Edible Coating to Extend the Shelf Life and Enhance the Quality of Fresh and Frozen Cut Fruits

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ABSTRACT

Edible coatings can protect food products from exposure to the environmental surroundings and favorably alter the physiological activities by providing necessary barriers against moisture loss and gas exchanges. Strawberry fruits in general are very perishable and in the cut form they become even more perishable due to the exposed internal tissues and induced mechanical damages. In response to the high demand of ready to eat cut fruits, a sodium alginate-calcium chloride edible coating formulation was used as a protective barrier to extend the shelf life of cut strawberries and enhance their sensory properties. The edible coating formulation was an aqueous mixture of 2% (w/w) sodium alginate and 2% (w/w) calcium chloride. The addition of edible coating helped in extending the shelf life of cut strawberries for up to 15 days at 4 ºC acting as a natural barrier by reducing both respiration and transpiration rates and retarding the further ripening and mold growth on the fruit tissue. Physiologically, it delayed the increase in pH and reduced the drop-in acidity. The sensory properties of the strawberry cut fruits were also enhanced by reduced surface darkening, oxidative browning and tissue softening. The calcium salts presumably contributed to structure formation and enhanced the fruit texture.

The usefulness of sodium alginate-calcium chloride edible coating was also tested for improving the quality and reducing the drip loss of dehydro-frozen pineapple cut fruits. A continuous medium flow microwave assisted osmotic dehydration under spray condition (MWODS) technique was first used to partially dehydrate the pineapple cut fruits. MWODS was carried for 10 min at 40 ºC using a 40 ºBrix sucrose solution. The MWODS semi-dried cut fruits were then alginate coated and frozen stored at -20 ºC for 10 and 50 days. The frozen stored coated cut fruits had a significant reduction in drip loss and improved quality

parameters such as appearance, color and texture which are normally associated with tissue softening and discoloration as a result of freezing.

RESUME

Les enrobages comestibles peuvent protéger les produits alimentaires de l'exposition à l'environnement et altérer favorablement les activités physiologiques en fournissant les barrières nécessaires contre la perte d'humidité et les échanges de gaz. Les fraises en général sont très périssables et en forme coupée, elles deviennent encore plus périssables en raison des tissus internes exposés et les dommages mécaniques induits. En réponse à la forte demande de fruits coupés prêts à manger, une formulation d'enrobage comestible à base d'alginate de sodium a été utilisée comme barrière protectrice pour prolonger la durée de conservation des fraises coupées, et améliorer leurs propriétés sensorielles. La formulation de revêtement comestible était un mélange d'alginate de sodium à 2% (p / p) et de chlorure de calcium à 2% (p / p). L'enrobage a contribué à prolonger la durée de conservation des fraises coupées jusqu'à 15 jours à 4 ºC, agissant comme une barrière naturelle pour réduire les taux de respiration et de transpiration et retarder la maturation et la croissance des moisissures sur le tissu du fruit. Physiologiquement, il a retardé l'augmentation du pH et réduit l'acidité résiduelle. Les propriétés sensorielles des fraises coupés ont été améliorées par une reduction de l'assombrissement de la surface, du brunissement oxydatif et du ramollissement des tissus. Les sels de calcium ont probablement contribué à la formation de la structure et amélioré la texture du fruit.

L'utilité de l'enrobage d'alginate de sodium et de chlorure de calcium a également été testée pour améliorer la qualité et réduire la perte de liquide des coupes de fruits d'ananas déhydro-congelées. Une technique de déshydratation osmotique micro-ondes à flux continu sous conditions de pulvérisation (MWODS) a d'abord été utilisée pour sécher partiellement les fruits d'ananas coupés. Le MWODS a fonctionné pour 10 min à 40 °C en utilisant une solution de saccharose à 40 ºBrix. Les morceaux de fruits semi-secs en utilisant MWODS ont ensuite été

enrobés en alginate et congelés à -20 ºC pour 10 et 50 jours. Les coupes de fruits enrobées et congelées présentaient une réduction significative de la perte de liquide et des paramètres de qualité tels que l'apparence, la couleur et la texture qui sont normalement associés au ramollissement et à la décoloration des tissus à à la suite du congelation.

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CHAPTER 1

INTRODUCTION

The number of people facing hunger and food insecurity around the world is increasing mainly in the developing countries facing wars, natural disasters and poverty. Finding a good solution to fight food insecurity is a priority for most humanitarian organisations. Increasing food production was provided as a solution (FAO, 2018). However, increased food production is associated with increased costs, higher energy use and lands cultivation. Another solution was provided, and it consists of reducing post-harvest losses instead of increasing food production.

Different methods were used to extend the post-harvest shelf life of fresh fruits and vegetables including refrigerated storage and modified atmosphere packaging (MAP). MAP consists of creating an atmosphere within the package, using synthetic polymers with selective permeability to O_2 and CO_2 gases as packaging materials. Another method is the controlled atmosphere storage (CA). CA is based on the creation of an atmosphere in the storage room, by lowering oxygen concentrations and increasing the concentrations of CO_2 gases, under high relative humidity and controlled low temperatures (Ramaswamy, 2015). Active or smart packaging is also a method that can be used to reduce post-harvest losses. Synthetic packages are made with variable active functions such as oxygen scavengers, carbon dioxide emitters, ethylene absorbents and humidity absorbers (Mehyar & Han, 2011).

Edible films and coatings are also preservation methods that were invented in the 12^{th} century by the Chinese by applying wax on citrus products. Today, wax layers are used on a variety of fruits and vegetables to delay ripening, enhance the appearances and reduce moisture loss. An edible coating is a thin layer of adhesive material that must be applied on the surface of

the produce in the liquid form by brushing, spraying or complete immersion. The coating layer should not affect the sensory properties of the fresh produce and it should also be colorless and odorless with less than 0.3 mm thickness. While an edible film is a self-standing material used to wrap or cover food products, and it acts as an effective barrier to gases and water vapor (Pavlath & Orts, 2009).

Edible films and coatings extend the shelf life of fresh produces and protect them from the external environmental damages in addition to the physical, chemical and biological changes. Research studies showed that edible films and coatings can act as natural barriers against moisture loss, gas exchanges, lipids and flavor compounds losses (Mahfoudhi & Hamdi, 2015).

