Effect of Edible Coatings on the Shelf-Life Extension of Fresh/Cut Fruits and Vegetables

By

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EDIBLE COATING FOR FRESH/CUT FRUITS AND VEGETABLES

ABSTRACT

Harvested fruits and vegetables are generally very perishable and they become more perishable when they are cut due to removal of their external protective layers and exposure of tissue to external atmosphere. There is an increase in demand for fresh cut fruits and vegetables which has opened real challenges to food producers to develop novel and sustainable preservation techniques. While many of the advanced packaging and storage techniques generally help to preserve fruits and vegetables, protective and edible films/coatings offer a better choice to cut fruits and vegetables as they provide an alternate and effective protective barrier to the cut surface. Food-grade biomolecules provide excellent coating properties with adequate physical-mechanical strength and can even supplement some nutrients. The main purpose of this research was to evaluate the suitability of selected edible coatings to extend the shelf life of both fresh whole and cut fruits and vegetables. The focus was on use of novel combination of sodium alginate, calcium chloride and ginger oil as well as a chitosan-based multilayer coating for extension refrigerated shelf-life of cut pears and whole mini cucumbers.

A coating formulation comprising of 2 % sodium alginate and 1% citric acid (SACC) was evaluated for cut pears for 15 days at 4 °C with samples without treatment as control. SACC coated pear fruits were then dipped in a 2 % calcium chloride to supplement as a firming agent cross link and enhance the film formation and surface binding of the coating. Another formulation was prepared with SACC blended with 0.5 % ginger essential oil (SAGEO) added as an antimicrobial agent. A multilayer coating with 2 % alginate and 1% chitosan was used for fresh mini cucumbers which were stored at 4°C for 20 days. Different quality parameters were evaluated to follow the course of physiological activity: tissue color, texture, total soluble solids,

titratable acidity, weight loss, respiration rate, chlorophyl content and mold growth. These were evaluated during the storage at selected time intervals.

Sodium alginate coating (SACC) extended the shelf life of cut pear fruits with better preserved quality parameters as compared to the control. SAGEO with ginger essential and citric acid exhibited even better results by preventing mold growth, suppressing oxidative browning of cut pear fruits during the entire storage period. Overall, SAGEO gave the best quality, although SACC was also better than control. The multilayer alginate and chitosan coating exhibited positive results and extended the storage life of fresh mini cucumbers with for 20 days with better preserved quality.

RESUME

Les fruits et légumes récoltés sont généralement très périssables et ils deviennent plus périssables lorsqu'ils sont coupés en raison de l'élimination de leurs couches protectrices externes et de l'exposition des tissus à l'atmosphère extérieure. Il y a une augmentation de la demande de fruits et légumes frais coupés qui a ouvert de véritables défis aux producteurs alimentaires pour développer des techniques de conservation innovantes et durables. Alors que de nombreuses techniques avancées d'emballage et de stockage aident généralement à conserver les fruits et légumes, les films/revêtements protecteurs et comestibles offrent un meilleur choix pour couper les fruits et légumes car ils fournissent une barrière protectrice alternative et efficace à la surface coupée. Les biomolécules de qualité alimentaire offrent d'excellentes propriétés de revêtement avec une résistance physico-mécanique adéquate et peuvent même compléter certains nutriments. L'objectif principal de cette recherche était d'évaluer la pertinence d'enrobages comestibles sélectionnés pour prolonger la durée de conservation des fruits et légumes frais entiers et coupés. L'accent a été mis sur l'utilisation d'une nouvelle combinaison d'alginate de sodium, de chlorure de calcium et d'huile de gingembre ainsi que sur un revêtement multicouche à base de chitosane pour prolonger la durée de conservation réfrigérée des poires coupées et des mini-concombres entiers.

Une formulation d'enrobage comprenant 2 % d'alginate de sodium et 1 % d'acide citrique (SACC) a été évaluée pour des poires coupées pendant 15 jours à 4 °C avec des échantillons sans traitement comme témoin. Les poires enrobées de SACC ont ensuite été trempées dans du chlorure de calcium à 2 % pour compléter en tant qu'agent de réticulation raffermissant et améliorer la formation de film et la liaison de surface de l'enrobage. Une autre formulation a été préparée avec du SACC mélangé à 0,5 % d'huile essentielle de gingembre

(SAGEO) ajoutée comme agent antimicrobien. Un revêtement multicouche avec 2 % d'alginate et 1 % de chitosane a été utilisé pour les mini-concombres frais qui ont été stockés à 4°C pendant 20 jours. Différents paramètres de qualité ont été évalués pour suivre l'évolution de l'activité physiologique : couleur des tissus, texture, solides solubles totaux, acidité titrable, perte de poids, rythme respiratoire, teneur en chlorophylle et croissance des moisissures. Ceux-ci ont été évalués pendant le stockage à des intervalles de temps sélectionnés.

Le revêtement d'alginate de sodium (SACC) a prolongé la durée de conservation des poires coupées avec des paramètres de qualité mieux préservés par rapport au témoin. SAGEO avec l'essentiel de gingembre et l'acide citrique a montré des résultats encore meilleurs en empêchant la croissance des moisissures, en supprimant le brunissement oxydatif des poires coupées pendant toute la période de stockage. Dans l'ensemble, SAGEO a donné la meilleure qualité, bien que SACC ait également été meilleure que le contrôle. Le revêtement multicouche d'alginate et de chitosan a donné des résultats positifs et a prolongé la durée de conservation des mini-concombres frais pendant 20 jours avec une meilleure qualité préservée.

CONTRIBUTION OF AUTHORS

Neelakanth A Lamani is the MSc candidate who supervised by Dr. Hosahalli S. Ramaswamy, planned and carried out all experiments, and gathered all data and analyzed the results and prepared the first draft of the thesis and manuscripts for scientific publications.

Dr. Hosahalli S. Ramaswamy is the research supervisor, under whose guidance and support the research was conducted, provided the research support, helped plan and execute the research activities as well as editing the thesis and manuscript drafts for publication.

This thesis is written in manuscript style with two chapter highlighting the area of research which are complete and can be suitably edited for publication.

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CHAPTER 1

INTRODUCTION

Edible coatings are among the novel methods of preserving the fruits and vegetables with no or very minimal use of chemical preservatives. Edible coatings were first used in 12th century in China on citrus fruits and wax was used as the coating material (Andrade et al., 2012). Although the Chinese did not realise the full function of edible coating contribution to lower the respiration and other metabolic activities, they found the difference, that the waxed citrus fruits could be stored longer than non waxed ones. Wax layer coating was only limited to fruits in 1930s to 1960s. With advancement wax coating was exploited on various fruits and vegetables for preservation and prolonging the storage life.

Food loses occur at each step of the supply chain, starting from the farm, during the transportation, during processing, distribution and finally at retailors until it reaches consumers (Kayikci et al., 2022). Reducing post harvest losses is better than increasing food production which needs lots of resources and environmental help (Kumar & Kalita, 2017). It will also add up better consumer satisfaction, post harvest quality maintenance and economical benefit for both consumers and producers. To feed growing population with healthy diet is a challenging approach due to short shelf life and quality loss of fresh produce during transportation and storage. Beside refrigerated storage, there are many technologies that helps to reduce the post harvest losses such as controlled atmospheric storage (CA), modified atmospheric packaging (MAP), active packaging and use of edible films and coatings. All these technologies not only help in minimizing the post harvest loses, but also, they help in extending the shelf life of fresh produce such as fruits and vegetables.

Fruits and vegetables are important for human diet. They are the source of nutrients, vitamins, minerals, flavonoids, aroma, alkaloids, and fibre etc. Fruits and vegetables are very important sources for nourishment, they may not be a major source of energy, but they provide palatability, taste and improve appetite. They neutralize the acid produced during the digestion. Most importantly, they improve general immunity of human body against diseases and deficiencies. The World Health Organisation and Food and Agriculture Organization of the United Nation recommends that adults should consume at least five servings of fruits and vegetables per day excluding starchy vegetables (Dhandevi & Jeewon, 2015). Fruits and vegetable help to maintain good health, as they are part of balanced diet and fruits do not possess cholesterol and low saturated fat, salts and sugars and help to lose weight. They are source to enjoy a variety of flavors and textures.

Concept of edible coating is new but one thing that makes it innovative method of preservation is it lets us manipulate the atmosphere on and around the fruit itself rather than occupying large space for storage. In the 15th century, Japan designed a taste less and odorless edible coating (EC) material made from boiled soybeans, and they called it yuba (Zaheer & Humayoun Akhtar, 2017). The first kind of ECs was water–wax microemulsions, used since 1930s (Estévez, 2009). In 1930s, hot-melt paraffin waxes used as ECs for apples and pears (Tugce Senturk Parreidt et al., 2018). With further development up to 1967, commercial use was mainly limited to wax layer on fruits (Pavlath & Orts, 2009). Edible coatings are nontoxic and generally considered as safe (GRAS), they are biodegradable and ecofriendly (Al-Tayyar et al., 2020).

General properties of Ecs and films are, when applied on the surface of fresh produce, it will form a barrier around it, which prevent moisture loss, lower gases exchange and ethylene production. They have the property of preserving the texture and other enzymatic reactions will also be lowered and which preserve the glossiness and color of the fresh and fresh cut produce. Research on edible packaging is going optimistically demanding every year. Although edible coatings and films help extend the shelf life of different food products, producers and researchers are still facing challenges of consumer acceptance. Due to the increased demand for the fresh produce, producers are looking into developing a novel and sustainable food packaging materials which should be ecofriendly and low cost of production along with reducing pollution caused by use of synthetic polymers such as plastics.

Most of the edible coating work has been focused on whole fruits and vegetables as a supplementary protective layer to provide additional benefits to slow down the respiration and other physiological activities to extend the shelf life. Nowadays there is huge increase in demand for minimally processed ready to eat fresh produce in various food market sectors. As the fruits are cut, the primary protective layer is completely removed there by exposing the internal tissue to atmosphere which accelerates the spoilage process. Secondly with the moist surface that exists after cutting, the adherence of the applied coating material on to the surface becomes problematic.

Researchers are focusing on developing packaging material with no use of chemical preservatives due to increased health consciousness among the people. Nowadays edible films and coating are applied for delivering bioactive compounds (Miteluț et al., 2021). Experts have found different biopolymers like polysaccharides, proteins and lipids could be used for producing various packaging materials and could applied through different methods. Studies have shown that sodium alginate and chitosan are very good polysaccharides for developing various edible films and coatings.

Sodium alginate produces edible coatings or films and but generally present poor water resistance due to their hydrophilic nature, which is a limiting factor. Sodium alginate coupled with calcium chloride produce excellent gel like film which was found to reduce moisture loss and reduce respiration in fresh and cut fruits like strawberry (Alharaty & Ramaswamy, 2020), pineapple and apple, etc. Use of sodium alginate is limited also because of its poor mechanical properties without the presence of divalent ions usually (Ca2+).

Chitosan edible films and coatings have exhibited excellent moisture barrier and found to preserve color of coated produce. Chitosan has a good characteristic feature without adding any type of additive and antioxidants such as it contains good O_2 barrier and CO_2 permeation and antimicrobial activity against microorganisms due to its cationic (+) property (Hassan et al., 2018). There are few limitations which hinders the use of chitosan as coating material due to poor mechanical resistance, difficulty in controlling pore size and low stability in neutral and alkaline pH also difficult manipulate multilayer deposition on fresh produce (Yousuf et al., 2018).

Composites are made of a combination of hydrocolloids and lipids to produce edible films and coatings with shared advantages from two different categories and also by introducing the various biproducts enhancing the effectiveness (Alharaty & Ramaswamy, 2020). Sodium alginate coating and chitosan films have poor mechanical properties, low water resistance, and limited antimicrobial action, which hinders their use in the food preservation sector. Different nano compounds like plant-based extracts and other functional ingredients could be incorporated into sodium alginate and chitosan coating to solve these problems along with enhancing their combining property and the safety attributes.

Pear fruits are a good source for minerals but also provide various phytochemicals such as, betanin, catechin, vanillic acid or gallic aid are present in appreciable concentration in the fruit and the remarkable health blessings are because of anti-inflammatory properties (Sabtain et al., 2021). It is also juicy and tender which attract consumers. As there is demand for ready to eat fresh produce consumers are looking for minimally processed ready to eat cut fruits and vegetables. The lack of availability of cut pear fruits due to its early perishability upon cutting as it losses outer protective layer.

Cucumber is the vegetable with high level of moisture (~90%). Cucumbers are being used in various food sectors like restaurants, school cafeterias as in cut and fresh forms for salads and making pickles, sandwiches, and hand burgers in fast food sectors. Cucumbers are tender very good source for dietary fibres. Big food industries are wrapping the cucumbers with plastic films which help in preserving the shelf life cucumber but creating plastic pollution after use. So that could be minimized by using different edible films and coating made from plant based biopolymers.

There is increasing demand for plant-based preservatives, such as herbal extracts like essential oils (ginger, cinnamon, thyme, mint, and lemongrass etc.) which exhibit excellent antimicrobial property and generally recognized as safe (GRAS). Essential oils also provide nutraceutical benefits to consumers. Different herbal extracts can be incorporated into edible coatings and films to improve effectiveness and impart antimicrobial property in biopolymers to create good packaging material (Miteluț et al., 2021). Essential oils have been proven to possess antimicrobial properties. In this study, ginger essential oil has been incorporated into coating solutions mainly because it is less expensive compared to other essential oils like cinnamon and mint etc. As like other herbal extracts it exhibits excellent antimicrobial, oxidative properties

(Al-Hilifi et al., 2022), and it adds up to health benefits for consumers and relieve several health complaints.

The study on use of ginger essential oil as antimicrobial agent in edible coating on cut pear fruits is very limited. There is limited research work on the application of ECs for cut fruits and vegetables. This research studies the effect of sodium alginate edible coating on fresh cut pear fruits as composite enhanced coating. The study on sodium alginate coating combined with chitosan as multilayer which forms composite electrostatic coating to extend the shelf life of fresh mini cucumbers is also limited. Based on these arguments objectives of thesis are formulated.

The first objective of the thesis is focused on cut pear fruit which is prone to enzymatic browning and discoloration upon cutting. External appearance plays very important role in case of cut fruits like pear and apple. Preserving the outer tissue could be done by using different antioxidants like ascorbic acid and citric acid, etc., which have impact on phenol oxidase enzymes which induces oxidative browning on cut fruits and vegetables. In this study citric acid has been used as anti-browning agent due to its inhibitory action on oxidase enzyme. The second objective concentrates on use of alginate and chitosan on whole mini cucumbers.

The latest development in application of edible coating and film on fresh and minimally processed fruits and vegetables is studied with extensive use of functional compounds added to enhance effectiveness of coating material not just limited to prolong the storage life of but also impart functional properties like antioxidant, antimicrobial and better quality attribute having.

OBJECTIVES

1. Study the effect of sodium alginate and ginger essential oil emulsion based edible coating on microbiological and physio-mechanical properties of fresh-cut pears.
2. Investigate the multilayer electrostatic coating comprising of chitosan and sodium alginate to extend the shelf life of mini cucumbers.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Introduction

Fruits and vegetables are considered protective food in nature. Our body requires vitamins and minerals for various life sustaining processes and for the formation of blood, bones, and teeth. Fruits and vegetables possess all the required nutritional components. Nearly 40% of food is wasted in one or another way, in various levels of handling and processing stages, most of them are fruits and vegetables (Chakraverty et al., 2003). There is a huge increase in demand for fresh-cut fruits and vegetables globally. With increased interest in ready-to-eat and nutritious food, cut fruits have become more popular and widely available in the supermarket, cafeterias, airline catering, schools, universities (Tugce Senturk Parreidt et al., 2018). Post-harvest factors play a major role in deciding the durability and shelf life of the fruits and vegetables. Fruits and vegetables go through various steps, from harvesting to consumption by consumers. During these processes, the preservation of the quality of the commodity is important.

Increasing population and demand for food supply have become a major concern for various researchers throughout the world. Extending the post-harvest life of fruits and vegetables through various preservative methods has been practiced for many centuries. The principles employed for the preservation of perishable foods even today are also the same, although the modern techniques in its application have been most fascinating and amazing (Thakur et al., 2000). In recent times based on scientific understanding preservation techniques have been developed. With knowledge of scientific research and techniques, there is a boost for the food preservation sector in today's world, perhaps the food industry has become a global industry (Thakur et al., 2000).

2.2 Post-harvest technology of fruits and vegetables

Post-harvest technology deals with post-harvest handling of the fruits and vegetables at various stages after harvesting till it reaches consumers. Post-harvest handling includes various preservation techniques for early perishable fruits and vegetables. There is an increase in the consumption of fresh fruits and vegetables all over the earth, as people are looking towards a healthy source of nutrition.

Once the fruits and vegetables are harvested there will be a change in the internal atmosphere, and which leads to early perishability. Hence various methods of preservation are brought into practice, such as conventional cold storage, modified packaging, edible coatings, and films, processing, or value addition. The best alternative is to reduce post-harvest losses, which earns consumer satisfaction, increase the economic benefits for both producers and consumers, and maintains post-harvest quality (Ramaswamy, 2014). One of the major reasons for the post-harvest losses is early perishability and that is directly related to the composition of various components in fruits and vegetables, such as moisture level and ethylene level, and also the effect of external factors such as surrounding temperature. By controlling the internal atmosphere there are chances of extending the shelf life of fruits and minimize post-harvest losses (Ramaswamy, 2014).

Depending upon the easiness of spoilage, the horticultural produce has been named into staple or non-perishable ones, which do not spoil easily when properly handled. The second category is semi-perishable and does not spoil if stored in ambient storage conditions. And the third one is perishable and spoils very quickly unless special methods of preservation are employed which requires very special care upon harvesting (Thakur et al., 2000).

2.3 Spoilage of fruits and vegetables

Spoilage is changes happening in fruits and vegetables which makes them inedible for humans. Fresh vegetables and fruits contain natural microflora coming from the soil, water, air, and other sources. The presence of air, high humidity, and high temperature as extrinsic factors during the storage of vegetables and fruits increases the chances of microbial growth and spoilage (Erkmen & Bozoglu, 2016). Once the fruits are harvested they become prone to numerous kinds of external factors causing damages.

Mechanical damage is one of the primary causes of spoilage, mechanical injuries will affect external protective barriers of fruits and vegetables causing them to decay faster than normal. The injuries hinder the components present inside and exposing internal tissues to external factors. The retention of vitamin C is lowered by bruising, and other mechanical injuries, such as chilling injury, and by excessive trimming. The microbiological growth and activity of microorganisms usually spoil the horticultural produce and make them not suitable for marketing and consumption. Physical damages are due to improper environmental and storage conditions; temperature, relative humidity which leads to quality decay. Other primary causes are physiological Sprouting, senescence, other respiratory and transpiratory changes. Physicochemical undesirable reactions between chemical compounds present in the food such as browning, rancidity, enzymatic changes, etc. (Ramaswamy, 2014).

2.4 Physiology of fruits and vegetables

Based on the type and nature (climacteric or non-climacteric) fruits and vegetables are involved in various physiological activities. Once the fruits and vegetables are harvested, the commodity completely sustains on its own moisture content and metabolic food reserves. Respiration and transpiration, and ethylene levels are in climacteric fruits and vegetables compared to non-climacteric ones upon ripening. Climacteric fruits such as mangoes, kiwi, peach, tomatoes, bananas and papaya are those whose ripening is associated with a rapid rise in the respiration rates (Fabi & Do Prado, 2019). Also, they experience an elevated production of ethylene and CO_2 during ripening (Bapat et al., 2010; Fenn & Giovannoni, 2021). Non-climacteric fruits and vegetables are grapes, cherries, melons, etc. Ripening is slow and the rate of respiration is very low and relatively less ethylene production compared to climacteric ones and should be harvested only after maturity (Ariwaodo, 2022; Fenn & Giovannoni, 2021).

Ripening is a metabolic process in fruits and vegetables that makes them more palatable (Fenn & Giovannoni, 2021). Due to metabolic changes, the outer protective barrier becomes softer, increases in sugar content, change in color, development of appetizing smell, and increase in acidity of fruits as ripening proceeds and finally starts decaying. A higher respiration rate indicates high metabolic activity happening inside the product (Pott et al., 2020).

2.4.1 Respiration

Respiration is the chemical process by which fruits and vegetables convert metabolic sugar and oxygen into carbon dioxide, water, and heat. The heat generated by the respiration process tends to increase the temperature of a commodity. This, in turn, increases the water vapour pressure just below the surface of a commodity, leading to increased transpiration. Thus, respiration can cause transpiration to occur in saturated environments (Rashmi & Negi, 2022).

Based on the type and temperature of the commodity the rate at which this chemical reaction takes place has been found to vary. More specifically, the rate of carbon dioxide production and heat generation due to respiration can be correlated to the temperature of the commodity.

In respiration glucose ($C_6H_{12}O_6$) is transformed into carbon dioxide (CO_2) and water (H_2O) using oxygen (O_2). This reaction releases energy as well which can be used for all sorts of processes in the plant. The reaction can be represented like this (Alharaty & Ramaswamy, 2020),

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + \text{energy} \qquad (2.1)$$

Glucose is a carbohydrate and is often stored in a plant as starch. Once all glucose has been converted no energy is being formed anymore to sustain the fruit or vegetable and starts decaying. The unit of respiration rate is $mg\ CO_2\ kg^{-1}\ h^{-1}$. The rate describes the amount of carbon dioxide produced per kg of produce in one hour. As it can be seen in the equation above, carbon dioxide is formed during respiration. If you know this amount you can also calculate how much glucose has been converted (Fonseca et al., 2002).

2.4.1.1 Factors affecting the respiration

A. **Temperature:** Temperature is a very important factor; the respiration is directly linked to temperature. The respiration rates of fruits can be controlled by storage in cool, dry places. Lower storage temperatures can slow down the respiration and ripening of fruit and vegetables (Moalemiyan & Ramaswamy, 2012).

B. **CO2 concentration:** The rate of carbon dioxide production and heat generation due to respiration can be correlated to the temperature of the commodity. Carbon dioxide, one of the waste products of the respiration process, also affects respiration (Lufu et al., 2019). The higher the concentration of carbon dioxide, the lower the rate of respiration. This can occur during times of reduced gas exchange in the atmosphere, such as when a plant is conserving water. Carbon dioxide is also used to measure the rate of respiration, described as the quantity of carbon dioxide (in mg) produced by one kilogram of plant material in one hour (Lufu et al., 2019; Rashmi & Negi, 2022).

C. **Oxygen concentration:** Respiration is a process where the involvement of oxygen is inevitable. Higher the oxygen concentration more is respiration, so low level oxygen concentration is reliable for fruits and vegetable during preservation and storage (Hussein et al., 2020; Soltani et al., 2015).

D. **Ethylene concentration:** Ethylene is known as a ripening hormone. Ethylene can accelerate the ripening process and reach the climacteric peak. And it is found that even very minute concentrations can influence respiration in fruits and vegetables. Introducing ethylene speeds the ripening process in stored fresh horticultural commodities (Farneti et al., 2022).

E. **Stress and injuries:** Mechanical injuries and other injuries like chilling injuries and physical injuries and bruising can induce respiration in fruits and vegetables (Hussein et al., 2020).

2.4.1.2 methods to measure respiration

The unit of respiration rate is *mg or mL* $CO_2\,kg^{-1}\,h^{-1}$. The rate describes the amount of carbon dioxide produced per kg of produce in 1 hour. Different methods can be used to measure the respiration rate in fruits and vegetables (Rashmi & Negi, 2022).

A. **Respirometer:** The atmosphere inside a container is monitored by sensors for O_2, CO_2, and temperature, and is refreshed with air or a special gas mixture once a specified amount of O_2 has been consumed by plant tissue and the rate of respiration can be calculated (Bower et al., 1998).

B. **Flow-through method:** The rate of respiration is measured by calculating the flow of gas inside and outside impermeable container with specific gas compositions (Calegario et al., 2001; Miranda et al., 2019).

C. **Permeable method:** The fruits and vegetables are placed in a package with known dimensions and film permeability to O_2 and CO_2 gases where the produced equilibrium concentrations of fruits and vegetables are measured, and the gas exchange rate can be calculated (Fonseca et al., 2002; Ghosh & Dash, 2020).

2.4.2 Transpiration

Postharvest phenomenon of moisture loss/mass transfer experienced by fresh fruits and vegetables (Bishnoi & Aharwal, 2022).This process includes the loss of moisture through the outer layer of the product, that is evaporation from the surface, and convective mass transport of the moisture to the surroundings (Bishnoi & Aharwal, 2022). Moisture migration is triggered by the concentration state of the fresh product surface and the surrounding air temperature and relative humidity have effect on the moisture loss. Transpiration or moisture loss from fresh food products like fruits and vegetable is an unavoidable process in ventilated storage and it leads to quality loss. Fresh/cut fruit and vegetables have a high-water (percent moisture) level and are susceptible to moisture loss.

2.4.2.1 Factors affecting transpiration

A. **Rate of respiration after harvesting**: Based on type of fruits and vegetables (climacteric and non climacteric) they use up the water in the cell after harvesting due to the increase respiration. The temperature within the fresh produce goes up and subsequently they lose some mass (Alam et al., 2021).

B. External conditions and temperature: When fresh produce is exposed to the high temperatures the moisture on the surface evaporates, which is induced due to the water vapour deficit and that will achieve a high rate of respiration in the fruits and vegetables up on harvesting and storage (Alam et al., 2021; Lentzou et al., 2021). Other external factor which has impact on transpiration along with temperature are humidity, air movement and pressure (Alam et al., 2021).

C. External damages and injuries: Post harvest damage to fresh fruits and vegetables can be due to the mechanical injuries caused by poor harvesting and careless handling. This will have impact on fresh produce causing bruising and scratches affecting the external skin and outer layers of the cell and leading to waster loss from damaged part, increased respiration, and internal heat production (Martinez-Romero et al., 2004).

D. Surface area of fruits and vegetables: Transpiration rate is directly influenced by the surface area of the fresh fruits and vegetables. The exposure of a large surface area of the fresh produce, which speeds drying on the outer layer causing water loss from external tissues (Alam et al., 2021).

2.5 Preservation of fruits and vegetables

The post-harvested physiological changes of respiration, transpiration, and biosynthesis are influenced by the internal properties (water content, chemical composition, pH level, contamination level) of food and the extrinsic properties (cold air temperature, air circulation, and relative humidity, products packing design, etc.) of the surrounding environment and in general, alterations in these causes the decline of the food product and limit its period of usability

Since ancient times various preservation methods have been practiced. Preservation can be done by delaying and preventing the early growth of microorganisms on fruits and vegetables by keeping out from microorganisms or maintaining aseptic condition and also by removing the microbes by various kinds of treatments and killing the microorganisms and preventing or protecting from self decomposition by inactivating the enzymes, delaying the chemical metabolic reactions happening and preventing all other kinds of injuries (Thakur et al., 2000).

