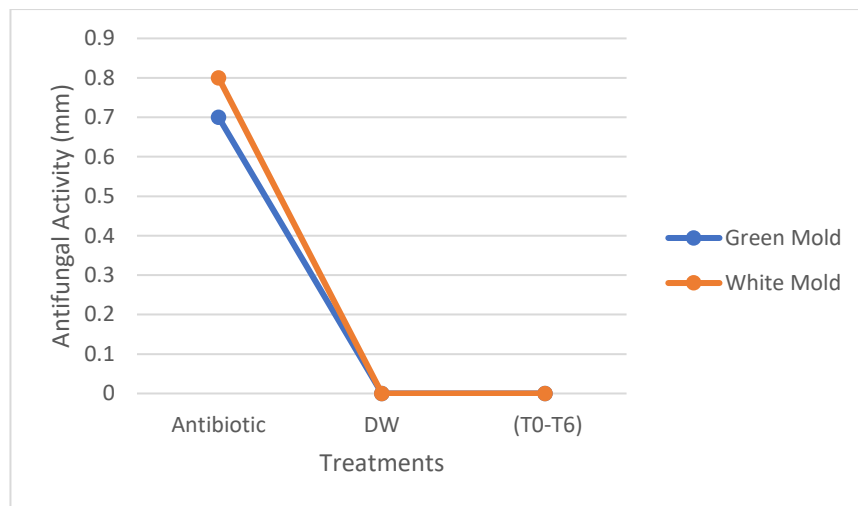


Figure 4.14: End Point Observation of Antifungal Activity



(a)



(b)

Figure 4.15: Antifungal Activity of prepared coatings (a) At Room Temperature (b) In Refrigerator

SUMMARY AND CONCLUSION

Edible Thin layers of coating, like pectin, are put to the surface of food products to protect them and enhance their quality. Pectin addition and other chemical processing yielded some encouraging results on the qualitative characteristics of ready-to-eat pomegranate arils. The effectiveness against the microorganisms that tend to give product its original scent was strengthened as pectin content increased. Pomegranate arils' microbiological quality was successfully maintained by coatings. Pectin should therefore be used in lower doses to prevent any negative effects on quality, notably the sensory qualities. Furthermore, adding more Pectin and other chemical treatments makes the coating viscous enough to make applying it to the surface of fresh pomegranate arils or barely processed food challenging. Then, this Edible Coating can assist growers make more profit by enhancing fruit characteristics while optimizing the storage conditions for pomegranate arils.

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