Edible coatings create an internal atmosphere when applied on the surface of the fresh products which reduces respiration and transpiration rates and delays quality deterioration and ripening (Pavlath & Orts, 2009). However, coating layers with high gas impermeability might cause anaerobic fermentation and produce undesirable off-flavors and odors that can affect the sensory properties of the fresh products (Mattheis & Fellman, 2000).

The development of new packaging technologies and the increased demand on high quality fresh produces, allowed the development of new packaging materials that doesn't harmfully affect the environment. Synthetic polymers are used in the manufacturing of plastic packaging materials and they are the following: Polyethylene (PE), Polyethylene terephthalate (PET), Polystyrene (PS), Polyvinyl chloride (PVC), Polyamide (PA) and Polypropylene (PP). A huge amount of used plastic materials which are not handled properly by recycling ends up in the oceans every year. In fact, 75 to 80 million tonnes of plastics have been disposed in the oceans in 2017, which triggers the need to find an eco-friendly alternative for the synthetic packaging materials (Andrady, 2011).

The production of edible coating using natural biopolymers has many advantages since they are bio-renewable, biodegradable and environmentally friendly. They are also considered as better alternatives to the synthetic petroleum-based polymers in term of edibility, biocompatibility and nontoxicity (Yousuf et al., 2018).

In the last decades scientists were able to identify different types of natural polymers that can be used in the making of edible films and coatings (Pavlath & Orts, 2009). These polymers can be derived from both plant and animal sources and they are divided into three different categories. The hydrocolloids category, which consists of both proteins and polysaccharides, the lipids category and the composites. Polysaccharides include cellulose derivatives, alginates, pectin, starches and chitosan. Proteins could be plant-based such as corn zein and wheat gluten or animal-based such as gelatin and caseins, while the lipids category includes waxes and paraffins, resins, fats and oils (Pavlath & Orts, 2009).

Composites are made of a combination of hydrocolloids and lipids to produce edible films and coatings with shared advantages from two different categories. A composite film can be formed as a bi-layer (one layer is a hydrocolloid and the other layer is a lipid) or as a conglomerate (lipids and hydrocolloid components are intermixed within the same film). Another remarkable advantage can be provided by edible films and coatings, is their ability to act as carriers to different additives such as colorant, flavor additives, vitamins, minerals and antimicrobial agent (Pavlath & Orts, 2009).

The fast and modern lifestyle and the increased demand on ready to eat and healthy snacks were the primary causes behind the success of the minimally processed fruits and vegetables in the market. Cut fruits and vegetables are successfully sold on the commercial scale especially in the developed countries, while in the developing countries the market is in its

growing stages. Minimally processed fruit and vegetables are damaged tissues with high respiration and transpiration rates, which makes them highly perishable and deteriorate faster than the whole fresh products. Food scientists are working on developing new technologies to enhance the quality of cut fruits and maintain their sensory properties in order to meet consumers' needs and expectations (Yousuf et al., 2018).

Due to the increased interest in edible films and coatings and the high perishability of the fresh cut fruits, this research studies the effect of sodium alginate-calcium chloride based edible coating on fresh and processed cut fruits. Based on previous studies, sodium alginate-based edible coatings showed positive results in extending the shelf life of fresh fruits.

The objectives of this thesis are the following:

1. Evaluate the effect of sodium alginate-calcium chloride edible coating on the quality parameters and shelf life extension of strawberry cut fruits stored for two weeks at 4 ºC.
2. Evaluate the effect of microwave-osmotic dehydration under continuous flow medium spray condition (MWODS) technique and sodium alginate-calcium chloride edible coating on the quality parameters of frozen pineapple cut fruits after thawing.

CHAPTER II

REVIEW OF LITERATURE

1. Introduction

Different physiological changes happen in fruits and vegetables after harvesting such as respiration, transpiration and ethylene gas production. It has been reported that about 20 to 50% of fruits and vegetables are lost along the food supply chain before even reaching the consumer (Chakraverty, 2003). Temperature, gaseous composition and the humidity of the storage room are the main factors in extending the post-harvest shelf life of fruits and vegetables, due to their effects on both respiration and transpiration rates. For this purpose, proper storage and transportation conditions are required to reduce post-harvest losses and maintain the sensory properties of the fresh produces including their color, flavor and texture (Rees et al., 2012).

The international trade of perishable food items has largely grown in the past few decades and consumers expect now to be able to eat fresh fruits and vegetables at any time of the year and from all over the world (Rees et al., 2012). Beside the conventional refrigerated storage, controlled atmosphere storage, modified atmosphere packaging and edible films and coatings are the technologies that can be used to extend the post-harvest shelf life of fresh fruits and vegetables (Chakraverty, 2003).

1.1. Post-harvest Changes in Fruits and Vegetables

Fruits and vegetables are good sources of various nutrients such as fibers, vitamins and minerals. To maintain a healthy lifestyle and reduce the risk of obesity and chronic diseases, consuming fruits and vegetables on a daily basis is recommended. In the last decade, the interest in a healthy lifestyle has become more popular due to the increased nutritional awareness and

education (Gopala Rao, 2015). Scientists provided multiple solutions to compensate with the increasing needs of the growing population, such as increasing food production by 3% every year. Since the early idea of adaptation of new lands for cultivation had pretty much extinct due to the urbanization and maximizing agricultural production, new methods such as intensified planting and the use of effective fertilizers etc., have been generally implemented. However, this solution is accompanied with additional expenses and energy use associated with the intensified cultivation and increased food production costs. The best alternative is to reduce post-harvest losses, which earns consumer satisfaction, increases the economic benefits for both producers and consumers and maintains post-harvest quality (Ramaswamy, 2015) .

According to the Food and Agriculture Organisation (FAO) (FAO, 2018), one third of the food produced around the world is lost along the food supply chain. Different methods are used to reduce food losses and minimise the number of people facing hunger and food insecurity. In fact, the number of food insecure people have reached 821 million in 2017, mainly in the developing countries experiencing wars and natural disasters (FAO, 2018). The supply chain of fruits and vegetables from field to the table comprises of different steps to allow the delivery of high-quality fresh produces, starting from the field, through transportation, storage, processing, packaging and distribution retailing until it finally reaches the consumer. Food losses occur at each stage of the food supply chain and the loss is cumulative -what is lost will never come back. New technologies and concepts need to be used to reduce post-harvest losses (Ramaswamy, 2015).