Freezing, canning, pickling, and drying or dehydrating are conventional methods followed for fruits and vegetables. Advancement in science and technology, various new preservative methods have been developed by researchers (Ramaswamy, 2014).

2.6 Shelf-life extension of fruits and vegetables

Delay in deterioration of fruits can be achieved by various preservative methods and deal with the internal and external atmosphere of the fruits and vegetables, such as controlled atmospheric, modified atmospheric packaging, coating the fruits and vegetables with protective layers or films. Controlled or modified atmosphere storage should be used as a supplement to, and not as a substitute for, proper temperature and relative humidity management (Bodbodak & Moshfeghifar, 2016).

2.6.1 Techniques/ methods of preservation

2.6.1.1 Controlled atmosphere storage

Controlled atmosphere storage is one of the innovative methods of storage for fruits and vegetables where low oxygen concentration and high carbon dioxide concentration is maintained along with the cold or refrigerated condition and no use of chemical preservatives to extend the shelf life of fruits and vegetables. CA needs continuous monitoring of these gases within the storage container (Moradinezhad & Dorostkar, 2020; Rama & Narasimham, 2003).

CA is more effective in combination with refrigeration, providing additional benefits. Most fruits and vegetables tolerate as low as 2% O_2 and as high as 5–10% CO_2. Studies have shown that CA has a relatively positive effect on fruits and vegetables with a high respiration rate by prolonging the pre-climacteric stage (Zheng & Wang, 2007). It has also been found that the ethylene production in stored commodities is minimized by reducing the oxygen level and increasing the carbon dioxide level (Lentzou et al., 2021). And other metabolic reactions such as ripening were also reduced in CA (Brizzolara et al., 2020). Further effect on sensory quality and flavor and aroma is preserved for fresh produce for a longer time. Due to delay in ripening under CA, normally a desirable texture is maintained for several fruit types like apples, pears, peaches, apricots, and tomatoes (Brizzolara et al., 2020; Erkan & Dogan, 2019). Retention of firmness is one of the most important advantages of the commercial CA of apples (Brizzolara et al., 2020; Paul & Pandey, 2014). Though high CO_2 and low O_2 contribute to the retention of flesh firmness, the effect of high CO_2 is more prominent than that of low O_2. The delayed ripening and senescence in such fruits by storing them in a controlled atmosphere allows them to retain greater resistance to postharvest diseases thus, CA can reduce disease development in crops by improving their physiological conditions (Brizzolara et al., 2020; Rama & Narasimham, 2003).

Inert gases like nitrogen (N_2) are used with the help of nitrogen generator as a filler in the storage. In the presence of O_2 the produce begins to show bacterial, mold and yeast growth making them not suitable for marketing (You et al., 2022). N_2 acts as O_2 scavenger which help prolong the storage life of fresh fruits and vegetables without any spoilage (Lee et al., 2015). Through this CA fresh produce can be sold all the year long, since this process doesn't involve any chemical preservatives, there is no harm on the quality of the produce.

2.6.1.2 Modified atmosphere packaging

Modified atmosphere packaging (MAP) technique. MAP can be defined as the enclosure of food in a package in which the atmosphere is modified or altered to provide an optimum atmosphere for increasing shelf life and maintaining food quality (Robertson, 2019). Modification of the atmosphere may be achieved either actively or passively. Gases used in MAP usually consist of O_2, CO_2, and N_2, which are essential for the respiration of fresh fruits and vegetables (Moradinezhad & Dorostkar, 2020). Among the gases used in MAP, just CO_2 has a significant nonselective antimicrobial influence on the product. MAP, a technique used to extend the shelf life of fresh or minimally processed foods, refers to the development of a modified atmosphere around the product using permeable polymeric films (Qu et al., 2022). The shelf life of the packaged products can be extended by 50–200% by using MAP if the nutritional consequences of MAP on the packaged food products become an issue (Bodbodak & Moshfeghifar, 2016).

MAP usually consists of O_2, CO_2, and N_2. It is the altered ratio of O_2 and CO_2 that makes a difference in the protection of fresh commodities (Opara et al., 2019). Ripening of fruits and vegetables could be delayed, softening could be retarded, respiration and ethylene production rates could be reduced, and various compositional changes associated with ripening could be decelerated by decreasing the O_2 level and increasing the CO_2 level (Brizzolara et al., 2020; Opara et al., 2019; Rizzolo et al., 2015).

Oxygen is essential for the respiration of fresh horticultural commodities. Atmospheres of more than 10 kPa CO_2 can be phytotoxic for many fresh horticultural commodities. N_2 is used as a filler gas since it has no direct biological effects on horticultural commodities (Feng et al., 2023; Opara et al., 2019). Therefore, N_2 is usually used as the inert component of MAP. The diffusivity of O_2, CO_2, and C_2H_4 may be raised by replacing N_2 with argon or helium, but they have no straight effect on plant tissues and are more expensive than N_2 as a modified atmosphere component (Bodbodak & Moshfeghifar, 2016).

The most widely used polymer films for MAP are synthetic polyolefins, low-density polyethylene (LDPE), linear low-density polyethylene(LLDPE), high-density polyethylene (HDPE), polypropylene (PP), polyvinyl chloride (PVC), polyesters, polyethylene terephthalate (PET), polyvinylidene chloride (PVDC), ethylene-vinyl alcohol (EVOH), polyamide (Nylon), polyvinyl alcohol (PVOH), ethylene-vinyl acetate (EVA),cellulose-derived plastics such as cellophane and natural biodegradable polymers like polylactic acid (PLA) (Al-Salem et al., 2019; Sandhya, 2010; Soltani M, 2015).

2.6.1.3 Edible coatings

The edible coating is defined as a thin layer that is applied to the surface of the fruit to create a barrier between the fruit and the environment which can be eaten as part of the whole product (E.A. Baldwin, 2012; Maringgal et al., 2020; Raghav et al., 2016).

2.7 Concept of edible coating

The first kind of edible coatings was water–wax microemulsions, used since the 1930s to increase brightness and color in fruits, as well as fungicide carriers (Saltveit, 2003; Zaheer & Humayoun Akhtar, 2017). Edible coatings are a new technique that is used to increase the shelf life of whole or fresh-cut fruits & vegetables. The edible coatings are applied on fresh or fresh-cut fruits and vegetables by different methods, dipping or spraying the coating solution. They are able to form a barrier against moisture, oxygen, and modify the atmosphere around the fruit by creating a barrier to gas exchange (Amin et al., 2021)

There are different kinds of edible coating based on their composition. Edible coatings are synthesized mainly from biopolymers, including proteins, polysaccharides (carbohydrates and gums) lipids (Pratap Singh & Packirisamy, 2022). The polysaccharides used in edible coatings are starches and modified starches, chitosan, alginates, gums, cellulose derivatives and Pectin (Alharaty & Ramaswamy, 2020; McHugh, 1994; Yousuf et al., 2018). The most used protein-based edible coatings are gelatin, corn zein, wheat gluten, soy protein and casein, and collagen. And wax and oil-based fatty acid and monoglycerides are some lipids-based edible coatings (Yousuf, Sun, et al., 2021).

2.7.1 History of edible coating

Edible coatings are used for extending the shelf life of food products. History says that the Chinese invented the first kind of edible coating made of wax on citrus fruit to enhance its appearance and prolong shelf life (Zaheer & Humayoun Akhtar, 2017). Later, larding (use of lards or fats) was practiced in England to enhance the shelf life of meat products (Dhaka & Upadhyay, 2018; Hassan et al., 2018). Although the Chinese did not understand that the full function of edible coatings was to slow down the respiratory gas exchange, they found that wax-coated fruits could be stored longer than non-waxed fruits (Raghav et al., 2016). In the 1930s hot-melt paraffin waxes became commercially available as edible coatings for fresh fruits such as apples and pears. During the 1960s edible coatings and films became popular in various food industries and got market demand. Since the early to mid 20^{th} century, coatings have been used to prevent water loss and add shine to fruits and vegetables (Alharaty & Ramaswamy, 2020). Edible films and coatings shape a barrier for chemical, bodily and biological changes. On the purchasing of fruit and vegetables, the user decides the healthy food item based on its freshness and appearance (Raghav et al., 2016)

2.8 Components of edible coating and composition

Active edible coatings are made from different kinds of sources that could be plant-based or animal-based biopolymers which have good film forming properties. Such as polysaccharides, proteins, lipids of various types and compositions (Alharaty & Ramaswamy, 2020; Hassan et al., 2018).

2.8.1 Polysaccharides

These are widely used naturally occurring, mostly used to produce or prepare edible films and coatings which include pectin, starch, cellulose, and derivatives of all these, such as pullulan, alginates, and chitosan (Dhall, 2013; Yousuf et al., 2018). Polysaccharide-based edible coatings have been studied by different researchers, these polysaccharide-based coatings are good oxygen blockers, due to the presence of hydrogen bonded molecular network structure (Hassan et al., 2018). Even though some coatings made by using polysaccharide polymers possess poor water vapor barrier properties, so use of these coatings can be modified with respect to avoid moisture loss (Amin et al., 2021; Hassan et al., 2018). Polysaccharide coatings are colorless, have no oily appearance, and can be applied to prolong the shelf life of fresh fruits, vegetables, other food commodities like meat, sea products and on processed products like candies, jellies etc. by significantly reducing moisture loss in fresh produce, lowering the surface darkening and oxidative reactions (Hassan et al., 2018; Nešić et al., 2019; Yousuf et al., 2018).

2.8.1.1 Chitosan

Chitosan is derived from chitin, it is an edible polymer. Chitin is mainly found in crustacean animal shells. Chitosan is the most common non-toxic and natural product after cellulose for the formation of edible films and coating (Maringgal et al., 2020). Chitosan has a good character feature without adding any type of additive and antioxidants such as it contains good O_2 barrier and CO_2 permeation and antimicrobial activity against microorganisms (Hassan et al., 2018) Chitosan molecular structure is like cellulose, the only difference is that the hydroxyl (OH) group on the 2nd carbon atom of the hexose repeat unit is substituted with the help of an acetamide group (Priyadarshi & Rhim, 2020). Chitosan is an important cationic (+)

polymer used in the synthesis of films and coatings as it has antimicrobial characteristics (Adhikari et al., 2022). Chitosan produces partial permeable coatings and films, which can control the interior atmosphere, by declining transpiration rates and retarding ripening in fruits and vegetables up on coating and help in extending the storage life (Alharaty & Ramaswamy, 2020; Maringgal et al., 2020).

2.8.1.2 Alginate

Alginate contains salts of Alginic acid. Alginate has some desirable properties including reduction of shrinkage, moisture retention, color, and odor of fresh produce (Alharaty & Ramaswamy, 2020). Strong edible coatings or films are made from alginate and present formally poor water resistance due to their hydrophilic nature (T. Senturk Parreidt et al., 2018). Alginates are polysaccharides acquired from brown seaweeds. They are composed of b-D-mannuronic acid (M) and a-l-guluronic acid (G) in varying degrees, arrangements, and molecular weight (Moussa & Aparicio, 2019). Alginate formation occurs when multivalent or divalent ions usually (Ca^{2+}) interact ionically with blocks of guluronic acid residues from two different chains resulting in a three-dimensional network (Lee & Mooney, 2012; Mantilla, 2012). The sodium alginate-calcium chloride edible coating was effective in reducing transpiration rates and respiration and detained the increase of the soluble solid content and pH in cut strawberry furthermore, the coating delayed surface mold growth for up to 15 days and preserved the sensory properties of the cut fruits such as color and texture (Alharaty & Ramaswamy, 2020).

2.8.1.3 Cellulose and derivatives

Cellulose is easily found in nature. It is made from a long chain of anhydrous-glucose polymer. Cellulose is reacted with alkali, then it is treated with an appropriate chemical reagent, and then it forms a substitute of cellulose chain of anhydrous-glucose monomers (Aziz et al., 2022). This process has been done under controlled conditions. Most of the cellulose derivatives consist of the following compounds such as hydroxypropyl cellulose, methylcellulose, carboxymethyl cellulose and the combination with hydroxypropyl methylcellulose and its commonly used for the preparation of edible coatings (Aziz et al., 2022; Ravishankar, 2018). These types of films/coatings are usually tasteless, bendy, odorless and are of low energy, transparent, resistant to oil and fat, hydrophilic in nature, moderate to oxygen diffusion and moisture (Iñiguez-Moreno et al., 2021). Methylcellulose (MC) is far from the bottom hydrophilic cellulose derivatives and highly resistant to water (Nasatto et al., 2015). Avocados coated with MC showed lower respiration rates, a greener color, and higher firmness as compared with the uncoated control during the entire storage. The brown spots and mesocarp discoloration normally associated with fruit ripening was detained in coated fruits (Maftoonazad & Ramaswamy, 2005).

2.8.1.4 Starch

Starch is the most common polysaccharide. Starches are obtained from cereal grains such as wheat, maize, rice, etc., and cereal grains have belonged to the Poaceae family (Golfam et al., 2021). Potato and other tubers, legumes are also good sources of starch (Tetlow & Emes, 2017). Corn starch-based films found to be and non-toxic, tasteless, odorless, and colorless and coatings exhibited physical attributes that resemble plastic films and coatings (Momin et al., 2021). Films and coatings made up of starch are extensively applied because they are transparent, and good CO_2 and O_2 barriers (Jiang et al., 2011; Momin et al., 2021).

However, due to their hydrophilic nature starch-based films and coatings show water solubility and poor water vapor barrier (Singh et al., 2022). The films made up of corn starch containing amylose in excess quantity (71%) had no measurable oxygen permeability at relative humidity levels fewer than a 100% (Kim et al., 2017). The addition of plasticizers and absorption of water molecules in films and coating leads to accelerate polymer chain mobility and gas permeability (Banker et al., 1966; Singh et al., 2022).

2.8.1.5 Pectin

Pectin is a group of polysaccharides that are derived from plants it is naturally found in fruits and vegetables. Pectin is good for low moisture fruits and vegetables but is not a good moisture barrier (Hassan et al., 2018; Moalemiyan et al., 2012). It is a heterogeneous group of acidic polysaccharides. It is commonly found in peels of citrus fruits and apple pomace. In one of the studies coated cucumber fruits by pectin reduced off color development, texture softening, weight loss, CO2 evolution, and acid production only pH and TSS increased compared with the control. The shelf life of the control sample was less than a week, whereas the coated fruits remained good for over 2 weeks (Moalemiyan et al., 2012).

2.8.2 Protein

Protein-based edible coatings are derived from animals and plants. The plant-based protein edible coating material is milk protein casein, whey protein, zein (from maize), gluten (from wheat), soy protein, etc (Mihalca et al., 2021). And the animal-based protein is egg albumen, collagen, etc (Hauzoukim & Mohanty, 2020). Protein-based edible coatings have shown many advantages and effects on preserving the quality and shelf life of fruits and vegetables (Budianto et al., 2022).

2.8.2.1 Gelatin

The functional use of gelatin polymers to synthesize edible films or coatings has been well documented since the 1960's (Hanani et al., 2012). Films or coatings produced by gelatin exhibit good transparency, mechanical barrier properties and can be manufactured by extrusion or casting processes (Hanani et al., 2012; Mihalca et al., 2021). Hydrolysis of fibrous hydrophobic protein produces gelatin. Collagen is naturally occurring as the main component of bone, skin and connective tissue. Usually gelatin based films and coatings are made of 20–30% gelatin, 10–30% plasticizer (e.g., glycerin or sorbitol), and 40–70% water (Ramos et al., 2016).

2.8.2.2 Casein and Whey Protein

Whey proteins are soluble proteins present in milk serum after caseinate coagulation during cheese processing. Whey proteins represent around 20% of total milk proteins (Pires et al., 2021). Casein is a milk protein; it is found in the form of micelles consisting of all casein species. Caseinate is the most common casein product, this is easily dissolved in water. Casein could be mostly used for edible coating because casein edible coatings are easy to form, this is because of their open secondary structure (McHugh & Krochta, 1994). These coatings are also exhibiting excellent barrier properties to aroma compounds and oil. However, due to the fact whey protein is hydrophilic in nature so these films have some limitations to moisture.

2.8.2.3 Zein Protein

Zein proteins are obtained from maize, made from corn gluten flour. Zein protein is immiscible in water, it dissolved in aq. Alcohol, glycol esters. Zein protein has good film and coating producing, adhesive and binding properties (Kim, 2008; Paramawati et al., 2001). All properties of zein coatings depend upon coating thickness (Park et al., 1994). The high content material of non-polar amino acids is a cause of its hydrophobic nature (Bayer, 2021). Biodegradable films and coatings are produced from zein due to its good film forming characteristics. Plasticizers are used in the synthesis of zein films and coatings to produce flexibility as they are highly brittle (Huo et al., 2018). Zein films or coatings are very good moisture blockers than other films. Fatty acids or cross-linking agents are used to improve the water vapor barrier characteristics of zein films and coatings (Paramawati et al., 2001; Sedlarikova et al., 2021).

2.8.2.4 Soya protein

Obtained by grinding of defatted soy flakes, by product from soy oil production. Transparent and flexible films. Films with a poor moisture barrier due to their hydrophilic nature (Hassan et al., 2018).

2.8.2.5 Wheat gluten

Wheat gluten is a hydrophobic protein of wheat flour which is a globular protein composed of a combination of polypeptide molecules. The main properties of gluten such as cohesiveness and elasticity facilitate the process of film formation (Cousineau, 2012; Kumar et al., 2022). Wheat gluten contains the two components of wheat flour protein, prolamine, and glutelin, commonly recognized as gliadin and glutenin, respectively. Wheat gluten is a mixture of two main proteins differing in their solubility in aqueous alcohols soluble gliadins and insoluble glutenin, gliadin is soluble in 70% ethanol but at the same time, glutenin is not soluble (Balakireva & Zamyatnin, 2016).

2.8.3 Lipids based edible coatings and films

The lipid-based edible coatings are used for many years for the preservation of fruits and vegetables. They provide a shiny and glossy appearance to food. The most common lipid-based coating materials are carnauba wax, beeswax, paraffin wax, and mineral or vegetable oil. Lipids are having good water barrier capacity (Hall, 2012). Lipid based compounds are applied as shielding film/coating comprise of herbal wax, acetylated monoglycerides, and surfactants. The simplest lipid compounds are paraffin and beeswax. Lipid based films and coating are highly effective to block the delivery of moisture due to their low polarity (Milani & Nemati, 2022).

The most common lipid-based coating materials used are:

A. Waxes

B. Lacs

C. Fatty acids

D. Acetylated glycerides

2.8.3.1 Waxes and Paraffins

Wax and oil-based coatings include paraffin wax, candelilla wax, beeswax, carnauba wax, polyethylene wax, and mineral oil. Paraffin wax-based films and coating are usually used for cheese, uncooked fruit and vegetable (Hall, 2012). Carnauba wax is obtained from Copernicia Cerifera (palm tree leaves). Beeswax is extracted from honeybees. Candelilla wax is extracted from candelilla plant (Amin et al., 2021). Films and coatings made from these waxes are used to block the moisture, gasoline and also to improve the outer surface appearance of different commodities (Kumar et al., 2021). If these types of coatings are applied as a thick layer, they should be disposed earlier than intake like sure cheese, while used in skinny layers, they're considered fit to be eaten. These waxes are the efficient healthy to eat compounds providing a maximum humidity barrier to high moisture containing produce such as fruits and vegetables (Hall, 2012).

2.8.3.2 Acetoglyceride

Reaction of acetic anhydride with acetylated glycerol monostearate produces 1-stearodiacetin (Vandenberg et al., 2011). The acetylated monoglyceride can be easily solidified into a bendy, wax-like strong from the molten state Most lipids can be extended up to 102% of their actual length before cracking very effectively (Feuge et al., 1953; Sofian-Seng, 2016). But acetylated glycerol monostearate have ability to be extended up to 800% of its length, these type of films and coating have less vapor permeability than polysaccharide films and coatings (Amin et al., 2021).

2.8.3.3 Shellac resins

Shellac resins are consisting of a complicated aggregate of aliphatic alicyclic hydroxyl acid polymers and obtained as a result of secretion through *Laccifer lacca* (insect) (Bar & Bianco-Peled, 2021; Buch et al., 2009). These resins are easily soluble in alcohols alkaline solutions. These resins aren't Generally Recognized as Safe (GRAS) substance, it's far simplest approved as a secondary chemical in edible coatings (Farag & Leopold, 2011). The major use of these films and coatings are in the pharmaceutical field and very few studies regarding its use in food products had been done (Li et al., 2022). The resins and its derivatives-based films and coatings are broadly utilized for fresh fruits and vegetables (Hassan et al., 2018). These coatings have been formed typically to produce extra shining on the food surface at the time of purchasing by the consumer. While films and coatings produce a further gas barrier through which gases need to cross (Hassan et al., 2018; Tauferova et al., 2021).

2.9 Composite edible coatings

These coatings are considered as new generation edible coatings. These coatings are heterogeneous in nature, consisting of a blend of polysaccharides, proteins and lipids (Dave et al., 2017; Moeini et al., 2022). These coatings combine beneficial properties of coating material to create superior quality layer or film. This approach enables one to utilize distinct functional characteristics of coating used (Ribeiro et al., 2021). For example the ediblecoating comprising of a mixture of carboxymethyl cellulose and chitosan onstrawberries with spearmint essential oil as antimicrobial agent maintained quality attributes of strawberry fruit and showed the antimicrobial effect (Shahbazi, 2018).

2.10 Methods of application edible coating

Once the edible coatings were prepared, the application involves various methods. Edible coatings are of different nature, based on the source of coating material. There are different methods being fallowed. The edible coatings are applied on fresh-cut fruits and vegetables by different methods, brushing, spraying, dipping, extrusion, a fluidized bed, panning, and solvent casting of the coating solution (Bizymis & Tzia, 2021; Suhag et al., 2020).

At a laboratory scale, coating and film formation are obtained by dipping and casting processes (Habibi et al., 2017). Dipping is method where product is dipped into coating solution to uniformly apply coating layer on to the fruits and vegetables. In case of spraying coating solution is sprayed on fruits and vegetables with the help of sprayers or electrospinning (Habibi et al., 2017). On commercial use, extrusion and spraying processes are desired methods for film formation.

2.11 Effect of edible coatings on fruits and vegetables

Effect on external appearance and glossiness, edible coatings offer physical barrier between fruit surface and external surroundings which eventually lead to preservation on post-harvest quality (Hassan et al., 2018). Effect on fruits firmness and softening, keeps firmness by evading excessive transpiration and respiration and edible coating has shown positive impact on reducing weight loss by minimizing the transpiration rate (Alharaty & Ramaswamy, 2020). These edible coatings control the entry of oxygen inside the fruits which prevents ethylene production and drops respiration rate and metabolic activities inside the fruits and vegetables (Rashmi & Negi, 2022). Edible coatings influence biochemical constituents which are responsible for the taste and shelf life of fruits and vegetables, these includes titratable acidity, pH, total soluble solids, and ascorbic acid content (Hosseini et al., 2019).

2.12 Advantages and disadvantages of edible coating

Generally, the potential benefits of edible coatings for early perishable produce (fruits and vegetables) is to stabilize the product and thereby extend product shelf life (Hassan et al., 2018; Raghav et al., 2016). Majorly edible coatings have ability to reduce moisture loss, once moisture loss is avoided that prevents loss of firmness and retards respiration rates, hinders solute movement and lower the ethylene production and slowdown the decay phenomenon (Pratap Singh & Packirisamy, 2022). Also prevents fruits from chilling injuries and storage disorders.

The major benefit of edible coatings is that they can be consumed along with food, can provide additional nutrients, may enhance sensory characteristics, and may include quality enhancing components like texture enhancers, antimicrobials and antioxidants (Dave et al., 2017; Guilbert, 1995). Edible coatings have some disadvantages. Thick coating can prohibit Oxygen exchange, causing off- flavour development. Edible coatings are very good gas barriers due to the restricted movement of oxygen and carbon dioxide which causes anaerobic respiration inside the coated atmosphere due to this normal maturation process is hindered in fruits and vegetables (Momin et al., 2021). Some edible coatings are hygroscopic in nature, which helps to increase microbial growth (Flores-López et al., 2016). Although edible films and coatings exhibit GRAS status, they do not assure complete product safety for consumers who have food allergies like lactose intolerance (milk) and from gluten (Pavlath & Orts, 2009).

2.13 Herb extracts used in edible coating

Recently, edible coatings are prepared by mixing or incorporating the extracts of herbs such as thyme, cinnamon, oregano, lemongrass, mint, and citral extract (Angane et al., 2022; Shweta et al., 2014; Thakur et al., 2020). The herbal extracts possess antimicrobial, antioxidant, therapeutic value and it also acts as a nutraceutical source to consumer.

The growing demand for minimally processed fresh cut fruits and vegetable has stimulated research on new ways to extend their shelf-life and preserve quality. Researchers have found categories of antimicrobials that can be potentially incorporated into edible coatings, including plant based essential oils (EOs) like (cinnamon, oregano, lemongrass and ginger), organic acids (acetic, benzoic, lactic, propionic acid), nitrites, and sulfites, among others (Raghav et al., 2016; Thakur et al., 2020). The natural plant based essential oils are outstanding options to minimize or stop the use of chemical preservatives, and their use in foods to meet consumer demands for minimally processed fresh products (Yousuf, Wu, et al., 2021).

2.14 Antimicrobial agents used in edible coatings on fresh fruits and vegetables

There are studies where chitosan and lemongrass essential oil has shown antifungal (*Botrytiscinerea*) activity of strawberry was enhanced and in grapes berries coating inhibited the growth of yeast maintain the good color of the product (Perdones et al., 2012). Composite coating of chitosan and alginate-based films containing garlic oil showed antimicrobial activity against *E Coli, Salmonella aureus,* and *Salmonella Typhimurium* (Irianto et al., 2021). Nano-chitosan-basedcoating with thyme essential oil on cucumbers lowered microbial counts and high radical scavenging activity and on tomato reduced growth of *E Coli* (Bautista-Baños et al., 2019).

Alginate-based coating with lemongrass extract on fresh-cut pineapples found to be effective against yeast and extending the shelf life (Prakash et al., 2020). Alginate combined with cinnamon and lemongrass on fresh-cut melons improved the shelf life of fruit and antimicrobial stability was found (Raybaudi-Massilia et al., 2008; Yousuf, Wu, et al., 2021). Sodium alginate plus cinnamon essential oil on apples and pear limited the fungal growth and ochratoxin A production (Kapetanakou et al., 2019).

Many studies have been performed where a combination of essential oils with various polysaccharides. Hydroxypropyl methylcellulose and oregano extract on fresh plum, which inhibited the total bacterial count on apples during storage (Choi et al., 2016). Carboxymethyl cellulose plus garlic essential oil on strawberries and lemongrass oil on papaya showed antifungal activity and enhanced the quality of fruits (F. Dong & X. Wang, 2017).

Pectin combined with oregano essential oil on tomatoes prevented the fungal decay and antioxidant property was enhanced (Rodriguez-Garcia et al., 2016). Pectin coating with cinnamon essential oil on fresh-cut peaches has a positive effect as complete bacterial growth decrease in peaches (Ju et al., 2019).

Gelatine combined with different plant-based extracts have been studied. Gelatine-based edible coating combined with mint essential oil showed effect by prohibiting the growth of *Botrytis cinerea* on guava cut fruits (Scartazzini et al., 2019). And there are studies where squash mint essential oil with gelatin-based coating on strawberries delayed deterioration up to 15 days and showed antimicrobial properties (Aitboulahsen et al., 2018). Gelatine along with some quantity of chitosan and mint essential oil on guava fruits inhibited the microbial growth (Silva et al., 2021). The antimicrobial activity of whey protein-based edible coatings by incorporation of oregano activity on cut fruits and vegetables (Hassan et al., 2018). Incorporating herb essential oil has shown relatively positive results with zein-based edible coatings, such as zein coating containing cinnamon oil destroyed cells membrane of *Alternaria alternata* and *Botrytis cinerea* in cut apples (Jiang et al., 2020). Thyme essential oil loaded in zein protein- based edible coating on strawberries lowered the total bacterial count and fungi and yeast at 4°C for 15 days (Ansarifar & Moradinezhad, 2021; Yousuf, Sun, et al., 2021).

Soya protein edible coating incorporated with thyme essential oil and oregano essential oil has shown great impact against *E Coli* O157; H7 and *Listeria monocytogen* on minimally processed fruits and vegetables (Valencia-Chamorro et al., 2011). Soya protein coating solution with thymol on strawberry showed antifungal activity and showed an antimicrobial effect on broad- spectrum microbes (Amal et al., 2010).

Wheat gluten-based edible coating with thymol showed the lowest count of microorganisms when applied on strawberry fruits and found that it has more antifungal activity (Aitboulahsen et al., 2018). Another study showed that wheat gluten with 1.5% cinnamaldehyde to the film producing coating solution stopped 33% of *Penicillium spp* and 28% & 16% of *Colletotrichum acutatum* and *Alternaria solani* on different fruits vegetables (Ravishankar, 2018). Commercial carnauba or shellac coatings with 0.5% *Cinnamonum zeylancium* essential oil provided protection against citrus green and blue molds (Khorram & Ramezanian, 2021).

A detailed look at the above literature illustrates some knowledge gap for extending the shelf-life of cut fruits using combination of sodium alginate coating, citric acid enhancement, calcium chloride fixing and possibly the enhancement of antimicrobial activity through natural essential oils (like ginger oil). This has been the driving force for this thesis research.

2.15 Global market of edible coatings

There are several factors that are driving the edible films and coating market growth include meeting the consumer demand and satisfaction and increased adaptability to nature-friendly packaging materials, rising inclination of consumers concerning towards environment-friendly way out, and the growing interest of food manufacturing sectors in edible films and coating (Size, 2021; Size, 2022). Edible films and coating are one of the best possible alternatives for food packaging and storage that can expand the shelf life of food, provide an alternative to existing packaging methods, bio-degradable (Size, 2021; Size, 2022; You et al., 2022).

Polysaccharides material accounted for the largest share of around 40% of global revenue in 2021 (Size, 2021; Size, 2022; Yousuf et al., 2018). Starch, cellulose, carrageenan, carboxymethylcellulose, gums, etc. are used for making edible films and coatings (Saha et al., 2022; Size, 2021; Size, 2022).

Fruits and vegetables categorised for the highest revenue share of over 35% in the global edible films and coating revenue in the year 2020 and 2021 and is expecting to be the fastest-growing division with a CAGR (compounded annual growth rate) of 8.1% in coming decade (Saha et al., 2022; Size, 2021; Size, 2022; Yousuf, Sun, et al., 2021). The meat, poultry, and seafood sector is the second largest segment where edible films and coating are used is expected to grow at a healthy growth rate from 2022 to 2028 (Size, 2022). Starch, carrageenan, chitosan, gelatin, etc. are used in meat, poultry, and seafood products (Saha et al., 2022; Size, 2021; Size, 2022; You et al., 2022).

North America accounted for the largest market share of around 35% in global revenue in 2021 and is expected to grow at a substantial growth rate from 2022 to 2030 (Size, 2021; Size, 2022). Increased awareness towards environmental friendly, long sustaining food preserving materials, the presence of global players and easy availability of edible films and coating products, and consumers' willingness to pay for products will drive the rapid market growth. Countries like China and Japan are the major contributors to the edible films and coating market in this region (Saha et al., 2022; Size, 2021; Size, 2022).

CONNECTING STATEMENT TO CHAPTER 3

Fresh cut fruits find great avenues in supermarkets, school, cafeterias, and university canteens, so there is huge demand for cut produce. These are highly perishable due to the induced mechanical damages as they lose their protective barrier up on cutting. Nowadays consumers are looking for minimally processed products with no chemical preservatives and provide healthy diet. This increase in demand for fresh ready to eat produce offers a real challenge to researchers and food producers to develop good and sustainable preservative techniques to meet consumer and market demands.

The fallowing chapter presents composite edible coatings where sodium alginate, citric acid, calcium chloride and ginger essential oil were manipulated to form a successful edible coating formulation for prolonging the shelf life of cut pear fruit stored at 4ºC for 15 days.

CHAPTER 3

EFFECT OF ALGINATE-GINGER ESSENTIAL OIL BASED EDIBLE COATING ON BIOLOGICAL PROPERTIES OF FRESH-CUT PEAR FRUITS

Abstract

Fruits are generally very perishable after harvesting, and they become even more so when they are stored as cut fruits due to the damage to their external protective skin, leading to acceleration of chemical and biochemical activities, respiration rate, ethylene production, texture softening and moisture loss. Several polymeric substances have been used as edible films and coatings to provide effective gas and moisture barrier properties to control respiration and transpiration of produce. Edible coatings can also modify the internal atmosphere of fruits and vegetables, and better maintain the quality of freshly cut produce. Sodium alginate has been successfully employed as a coating material in several studies. This study was focused on evaluating the effect of alginate and ginger essential oil based edible coating in combination with citric acid and calcium chloride for controlling physiological and microbiological activity in fresh-cut pear fruits during refrigerated storage. A 2% sodium alginate solution, 0.5% citric acid as a synergy enhancer, 0.5% ginger oil as an herbal antimicrobial agent as coating material and 2% calcium chloride for cross linking and firming was used. Coated and control cut fruits were sealed in plastic containers and stored at 4°C for 15-20 days. Respiration rate, color, texture, moisture loss and other quality parameters were evaluated during the storage. Coated fruits had a significantly ($p < 0.05$) better retention of product quality with no microbial spoilage up to 15 days as compared to control samples which spoiled within a week. Overall, the sodium alginate

- ginger oil - calcium alginate coating extended the fungus controlled, good quality refrigerated shelf-life up to 20 days.

3.1 Introduction

Fruits and vegetables play very important role in human diet. They are sources of many nutrients for the huma diet. Food security is therefore important. It essential that we conserve the food we grow. Food losses occur at every step of the postharvest supply chain management starting from the field through harvesting, handling, storage, processing, transportation, distribution and finally the consumer use (Ramaswamy, 2014). Nearly 40% of food is wasted overall in one way or the other at different stages of fruits and vegetable handling (Raghav et al., 2016). There is a huge global demand for fresh-cut fruits and vegetables, but their perishability rate is very high. Post-harvest spoilage factors include several physiological activities like respiration and transpiration rates, ethylene production as well as microbial, physical, chemical, and other environmental conditions during the postharvest handling. Postharvest applications such as refrigeration, control and modified atmosphere storage / packaging, irradiation, application of edible coatings etc., have been explored for effective control of spoilage and yield shelf-life extension (Ramaswamy, 2014).

Edible coatings have been found to be promising preservative methods and they have effect on external appearance and glossiness. Edible coatings offer a physical barrier between fruit surface and external surroundings which eventually lead to preservation of the post harvest quality (Maringgal et al., 2020). Recent studies of edible coatings with plant extracts (ginger, cinnamon, oregano, lemongrass essential oils), organic acids (acetic, benzoic, lactic, propionic acid), nitrites, sulfites and other components have been found to extend their effectiveness(Suhag et al., 2020; Thakur et al., 2020). Edible coatings have been found to effectively control both respiration and transpiration activities, and therefore controls the physiological activities as well as moisture loss. However, the active films/coatings formed by these pure biomacromolecules often do not meet the real needs for food packaging and preservation (Jiang et al., 2020).

The edible coating is defined as a thin layer that is applied to the surface of the fruit to create a barrier between the fruit and the environment, but can be eaten as part of the whole product (Maringgal et al., 2020; Raghav et al., 2016). In explorative studies, coating and film formation and applications are usually done by dipping and casting processes and on commercial scale, extrusion and spraying processes are used for the film formation (Suhag et al., 2020). Different biopolymers are used such as proteins, polysaccharides and gums, lipids (Hassan et al., 2018; Thakur et al., 2020).The polysaccharides used include various starches including the modified starches, chitosan, alginates, gums, cellulose derivatives, and pectin (Hassan et al., 2018; Thakur et al., 2020). The most used protein-based edible coatings are gelatin, corn zein, wheat gluten, soy protein and casein, and collagen. Shellac and oil-based, fatty acid, and monoglycerides are some lipids-based edible coatings (Hassan et al., 2018).

Sodium alginate has been widely used in coating applications. Alginate is obtained from brown seaweed and contains salts of alginic acid. Alginate has some desirable properties including reduction of shrinkage, moisture retention, color, and odor of food commodities. Alginate-based coating with cinnamon extract has been found effective on fresh cut apple and pear against *Aspergillus carbonarius* growth and ochratoxin A production and extending the shelf-life (Kapetanakou et al., 2019). In one of the studies, sodium alginate coating with a subsequent calcium chloride dip has been shown to extended the shelf life of strawberry cut fruits stored at 4°C for up to 15 days (Alharaty & Ramaswamy, 2020). Presently there has been an increased interest in the development of natural edible coatings, using the herb extracts such as cinnamon, clove, lemongrass, Oregon, rosemary, and mint because these can contribute antimicrobial properties to edible coatings. Many herb extracts have proven to possess antimicrobial activities, and these are generally recognised as safe (GRAS) (Alharaty & Ramaswamy, 2020; Angane et al., 2022). The incorporation of essential oils into edible coatings has been widely studied in the literature, with reports of reduced postharvest losses in plums as well as peaches (Riva et al., 2020; Thakur et al., 2020).

Composite coatings have been considered as the future of these coatings and are made by the incorporation of combinations of polysaccharides, proteins and lipids to deliver an effective barrier (Dave et al., 2017). Oms-Oliu (2008) used edible coatings with anti-browning agents to maintain sensory quality and antioxidant properties of fresh-cut pears. Composite edible coatings are most suitable coatings for cut fruits and vegetables for prolonging the shelf life.

Pear belongs to the family *Rosaceae*, also called as pome type/false fruit. Like all other fruits, pear is consumed fresh or processed in to cut pieces, puree, juice, fiber and other products and preserved in all different forms; fresh, canned, frozen, dehydrated etc. Refrigerated and controlled atmosphere storage / packaging are the best approaches used for fresh storage. Cut fruits are sold in limited format in packaged refrigerated format. Fresh-cut pear has become an important food commodity which is growing quickly due to the preserved freshness, being part of healthy diet and increased demand in various food sectors. Fresh-cut pears have very high susceptibility to enzymatic browning and tissue softening after cutting (Passafiume et al., 2021; Pleşoianu & Nour, 2022). Many studies have been carried out on coating of fresh fruits and very few coating materials have been successfully employed on fresh cut pears, noticeably using starch (Dai et al., 2020; Hashemi et al., 2016), pectin, (Pleşoianu & Nour, 2022) and aloe vera based coating (Passafiume et al., 2021). A few studies have also been carried out using whey protein for cut pears (Galus et al., 2021; Pizato et al., 2015). In general, the shelf-life has been extended by 10-12 days under refrigerated conditions for cut pears.

Edible coating using sodium alginate and ginger oil has not been explored with cut pear cylindrical pieces especially with calcium dip and citric acid enhanced medium. Therefore, this study was focused on evaluating the efficiency of sodium-alginate-calcium chloride treatment together with Tween-80 stabilized ginger oil for controlling physiological, physicochemical, textural, microbiological and appearance quality of fresh cut pear fruits.

3.2 Materials and methods

3.2.1 Sample preparation

Fresh pear fruits were purchased from a local market in bulk and selected based on similar appearance, color and shape/size and stored at 4^{o}C and used within two days. Fruits were washed in water and cut into round cylindrical slices of 1 cm thickness and 2 cm diameter and dipped in 1% ascorbic acid solution for 5 min and drained for 3 min before coating to prevent discoloration. Cut fruit pieces were again washed with water, drained and left for 10 min at room temperature, divided in to three lots and held in plastic containers at 4°C for storage.

3.2.2 Preparation of sodium alginate and calcium chloride solutions

Sodium alginate (Sigma, Oakville, ON, Canada) solution 2% (w/w) was prepared by dissolving sodium alginate into water with mixing facilitated by a magnetic stirrer at 300 rpm with no heat until complete dissolution (Alharaty & Ramaswamy, 2020) and the solution was autoclaved. A 2% calcium chloride (Sigma, Oakville, ON, Canada) was used as the firming agent which was prepared by dissolving the required amount of calcium chloride salt in water.

3.2.3 Preparation of coating solution/emulsion

Two edible coating solutions were prepared, one with ginger essential oil and the other one without. The ginger essential oil (Hana, Paul Street, London, UK) (at 0.5%) was solubilized by adding Tween 80 (Croda Inc, Mill Hall, USA) as emulsifier. Citric acid (Milliard, Lakewood, NJ 8701, USA) was added to both to provide an acidic sensorial property and also enhancing synergistic functional properties of the coating solution. A high-speed homogenizer was used at 18000 rpm for 5 min to create stable emulsion. Prepared coating solutions with and without ginger essential oil and these were labelled SACC and SAGEO for identification purposes.

3.2.4 Sample treatment

The prepared coating solutions were poured into marked treatment containers and fresh cut pear samples that were pre-dipped in ascorbic acid (Sigma, Oakville, ON, Canada) added and held immersed in the coating solutions on for 5 min, drained for 3 min and dipped into 2% calcium chloride solution for 5 min in order to better fix the coating material and finally drained again for 3 min. They were then spread on aluminium foil at room temperature for surface drying for 10-15 min. Coated cut fruits were placed in plastic containers with 3-5 small holes (to prevent anaerobic conditions) on top cover and stored in a refrigerator at 4°C for 15 days. Samples were taken out every 3 days intervals for testing and analysis of different physiological and physio-chemical quality parameters.

3.2.5 Sample packaging and storage

Samples were divided into the three groups: control, and treated samples, coated with sodium alginate and calcium chloride (SACC) and coated with sodium alginate emulsion containing the ginger essential oil and citric acid (SAGEO). Once the samples were treated according to the procedure, around 15 sample from each treatment were stored in transparent plastic containers with 3-5 holes on top (2 mm each) to create balanced gas composition and to avoid anerobic respiration within the container. Finally, the containers of each treatment (control, SACC, SAGEO) were stored in refrigerator at 4°C for 15 days for further study and analysis.

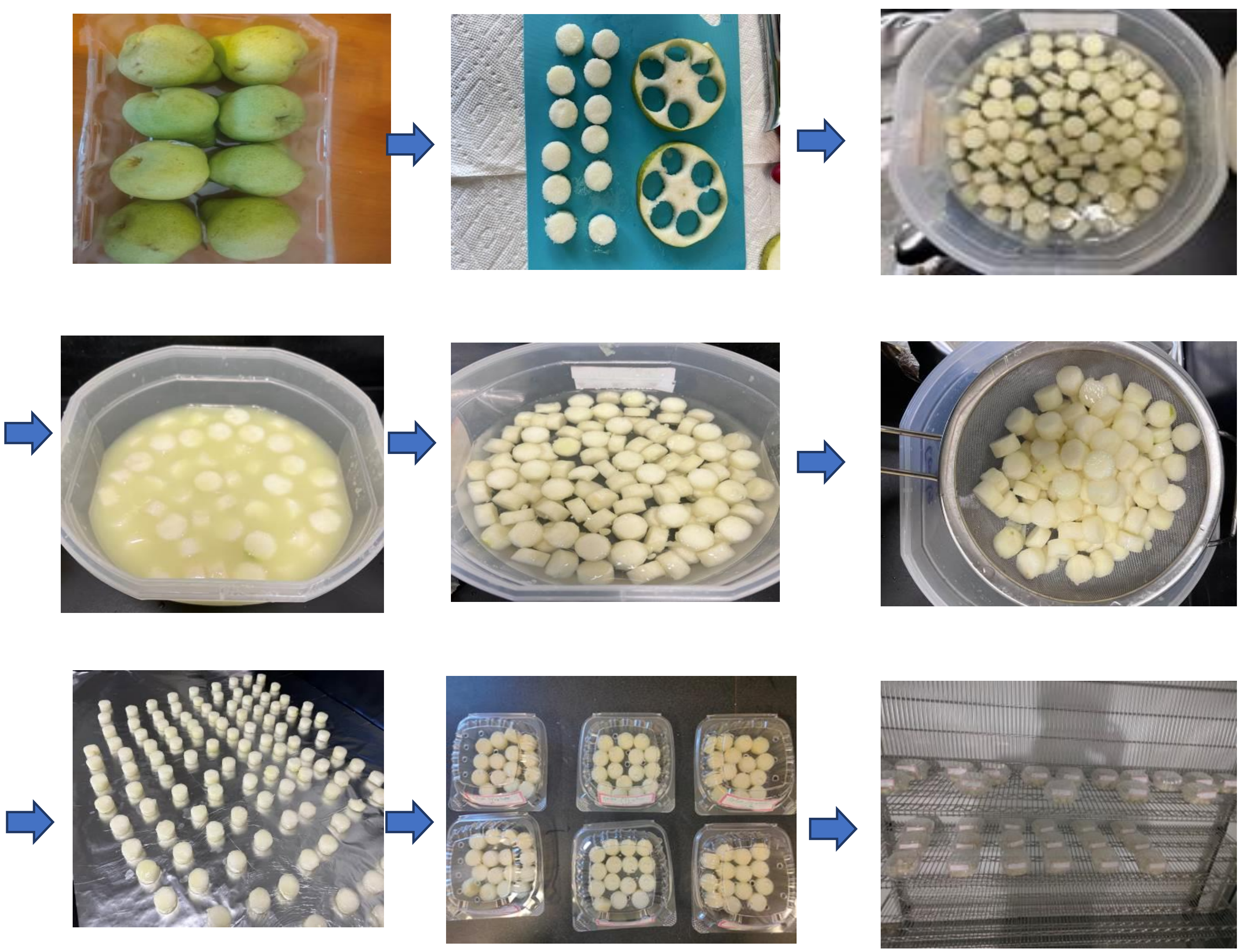

Figure 3.1: Experimental flow diagram

3.3 Physiological properties

3.3.1 Respiration rate

50 g of control and coated samples (M, expressed in kg) were kept in an airtight Plexiglass chamber (18 cm × 12 cm × 27 cm) at room temperature (22 °C) for 2 h (Alharaty & Ramaswamy, 2020; Maftoonazad & Ramaswamy, 2005). The chamber was fitted with a CO_2 sensor (ACR Systems Inc, St-Laurent, QC, Canada) data from which were transferred to a computer through a data acquisition system (Smart Reader plus 7). CO_2 evolution data were gathered every 1 min up to 2 h and the rate of change in concentration was used to compute the respiration rate using Equation 3.1 based on the CO_2 evolution rate (mLCO_2 evolved per hour). Tests carried out in triplicates. Respiration rate is a physiological activity and it is vital for the living produce. The natural respiration rate can be expected to increase once the pear fruit is cut thereby exposing the internal surface to the environment. Edible coating is applied in order to reduce the respiration rate in cut fruits by covering the open fruit tissues with a protective layer.

$$\text{Respiration rate} = [(CO_2 \text{ mL / h}) / M] \text{ mL}CO_2 \text{ kg}^{-1} \text{ h}^{-1} \quad (3.1)$$

3.3.2 Transpiration rate

Weight loss in cut pears with time was taken as a measure of the transpiration loss under the conditions of storage. Control and coated samples were weighed on day zero and again every 3 days during the storage. Using a balance, (Denver instrument, APX-323, NY, USA) weight loss or the moisture loss was recorded and presented as percentage of initial weight of sample (Pleșoianu & Nour, 2022) as shown in Equation 3.2; tests were conducted in triplicates.

$$\text{Weight loss (\%)} = [(W1 - W2)/W1] \times 100 \quad (3.2)$$

where, W1 is initial weight, W2 is weight measured after the specific storage time.

3.3.3 Color and appearance

Color parameters were measured using a colorimeter, a tristimulus Minolta chroma meter (Minolta crop, Ramsey, N). The colorimeter was calibrated with white standard and calibration was done every time while taking readings. 15 samples from each treatment; control, SACC and SAGEO, were used to evaluate the L value (lightness, loss of whiteness or brightness), a* value (green to red shade) and b* value (yellow-blue shades) which were used to calculate the hue angle and chroma at room temperature. All the samples were kept in storage containers for 15 days at (4°C) and external surface change was evaluated such as browning of samples.

3.4 Physio-chemical and textural properties

3.4.1 pH

50 g control and coated samples were separately homogenized with 150 mL distilled waster using blender and followed by filtration, filtrate was divided in to three replicates in 30mL small beakers. pH was measured with a calibrated pH meter (Brinkman Co., Mississauga, ON, Canada).

3.4.2 TSS (Total soluble solids) (° Brix)

A digital refractometer (ATAGO N1, Kirkland, DC, USA) was used to measure the total soluble solids of control and coated samples. Test was conducted in triplicate, and TSS was expressed in degree Brix. 50 g control and coated treatments samples were blended and filtered using sieve, filtrate was used to measure TSS.

3.4.3 Titratable acidity (%)

50 g control and coated samples were separately blended with 150 mL distilled water and filtered using sieve, 10 mL of filtered juice of control and coated was titrated with 0.1Normal NaOH using two drops of phenolphthalein indicator were added to 10 mL pear juice as an indicator. AOAC procedure of TA measurement for fruits was fallowed (Chemists & Cunniff, 1990). Titratable acidity (%) was calculated using volume of NaOH used, applying multiplication factor (0.064) for citric acid and results were expressed as citric acid (%).

3.4.4 Texture & Elasticity

Texture measurements are in Newton (N) and it was done using the Texture analyzer (Model TA XT Plus, Texture Technologies corporation, Scarsdale, NY, USA/Stable MicroSystems, Godaminig, Surrey, UK). 15 cut pear samples from control and coated were subjected to test, round tipped puncture TA52 2mm probe with a speed of 10 mm/s. And firmness (N) and elasticity (mm) values were collected from the TA software.

3.4.5 Mold count

10 g of samples from each treatment were blended with 0.1% peptone water and filtered. Serial dilution method was fallowed. PCA (Plate count agar) media was used and pour plate method was fallowed. Every 3 days interval mold colonies were counted, test was done in triplicate and counts were represented as log CFU/mL.

3.5 Statistical analysis

Minitab Statistical software was used to conduct one-way Analysis of Variance (ANOVA) at 95% level of confidence and 5% level of significance. Tukey's method of comparison was used to indicate the significant difference between the control and coated samples during storage as well as between the different storage times for all the three treatments the control and two coated samples ($p < 0.05$).

3.6 Results and Discussion

3.6.1 Preliminary experiments

During the preliminary experiments (Figure 3.2), 5 different combinations of sodium alginate coatings solutions were prepared, where concentrations of sodium alginate (2%) and ginger essential oil (0.5%) was unchanged for all the combinations, only citric acid concentration was varied (0, 0.5%, 1%, and 1.5%). 30mL from each combination of coating media were transferred to centrifuge test tube (Fisher brand) (Figure 3.2) for week to observe stability of solution with different concentration of citric acid in it. After one week the stability was checked based on the separation inside the tube. Along with the stability check all the 5 combinations were used to coat cut pear fruits. Different quality parameters like color, texture and antimicrobial property were tested and analyzed for 12 days storage period at 4°C.

Addition of ginger essential oil exhibited excellent antimicrobial property in all the combinations during preliminary experiments. Whereas addition of citric acid showed varied results and data. 1st combination with 2% alginate 0.5% ginger essential oil without citric acid produced an unstable coating solution after week as 5mL separation was noted in the tube. When this combination was applied on cut pear fruits early browning was seen. The second combination with 2% alginate 0.5% ginger essential oil and 0.5% citric acid also showed separation in tube after one week. Cut pear fruits stated showing early browning and low texture retention and moisture loss when coated.

The third coating solution with 2% alginate 0.5% ginger essential oil and 1% citric acid resulted in producing better and stable combination after one week no separation was seen in coating mixture inside tube. When applied on cut pear fruit the better color retention along with moisture and texture was recorded compared to other.

The fourth combinations with 2% alginate 0.5% ginger essential oil and 1.5% citric acid showed large separation of 10mL in the tube resulting unstable coating solution. Samples coated with this combination showed early softening of tissue and rapid decrease in texture along with more moisture loss after 6 days of storage. Whereas last combination with 2 % citric acid produced very thick coating solution after 3 days and considered not suitable for coating the cut fruits.