1.2. Causes of Post-Harvest Losses

The sensory properties of fresh fruits and vegetables such as color, aroma and texture are very important parameters that should be maintained after harvesting to ensure consumers'

acceptance. However, fruits and vegetables are made of living tissues and have different metabolic activities which continue even after harvest and affect their quality characteristics as well as deterioration (Angelo et al., 2015). Chemical and biochemical activities including enzymatic reactions, rancidity and browning, in addition to the mechanical injuries resulting from bruising, puncturing and crushing are common causes of food losses along the food supply chain. Also, biological and microbiological damages caused by insects, pests and microorganisms can lead to quality deterioration. Furthermore, physiological reactions including respiration, transpiration and sprouting are considered as the main causes of post-harvest losses (Barrett et al., 2005).

1.2.1. Physiological Causes of Post-Harvest Losses

1.2.1.1. Respiration

Respiration is a metabolic process based on the oxidation of energy rich organic compounds found in fruits and vegetables such as starch, sugars and organic acids into small molecules (CO_2 and H_2O). During respiration, energy is released and intermediate products that are needed for cellular synthetic reactions are produced (Gopala Rao, 2015). Respiration rates differ among fruits and vegetables and are used as shelf life indicators. In fact, the higher the rate of respiration is, the shorter is their shelf life. For example, thin or thick skinned or peeled fruits such as oranges have much lower respiration rates than unskinned fruits such as strawberries, which explains the extended shelf life of oranges over strawberries (Ramaswamy, 2015).

Respiration in the presence of oxygen in known as aerobic respiration while it is anaerobic when the required amount of oxygen is insufficient. During anaerobic respiration which is also known by fermentation, glucose is converted into acetaldehyde and ethanol, CO_2 is accumulated, and a low energy level is produced (only 2 ATP). Anaerobic respiration is also

coupled with off-flavors and alcoholic odors that makes it an undesirable process (Gopala Rao, 2015).

During aerobic respiration and in the presence of O_2, the hexose sugar found in fruits and vegetables (glucose) acts as a substrate. The oxidation of one molecule of glucose will allow the release of 38 moles of ATP energy, with 673 kcal of heat. Giving the following equation:

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

Respiration activity should be kept at a minimal level using good storage conditions to minimize the deteriorating effects associated with it including senescence, quality loss and weight reduction. Different factors are known to affect the respiration rates and these factors should be controlled to extend the post-harvest shelf life of fruits and vegetables during storage (Ramaswamy, 2015).

Temperature: Low temperatures are preferred during post-harvest storage. Also, the rapid lowering of temperatures after harvesting is an effective way to reduce respiration rates. Based on Van Hoff's rule, a two folds increase in the respiration rate can be induced by a 10 ºC increase in the temperature (Ramaswamy, 2015).

Gas concentrations: Controlling the concentrations of oxygen (O_2) and carbon dioxide (CO_2) in the storage area is important to reduce respiration rates and prevent the undesirable anaerobic respiration. To reduce respiration rates, O_2 concentration below 10% and CO_2 concentration up to 5% should be maintained in the storage area. However, oxygen levels should be kept above the extension point (EP) which is the minimal level of O_2 needed to maintain aerobic respiration which is generally around 2% (Ramaswamy, 2015).

Maturity of the fresh produce: The degree of maturation of fruits and vegetables can affect the respiration rates, which are high at tender stages and decreases with the maturity of the plant (Thompson, 2016).

Nature of the fresh produce: Fruits and vegetables belong to two different classes which are climacteric and non-climacteric. Climacteric fruits such as tomatoes, mangoes and bananas are those whose ripening is associated with a rapid rise in the respiration rates (Gopala Rao, 2015). Also, they experience an elevated production of ethylene and CO_2 during ripening. On the other hand, non-climacteric fruits such as citrus, grapes and strawberries does not show an increase in ethylene production and show a gradual decrease in the respiration rates during ripening. Also, they should be harvested at the maturation point (Thompson, 2016).

Ethylene hormone: Ethylene occurs in all plant tissues at a minimal level and plays an important role in increasing the respiration rates during storage since it is physiologically active even at trace amounts (Gopala Rao, 2015).

Injuries and stress: Chilling injuries, diseases and mechanical damages can increase both respiration rates and ethylene production, which causes undesirable changes in the flavor, color and texture of the fresh produce (Gopala Rao, 2015).

1.2.1.1.1. Methods to Measure Respiration Rates and Gas Exchange

The respiration rates can be expressed by the amounts of O_2 consumed or the amounts of CO_2 produced by the fresh produces. Different methods can be used to determine the respiration rates (Fonseca et al., 2020).

- **Closed system or static system:** Fresh products are stored in an airtight container, where the ambient air is considered as the initial atmosphere. Gas composition is measured at the beginning and after a period of time to determine the respiration rate.

- **Flow through or flushed system:** A mixture of gas is created and flow at a constant rate through an impermeable container where the product is enclosed. The respiration rate is calculated from the difference of gas composition of the inward and outward flow.
- **Permeable system:** The product is placed in a package with known dimensions and film permeability to O_2 and CO_2 gases where the produced equilibrium concentrations are measured, which helps in calculating the gas exchange rates (Fonseca et al., 2020).

1.2.1.2. Transpiration

Water is essential for the development of biochemical reactions in plants, and it is available in high amounts forming 80 to 90% of the plant's body. Before harvesting, the plant can maintain a balance between water lost through evaporation, which is also known by transpiration and the water absorbed by the plant through the roots. Transpiration at this point is desired since it provides a cooling effect to the plant, and allows mineral and water absorption from the soil and mineral distribution in the plant's body (Díaz-Pérez, 2019).

Transpiration follows the basic Fick's law of diffusion represented by the following equation:

$$J = AD \frac{(\Delta C/\Delta x)}{[RT]} = AD \frac{[(Pi - Pa)/x]}{[RT]} \quad (2.2)$$

where J is the gas flux expressed, A is the area of the surface, D is the diffusion coefficient, R is the gas constant and T is the Temperature (absolute). $\Delta C/\Delta x$ is the concentration gradient which is the gradient of water vapor pressure [(Pi - Pa)/x] where x is the thickness of the surface layer providing resistance to moisture migration (Narayanapurapu, 2012). Pa is the atmospheric pressure and Pi is the fruit's internal water vapour pressure (Montanaro et al., 2012).