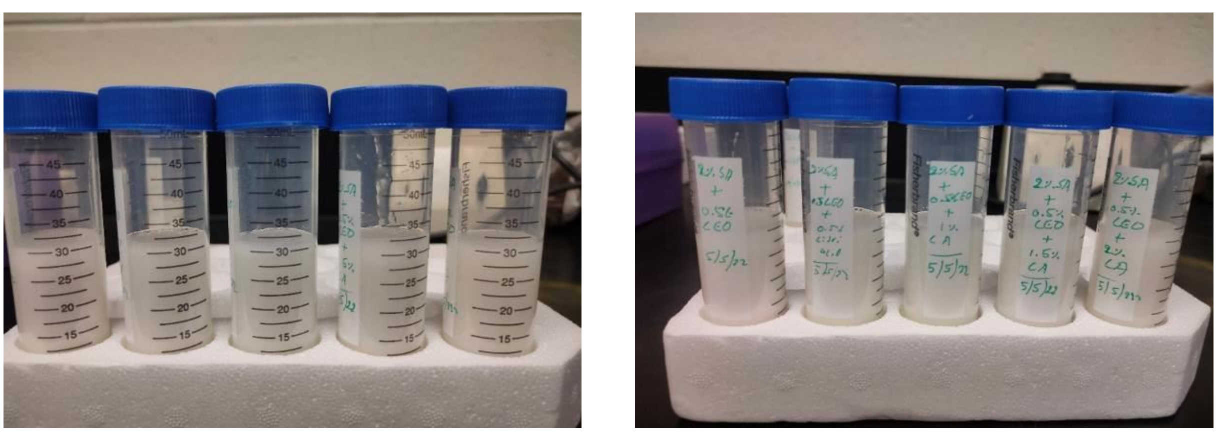

Figure 3.2. Different Combinations of 2% sodium alginate with 0.5% ginger EO and different citric acid concentrations (0, 0.5%, 1%, 1.5% and 2%)

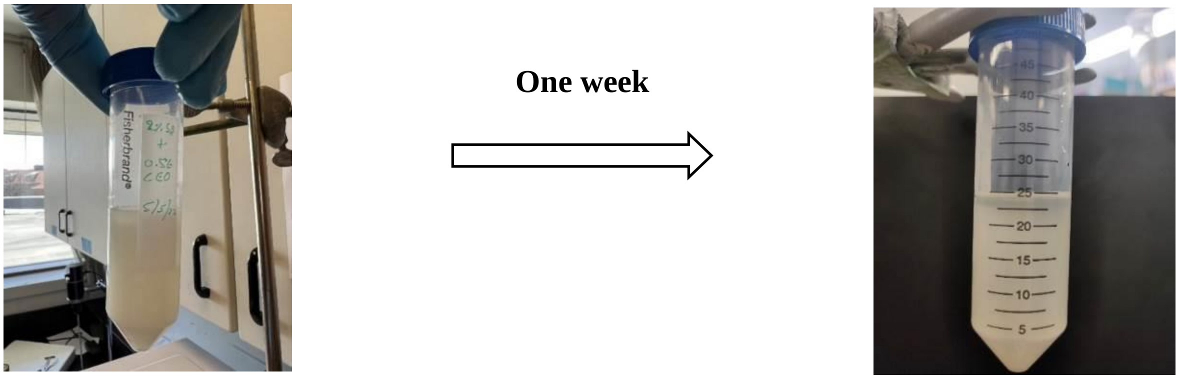

Figure 3.3: Changes in 2%Sodium alginate+ 0.5% GEO solution after one week

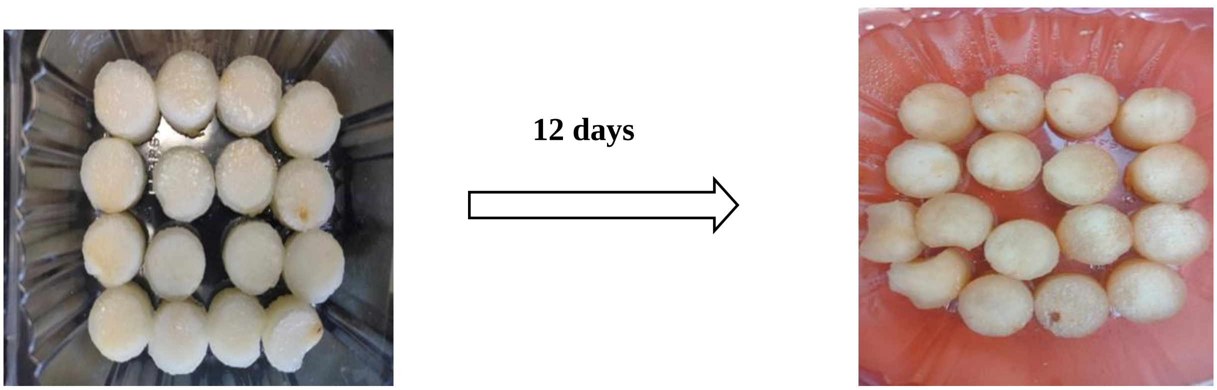

Figure 3.4: Changes in appearance of samples coated with 2%Sodium alginate+ 0.5% GEO solution after 12 days

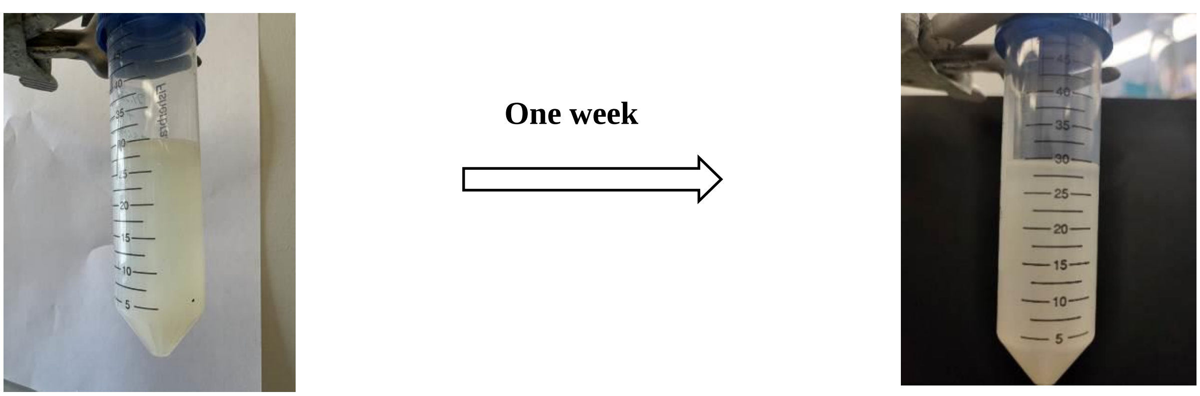

Figure 3.5: Changes in 2%Sodium alginate + 0.5% GEO +0.5% citric acid solution after one week

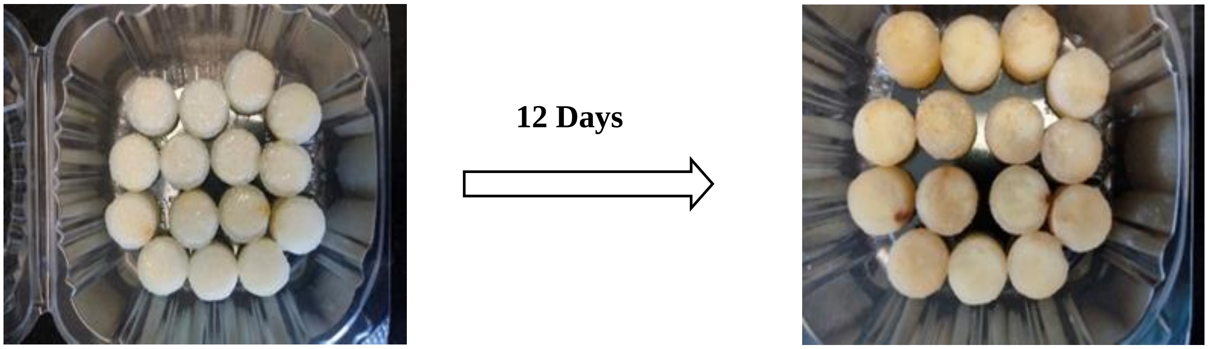

Figure 3.6: Changes in appearance of samples coated with 2%Sodium alginate+ 0.5% GEO + 0.5% Citric acid solution after 12 days

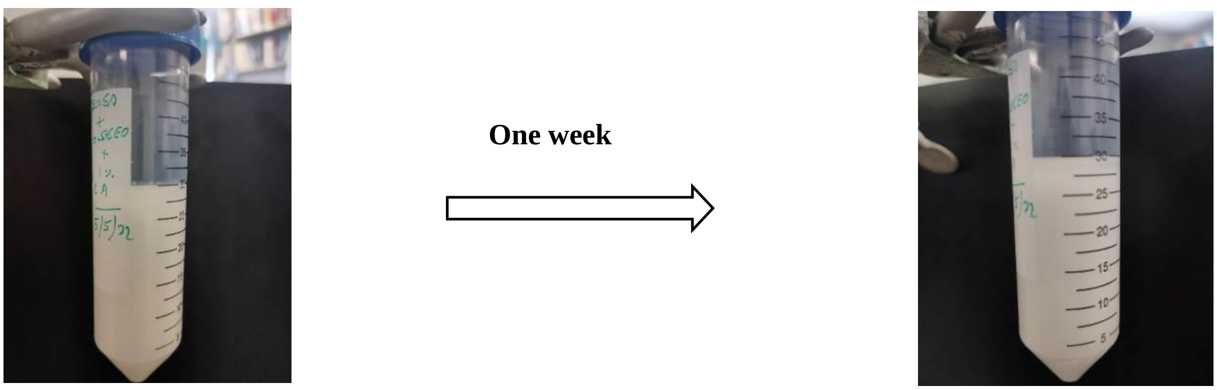

Figure 3.7: Changes in 2%Sodium alginate + 0.5% GEO +1% citric acid solution after one week

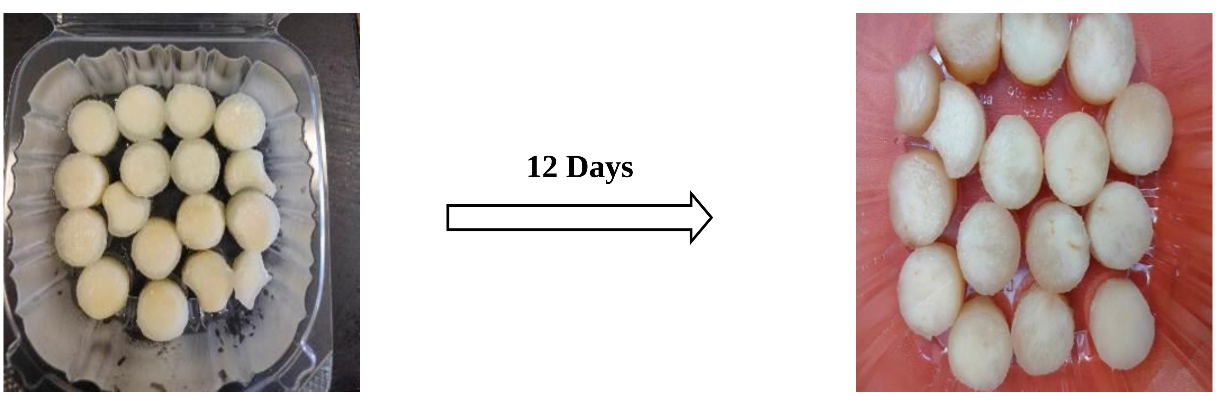

Figure 3.8: Changes in appearance of samples coated with 2%Sodium alginate+ 0.5% GEO + 1% citric acid solution after 12 days

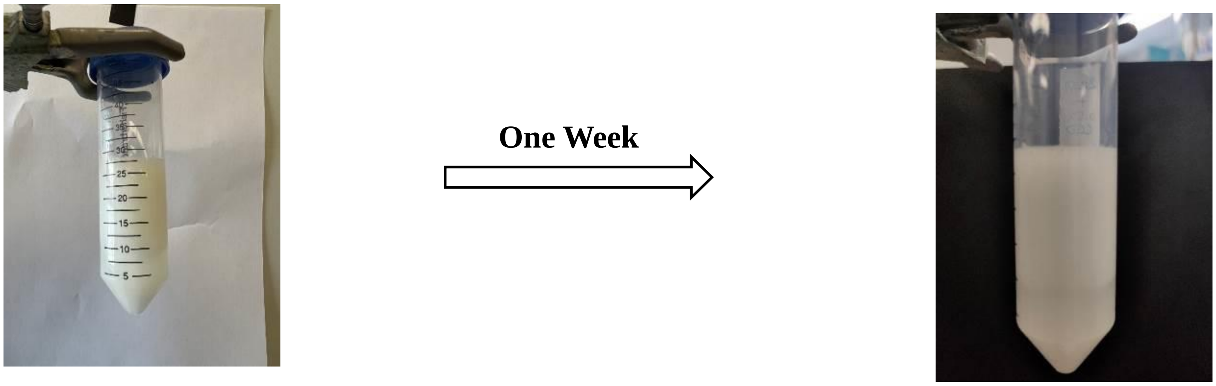

Figure 3.9: Changes in 2%Sodium alginate + 0.5% GEO +1.5% citric acid solution after one week

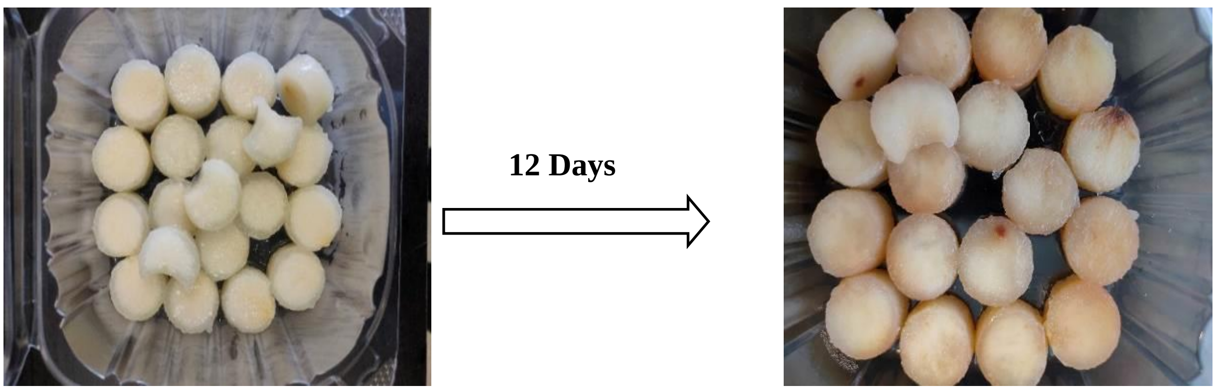

Figure 3.10: Changes in appearance of samples coated with 2%Sodium alginate+ 0.5% GEO + 1.5% citric acid solution after 12 days

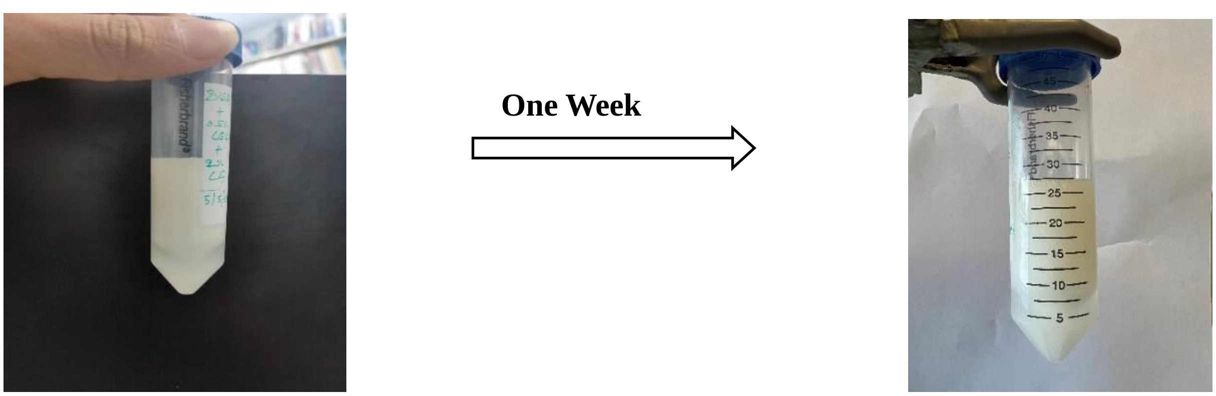

Figure 3.11: Changes in 2%Sodium alginate + 0.5% GEO +2% citric acid solution after one week

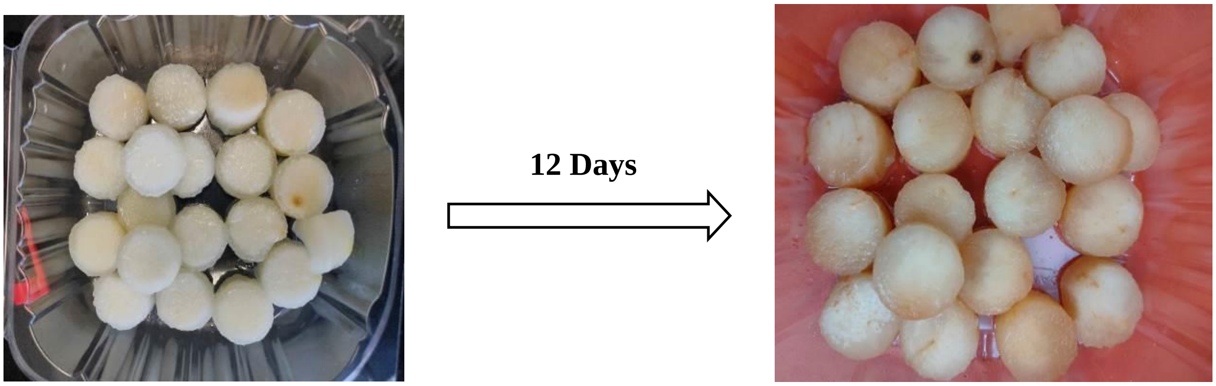

Figure 3.12: Changes in appearance of samples coated with 2%Sodium alginate+ 0.5% GEO +2% citric acid solution after 12 days

Among the different treatments, it appears 1.0% citric acid provided the best acidic environment for effective coating formulation and was used in further studies. These were employed for further testing and product was analyzed for different physiological, physico-chemical, color and textural analysis.

3.6.2 Respiration rate

One way ANOVA results showed no significant difference ($p \geq 0.05$) between control and coated samples Figure 3.13. Control and SACC samples showed similar CO_2 data of 64.3 $mLCO_2\ kg^{-1}\ h^{-1}$ and 64.4 3 $mLCO_2\ kg^{-1}\ h^{-1}$ respectively on day 0 and slightly higher respiration rate was recorded in SAGEO samples with 79 $mLCO_2\ kg^{-1}\ h^{-1}$. A significant increase in CO_2 level was observed with control samples on Day 3, while no increase in CO_2 was observed in samples coated with SACC, and a decrease in respiration rate was noted in samples of SAGEO with lower respiration rate of 63.5 $mLCO_2\ kg^{-1}\ h^{-1}$. By day 6, the respiration rate increased to over 103 $mLCO_2\ kg^{-1}\ h^{-1}$ in control, 85 $mLCO_2\ kg^{-1}\ h^{-1}$ in samples coated with SACC and 69 $mLCO_2\ kg^{-1}\ h^{-1}$ in SAGEO. Samples coated with SACC and SAGEO continued to show low CO_2 level till Day 9, while it increased with control to 149 $mLCO_2\ kg^{-1}\ h^{-1}$. On Day 12, an increase was seen all three treatments, with control treatment samples reached climacteric peak reaching the highest rate of 242 $mLCO_2\ kg^{-1}\ h^{-1}$ while SACC and SAGEO samples increasing to 152 $mLCO_2\ kg^{-1}\ h^{-1}$ and 122 $mLCO_2\ kg^{-1}\ h^{-1}$, respectively.

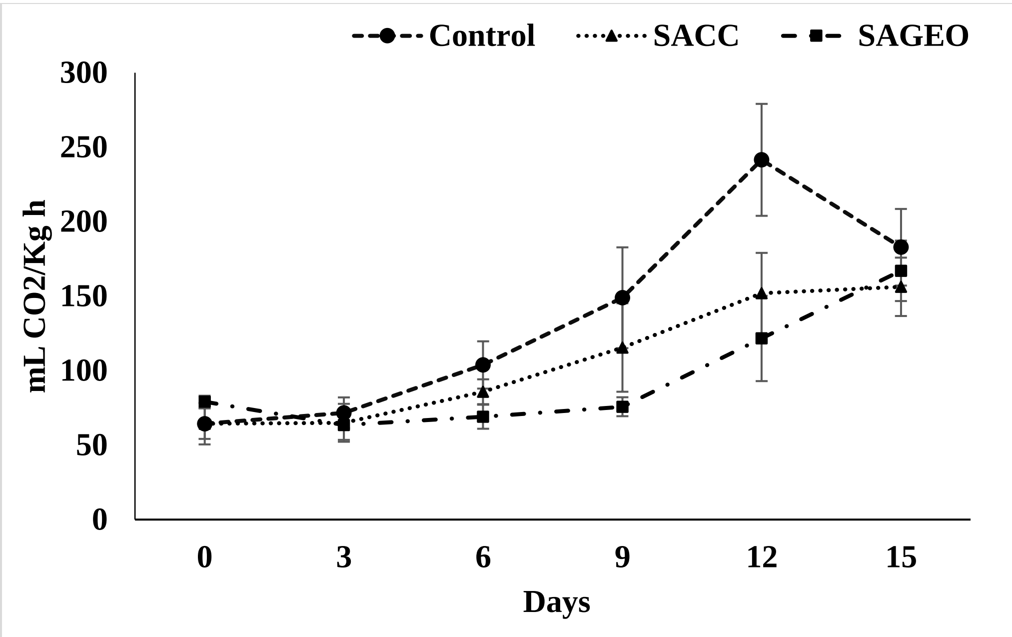

Figure 3.13: Changes in respiration rate of control, SACC and SAGEO during the 15 days of storage period

A decrease in CO_2 was observed on day 15 in control samples but no change in samples coated with SACC. Slight increase in CO_2 was recorded in SAGEO treated samples but it was still lower than control. One way ANOVA showed significant ($p \leq 0.05$) differences in respiration rate among the treatments during the 15 days of storage time. Physiological factors such as transpiration and gas exchange show higher metabolic activity inside upon harvesting and cutting of samples due to exposure of cut internal surface. The use of modified atmospheric packaging along with postharvest techniques is employed by industry to combat the high respiration rate (Gomes et al., 2017). Some studies have shown that presence of Ca^{2+} ions in film forming solution reduced initial respiration rate and ethylene production of coated cut apples and cut strawberry fruits (Alharaty & Ramaswamy, 2020; Oms-Oliu et al., 2008), in agreement with these studies dipping cut pear fruits into $CaCl_2$ lowered the respiration rate.

The obtained data are in agreement with some published studies demontrating how incorporation of essential oils and other additives improved the effectiveness of edible coating. Rojas-Graü et al. (2007) reported that concentration of essential oil can affect the headspace gas composition on fresh cut apple coated with sodium alginate edible coating containing essential oil. The gas barrier properties of the edible coating was improved due to the interactions between the essential oils and coating material (Yousuf, Wu, et al., 2021). Edible coatings helps in reducing the respiration and diffusion of gases but, special care has to be taken for maintaining the internal gas composition, very low O_2 concentration may lead to the anaerobic respiration which will lead to ethanol and off flavor production (Yousuf, Wu, et al., 2021). In one of the studies (Gardesh et al., 2016) authors reported that 0.5% nano chitosan coatings could slow down ripening and climacteric respiration peak This study also confirmed that sodium alginate coating lowered the respiration rate of coated samples compared to control. Significant differences were also noted in CO_2 level among the cut apples coated with alginate coating with and without essential oils (Chiabrando & Giacalone, 2015).

3.6.3 Moisture loss (transpiration rate)

A small change in moisture loss was observed on Day 3 (1.4% with control, 0.76% with SACC and 0.69% with SAGEO). Significantly high moisture loss of 5.35% was observed in control samples on Day 6 while only 1.84% SACC and 2.4% SAGEO were observed with coated fruits. By Day 9 moisture loss in control increased to 8.78% and reached 12.6% on Day 12. Cut fruit samples coated with SACC and SAGEO showed no significant difference in moisture loss ($p \geq 0.05$), and the loss was less than 5% until Day 9 and moisture loss of 5.4% and 5.3%, respectively on day 12 was recorded. After 15 days, a 15.7 % moisture loss was observed in control samples and 7.4% loss in sample coated with SACC and 7.2 % loss in samples coated with SAGEO. Studies have reported lower moisture loss when essential oil is incorporated into the coating material.

While high moisture loss occurs in control due to increased transpiration rate, coating with essential oil lowers the weight loss due to the hydrophobic part in emulsion based coating which act as barrier against the transpiration rate (Hassan et al., 2018; Peralta-Ruiz et al., 2021; Yousuf, Wu, et al., 2021). One of the studies where strawberries were coated with carboxyl methyl cellulose and garlic's essential oil, weight loss was prevented and the shelf life was extended (Feng Dong & Xiaolin Wang, 2017). Some studies suggest that lower weight loss in coated cut fruit samples can also be attributed to the slower ripening rate of the coated samples (Ivanova & Philipchenko, 2012; Perdones et al., 2012).

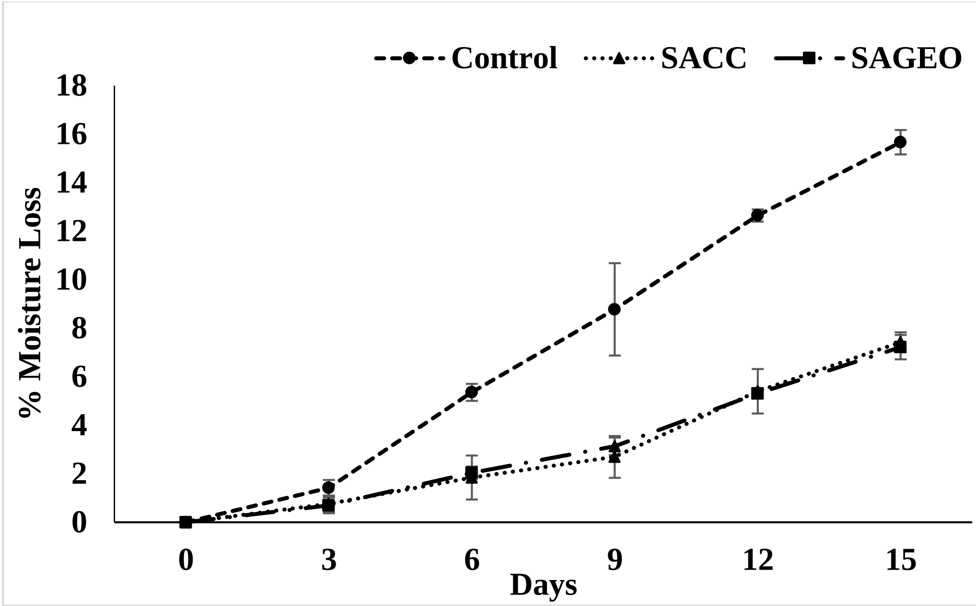

Figure 3.14: Changes in % moisture loss in control, SACC and SAGEO during the 15 days of storage period

One way ANOVA showed significant difference in moisture loss among the treatments control and coated ($p<0.05$) (Figure 3.14). Loss in weight results from moisture loss and also due to the other components which are considered negligible (Yousuf, Wu, et al., 2021). Moisture plays a very important role in shelf life of fresh fruits and vegetables. Moisture is directly related to the texture (turgidity) and other metabolic activities and freshness of produce. Figure 3.14 shows the percent moisture loss in all the three treatments during 15 days of storage. Based on the data collected sodium alginate prevented the moisture loss in coated samples compared to control. One way ANOVA showed significant difference in weight loss as compared to control and coated treatments and during the 15 days of storage period ($p<0.05$) and no significant difference was observed in weight loss of between the two coated treatments ($p\geq0.05$) during complete experiment.

3.7.3 Mold activity

Coated samples showed lower mold counts of than control Figure 3.15. The ginger essential oil was incorporated into the coating matrix in SAGEO to provide some natural antimicrobial property. SAGEO samples showed excellent antimicrobial property, with less than 3 log CFU/mL mold growth during the study. On Day 0, control and SACC samples showed the 4 log CFU/mL, whereas SAGEO samples showed 2 log CFU/mL which was half compared to other two treatments. CFU value of SACC treatment remained same on day 3 while an increase in count was observed in control samples, it increased to 4.4 log CFU/mL. Where in case of samples coated with SAGEO emulsion showed 3.3 log CFU/mL on day 3. As shown in Figure 3.15 more than 5 log CFU/mL was observed in control on day nine, while no increase in count was associated with both coated treatments.

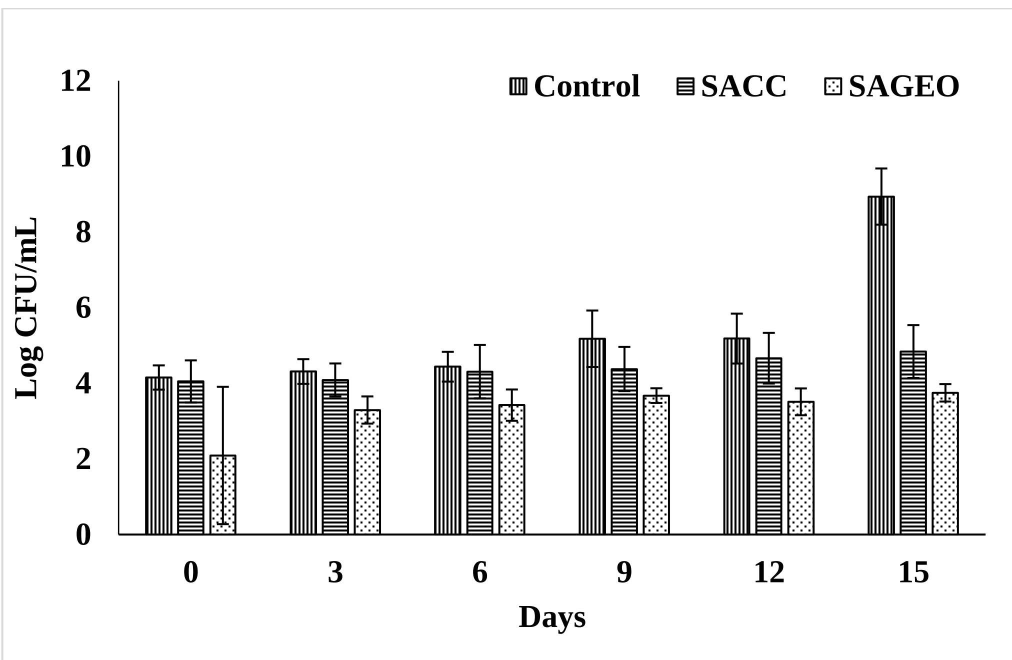

Figure 3.15: changes in mold growth (logCFU/mL) in control SACC and SAGEO during 15 days of storage period

One way ANOVA showed no significant difference in microbial count of two coated treatments until day 12 ($p \geq 0.05$). On day 12 slight increase in count was observed in samples coated with SACC which was less than control samples which crossed the threshold level (3-5 log CFU/mL) but there was no increase in count and below threshold level was observed in SAGEO samples. One way ANOVA showed no significant differences in microbial growth between the control and samples treated with SACC ($p > 0.05$), but there was significant difference in control and SAGEO treatment during the storage ($p < 0.05$). The edible coatings incorporated with different combinations of essential oils proved to have lower gas transfer which lead to decline in availability of oxygen to the fruits tissue which could lower microbial growth on surface (Gomes et al., 2017).

Control samples showed count of 8.9 log CFU/mL much higher than threshold level on day 15. Slight increase in count was observed in both coated treatments, 4.8 log CFU/mL and 3.7 log CFU/mL respectively. Based on the data collected use of herbal extract showed very good role in improving the effectiveness of edible coating and minimize the use of chemicals. Hashemi et al. (Hashemi et al., 2016) observed the positive effect of starch based edible coating with *adiantum capillus veneris* extracts (0, 0.1, 0.2 and 0.3% v/v) enhanced their antioxidant load and decrease microbial contamination. When fruits are cut, they lose their natural skin barrier and tissues are exposed to external conditions. As cut fruits and vegetables consumed raw, their microbiological safety is a concern for both producers and consumers. Active coating with improved antimicrobial properties can safeguard produce from microbial spoilage and extending the shelf life of cut fruits and vegetables (Yousuf et al., 2021).

No mold growth was seen throughout the experiment. Minor mold growth was seen on day nine on the surface of control samples. Mold growth developed rapidly on control samples after thart, where in case of coated samples only slight change in color was observed. On day twelve browning of control samples was observed with decreased textural values demontrating tissue softening. Coated samples showed no sign of mold growth and browing. As shown in Figure 3.15 encorpoartion of ginger essential oil enhanced the antimicrobial property of sodium alginate coating and citric acid prevented browning in cut pear fruits in third treatmemts.

3.7.4 Physico-chemical changes

3.7.4.1 Color

Since alginate coating creates a semi- permeable barrier that controls gas exchange, reducing the contact of the exposed fruit surface to oxygen, the combined effect of coating plus ascorbic acid resulted in an effective way to control browning in cut pear samples. Moreover, the addition of citric acid enhanced anti-browning capacity of the sodium alginate. Color was influenced by sodium alginate based edible coating on fresh cut pear fruits and color retention of coated samples compared with control (OLIVAS et al., 2003). Enzymatic browning is frequently the major limiting factor of shelf life of fresh cut susceptible fruits like pear and apples caused by an enzyme (polyphenol oxidase) that is found in many fruits. After certain days of storage control samples started to lose lightness, whereas samples coated with SACC and SAGEO significantly delayed tissue browning. Sharma & Rao (2015) and Xiao et al (2010), (Sharma & Rao, 2015; Xiao et al., 2010) reported that fresh cut pears coated with essential oils delayed enzymatic browning by inhibiting the effect of polyphenol oxidase reaction.

Natural anti-browning agents play very important role and help in modification of the internal gaseous atmosphere in fruit when they are coated. Ascorbic acid is well known antioxidant that has been successfully used to reduce enzymatic browning in pome fruits (Gorny et al., 2002; Krasnova et al., 2013; Olivas & Barbosa-Cánovas, 2005). Decrease in L values indicates emergence of browning/darkening of samples and increase in a* values (positive) state that samples are turning more brown/red which reflects the ripening status of the fruits.

3.7.4.2 L value

Same L value was recorded on day zero for all the three treatments. L value decreased to 73.7 from 74.4 in control, 74.1 from 75.6 in samples coated with SACC and slight decrease in L value was seen in samples of SAGEO from 75.6 to 74.8 day 3 (Figure 3.16). Rapid decrease in L value of control was observed on day 6, it went down to 66.4 and no significant decrease was seen in both the coated treatments (SACC and SAGEO) L value of 72.5 and 73.5 were recorded respectively. A similar trend was observed in study done by Passafiume et al (2021) on cut pear fruits using aloe vera based edible coating on cut Italian pear fruits. Coated samples did not show much decrease in L value compared to control on day 9 and 12, L value of 59.5 and 57.0 was observed respectively on these days in control, addition of citric acid showed positive impact on preserving the lightness of cut pear fruits (Guerreiro et al., 2016; Kumar et al., 2018; Robles-Sánchez et al., 2013). One way ANOVA showed no significant difference L value of control and coated ($p>0.05$). On day 15 control recorded lowest L value of 54.6 and coated treatments (SACC and SAGEO) had L value of 67.4 and 68.2 respectively.

Figure 3.16 shows the data collected during the study. High L value was observed in samples coated with edible coating containing ginger essential oil during storage period, same trend was also seen in study done by Chiabrando & Giacalone (2015) on cut apples samples treated with coating containing rosemary essential oil.

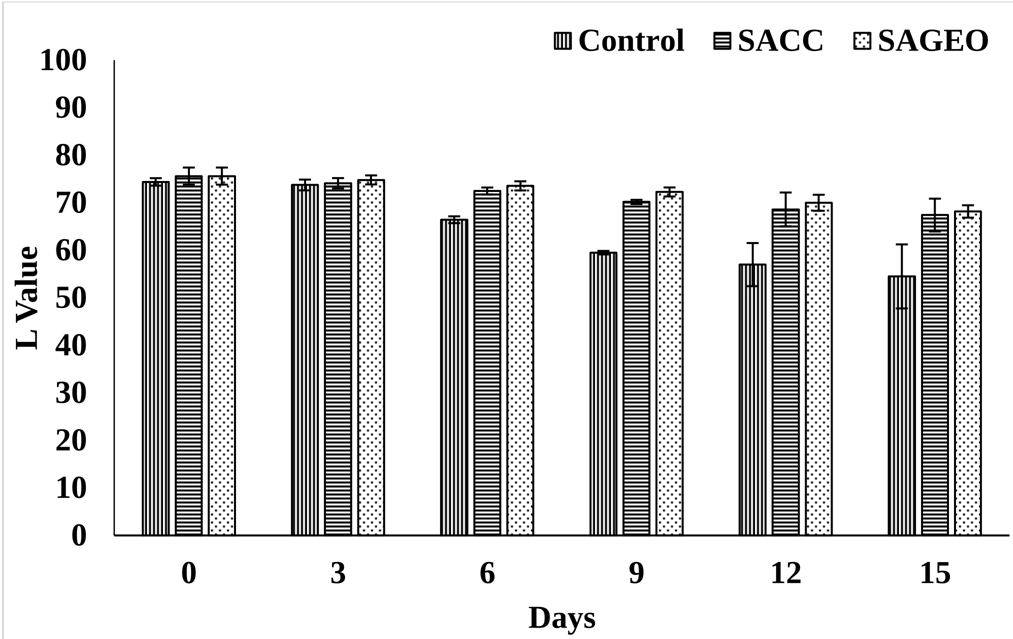

Figure 3.16: Changes in L values of control, SACC and SAGEO during 15 days of storage period

3.7.4.3 a* value

Figure 3.17 shows the a* values of all the three treatments during storage. It was observed that the storage time significantly influenced the change of the parameter a*. Negative a* values were observed on day 0 in control as well as coated samples as fruits samples were freshly cut and negative value shows increased proportion of green (Galus et al., 2021). a* value of control increased to 1.01 on day 3 and negative a* value of coated samples of SACC and SAGEO treatments noted. Slight decrease was seen in control samples on day 6 and increase in a* value of samples coated with SACC was observed, whereas samples of SAGEO showed negative a* value Presence of citric acid as anti-browning agent prevented darkness of cut fruits during the storage. On day 9 sudden increase of 2.45 was noted in control samples and very minute increase in coated samples was recorded with a* values 1.01 and 0.03 in samples coated with SACC and SAGEO respectively. One way ANOVA showed significant difference ($p<0.05$) in a* value of control and coated samples during the storage. a* value of control increased to 2.8 and 2.80 on day twelve and fifteen, whereas coated samples showed 2.2 and 2.3. lowest a* values of 1.18 and 1.76 was observed in SAGEO samples.

3.7.4.4 b value

The value of the b parameter (yellow and blue) for all samples showed a positive value, for coated treatments. No significant ($p>0.5$) difference in b value of control and coated sample was observed for 15 days storage time. Control samples showed higher b value on day 0 with 16.6 and coated treatments showed similar data with b value 11.9. b value of control and coated samples increased to 18.5, 14.3 and 15.0 respectively on day 3. On day 6 and day 9, b value of control samples decreased to 17.94 and 16.01, slight increase of 15.8 and 15.7 was observed in samples coated with SACC whereas samples coated with SAGEO 16.6 and 16.9.

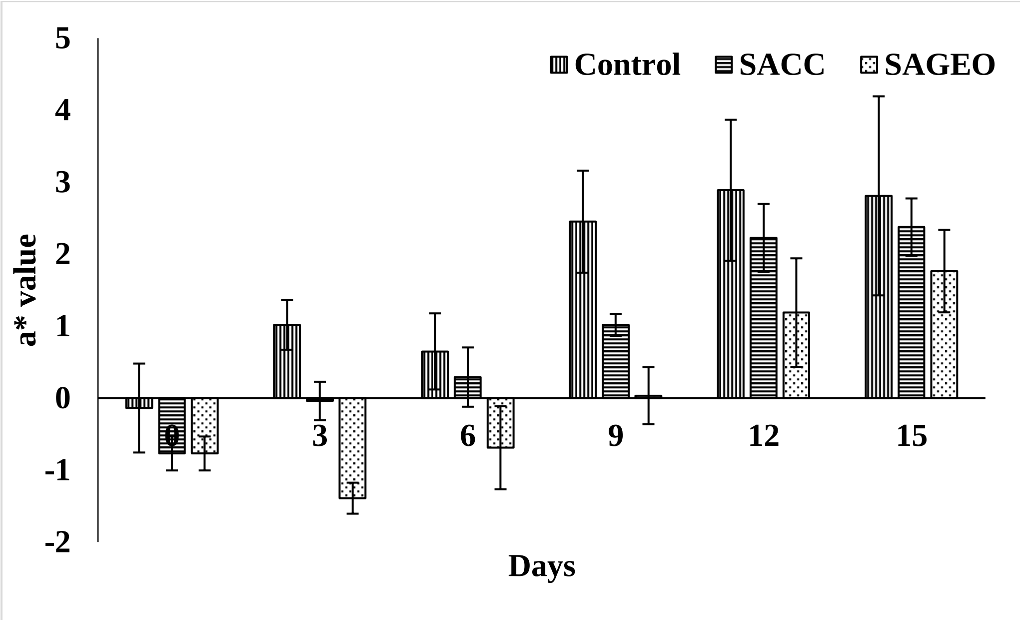

Figure 3.17: Changes in a* values of control, SACC and SAGEO during 15 days of storage period

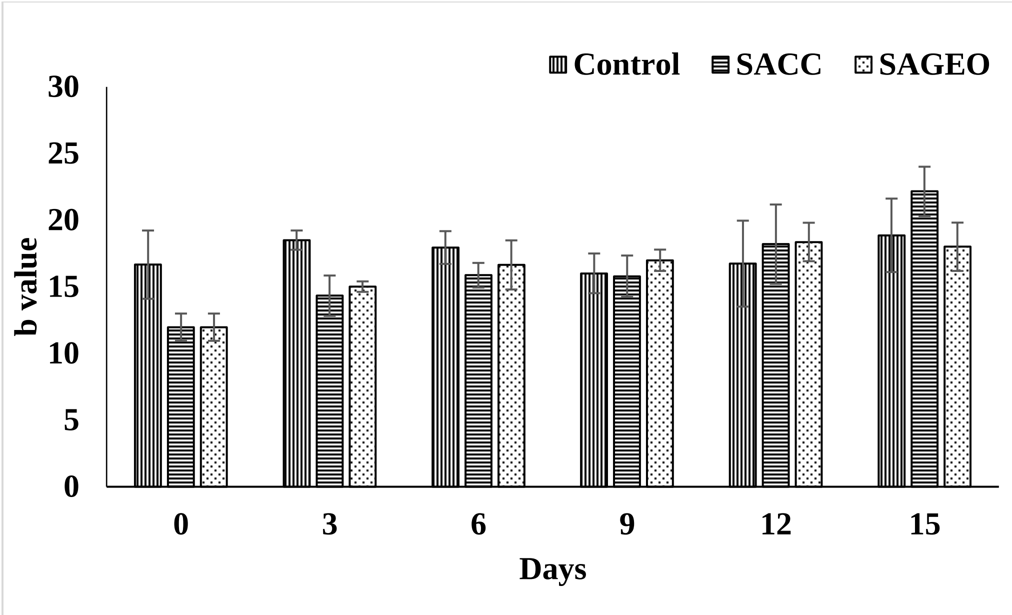

Figure 3.18: Changes in b values of control, SACC and SAGEO during 15 days of storage period

Increase in b value was recorded in coated samples on day 12 and 15, samples coated with SACC showed 18.2 and 22.2 b values. In case of SAGEO treatment b value of 18.4 and 18.0 was observed. Day 15 control samples showed increase in b value to 18.9. Progressive change in color can be seen in this parameter has the highest value in the case of the test without the addition of ginger essential oil. The type of coating used had no significant statistical differences between the value of the parameter (b) for the coatings. One way ANOVA showed no significant difference in color value among the control and samples coated with SACC and SAGEO during the storage time ($p>0.05$). Figure 3.18 shows the data of 15 days.

3.7.4.5 Effect on pH

pH was measured during the 15 days experiment, based on the data collected control samples showed more increase in pH than the coated samples. Control samples and samples coated with just sodium alginate and calcium chloride showed similar readings, samples coated with sodium alginate incorporated essential oil coating showed very less pH reading on day 0 due to the presence of citric acid.

On day 3 pH of control samples increased to 5.02 and pH then increased to 5.34, 5.85 on day 6 and day 9 respectively, then it went up to 6.30 and 7.94 on day 12 and day 15 respectively, this increase in pH showed decrease in acidity of the samples. Whereas samples coated with SACC did not show much increase in the pH, on day 3 and day 4 the pH level of 4.53, 4.64 was recorded and very slight increase was found on day 9 and reached 5.01 and 5.2 on day 12 and day 15, and low pH and high acid content lower the mold growth. There was no significant difference between the pH of both coated treatments (SACC and SAGEO) until day 12 ($p>0.05$).

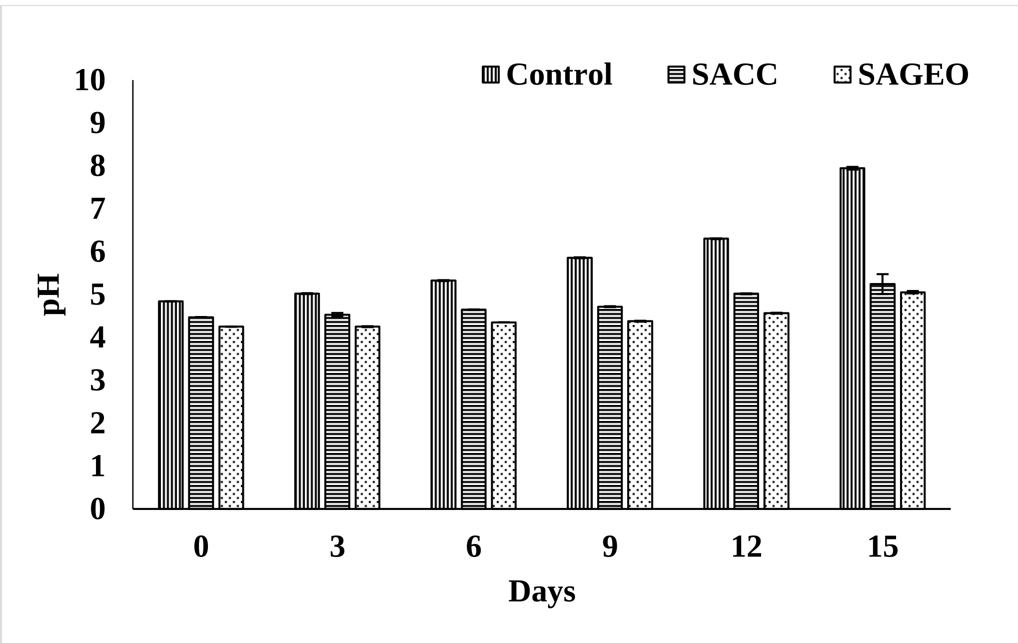

Figure 3.19: Changes in pH values of control, SACC and SAGEO during 15 days of storage period

In comparison with the control samples there wasn't much increase in the pH in coated treatments, which indicated that the sodium alginate coating lowered the increase in pH of coated samples. One of the studies stated that low pH could be maturity stage at the time of minimal processing (Sharma & Rao, 2015).

Finally, the third treatment samples coated with SAGEO showed very less increase in pH level even compared to the samples coated with sodium alginate without ginger essential oil (SACC). One way ANOVA showed significant difference in pH of control and coated treatments during 15 days storage time ($p<0.05$). For product quality acceptance all these values have to be in the 10 % limit (Saquet, 2019) and it was achieved during experiment.

3.7.4.6 Effect on total soluble solids (° Brix)

On day 0 both control and samples coated with sodium alginate showed similar readings whereas TSS of samples treated with SAGEO was 7.1°Brix (Figure 3.20). On day 3 there wasn't much increase in TSS content of both control and coated samples. Rapid increase in TSS content of control samples was seen it went to 9.3 °Brix and coated samples showed no increase in the TSS value until day 9 and it was less in samples treated with SAGEO emulsion. On day 12 TSS content of control samples reached 12°Brix and it increased to 16.5°Brix on day 15. Coated sample showed <10°Brix increase in the TSS content till day 12, the increase in TSS is not associated by influence of coating or essential in coating (Yousuf, Wu, et al., 2021). Slight increase was recorded in samples coated with SACC. Whereas samples with SAGEO coating showed very less increase in TSS. One way ANOVA showed Significant difference with TSS of control and coated samples of both the coated treatment ($p\leq 0.05$) and no significant difference in TSS among the two coated treatments till day 12.

Edible coating slow down the degradative biochemical changes as compared with the control samples by lowering the metabolic activity of the cut fruits and prevented the increase in sugar content at 4°C during 15 days of storage time (Passafiume et al., 2021; Pleșoianu & Nour, 2022). Edible coating prevented the increase in TSS content of coated samples by lowering the metabolic activity of the cut fruits and prevented the increase in sugar content at 4°C during 15 days of storage time. A study done by Gomes et al and Souza et al, (Gomes et al., 2017; Souza et al., 2015) justified that increase in TSS is associated with moisture loss during the storage. The one-way ANOVA showed significant difference between the TSS of control and coated treatments during 15 days of storage time ($p<0.05$).

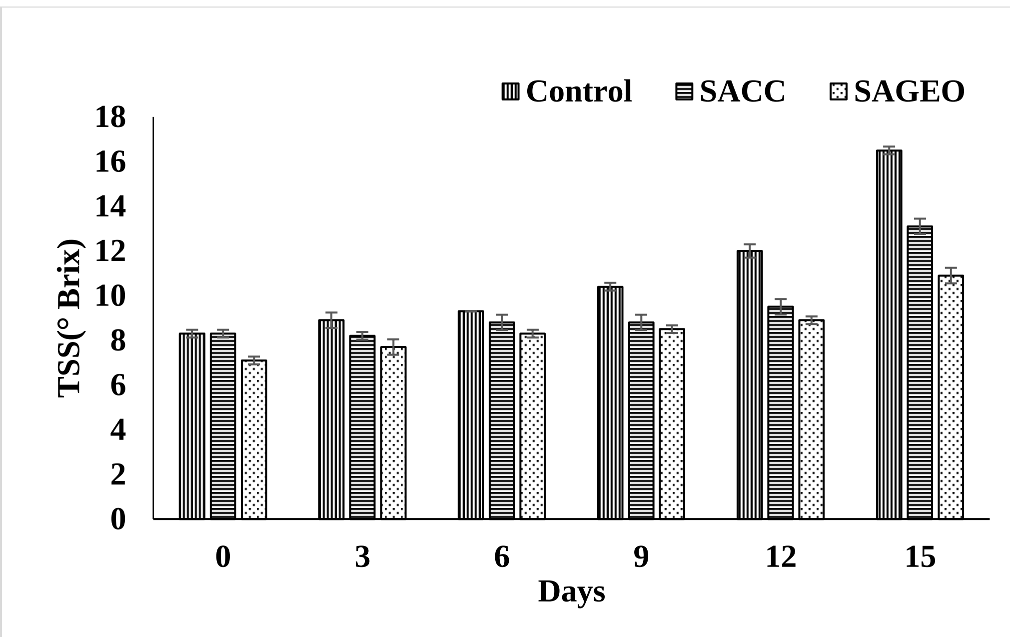

Figure 3.20: Changes in TSS (Total soluble solids) values of control, SACC and SAGEO during 15 days of storage period

3.7.4.7 Effect on titratable acidity

The one way ANOVA showed no significant difference in titratable acidity (TA) on coated treatments ($p \geq 0.05$) until day 9. The TA content is related to the presence of acids in the fruits and conversion of organic acids into sugars and development of microbial growth on fruits, higher the acid content lowers the microbial growth potential of molds and yeast (Yousuf, Wu, et al., 2021). There are several studies which report effect of essential oils on the chemical composition of fruits. study done by Hosseini et al., (2019) (Hosseini et al., 2019) carried out on kumquat fruit treated with chitosan incorporated with essential oil of different combinations showed similar decrease in acidity of fruits. Even on day 0 all three treatments showed some small distribution in variable data which is natural representing a fruit variability. Slightly higher TA was recorded in pear fruits coated with SAGEO edible coating due to the presence of citric acid.