After harvesting and together with respiration, transpiration becomes one of the major factors behind the deterioration of plants during storage. Transpiration is the water loss through evaporation from the tissues and organs of the plant to the surrounding air. Moisture loss in fruits and vegetables should be minimized during post-harvest storage since it causes quality deterioration, discoloration, shrinkage, weight loss and texture softening (Díaz-Pérez, 2019). Many factors are known to affect transpiration rates and they are the following:

- **Respiration**: Respiration allows the production of heat and energy that rise the temperature of the produce and increases the transpiration rate (Fallik & Ilic, 2018).
- **Injuries and mechanical damages**: Injuries that are commonly observed in the products with thin skin can accelerate the transpiration rates. The exposure of the internal tissues to the surrounding environment allows the entrance of pathogens, and increases microbial growth and transpiration rate (Fallik & Ilic, 2018).
- **Size of the fresh product:** Transpiration rate decreases with the increasing size of the fresh produce and it is higher in products with large surface area to volume ratio (Díaz-Pérez, 2019).
- **Surrounding environment:** Maintaining low temperatures and high relative humidity in the storage room is very effective in reducing transpiration rates. Also, moderate speed of air circulation is required to remove the heat and moisture produced during the respiration and transpiration of the neighboring products in the storage area (Ramaswamy, 2015).

1.2.1.3.Ethylene Production

Ethylene gas (C_2H_4) is a colorless gas produced by fruits and vegetables as a growth stimulating hormone, and it plays an important role in the ripening of fruits both climacteric and non-climacteric (in different ways). Ethylene gas is commercially used to accelerate the ripening of bananas by the degradation of chlorophyll, which allows the change of the color from green to golden yellow in a short period of time. Also, ethylene is active in trace amounts (less than 0.1 ppm) and it is produced by the plants even after harvesting as a natural product of respiration. The presence of this gas in high concentrations in the storage room can negatively affect the quality of the products by causing tissue degradation, premature decay and color changes. Activated charcoal and potassium permanganate can be used to remove the excess ethylene from the storage area (Ramaswamy, 2015).

1.2.1.4. Post-Harvest Physiological Breakdown

Physiological breakdown is mainly related to the storage conditions of the fruits and vegetable, and it can be in the form of chilling and freezing injuries.

Chilling injuries are observed in tropical and subtropical fruits stored at temperatures between 5 ºC and 15 ºC, and causes surface discoloration, softening, increased respiration rates and high ethylene production (Gopala Rao, 2015).

Freezing injuries can be observed in commodities stored at temperatures below their freezing points, which delays the ripening of the product and causes fungal spoilage. Controlling the temperature of the storage area is very important (Gopala Rao, 2015).

2. Post-Harvest Technologies for Food Loss Reduction

In a proper storage environment, fruits and vegetables should be protected from injuries, diseases and mechanical damages. Additionally, the temperature, relative humidity, gas

compositions and air movement should be controlled in the storage area. The control of these factors allows the shelf life extension of fresh produces and quality preservation by reducing the undesirable changes such as tissue softening, water loss and oxidative browning. Beside the refrigerated storage these are the different technologies that can be used to reduce post-harvest losses (Zhuang, 2011).

2.1. Controlled Atmosphere Storage

Controlled atmosphere storage (CA), is considered as a major invention in the refrigerated storage. CA is a non-chemical method used to extend the shelf life of fruits and vegetables after harvesting (Gopala Rao, 2015). It preserves the flavor and aroma of the fresh produce; retards aging and reduces respiration rates and ethylene production. Furthermore, CA is mainly effective on climacteric fruits and preferred to be used as fast as possible after harvesting (Valdez Fragoso & Mújica-Paz, 2016).

The main concept of CA is to create an atmosphere within an airtight storage room by lowering the concentration of oxygen gas and increasing the concentration of carbon dioxide. High relative humidity and controlled low temperatures should also be maintained (Valdez Fragoso & Mújica-Paz, 2016). Studies have showed that the concentration of oxygen gas is lowered to 2% to 3% by flushing O_2 using gaseous or liquid nitrogen, while the concentration of CO_2 gas is increased to 5% in the storage room by injection through gas generators. Also, gas concentrations should be monitored, and different gas percentages and compositions can be used. For example, carbon monoxide gas can be added into the storage room for its fungistatic effect (Ramaswamy, 2015).

The prevention of ethylene accumulation is also important and it is achieved by ventilation and the use of oxidizing agents such as potassium permanganate ($KMNO_4$) (Valdez

Fragoso & Mújica-Paz, 2016). $KMNO_4$ can be mixed with aluminium silicate spheres to form absorbent bead scrubbers that oxidize ethylene into ethylene glycerol. Ethylene glycerol is converted later into CO_2 and water (Zhuang, 2011).

2.2. Modified Atmosphere Packaging

Modified Atmosphere Packaging (MAP) creates an environment within the package of the fresh produce where the gaseous composition needs to be modified. Synthetic polymers with a selective permeability to O_2 and CO_2 gases are used as packaging material (Gopala Rao, 2015). This technology was invented in the 1970s and it is mainly used to reduce respiration rates, delay enzymatic reactions and decrease microbial growth (Bodbodak & Moshfeghifar, 2016).

MAP allows the change of the gas compositions within the package, which are normally formed of 20.95% of O_2, 78.09% of N_2, 0.93% of argon and 0.038% of CO_2 to provide new gas compositions (Zhuang, 2011). The atmosphere creation is done through oxygen diffusion into the package and CO_2 diffusion out of the package, since O_2 is consumed during respiration and needs to be brought in, and CO_2 is normally produced by respiration and needs to be moved out of the package (Ramaswamy, 2015). Also, N_2 gas can be appropriately added as a filler to prevent the collapse of the package by the creation of a pressurised atmosphere (Bodbodak & Moshfeghifar, 2016).

2.3. Active Packaging

Active packaging, also known by smart or intelligent packaging is a novel technology used to extend the shelf life of fruits and vegetables and it consists of using packages with different active functions. Many types of active packaging are available such as oxygen scavengers, carbon dioxide emitters, humidity absorbents and ethylene absorbers (Mehyar & Han, 2011).