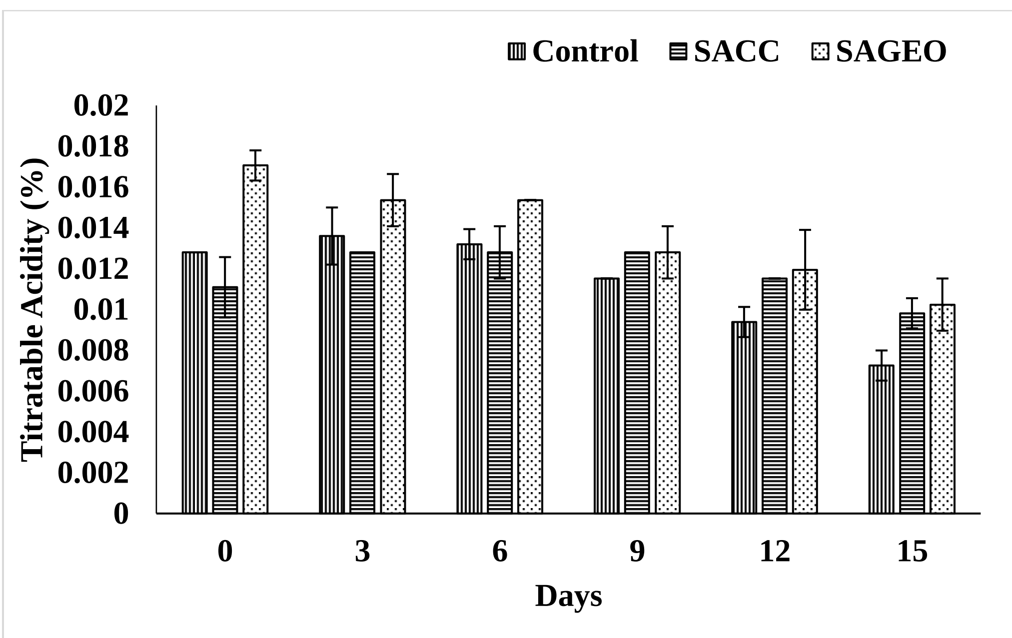

Figure 3.21: Changes TA (Titratable acidity) values of control, SACC and SAGEO during 15 days of storage period

Similar trend was observed in another study by Pleșoianu and Nour (2022) where it was observed that addition of ascorbic acid and citric acid due sto chemical dip resulted in significantly higher titratable acidity pf cut pear fruits during 15 days of storage and they reported due to the delayed fermentation process in the fruit by the addition of preservative (Pleșoianu & Nour, 2022). Whereas slightly higher TA was recorded in control compared to the samples coated with SACC. Gradual decrease in TA level was observed in control 0.014% on day 3 and 0.013% on day 6. Coated samples showed TA of 0.013% and it remained same till day 9. Very less decrease in TA was recorded in third treatment compared to control.

On day 12 titratable acidity decreased to 0.0094% in control. 0.012% in SACC treatment and 0.012% in SAGEO. On day 15 TA in control decrease to 0.0073% in whereas the coated treatments showed lower decrease in TA. One way ANOVA showed no significant difference on SACC treatment and SAGEO ($p \geq 0.05$). The one way ANOVA also showed no significant difference in between the control samples and coated samples during 15 days of storage ($p \geq 0.05$).

3.7.5 Effect on texture/firmness

The pear samples treated with sodium alginate showed very good retention of original firmness during the 15 days storage. Firmness was measured as the maximum force (N) at failure as the probe passes through the fruit tissue. All three treatments showed similar results on day 0 there with demonstrating some variability. The one way ANOVA showed no significant difference in control and coated treatments ($p > 0.05$). Decrease in firmness value in control samples observed on day 3 it lowered from 5.6 N to 5.1 N. Whereas samples coated with SACC showed very minute decrease from 5.5 N on day 0 to 5.3 N on day 3, in case of samples coated with SAGEO edible coating 5.6 N on day 0 and 5.4 N on day 3. No significant difference was observed between the coated treatments. Coated samples showed high firmness value till day 12 compared to control in which the firmness was reduced to 4.7 N on day12. Figure 3.23 shows the graphical information on loss of firmness in all three treatments during the storage.

By day 15, firmness value of control samples manifested even more due to loss of moisture and increased respiration rates with firmness of 4.7 N for control versus 5.0 N for coated samples. The sodium alginate coating lowered the moisture loss and preserved firmness of cut pear fruits samples.

A recent study (Carvalho et al., 2016). using chitosan and incorporating it with trans-cinnamaldehyde showed positive influence on firmness by decreasing the damage by free radical to membrane structure of cut melons. The study also found that edible coating with essential oil lowered respiratory metabolic activity of cut fruits. Oms-Oliu et al. (2008) reported that the texture of control fresh-cut pears was retained over the storage period and based on this observation calcium chloride may not even needed to maintain firmness of cut pear fruits. The somewhat similar results were observed in study.

Texture is an important factor to determine the quality and the shelf life of fresh cut pear. During the storage period the feel quality of cut fruits is affected by the softening of tissue and reduce shelf life, and the use of essential oil may promote some protection activity which lead to better retention in firmness of cut fruits (Yousuf et al., 2021).

 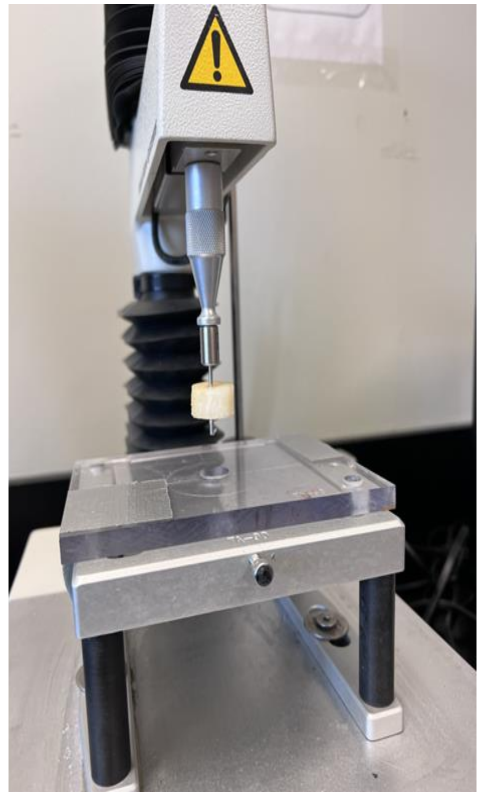

Figure 3.22: Puncture test on cut pear fruit samples

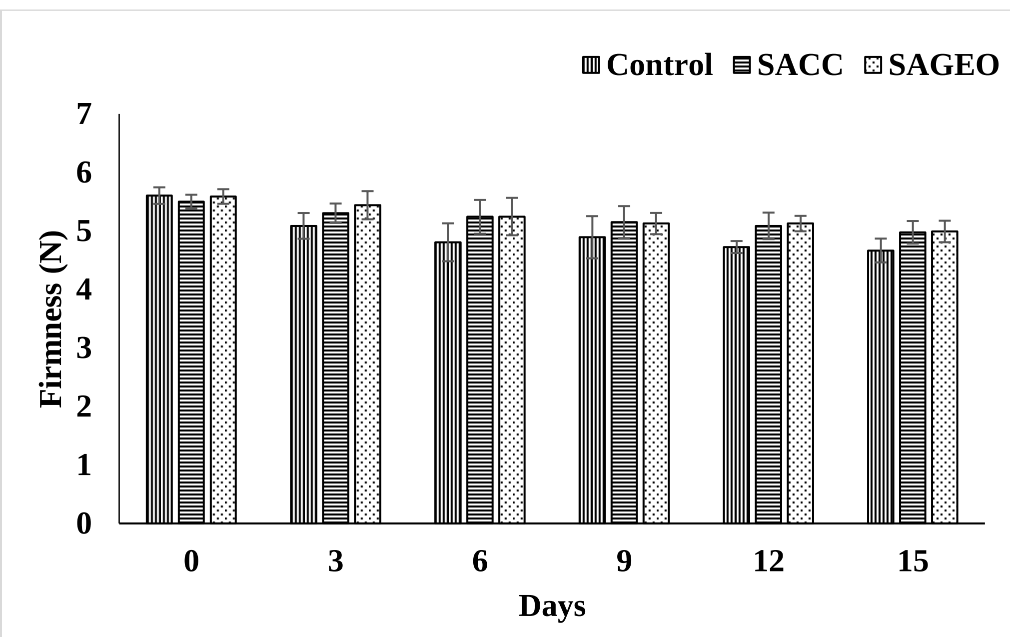

Figure 3.23: Changes in firmness values of control, SACC and SAGEO during 15 days of storage period.

One of the primary properties of edible coatings is to preserve the texture by preventing the moisture loss and retarding the metabolic activity in cut fruits. Addition of acetic acid in to chitosan coating has been beneficial to preserve or maintain firmness in cut prickly pears for 12 days (Guerreiro et al., 2016; Ochoa-Velasco & Guerrero-Beltrán, 2014).

3.7.6 Elasticity

Elasticity plays an important role in cut fruits and vegetables. This parameter was measured along with the firmness as the probe distance (mm) travelled into the fruit tissue at the maximum firmness. A small decrease in elasticity values was observed in coated samples of SAGEO treatment and slight decrease in samples coated with SACC, it lowered form 9.4 mm to 8.9 mm on day 3, whereas control samples showed higher loss from 10 mm from on day 0 to 8.1 mm on day 3. The one way ANOVA showed no significant difference in elasticity values of control and coated samples till day 6 ($p>0.05$). Elasticity value of control samples was 7.9 mm on day 6 which eventually was further lowered on day 9 to 6.6 mm. There was not much in elasticity in coated samples compared to control, 8.3 mm and 8.1 mm elasticity values were observed, respectively, and it decreased to 7.8 mm and 7.5 mm on day 9 in both coated treatments respectively. Further loss in elasticity was observed in control on day 12, with the elasticity of 5.7 mm was observed, in case of samples of each coated treatment was 7.3 mm and 7.5 mm was observed respectively, which was much higher than the control. On day 15 lowest value of 4.7 mm elasticity was recorded in control and SACC and SAGEO yielded 6.8 mm and

6.5 mm respectively. Edible coating of sodium alginate coating therefore demonstrated positive impact on preserving the elasticity of cut pear fruits.

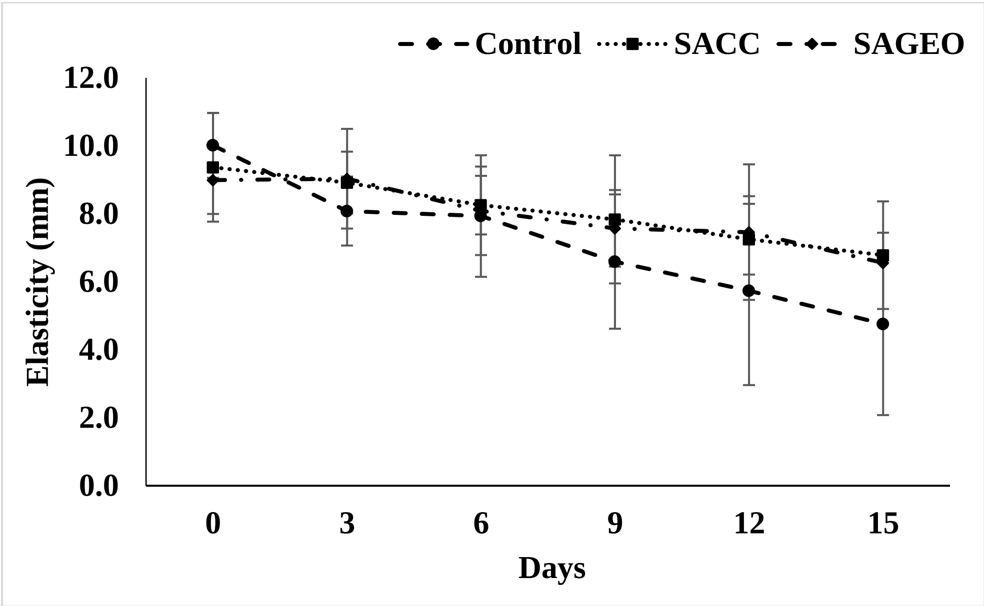

Figure 3.24: Changes in elasticity values of control, SACC and SAGEO during 15 days of storage period

3.7.7 Effect on Appearance

Figure 3.25 shows the appearance of samples both control and coated between time zero and 15 days of storage. The control samples developed progressive browning to a much greater extent during storage and allowed the manifestation of mold growth whereas the coated SACC and SAGEO samples maintained better appearance with no mold growth on the cut fruit surfaces.

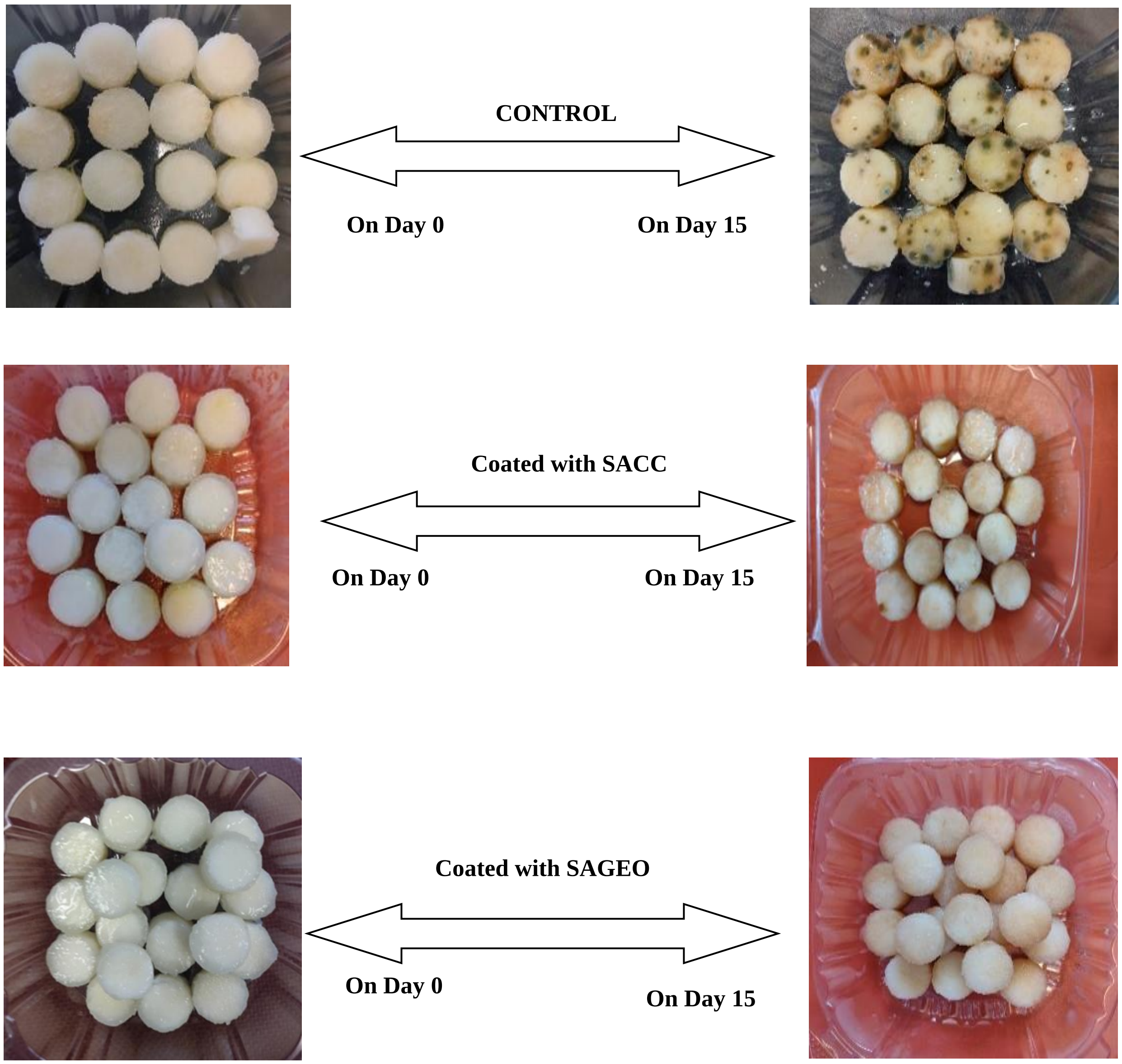

Figure 3.25. Changes during the 15 days of storage period. (SACC) treatment, samples coated with sodium alginate only, (SAGEO)

3.8 Conclusions

This study demonstrated the effect of sodium alginate coating with or without ginger essential oil (SAGEO and SACC) on extension of the shelf life of cut pear fruits for 15 days. Study showed positive results in terms of preserving the post harvest quality parameters. Sodium alginate coating with calcium chloride as firming agent preserved the texture and colour of coated cut pear fruits. Coated samples also showed low respiration rates, low moisture loss and titratable acidity and during the 15 days storage(4°C). Also, there was lower increase in pH, total soluble solids of cut pear fruits of both coated treatments. SAGEO showed better performance in controlling the mold growth. Overall, incorporation of ginger essential oil improved the antimicrobial property of sodium alginate coating and helped to increase the shelf life of cut pear fruits.

CONNECTING STATEMENT TO CHAPTER 4

The second objective of the thesis makes use of electrostatic (anionic and cationic) edible coatings to extend the shelf life of mini cucumbers. The mini cucumbers are high moisture content vegetable and there is big demand in various food sector like restaurants, schools and universities. Cucumbers play very important role in providing dietary fibers.

In Chapter 4 the multilayer coating with sodium alginate couped with calcium chloride dipping fallowed by dipping in chitosan edible coatings to develop multilayer electrostatic coating on mini cucumbers to preserve post harvest quality factors, tenderness or texture, moisture, and color.

CHAPTER 4

EFFECT OF MULTILAYER ELECTROSTATIC EDIBLE COATING ON SHELF-LIFE EXTENSION OF MINI CUCUMBERS

Abstract

This study is focused on use of composite coating materials applied by layer by layer/alternative deposition of oppositely charged polysaccharides around the mini cucumbers (*Cucumis sativa*) by dipping method followed by storage at 4°C. The combined effect of edible coating composing of 2% sodium alginate, 1% chitosan, and 2% calcium chloride as a interlinking agent between two coating materials, was investigated. It was found that multilayer edible coating could preserve the post harvest quality parameters of mini cucumber at 4°C and extended the shelf life up to 20 days. Coating the mini cucumbers with sodium alginate and chitosan in layer by layer fashion lowered the moisture loss and respiration rate. Color and chlorophyll components were preserved for longer time. Low increase in pH and total soluble solids was observed compared to control during the storage time. Along with sodium alginate and chitosan coating preserved the textural properties like firmness and tenderness of the fresh mini cucumbers for longer time.

4.1 Introduction

Physiological factors such as transpiration and gas exchange could be higher due to increase in metabolic activity inside the fruits and vegetables, upon harvesting. Likewise, quality deterioration is higher in harvested cucumber as respiration, ripening, and softening of outer protective barriers increases. Quality preservation and extending shelf life can be achieved by edible coating. Edible coatings were found to be promising alternative preservative methods. By effecting on the appearance and glossiness, edible coatings offer a physical barrier between fruit surface and external surroundings which eventually lead to preservation of the post-harvest quality (Alharaty & Ramaswamy, 2020; Hassan et al., 2018). When a layer of edible coatings is applied on the surface of a product it will form a protective layer which prevent moisture loss and reduce gases exchange. As moisture loss is avoided, that will affect on fruits firmness and softening. Keeps firmness by evading excessive transpiration and respiration (Mounika et al., 2022). Edible coatings have been found to lower other enzymatic reactions as well, and prevent browning, discoloration by avoiding degradation of cell wall pigments, provide shininess to the surface of the commodity. Edible coatings possess antimicrobial property which help in lowering the mold and yeast growth on fresh fruits and vegetables during the storage and avoid the use of chemical preservatives (Al-Tayyar et al., 2020).

Beside refrigerated storage shelf-life extension of fruits and vegetables was done by different innovative technologies such as controlled atmosphere storage (CA), fresh produce is stored in room like storage place where oxygen and carbon dioxide concentration are regulated, high CO_2 level and low O_2 level is maintained with no use of any chemical (Moradinezhad & Dorostkar, 2020)**.s**

With further advancement modified atmospheric packaging (MAP) became popular, and it is defined as enclosure of food in a package in which the atmosphere is modified or altered to provide an optimum storage atmosphere (Robertson, 2019). MAP can be created in two different ways active MAP and passive MAP, in active MAP the displacement of gases in the package is done which is later replaced by a desired mixture of gases base on type of commodity, where in case of passive MAP the fresh produce is packaged using the selected film and the desired atmosphere develops naturally due to respiration and diffusion of gases through the film (Qu et al., 2022).

Active packaging (AP) is other innovative techniques followed in extending the shelf life of fresh fruits and vegetables by wrapping them with polythene films. This type of preservation is being practiced by many producers in preserving the cucumber, but this method has its own drawbacks, as high-density polythene films are used and these are nonbiodegradable (Saha et al., 2022). Edible coatings can be prepared from different sources, scientists have been able to identify various natural biopolymers derived from different sources such as plants, animals and also from useful micro-organisms (Alharaty & Ramaswamy, 2020). Namely polysaccharides, lipids, proteins are often used (Yousuf et al., 2018). All these different sources have their own properties, effective gas barriers with selective permeability to gases such as oxygen and carbon dioxide and help in extending the shelf life of fresh fruits and vegetables when applied on the surface as coating material (Yousuf et al., 2018).

Even though there are different components to prepare edible coating of interest, the effectiveness may not meet experimental requirement as each component have their own advantages and limitations. To develop more effective and stable edible coating the disadvantages of that accompanying should be diminished. That can be done by use of multiple components or mixing and combining the two components together developing composite edible coatings. These edible coatings are blend of polysaccharides, proteins, lipids and other hydrocolloids. This method will add up all the beneficial properties of each component and help in developing superior quality edible coating (Budianto et al., 2022). For examples, in some studies it is proven that adding lipids to polysaccharides which are hydrophilic in nature to increase the hydrophobicity of edible coating (Yousuf, Sun, et al., 2021). The researchers believe that studies have to go beyond just use of mono edible coating made from one source and gaining the understanding on how coating could produce new atmosphere around the product during the storage and affect the internal composition of the commodity (Sharma et al., 2019).

There are various approaches in application of edible coatings on fresh produce. Studies have shown that multilayer or layer by layer deposition technique leads to alteration of nanolayers of polyelectrolytes on the solid substrate, due to attractive forces of oppositely charged polymer on solid surface (Adhikari et al., 2022). The electrostatic interaction binds the neighbouring molecular layers by inhibiting the additional adsorption of similarly charged polyions (Homsaard et al., 2021). It has been reported that high hydrophobicity is increased with layer by layer/multilayer application of sodium alginate and chitosan along with lower water vapour permeability, water solubility and swelling degree (Li et al., 2019).

However, the study on extending the shelf life of mini cucumber is lacking. Therefore, in this study, the focus has been on the use of multilayer method applying the coating material, alternative deposition of oppositely charged polysaccharides around the mini cucumbers and store at 4°C for further studies.

4.2 Materials and method

4.2.1 Sample selection and preparation

Fresh mini cucumbers were purchased from the local store and stored inside the refrigerator for over night. The day after, samples were washed with tap water and then distilled water to remove any dirt from the surface. Then samples were sorted uniformly based on the size for father treatments.

4.2.2 Preparation of coating solutions

A 2% (w/w) solution was prepared by dissolving 2 g sodium alginate (Sigma, Oakville, ON, Canada) in 98 g distilled water while stirring at 300 rpm using a magnet stirrer. Later, a 2 % (w/w) calcium chloride (Sigma, Oakville, ON, Canada) solution was prepared by dissolving 2 g in 98 g distilled water (w/w) using a volumetric flask (Alharaty & Ramaswamy, 2020). A 1 % (w/w) chitosan solution was also prepared by dissolving 1g chitosan into distilled water containing 0.5% (v/v) acetic acid using magnetic stirrer (Le et al., 2021). All solutions were prepared at room temperature.

4.2.3 Sample treatment and packaging

For control, mini cucumbers of uniform size were selected, samples were washed with distilled water and set aside for 15 min at room temperature for surface drying. Afterward, the samples were transferred into the plastic containers and store at refrigerated condition (4°C) for 20 days. The coated samples were prepared by dipping the mini cumbers of uniform size into the anionic 2% (w/w) sodium alginate solution for 5 minutes followed by 15 min surface drying at room temperature. The coated sample were then dipped into the 2% calcium chloride solution for 5 minutes which led the development of jelly like firming as first layer around the samples. After surface drying for 15 min at room temperature, the samples were dipped into 1% (w/w) cationic chitosan solution for 5 min forming the second layer via electrostatic interaction.

Once samples were prepared and treated then transferred into the plastic containers containing 3-5 holes on top and stored at refrigerated condition (4°C) for further investigation (Figure 4.1).

Figure 4.1: Sample storage in plastic containers

4.3 Quality parameters analysis

4.3.1 Weight loss

200 g of samples from control and coated treatments were kept in plastic containers with few holes on top for gas exchange. Weight loss was measured (Denver instrument, APX-323, NY, USA) by percent moisture loss which was recorded within every four days for a total of 20 days. All tests were carried out in triplicates.

4.3.2 pH

pH was measured using calibrated pH meter (Brinkman Co., Mississuaga, ON, Canada) in four days interval. A100 g control and coated samples were blended in 100 mL distilled water, followed by filtration through the metal screen. The filtrate was then divided in three beakers and pH was recorded 3 time for each sample.

4.3.3 Total soluble solids (TSS)

Digital refractometer (ATAGO N1, Kirkland, DC, USA) was used to measure the total soluble solids of control and coated samples. Three reading was recorded 3 times for each sample every four days, and TSS was expressed as degree of Brix.

4.3.4 Color

Color parameters (L, a, and b values) of 10-15 coated and uncoated samples were measured each four days using a tristimulus Minolta chroma meter (Minolta crop, Ramsey, NJ, USA). The color parameters of L value (lightness, loss of whiteness or brightness) and b value (yellow-blue shades) were used to compare the changes in samples color for this work.

4.3.5 Chlorophyl content

Chlorophyll content of control and coated mini cucumbers were measured based on the procedure followed by Moalemiyan & Ramaswamy (2012) with the slight modification. The follwing Equations (4.1, 4.2, and 4.3) were used to calculate the chlorophyll a, chlorophyll b and total chlorophyll.

$$\text{Chlorophyll a} = [12.7\ (A663) - 2.69\ (A\ 645)] \times \frac{V}{1000 \times W \times a}\ (\text{mg g}^{-1}\text{fresh weight}) \quad (4.1)$$

$$\text{Chlorophyll b} = [22.9\ (A645) - 4.68\ (A663)] \times \frac{V}{1000 \times W \times a}\ (\text{mg g}^{-1}\text{fresh weight}) \quad (4.2)$$

$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b} \quad (4.3)$$

where *A* is the absorbance at specific wavelengths (645 and 663 nm), v is the final volume of the chlorophyll content (mL), *W* is fresh weight of the sample (g), and *a* path length of light (1 cm).