- Oxygen scavengers can be added to the films to control oxygen concentrations and reduce respiration rates and ethylene production. Also, it allows color preservation by preventing oxidative browning caused by the oxidation of vitamins and natural pigments.
- Carbon dioxide emitters can reduce respiration rates and the growth of aerobic microorganism within the package.
- Humidity absorbents such as silica gel, calcium oxide, calcium chloride and natural clays can be added to absorb the moisture produced, and it is generally used to keep the products dry.
- Ethylene absorbers like potassium permanganate ($KMNO_4$), can be inserted into silica gels within the polymeric films to absorb the ethylene produced within the package. However, studies suggested that the use of $KMNO_4$ in the packaging material in contact with food surfaces should be restricted due to its toxicity (Mehyar & Han, 2011).

2.4. Edible Films and Coatings

The use of synthetic polymers in the production of packaging materials can negatively affect the environment due to the massive disposal of plastic into the oceans. To solve this problem, biodegradable and bio-renewable natural polymers have been used in the last two decades in the manufacturing of edible films and coatings. Edible films and coatings are commonly used to extend the post-harvest shelf life of fruits and vegetables (Pavlath & Orts, 2009).

3. Edible Films and Coatings

3.1. History of Edible Films and Coating

With the growth in food science and technology concepts in the food packaging area, edible films and coatings have been introduced to reduce post-harvest food losses and effective

use of packaging materials. Edible films and coatings preserve highly perishable produces by providing a gas barrier to oxygen, carbon dioxide and ethylene, with the intention of reducing respiration rates. Also, they act as barriers to water vapor and volatile compounds, which reduces transpiration as well as flavor and aroma losses. Edible films and coatings can also protect the food products from microbes, insects and mechanical damages that can be mainly caused during transportation and storage (Pavlath & Orts, 2009).

Edible coating was invented in the 12^{th} century by the Chinese by applying wax on citrus to reduce moisture loss and enhance the appearance. The use of paraffin wax on food surfaces began in the 1930's after it was announced as edible and became commercially available (Dhall, 2013).

The interest in edible coating has increased over the years and became highly used by the food industry mainly in 1960, when wax layers were applied on different fruits to keep them shiny and reduce moisture loss and quality deterioration. Today, edible coating is used on a variety of food products with an annual revenue exceeding $100 million. Highly perishable fruits such as berries, apples and avocadoes that experience rapid quality deterioration can benefit from the use of edible coatings which helps both producers and consumers save money and reduce food losses (Pavlath & Orts, 2009).

3.2. Methods of Coating

Edible packaging is available in two different forms which are coatings and films. Edible coating is a thin layer formed on the surface of the food produce. The coating solution is applied by immersion, spray coating or brushing and it must have a thickness of 0.3 mm. Spray coating is used to apply a uniform coating on food surfaces, and it is preferred to be used on food products with a large surface area, while dipping is commonly used on food products with

irregular shapes. However, less uniform coating might be formed using the dipping technique (Mantilla, 2012). Also, an edible coating can be safely consumed without the need to peel it off.

An edible film is a thin self-standing material used to wrap or cover the food products after the drying of the film-forming solution (Angelo et al., 2015). Based on the hydrophilic and hydrophobic natures of the materials used in the making of edible films and coatings, only water and ethanol or a combination of both can be used as solvents (Salgado et al., 2015).

3.3. Advantages and Disadvantages of Edible Films and Coatings

Edible films are used to wrap or cover the food produce and can be easily removed. They are ecofriendly and biodegradable which reduces environmental pollution. Also, they can act as carriers for different additives such as anti-browning agents, antimicrobial agents and color additives. However, edible films might have low mechanical stability such as weakness, rigidity and brittleness (Angelo et al., 2015).

Edible coatings are directly applied on the food surface and can improve the appearance of the fresh produce. Also, they act as carriers for colorants, odor and flavoring agents to improve the appearance and sensory attributes as well as antioxidants and antimicrobial agents to provide enhanced protection. The main disadvantage of edible coating is the possible consumer unacceptance. Consumers might refuse the change in the sensory properties caused by the coating thickness and the addition of different additives (Angelo et al., 2015).

The following Table 2.1 shows the common advantages and disadvantages that can be provided by both edible films and coatings.

Table 2.1: Common advantages and disadvantages between edible films and coating (Angelo et al., 2015)

Common Advantages between Edible Films and Coatings	Common Disadvantages between Edible Films and Coatings
- Shelf life extension of the fresh produces. - Natural biopolymers are highly available. - Good barrier properties: Barrier to gas exchange, moisture loss and ethylene production.	- Lack of machinery (research stages). - Production might be costly. - Food safety concerns: all used substances should be food grade and GRAS.

3.4. Properties of Edible Films and Coatings

Different properties should be considered to produce edible films and coatings with good qualities and enhanced properties. Components used should be safe and food grade (generally regarded as safe, GRAS), widely available and affordable. Films and coatings must have good barrier properties, represented by gas selectivity and adequate water vapor and solutes permeability (Vargas et al., 2008).

Edible films should have good mechanical properties such as elasticity and flexibility and should not be rigid and brittle. Also, they should be biodegradable and eco-friendly with a non-polluting nature (Vargas et al., 2008).

Sensory properties are also very important and play an essential role in the consumer acceptance of the food product. Colorless, flavorless, odorless and thin coatings should be applied on the food surface. However, in some cases colorants and flavor additives can be used to enhance the sensory properties of the produce. Coatings must also adhere and spread uniformly on the food surface and must be suitable as carriers to different additives such as antioxidants and antimicrobial agents (Zhuang, 2011).

3.5. GRAS

GRAS is an acronym that means Generally Recognised as Safe and it is introduced by the Food and Drug Administration (FDA) of the United States. GRAS is based on scientific evidence and there is a list of all the chemicals and substances that are considered safe and can be used as food additives in the food industry. During the production of edible films and coatings we should make sure that all the used substances are GRAS, safe and poses no adverse effect on the human health (Pavlath & Orts, 2009).

3.6. Composition of the Edible Films and Coatings

The materials that can be used in the production of edible films and coatings are classified in three different categories.

- Hydrocolloids including proteins and polysaccharides
- Lipids
- Composites: Composite films consist of a mixture of both hydrocolloids and lipids. The components are combined to form a film with a homogeneous layer, or multi-layers known as a bilayer (Galus & Kadzińska, 2015).

Studying the properties of the three major components used in making of edible films and coatings is very important. These components have different chemical structures, which will affect the functional properties of the produced films and coatings (Pavlath & Orts, 2009). The functional properties are also affected by the variable physical and chemical characteristics of the used materials (Talens et al., 2010). A general rule, fats are used to add gloss to the product's surface and reduce moisture loss due to their hydrophobic nature. Polysaccharides are mainly used to control gas transmission, while proteins have advantages for their mechanical stability

(Pavlath & Orts, 2009). Also, both polysaccharides and proteins are hydrophilic with good barriers against oxygen, lipids and aroma (Salgado et al., 2015).

3.6.1. Polysaccharides

Polysaccharides are polymers of monosaccharides connected to each other by glycocidic bonds and used in the production of edible films and coatings. Also, polysaccharides are suitable to be used in the coating of fruits and vegetables due to their effective gas barriers. Their selective permeability to O_2 and CO_2 gases allows the creation of a modified atmosphere. Other advantages are their low cost and high availability since they are mainly found in plants and seaweeds. However, polysaccharides are hydrophilic with a high-water vapor permeability. For this purpose it is preferred to combine them with multivalent ions to reduce their solubility, or mix them with hydrophobic components such as oils and wax (Vargas et al., 2008). The following are the different types of polysaccharides.

Starch and derivatives: Starch is a polysaccharide derived from tubers and cereals (Talens et al., 2010). Modified starches are widely used in the food industry and in the formulation of edible films and coatings since they are biodegradable, cheap and highly abundant (Vargas et al., 2008). Also, they provide odorless, tasteless and transparent films with good gas barriers (Hassan et al., 2018). Starch is formed of glucose molecules connected to each other by glycosidic bonds. Linear amylose and the branched amylopectin are the two molecules found in starch and they have different distribution percentages in plants. 20% to 25% of amylose with 75% to 80% of amylopectin are the constituents of starches derived from wheat and potatoes (Talens et al., 2010). Maize starches that contain more than 50% to 80% of amylose can produce stronger films than those containing less amylose percentages (Baldwin et al., 2012). The main

disadvantage of starch based edible films and coatings is the poor moisture barrier and the possibility of getting brittle at high environmental humidity (Vargas et al., 2008).

Pectin: Pectin is a complex polysaccharide mainly found in the cell walls of plants and it is made of D-galacturonic acid molecules. Pectin is widely used in the food industry in the manufacturing of jams, jellies and marmalade, due to its ability to form gels in aqueous mediums with the presence of sugar and acids. Apple pomace and citrus peel are the main sources of the commercially available pectin that can be used in the making of edible films and coatings. Pectin based edible films and coatings help reducing moisture, aroma and flavor losses. Also, they act as gas barriers and reduce fat migration (Talens et al., 2010).

Chitosan: Chitosan is a water-soluble polysaccharide obtained by the diacylation of chitin. Chitin is mainly extracted from the fungal cell walls, invertebrates and exoskeleton of crustaceans such as crabs and shrimp shells. Chitosan based films and coatings are semi-permeable, have good mechanical properties and a selective gas permeability that can alter the atmosphere within the package. Also, due to its polycationic nature, chitosan is commonly used on fruits and vegetables as an antimicrobial and antifungal agent. Chitosan was applied on the surface of different commodities including cucumbers, bell pepper and strawberries where they acted as antimicrobial agents (Hassan et al., 2018). However, it has a poor moisture barrier which affects its uses on the surface of fruits and vegetables (Vargas et al., 2008).

Cellulose and derivatives: Cellulose is the most abundant natural polymer on earth, and it is made of glucose molecules connected to each other by ß-1,4 glyosidic linkages. Cellulose is found in animals and green plants as one of their major cell walls component. During the making of edible films and coatings, cellulose derivatives obtained by the chemical modification of cellulose can be used due to their good film forming properties. Cellulose derivatives are the

following: Methylcellulose (MC), carboxymethyl cellulose (CMC), hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC) and microcrystalline cellulose (MCC). Cellulose is water insoluble while its derivatives have high water solubility. Low energy is used to produce odorless, transparent, tasteless and flexible cellulose-based films and coatings with moderate oxygen barrier and high oil barrier. Also, MC and HPMC were used as coating materials to decrease fat absorption in fried food products, and they are very effective especially when the food products are pretreated with calcium chloride (Hassan et al., 2018).

Seaweeds extracts: Seaweeds extracted components can also be used in the making of edible films and coatings and these materials are alginates, agar and carrageenan.

Alginates are the salts of alginic acid and they are made of D-mannuronic and L-guluronic acid monomers (Vargas et al., 2008). They are mainly derived from brown algae, species of the family *Phaeophyceae* (Hassan et al., 2018). Also, they are produced by some bacteria such as *Azotobacter vinelandii*. Alginates-based edible films and coatings were applied in different research studies on the surface of fruits and vegetables, poultry, meat, fish and cheese (Senturk Parreidt et al., 2018). Films made by alginic acids are heat stable, transparent, strong, tasteless, flexible and water soluble with low permeability to oxygen and oil. Due to their high water solubility, multivalent ions such as calcium ions can be added during the formulation of the alginates-based films and coatings, to reduce their water solubility and increase their strength (Campos et al., 2011).

Carrageenan is a polysaccharide made of a mixture of water-soluble galactose polymers and it is mainly extracted from red seaweeds. In the food industry carrageenan act as an emulsifier, a thickener and a stabilizer. Also, it can be used in the formulation of edible films and coatings since it helps in reducing moisture loss. It is mainly used in the making of multilayered

films carrying antimicrobial agents and antioxidants. These films can also be applied on the food surfaces to retard the oxidation of lipids (Vargas et al., 2008).

Gums are complex polysaccharides used in the making of edible films and coatings and they are mainly combined with starches. There are different types of gums including exudate gums (Arabic and karaya gums), seed gums (locust bean and guar gum) and the gums obtained by microbial fermentation such as Xanthan and Gellan gums (Campos et al., 2011).

3.6.2. Proteins

Proteins are polymers made of amino acid chains and have variable molecular characteristics. The diverse biological sources they are extracted from, their different structures and the variable amino acids sequences are the main factors affecting their characteristics (Guerrero & Caba, 2015). Proteins might also have different conformations, weights, elasticity and thermal stabilities which have an impact on the properties of the produced films and coatings (Salgado et al., 2015).

Proteins, solvent and plasticizers are the components that should be used during the making of protein-based films and coatings, which can act as good oxygen barriers at low relative humidity. However, they have poor moisture barriers due to the dominant hydrophilic nature of proteins, which requires the addition of hydrophobic components such as oils to reduce water vapor permeability (Salgado et al., 2015). An additional advantage is the release of nitrogen after the biodegradation of the protein-based edible films, which can be very helpful since nitrogen acts as a fertilizer in nature (Dangaran et al., 2009).