Approximately, 1 g of sample was cut from control and coated samples, separately and placed in a test tube. 10 mL of dimethyl sulfoxide (DMSO) and 80 percent acetone mixture (1:1 ratio) was poured into the test tubes containing the comminuted fruit pieces and incubated overnight. The colored solution was then decanted into measuring cylinder and the volume was made up to 25 mL with DMSO-Aceton mixture. Using a spectrophotometer (Visible Spectrophotometer, Novaspec II, Biochrom Ltd., Cambridge, England) the OD values were recorded at 645 nm and 663nm. The chlorophyll a, chlorophyll b, and the total chlorophyll content were obtained and expressed as mg/g fresh weight (Moalemiyan & Ramaswamy, 2012).

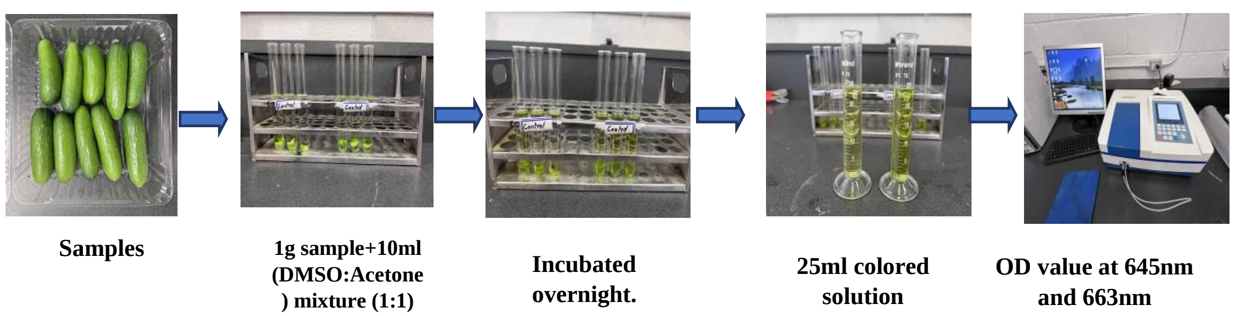

Figure 4.2: Chlorophyll extraction and analysis

4.3.6 Respiration rate

The respiration rate was assessed every four days. A 50 g of control and coated samples were placed in an airtight Plexi-glass chamber (18 cm × 12 cm × 27 cm) connected to the carbon dioxide (ACR Systems Inc, St-Laurent, QC, Canada) for 2 hours. The data were collected during 2 hours at room temperature and transferred to a data acquisition system. The data logger was programmed to collect real time data from the regression slope of CO2 concentration. The respiration rate was then obtained and evaluated as mL $CO_2kg^{-1}h^{-1}$ using Smart Reader plus 7 installed inside the chamber (Alharaty & Ramaswamy, 2020; Maftoonazad & Ramaswamy, 2005).

4.3.7 Texture

Textural properties were assessed using a Texture analyzer (Model TA XT Plus, Texture Technologies corporation, Scarsadale, NY, USA). About 12-15 samples from each treatment were analysed using puncture probe TA52 (2mm) at a speed of 10 mm/s. Test were conducted every 4 days interval for 20 days storage time.

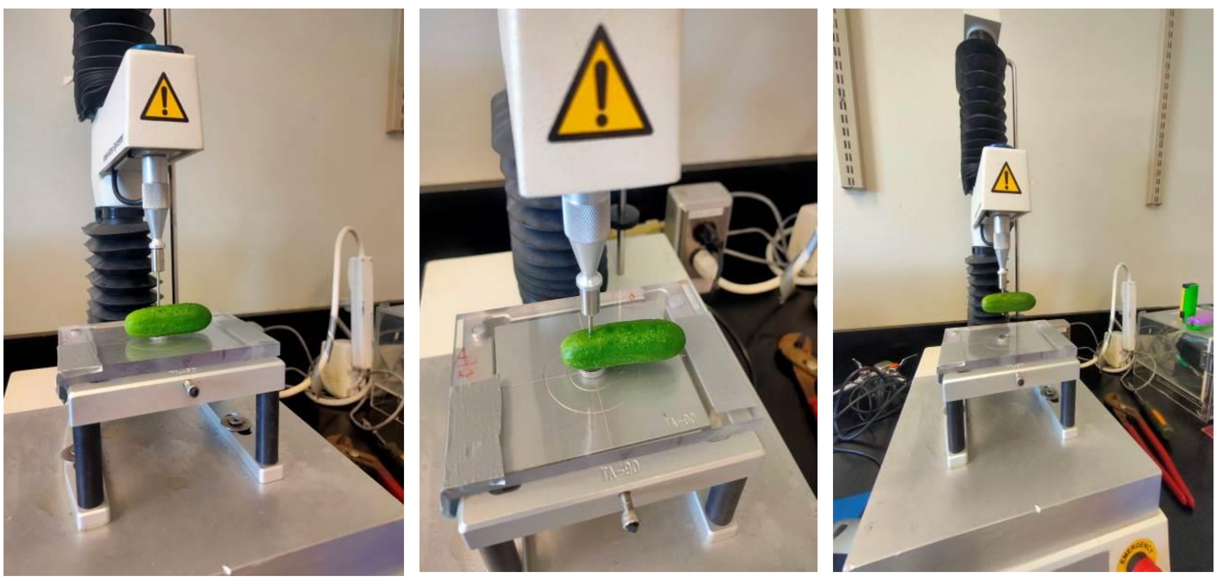

Figure 4.3: Puncture test on control and coated mini cucumbers

4.3.8 Microbiology

Serial dilution method was fallowed using PCA (plate count agar) media and pour plate method. Every four day’s interval mold and yeast colonies were counted, test was done in triplicates and total microbial count was represented in log CFU/mL.

4.3.9 Statistical analysis

Minitab Statistical software was used to conduct one-way Analysis of Variance (ANOVA) at 95% level of confidence and 5% level of significance. Tukey's method of comparison was used to indicate the significant difference between the control and coated samples during storage, as well as, between the different storage times for control and treated samples ($p < 0.05$).

4.4 Results and discussions

4.4.1 Weight loss

The weight loss was measured and expressed as the percent of moisture loss. The moisture loss of control samples was higher than coated samples at refrigerated condition. The one way ANOA showed significant ($p<0.05$) difference in moisture loss of control and coated samples for 20 days storage time. The difference in moisture loss was observed only after 4th day of storage. Rapid moisture loss was observed in control and relatively low moisture loss observed in coated samples until day 12, less than 10 percent loss was recorded. Multilayer coating showed positive impact on preserving the moisture in coated samples compare to control samples. This could be due to multilayer electrostatic coatings prevent migration of water molecules from the matrix. Similar trend was observed in the study done by Medeiros et al (2012) on Tommy Atkins mangoes.

Electrostatic interaction is due to the presence of interlinking agent calcium chloride, which enhanced the barrier properties of sodium alginate and chitosan as biopolymeric layers, developing the increment of the tortuosity pathway (Mantilla et al., 2013). Day 16 onwards, more moisture loss was observed and it was 14.5% in control and 10.9% in coated samples was recorded. The maximum weight loss was associated with the coated samples on the day 20 (14.3%), however, this loss was more for the control samples (16.2%). Overall, in this study, the percentage weight loss was found to significantly ($p<0.05$) increase with storage time for control as well as coated mini cucumber. With this phenomenon, it was revealed that the alternative deposition of sodium alginate and chitosan had most efficient effect on coated samples and exhibited superior moisture barrier property compared to uncoated ones. Similar trend was reported by Saha et al. (2016) and Adetunji et al. (2014) where guar gum based edible coating delayed the moisture loss in cucumbers (Saha et al., 2016) and chitosan in combination with aloe-vera significantly delayed the moisture loss by reducing the transpiration rate and lowering the respiration rate in coated cucumbers respectively (Adetunji et al., 2014).

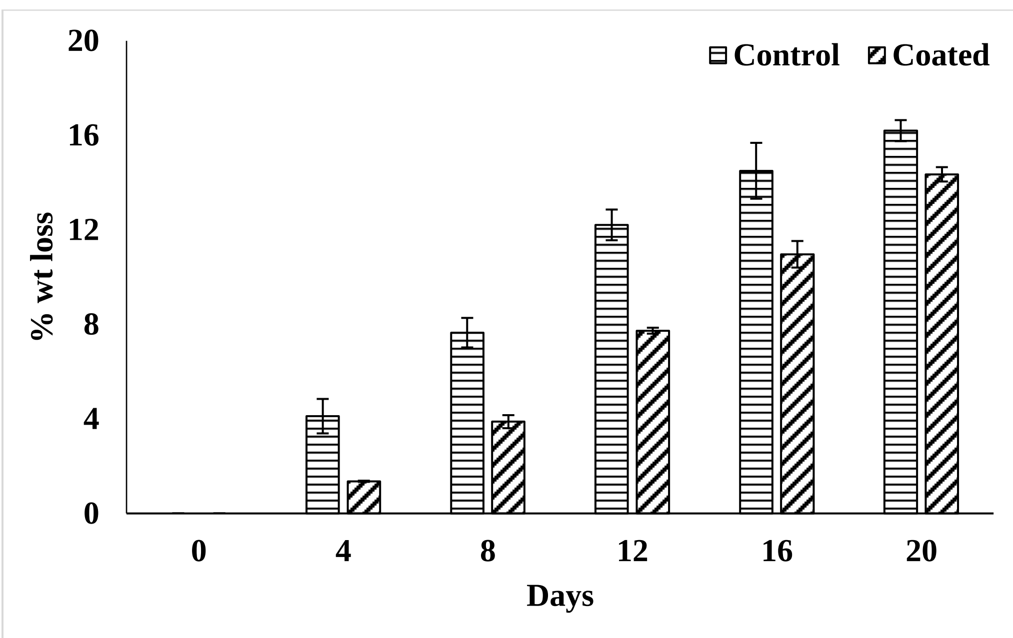

Figure 4.4: Changes in moisture loss of coated and uncoated mini cucumbers as influence of storage time at 4°C

4.4.2 pH

Figure 4.5 shows the increase in pH value of control and coated samples as influence of storage time. The one-way NAOVA showed that the pH value of control and coated treatments did not change significantly ($p>0.05$) during the storage period. Most of the vegetables are basic in nature (pH, ≥5.5 - ≥7.7), cucumber fall in that category. Changes in pH could be associated with many factors, it might be due to the slow or fast metabolic reaction happening inside or the effect of treatments on the commodity which slow down the biochemical reaction like respiration which involves conversion of organic acid like malic acid and citric acid into sugars (Omoba & Onyekwere, 2016). Same pH of 5.6 and 5.1 was recorded in control and coated samples on day 0 and day 4 respectively. Increasing trend was observed in pH of control sample, this due to the decrease in acidity which triggered by conversion of organic acids into sugars and increased respiratory metabolic rate and decrease in acidity can be attributed to the utilization of accumulated citric acid in the endocarp of cucumber. (Saha et al., 2016; Yin et al., 2019). On the days 8 and day 12, pH of 6.1 and 6.4 was observed in control samples, whereas coated samples showed relatively low increase in pH values from 5.6 to 5.8 respectively.

The highest pH value of 7.6 was recorded for control on the day 20, while the pH value of 6.2 was observed in samples coated with sodium alginate and chitosan on the same day. It was concluded that multilayer deposition of edible coating prevented rapid increase in pH by developing a modified atmosphere around the fruit. However, control mini cucumbers had greater pH increase during storage it could be due to greater utilization of organic acids during the ripening process which makes fruit less acidic (Omoba & Onyekwere, 2016). Hence coating retained the acidic components in the coated mini cucumbers and retention of acidity contributed toward the shelf life during the storage at 4 °C.

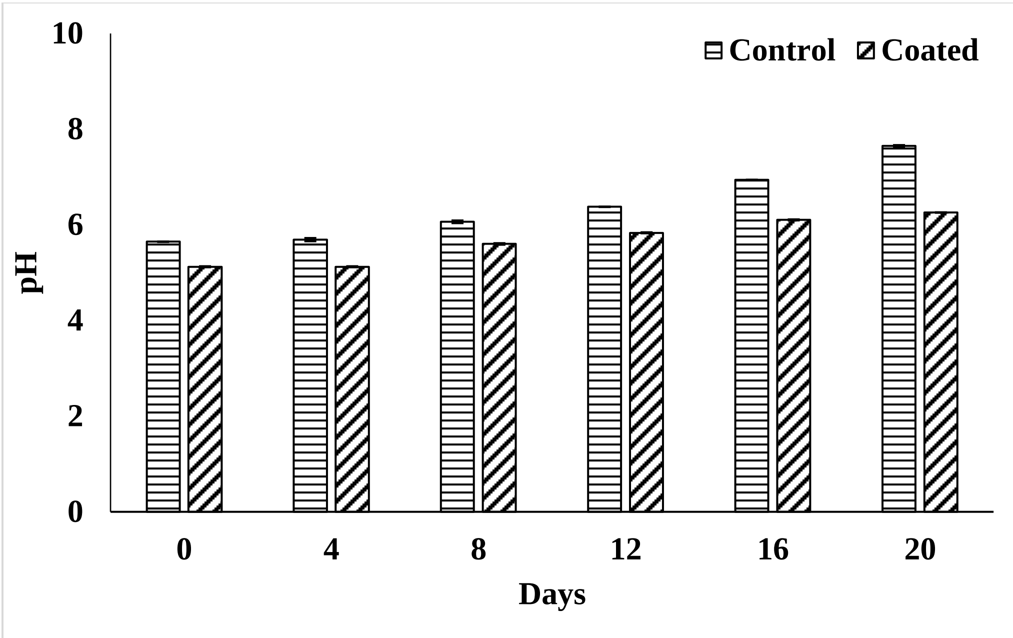

Figure 4.5: Changes in pH of coated and uncoated mini cucumbers as influence of storage time at 4ºC

4.4.3 Total soluble solid (TSS) °Brix

Figure 4.6 shows changes in TSS (Total Soluble Solids). Initially, the total soluble solid was 1.6 °Brix and 1.3 °Brix for control and coated samples, respectively. There was significant ($p<0.05$) increase in TSS level of control and coated samples was recorded until the day 12 and similar trend was monitored in study done by Moalemiyan and Ramaswamy (2012) on cucumber using pectin. Medeiros et al.(2012) reported that increase in total soluble solids might be due to sugar level increased which is result of degradation of polysaccharides and water loss which induced the ripening process in fruits and vegetables (Medeiros et al., 2012). These have impact on the increase of sugar concentration in the fruit tissue due to breakdown of starch into sugar by respiration process as sugars are main substates utilized during the process.

One way ANOVA showed significant ($p<0.05$) high TSS of control sample on day 8 onwards due to increase respiration and TSS of coated samples was significantly lower than that of control as coating decreased the respiration rate which slows down the synthesis and metabolites resulting low TSS (Omoba & Onyekwere, 2016). As shown, the TSS levels of control sample increased to 3.6 °Brix and 4.5°Brix on the day 16 and day 20, respectively, whereas the TSS level of coated samples had the short increases of 1.8 °Brix, 2.6 °Brix on the similar days In agreement with this study that the synthesis of sugar was at lower rate in coated samples. Table 4.1 shows the data collected during the experiment.

Table 4.1: TSS data of control and coated samples for 20 days storage period

Treatment	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
Control	1.6±0.1	1.6±0.17	1.7±0.17	1.9±0.15	3.6±0.05	4.5±0.17
Coated	1.3±0	1.3±0.05	1.6±0.05	1.6±0.05	1.8±0	2.6±0.05

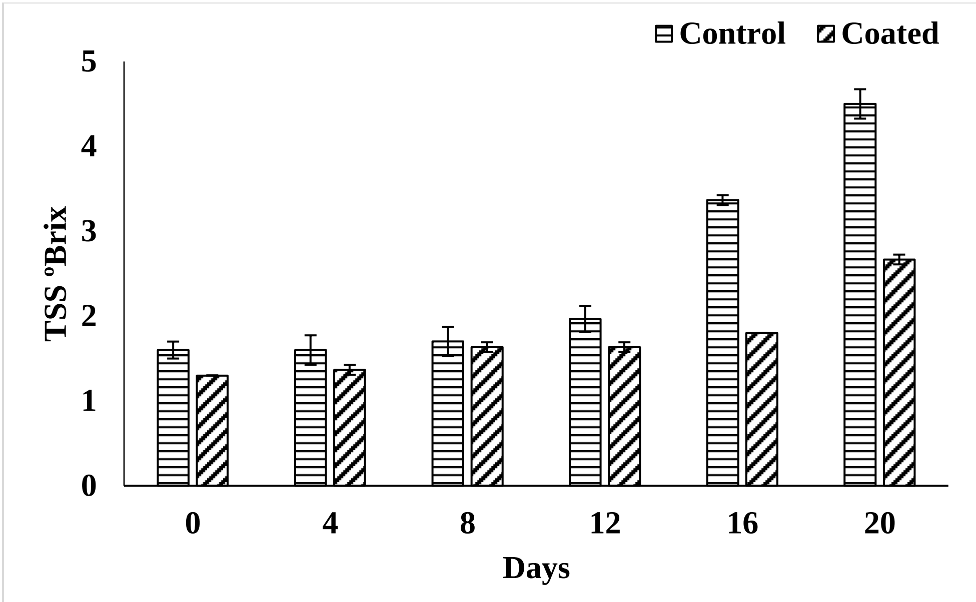

Figure 4.6 Changes in TSS (Total Soluble Solids) content of coated and uncoated mini cucumbers as influence of storage time at 4ºC

4.4.4 Color

Most of the green vegetables including the cucumbers stop producing the chlorophyll when stored for a long time. The main reason is the development of yellowness on the surface, even at lower storage temperatures and also due to the loss of moisture during the storage which causes shriveling (Zapotoczny & Markowski, 2014). Edible coatings found to be effective in preserving green color and delaying the yellowness of cucumber. This color preservation occurs by creating an environment with the low oxygen and high carbon dioxide as reported by Moalemiyan & Ramaswamy (2012). In this study, the effect of multilayer deposition of sodium alginate and chitosan as composite coating was effective in preserving green color and delaying yellowness in cucumbers especially at lower temperatures and different parameters of color chroma including L value and b value were investigated. In the current study,

4.4.4.1 L value

The higher the value of L the clearer the sample, decrease in L values indicates emergence of browning for cucumbers as shown in Figure 4.7. On the starting day, both control and coated samples showed similar L value. The decreasing trend was observed after 4 days storage at 4ºC, and measurement was done at room temperature and the coated samples showed relatively lower decrease in L value compared to that of control. One way ANOVA showed no significant difference in L value between the control and coated sample during the storage period ($p \geq 0.05$).

The L value decreased from 41.41 to 35.69 after 8 days for control samples. In case of coated samples on the same day, the L value decreased from 41.08 to 39.20 which was less than control. The fungal decay was observed in control sample after 12 days, which lowered the L value to 33.65 on day 16. That lead to degradation of surface color pigments and softening of tissue. On the other hand, coated samples showed no fungal decay with the L value of 36.97 indicating lower decrease compared to the control samples.

Due to the increased mold growth on the surface of control samples rapid drop in L value was noted on the day 20, while coated samples showed no or very low mold growth (Figure 4.12) with a decrease in the L value down to 35.44. The decrease in the L value of control was higher down to 30.25. sodium alginate and chitosan exhibited good antimicrobial properties by inhibiting the mold growth on outer layers of mini cucumber.

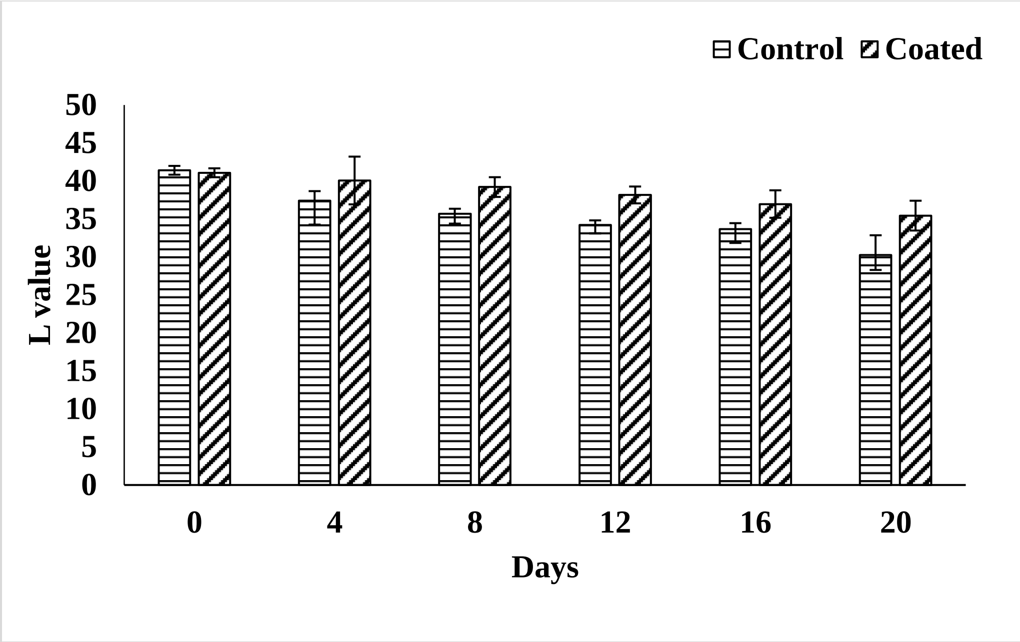

Figure 4.7: Changes in color components of coated and uncoated mini cucumbers stored at 4ºC (L value)

4.4.4.2 b value

The Chroma b values also declined as time of storage went by and the treatments presented smaller yellowing. A significant decrease in the b value of control was noted, whereas coated samples maintained stable b value during the storage at 4ºC. On the first day, the control and coated samples showed similar b values of 26.05 and 26.02 respectively. After 4 days of storage at 4ºC, a slight increase was associated with the coated samples from 25.98 to 29.63, while the control samples displayed a decrease in b value from 26.06 to 23.1. Similar b values of 22.07and 22.11 was recorded in control sample on day 8 and day 12 respectively.

Coated samples showed slight decrease in b value of 27.44 and 22.75 on day 8 and day 12 respectively. A rapid decrease in b value of 18.04 was recorded for control samples after 16 days but the coated samples showed an increase up to 27.6. Moalemiyan & Ramaswamy (2012) reported similar trend in their study by using pectin as coating material. The b values of 17.4 and 25.38 was recorded on the day 20 in control and coated samples, respectively.

The one way ANOVA showed significant ($p<0.05$) difference in b values between the control and coated samples during the storage period. Figure 4.8 shows the data collected during the experiment. The results found in this study agree with (Pizato et al.(2020) who worked with broccoli coated with carboxymethyl cellulose and chitosan and they kept the green color.

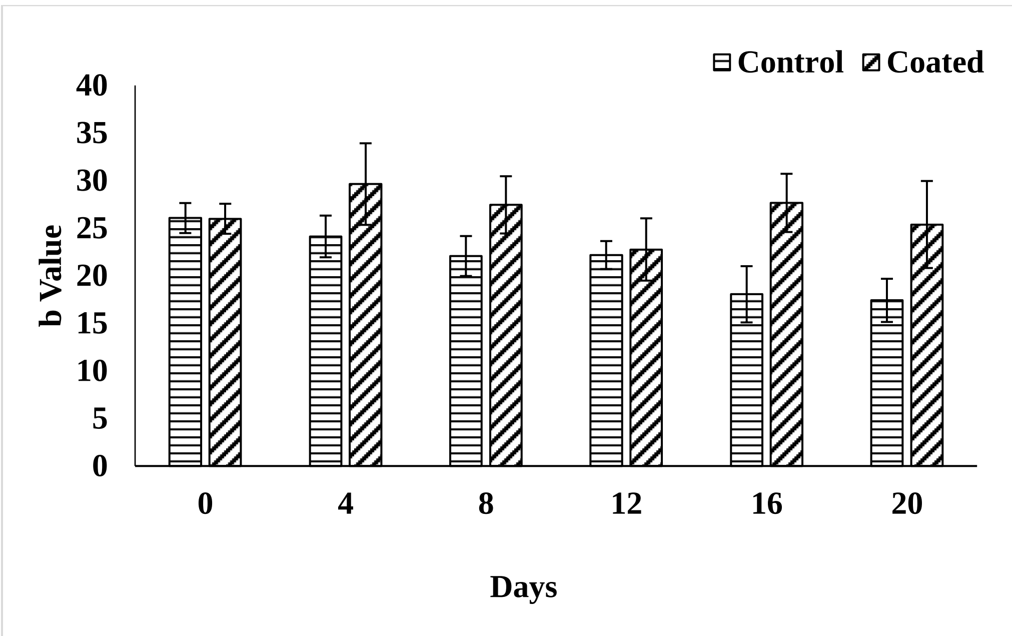

Figure 4.8: Changes in color components of coated and uncoated mini cucumbers stored at 4ºC (b value)

4.4.5 Chlorophyll

One way ANOVA showed significant ($p<0.05$) difference in chlorophyll content of control and coated samples during the 20 days storage time. chlorophyll degradation began immediately with storage. There was relatively higher loss in chlorophyll content of control samples compared to the coated samples. Layer by layer/ multilayer coating of sodium alginate and chitosan preserved the chlorophyll a chlorophyll b contents in coated mini cucumbers stored at 4ºC. Among the chlorophyll a and chlorophyll b loss was higher in chlorophyll b of control samples, whereas it was significantly ($p<0.05$) lower in coated samples. Figure 4.9 and Figure 4.10 shows the changes in chlorophyll a and chlorophyll b during 20 days of storage time in control and coated mini cucumbers. The changes study done by Moalemiyan & Ramaswamy (2012) showed similar results where loss of chlorophyll b was higher in control cucumber stored at different temperature.

Based on the collected data, the total chlorophyll content of coated mini cucumbers was higher than control/ uncoated mini cucumbers. Figure 4.11 shows the changes in total chlorophyll degradation of control and coated mini cucumber. Similar trend was reported by Moalemiyan & Ramaswamy (2012), where cucmbers coated with pectin based edible coating/film preserved the chlorophyll content during 13 days at 4ºC storage. Retardation of changes in chlorophyll of coated fruits may be due to high CO_2 and or low O_2 levels in the internal atmosphere of the fruits (Moalemiyan et al., 2012). In this study coating manipulated internal atmosphere with low oxygen level and high carbon dioxide level inhibiting the yellowness of mini cucumber. Pizato et al.(2020) who worked with green broccoli coated with carboxymethyl cellulose and chitosan and reported the preservation of green color and the degradation of chlorophyll occurred in lower proportion when compared with the control sample (Pizato et al., 2020).

There are various factors which influence the degradation of chlorophyll on mini cucumber. Mainly the loss of moisture which induces the shrinkage of samples and softening of tissue. If stored for a long time green vegetable goes through rapid senescence inducing chlorophyll degradation, exposing the lighter yellow pigments and most green vegetables will undergo unmasking of chlorophyll (Moalemiyan et al., 2012). Chlorophyll data collected during the experiment are listed in fallowing tables (Table 4.2, Table 4.3, Table 4.4).

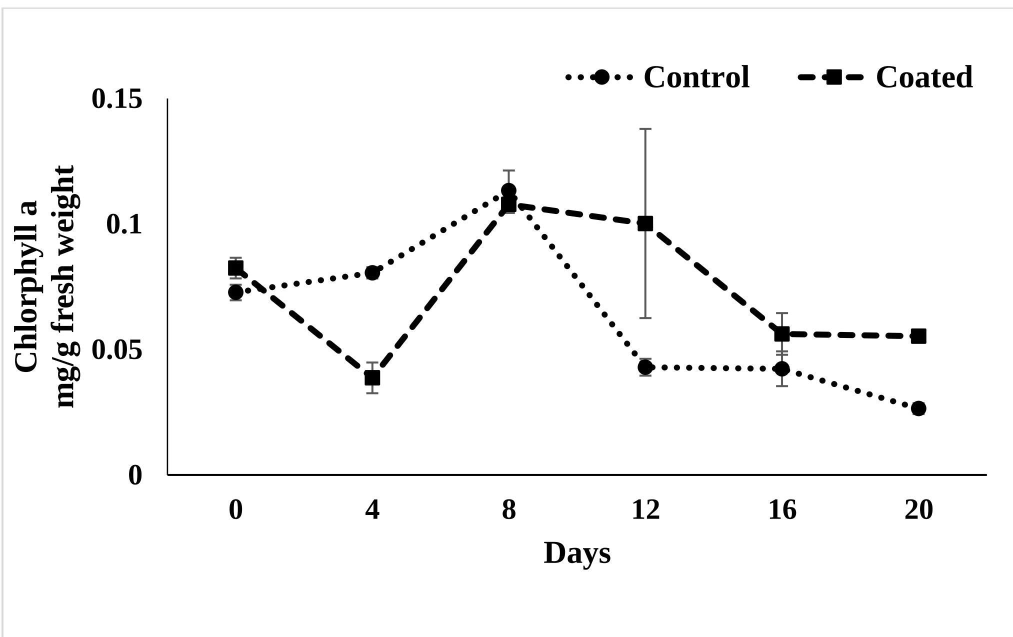

Figure 4.9: Changes in chlorophyll a content of coated and uncoated mini cucumbers stored at 4ºC

Table 4.2: Chlorophyll a data of control and coated mini cucumber

Treatment	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
Control	0.072±0.0030	0.080±0.0022	0.113±0.0080	0.042±0.0033	0.042±0.0069	0.026±0.0021
Coated	0.082±0.0041	0.038±0.0061	0.107±0.0034	0.100±0.037	0.056±0.0083	0.055±0.0011

Table 4.3: Chlorophyll b data of control and coated mini cucumber

Treatment	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
Control	0.035±0.0038	0.088±0.0051	0.120±0.033	0.025±0.0034	0.025±0.0053	0.021±0.0054
Coated	0.040±0.0021	0.025±0.00011	0.180±0.0028	0.185±0.0043	0.091±0.0014	0.083±0

Table 4.4: Total Chlorophyll data of control and coated mini cucumber

Treatment	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
Control	0.107±0.0026	0.168±0.0019	0.234±0.0077	0.068±0.00079	0.067±0.00069	0.0486±0.00
Coated	0.122±0.0053	0.063±0.0019	0.288±0.0086	0.285±0.0042	0.147±0.0019	0.138±0.00

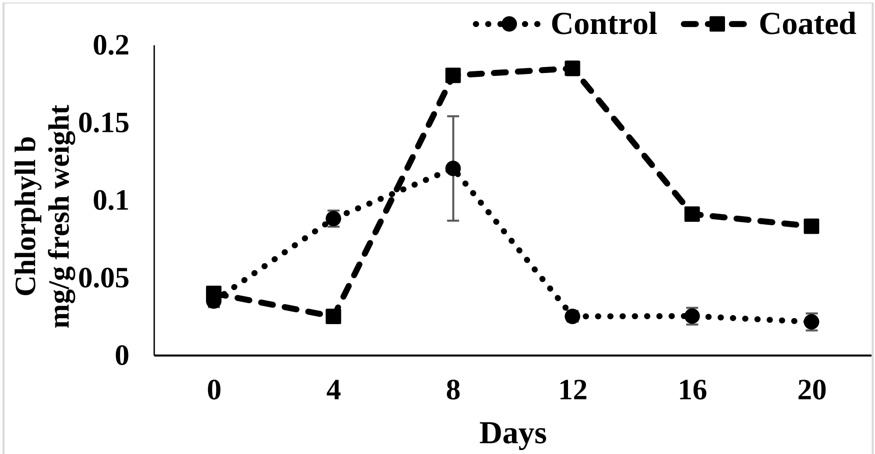

Figure 4.10: Changes in chlorophyll b content of coated and uncoated mini cucumbers stored at 4ºC

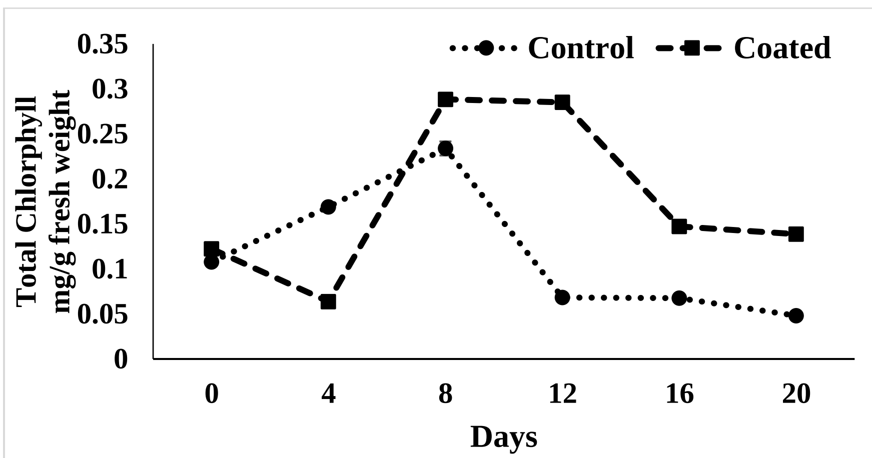

Figure 4.11: Changes in total chlorophyll content of coated and uncoated mini cucumbers stored at 4ºC

4.4.6 Respiration

Respiration rate was measured in $mLCO_2$ kg^{-1} h^{-1} for control and coated samples. Then rapid decrease in respiration rate was observed for control samples from 125 $mLCO_2$ kg^{-1} h^{-1} down to 98.2 $mLCO_2$ kg^{-1} h^{-1}, this decrease was 94.1 $mLCO_2$ kg^{-1} h^{-1} for coated sample on the day 4. The one way ANOVA showed significant difference in respiration rate of control and coated treatments ($p \leq 0,05$). Respiration rate in control samples raised up to 133 $mLCO_2$ kg^{-1} h^{-1} in control, whereas coated samples had low respiration rate of 79.8 $mLCO_2\ kg^{-1}\ h^{-1}$. An increasing trend was recorded in control samples, in case of coated samples respiration rate was stable until day 12 and lower compared to control as shown in Figure 4.12. Sodium alginate coating coupled with calcium chloride followed by chitosan coating played an efficient role in reducing the respiration rates in coated mini cucumbers by dropping the amounts of CO level. This can be supported by study done by Alharaty & Ramaswamy (2020) on cut strawberry coated with sodium alginate and calcium chloride. The edible coating lowered the respiration rate by preventing gases exchange and decreasing the metabolic activity of the coated samples. Previous studies have also shown that sodium alginate and chitosan have very good gas barrier properties (Kopacic et al., 2018). Respiration rate of 141 $mLCO_2\ kg^{-1}\ h^{-1}$ and 132 $mLCO_2\ kg^{-1}\ h^{-1}$ was recorded in control on day 12 and day 16. The coated samples had respiration rate of 98.7 $mLCO_2\ kg^{-1}\ h^{-1}$ and 99.9 $mLCO_2\ kg^{-1}\ h^{-1}$ on similar days. All samples reached their respiration peak after 20 days storage at 4 °C and the peak values of 143 $mLCO_2\ kg^{-1}\ h^{-1}$ and 124.97 $mLCO_2\ kg^{-1}\ h^{-1}$ were recorded for control and coated samples, respectively.

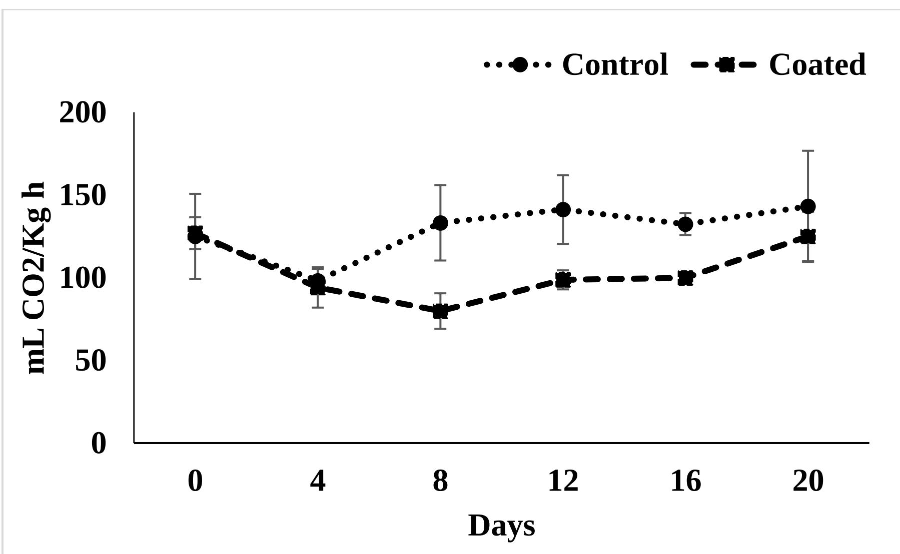

Figure 4.12: Comparison of changes in respiration rate for control and coated mini cucumbers

4.4.7 Appearance

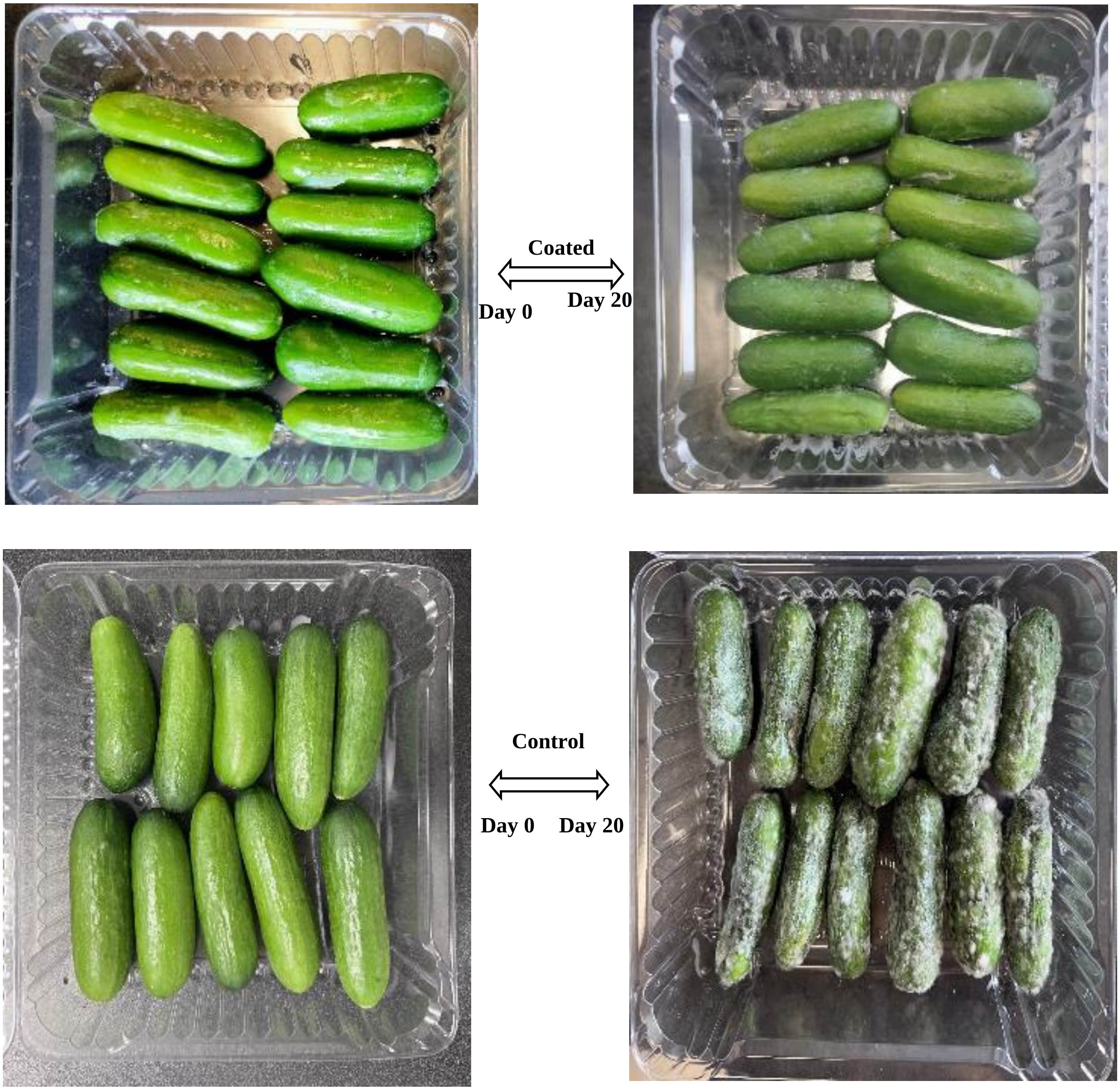

Figure 4.13: External appearance of coated and uncoated mini cucumbers after 20 days of storage period

4.4.8 Texture

In this study, multilayer electrostatic coating composed of sodium alginate and chitosan maintained firmness of coated samples and very minor loss of firmness was observed for coated samples. Edible coating showed significantly ($p<0.05$) higher firmness retention in coated samples compared to control samples. The data presented here are in newton (N). The firmness values were recorded in both the treatments on day 0 are, 8.2 N and 8.7 N respectively. The gradual decrease in firmness was recorded in control with 7.2 N and 7.3 N on day 4 and day 8, whereas coated samples had 8.4 N and 8.1 N on same days which was higher compared to control. Studies have shown that reducing the oxygen concentration and lowering the carbon dioxide concentration by modification of internal gas composition created by coating material will have impact on texture changes in coated fruits and vegetables (Kocira et al., 2021).

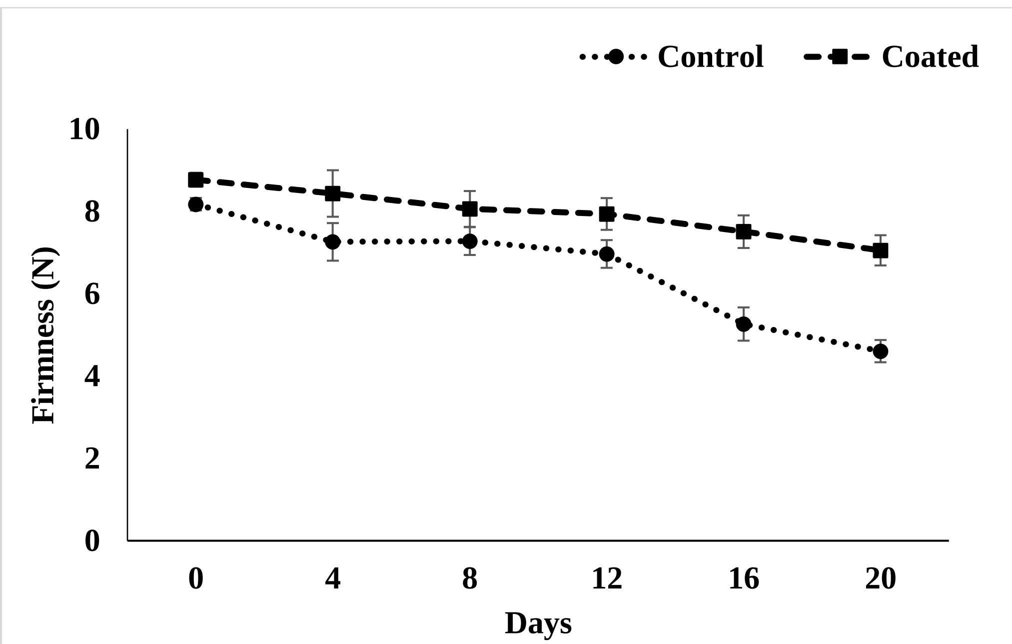

Figure 4.14: Changes in firmness of coated and uncoated mini cucumbers stored at 4ºC

On day 12 and day 16 more decrease in firmness was recorded in control samples with 6.9 N and 5.3 N firmness values respectively, whereas coated samples had 7.93 N and 7.5 N on these days. On last day firmness value of control samples went down to 4.6 N and coated samples had higher value of 7.1 N. The control samples showed significant ($p<0.05$), decrease in firmness during the storage time may also associated to various other parameters such as moisture loss, fungal contamination on surface and increased respiration rate.

In agreement with the previous studies, coating enhanced intermolecular bond between the layer-by-layer deposition and multilayer coating led electrostatic filming and prevented loss of firmness by preserving the moisture loss and lowering the fungal growth (Adhikari et al., 2022).

4.4.9 Microbiology

Due to the cationic nature, chitosan has antimicrobial properties which is not found in other biopolymers such as proteins and lipids (Priyadarshi & Rhim, 2020). Figure 4.15 shows the log CFU/mL data for control and coated samples, significantly ($p< 0.05$) lower log CFU/mL value was recorded for coated samples compared to control where more than 5 log CFU/mL. Control samples had slightly higher count compared to coated samples, 4.35 log CFU/mL and 4.71 logCFU/mL and lower count in case of coated samples with 3.54 log CFU/mL and 3.84 log CFU/mL on day 0 and day 4 respectively. Increasing trend was observed in case of control samples, mold count went up to 4.83 log CFU/mL and 5.5 log CFU/mL on day 8 and day 12. And very minute increase was recorded in coated samples which was less than the control samples in these days.

On day 16 day 20 control samples had significantly higher count with 5.72 log CFU/mL and 5.96 log CFU/mL, whereas coated samples showed 4.40 log CFU/mL and 4.53 log CFU/mL on these days respectively. Overall coated samples had less than 5 log CFU/mL count, and control samples more than 5 log CFU/mL count was recorded during the experiment. During the period of storage coating hindered the increase in aerobic yeasts and moulds count compared with the control. In associated with studies done by Saha et al. (2016) (Saha et al., 2016) showed clear zone of inhibition against food pathogenic bacteria like *S. aureus, P. aeruginosa, B. substilis, S. flexineri, B. cereus and E-coli* on cucumber coated with different combination of edible coatings like carboxymethyl guar gum (1%)+potassium sorbate (0.4%)+glycerol (35%w/w)+tween 80 (0.1%, carboxymethyl guar gum(1%)+ cinnamon oil(0.1%)+glycerol+tween80 (0.1%) and guar gum(1%)+potassium sorbate (0.4%)+glycerol (35% w/w)+tween-80 (0.1%).

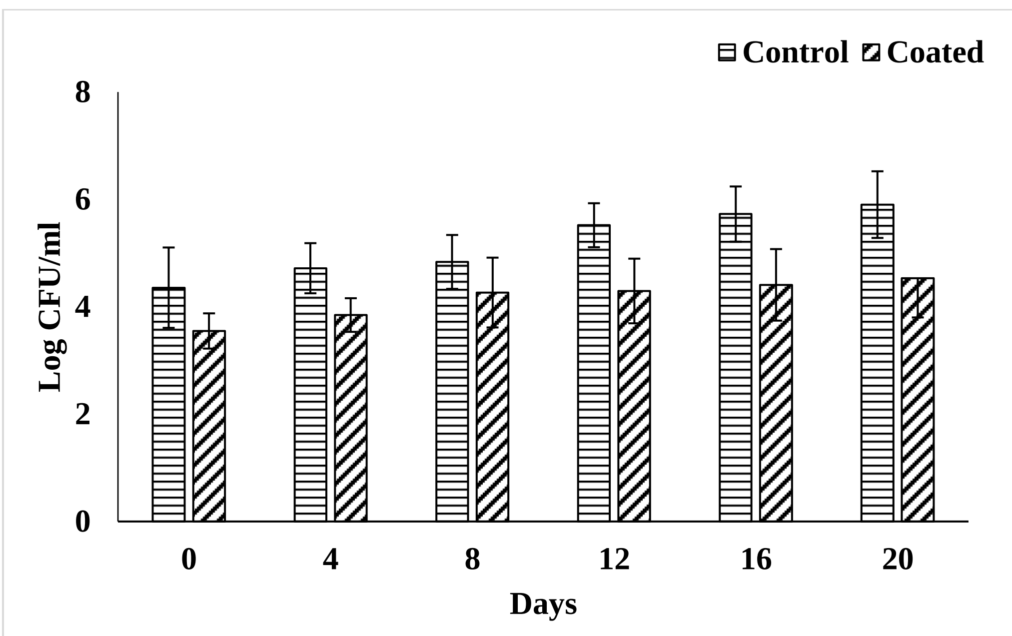

Figure 4.15: Changes in microbial count log CFU/mL of coated and uncoated mini cucumbers stored at 4ºC

Rapid increase in microbial mold was observed on the surface of control samples during the experiment. The one way ANOVA showed no significant ($p>0.05$) difference between control and coated samples during storage time. In this study multilayer deposition of edible coating found to be effective in preventing the microbial growth mold and extending the shelf life of coated product during 20 days of storage time.

4.5 Conclusions

Multilayer electrostatic layer by layer deposition of sodium alginate and chitosan found to be very effective in preserving all the post harvest quality parameters by reducing moisture loss during the storage. External quality parameters such as color and firmness which are noticeable to consumers, were also retained. Low increase in physiochemical parameters like TSS and pH was observed in coated mini cucumbers compared to uncoated at refrigerated condition (4ºC). In case of whole fruits and vegetables texture plays very important role and this coating preserved the texture of coated samples during the storage. Multilayer coating prohibited loss of chlorophyll content in coated mini cucumbers, and sodium alginate and chitosan exhibited excellent antimicrobial property by preventing mold and yeast growth and found to be effective in extending the shelf life of mini cucumbers at (4ºC).

CHAPTER 5

GENERAL SUMMARY AND FUTURE RESEARCH

The main objectives of this thesis are focused on evaluating the satbility of composite coatings and formaulation to extend the shelf life of fresh and cut fruits vegetables. The first objective is concentrating on use of sodium alginate caotinng coupled with calcium chloride dipping to prolong the shelf life of cut pear fruits. To imrove effectiveness of edible coating media additive like ginger essential oil and citric acid were incorporated into in to alginate solution.

Furthermore the experiment was conducted with one control and two treatments, one is control, secondly the treatment with sodium alginate coating coupled with calcium chloride (SACC), third one is composite coating with ginger essential oil and citric acid encapsulated emulsion applied on cut pear fruits and stored at 4℃ for 15 days.

Sodium alginate coating (SACC and SAGEO) on cut pear fruits effectively lowered the transpiration rate and avoided mositure loss, which delayed the softening of the tissue in coated cut fruits. Other physiological activities like respiration rate was reduced and respiration peal (climactric peak) was hindered, whereas control samples had high moisture loss, rapid saftening of the tissue and siginificantly high respiration rate.

Phisico- chemical parameters like pH , TSS and titratable acidity were reduced in coated treatmeants. Composite coating showed excellent impact on preserving the color or external appearance of the cut pears samples. Antibrowing agent delayed browning of out surface in coated pear fruits. Whereas incorporation of ginger essential oil was to impart antimicrobial property and it played essential role and delayed mold growth up to 15 days and no sign of mold growth was seen on the surface of coated cut pear fruits. Whereas in control samples mold growth appeared very early days of storage period.

Based on the data obtained during the experiment, coating the cut fruist with ginger essential oil and citric acid emulsion based edible caoting using alginate can be utilized in extending the shelf life of cut pear fruits.

The second objective is focused on use of multilayer coating on mini cucumbers. The electrostatic caoting was produced by using anionic sodium alginate as first layer and cationic chitosan as second layer. Cationic property of chitosan showed good antimicrobial property which was seen on coated mini cucumbers with no mold growth.

The multilayer coating effectively reduced physilogical and physico chemical activities of coated mini cucumbers. The coating significantly reduced the moisture loss and respiration rate. It also slowed down the chemical changes like increase in pH, total soluble solids. Whereas control mini cucumbers had high mositure loss and respiration rate. The multilayer coating presereved the firmness and delayed skin softening. Degardation of chlorophyll was avoided in coated mini cucumbers. Coated mini cucmbers had better quality retaintion than uncoated mini cucumbers. Futhermore coating effectively reduced metabolic activities inside the fruit and prolonged the shelf life of mini cucumbers.

FUTURE RESEARCH

There is increase in the demand for ready to eat fresh produce like cut fruits and vegetables. Edible coatings are very good feasable options in improving the post harvest and processed parameters to meet market demand.

The studies on edible coatings have to go beyond just analysing the changes in quality parameters, along with that the research should focus on improving the properties of edible coating componenets and produce modified fromulation and create superior quality edible coating. Which will have influence on factors which affect the performance of the coating.

Research can be narrowed into use of natuaral preservatives and say no to chemical, because nowadys consumners are concerned about their health. To minimize the use of chemical compounds in coating research can be extended to include incorporation of herbal extracts like essential oils from various sources like medicinal and aromatic plants like ginger, clove, rosemarry, thyme, lemongrass, and mint, etc.

Satbility of composite coating should be focused on to ensure good performance upon coating. In addtion to that research has to focus on cost of prodcution and consumers acceptability and marketablity.

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