3.6.2.1. Animal Based Proteins

The variable sources, properties and uses of animal-based proteins are shown in Table 2.2.

Table 2.2: Animal-based proteins used in the making of edible films and coatings

Animal-based proteins	Sources	Properties and Uses
1. Collagen	-Main component of connective tissues: bones, skin and tendons (Hassan et al., 2018).	- Flexible films - Water insoluble - Used in edible sausage casing (Dhall, 2013).
2. Gelatin	- Obtained by partial acid or alkaline Hydrolysis of collagen at high temperatures (Hassan et al., 2018).	- Good gelling properties - Low cost and high availability - Transparent films with good mechanical and barrier properties - Common uses in pharmaceutical: drug capsules (Hassan et al., 2018).
3. Caseins	- Major milk protein (Dhall, 2013).	- Low oxygen permeability - Stretchable and tasteless films with opaque appearance - Expensive - High nutritional quality (Dhall, 2013).
4. Whey proteins	-By product from cheese making (Vargas et al., 2008).	- Films have good gas, aroma and oil barrier - Water soluble films - Films are brittle: needs plasticizers - Transparent films - additive in pharmaceutical and in human and animal food (Vargas et al., 2008).
5. Keratin	- Extracted from chicken's feather (Dhall, 2013).	- Cheap - Biodegradable films - Difficult to process - Water insoluble with poor mechanical properties (Dhall, 2013).

3.6.2.2. Plant Based Proteins

The variable sources, properties and uses of plant-based proteins are shown in Table 2.3.

Table 2.3: Plant-based proteins used in the making of edible films and coatings

Plant-based proteins	Sources	Properties
1. Soy protein	- Obtained by grinding of defatted soy flakes. - By product from soy oil production (Hassan et al., 2018).	- Transparent and flexible films - Films with poor moisture barrier (hydrophilic) - Reduces fat intake in deep fried foods and lipid oxidation in frozen salmon (Hassan et al., 2018).
2. Corn Zein	- Found in corn endosperm. - Excellent film forming properties (Hassan et al., 2018).	- Hydrophobic: films with good moisture barrier - Brittle film: needs plasticizers (Hassan et al., 2018) - Film have a strong yellow color - Applied on nuts, candies and confectionary (Olivas & Barbosa-Cánovas, 2009).
3. Wheat gluten	- Hydrophobic or water insoluble protein of wheat flower. - Made of gliadin and glutenin (Hassan et al., 2018)	- Film with a good elasticity (Hassan et al., 2018). - Transparent and strong film with a good water barrier. - Water insoluble film (Vargas et al., 2008)

3.6.3. Lipids and Resins Used in Edible Films and Coatings

Lipids were the first components used in the production of edible coatings. They were applied on food products almost 800 years ago by Chinese people, and they have excellent moisture barriers due to their hydrophobic nature. Lipids can be added to the hydrocolloid materials such as proteins and polysaccharides to reduce their water solubility and produce composite films with enhanced mechanical and barrier properties. Lipids used in edible films and

coatings can be divided into different categories including waxes and paraffins, resins, fats and oils (Dhall, 2013).

3.6.3.1. Waxes and Paraffins

Different kinds of wax can be used as coating materials since they are GRAS and edible when applied in thin layers on the surface of the food produces. Waxes act as moisture barriers because of their hydrophobic nature and can also act as effective gas barriers. They have been used for many decades in enhancing the appearance of the food products by making it shinier and glossier. Also, they can be mixed with fungicides to extend the shelf life of the fresh products. Waxes are derived from different sources and they are the following (Dhall, 2013).

Animal wax includes bees wax, shellac wax and carnauba wax. Bees wax is made by honey bees, it is solid at room temperature with a light-yellow color (Dhall, 2013) and it is mainly used in the formulation of composite films in combination with pea starch.

Vegetable wax consists of cotton seed wax and the carnauba wax removed from a Brazilian tree known by the carnauba palm (Olivas & Barbosa-Cánovas, 2009).

Mineral and synthetic waxes such as microcrystalline wax and paraffin wax. Paraffin wax is synthetic and derived from the distillate fraction of crude petroleum and it is one of the most effective components used in the making of edible coatings. Also, paraffin is consumer safe and can be applied on the surface of fruits and vegetables (Dhall, 2013).

3.6.3.2. Resins

Resins are lipid-based materials that can be applied on food surfaces and act as good gas barriers, good adhesive agents and emulsifier. Resins can be either natural or synthetic.

Natural resins are hydrophobic and acidic substances derived from trees, mainly from the coniferous trees and they are secreted by the cells of the plant in response to an injury or an

infection. Resins that are most commonly used are shellac resin, wood rosin, coumarone indene and terpene resin. Shellac resin is secreted by the insect *Laccifer lacca* found in India and it can be applied on food surfaces to give them a glossy appearance.

Synthetic resins are petroleum-based products that are very stable and homogenous and can be used in the making of plastics, food containers, paints and textiles (Aguirre-Joya et al., 2015).

3.6.3.3. Fats and Oils

Different kinds of oils can be added to the edible films and coatings to enhance their hydrophobicity, and the most commonly used oils are sunflower oil, coconut oil, palm oil, and cocoa butter (Aguirre-Joya et al., 2015). Also, vegetable oils and fatty acids such as corn oil, olive oil, oleic acid and stearic acid can be incorporated during the making of films and coatings and act as emulsifiers (Galus & Kadzińska, 2015).

3.6.4. Other films and Coatings

Aloe Vera, cactus mucilage, pullulan and fruit puree also have been used in the making of edible films and coatings for fruits and vegetables. Aloe Vera was shown to have an anti-inflammatory activity since it contains malic acid-acetylated carbohydrates and it showed an increase in the shelf life of grapes by 35 days at 1 ºC by acting as a good gas barrier (Olivas & Barbosa-Cánovas, 2009).

3.7. Functional Ingredients

Films and coatings act as carriers to nutrients and functional components such as vitamins, antioxidants, anti-browning and antimicrobial agents. Furthermore, flavor additives, colorants and nutraceuticals can be added to the films and coatings to enhance their properties and improve their quality and stability (Ansorena et al., 2018).

3.7.1. Anti-browning Agents

Anti-browning agents are mainly added to protect the food products from biochemical reactions such as enzymatic browning, and they can reduce the oxidative damages that might occur in fruits and vegetables. Oxidative rancidity and the formation of melanin brown pigments in fruits and vegetables are caused by the exposure of the polyphenol oxidase enzyme to oxygen. It is very important to prevent oxidative browning since the color of fruits and vegetables is a major parameter in the acceptance of the produce by the consumers. The main antioxidants or anti-browning agents that can be added are ascorbic acid, citric acid and oxalic acid, in addition to tocopherol and sulfur-containing compounds such as n-acetylcysteine and glutathione (Pereira & Pintado, 2015).

3.7.2. Antimicrobial Agents

Antimicrobial agents can be added to prevent microbial growth and extend the shelf life of the products by acting against yeast, molds and aerobic bacteria (Pastrana et al., 2015). Films and coatings can also be named as active packaging since they carry an additional function. Different substances can be used as antimicrobial agents such as organic acids (acetic, sorbic and lactic acids), fatty acid esters (glyceryl monolaurate), polypeptides (lysozyme, peroxidase, lactoferrin and nisin), plant essential oils (cinnamon, oregano, lemongrass and rosemary oils) in addition to nitrites and sulphites (Mantilla, 2012). The most commonly used antimicrobial agents are chitosan, lysozyme and nisin which are safe and can be applied on the surfaces of fruits and vegetables. However, benzoic acids, sodium benzoate, potassium sorbate and propionic acids are the antimicrobial agents that are not preferred to be used on food surfaces (Tavassoli-kafrani et al., 2016).

3.7.3. Multivalent Ions

Based on the hydrophilic nature of polysaccharides, ions can be added to formulate insoluble gels by creating a three-dimensional network that helps reducing the solubility of the material. Zink and calcium salts such as calcium chloride and calcium lactate can be added to enhance the texture of the film, stabilize it and improve its mechanical properties (Pereira & Pintado, 2015).

3.7.4. Emulsifiers and Surfactants

Surfactants are surface acting agents that can increase the wettability of the products, stabilize the components of the edible coating and enhance it adherence on the hydrophobic rough surfaces of the products to obtain a uniform edible coating. Sorbitan monolaurate (Span 20) is commonly used as a surfactant in different research papers (Salgado et al., 2015).

Emulsifiers such as acetylated monoglyceride, lecithin, glycerol monostearate, sorbitan fatty acid esters (TWEENS), and palm oil can be added to reduce surface tension and enhance the wettability, the thickness uniformity and the spreading coefficient of the coating. Also, both surfactants and emulsifiers can be effective in reducing water vapor permeability and moisture loss from the coated food products (Pereira & Pintado, 2015).

3.7.5. Plasticizers

Plasticizers are low molecular weight compounds that are mainly added to the edible films and coatings to improve their mechanical properties (Olivas & Barbosa-Cánovas, 2009). They can enhance the strength, the flexibility and the elongation of the edible films, and they are always added to the protein powder during the formulation of protein-based edible films (Pereira & Pintado, 2015). Plasticizers help in the separation of the adjacent polymeric chains and reduce their intermolecular interactions, which results in a minimisation of the film rigidity and prevent

the formation of a brittle and non-homogenous films (Olivas & Barbosa-Cánovas, 2009). The most commonly used plasticizers are polyol including glycerol, sorbitol, ethylene glycol (ETG), diethylene glycol (DTG), mannitol, and sucrose (Galus & Kadzińska, 2015). In addition to the hydrophobic compounds derived from citric acid such as Acetyl-tributyl citrate and Triethyl citrate (Pereira & Pintado, 2015).

3.8. Applications of Edible Coatings on Fresh Produces

Despite their hydrophilic nature and low moisture permeability, polysaccharides have been usefully used in the coating of minimally processed fruits and vegetables, since they can act as effective gas barriers that modify the internal atmosphere and extend the shelf life of the fresh products. Different studies showed how proteins can also be used in the coating of fruits and vegetables since they act as excellent gas barriers (Yousuf et al., 2018).

Whey proteins-based edible coating and calcium caseinate-based edible coating showed beneficial effects in preventing oxidative browning in apples and potato cuts. However, whey proteins have low mechanical properties, which makes them not very suitable to be used on the surfaces of fresh fruit and vegetable (Le Tien et al., 2001).

Sodium alginate-based edible coating with the incorporation of cinnamon and lemongrass essential oils showed a shelf life extension and decrease microbiological activity in fresh cut melons (Raybaudi-Massilia et al., 2008). Alginates and gellan-based edible coating with the incorporation of ascorbic acid enhanced the firmness of papaya cut fruits, in addition to moisture loss reduction and ascorbic acid retention (Tapia et al., 2008). While the use of alginate-based edible coating and gellan-based edible coating with the incorporation of cinnamon oil and rosemary oil on fresh cut pineapples, showed a decrease in both respiration and transpiration

rates and maintained the firmness of the coated samples during storage at 10 ºC and 65% RH (Chiabrando & Giacalone, 2015).

Bilayer coating made of a combination of alginates and chitosan in a study in 2014 was applied on fresh cut melons and showed a reduction in tissue texture degradation, beside the reduction in yeasts, bacteria and fungi counts (Poverenov et al., 2014). Also, alginate-based edible coating with the incorporation of N-acetylcysteine and glutathione applied on pear cut fruits, showed effective results in reducing ethylene production and increasing the resistance to water vapour permeability, in addition to retarding mold growth and oxidative browning (Oms-Oliu et al., 2008).

Calcium-alginate coating enhanced the sensory properties and reduced mold growth in fresh cut carrots (Costa et al., 2012). Also chitosan coating reduced moisture loss and extended the shelf life of peeled litchi fruits by delaying the increase in total soluble solids content during storage (Dong et al., 2004).

It has also been reported that methyl cellulose and sodium alginate-based edible coating extended the shelf life of peaches stored at 15 ºC, up to 21 and 24 days compared with 15 days in control samples. The edible coatings reduced the respiration and transpiration rates and delayed the increase in total soluble solids content (Maftoonazad, 2006).

In another study, the effect of pectin-based edible coating on mango fruits stored at room temperature was evaluated. The coating formulation included pectin, beeswax, sorbitol and monoglyceride. A shelf life extension was observed in coated fruits due to the reduction in respiration and transpiration rates. Also, the firmness and color of the coated samples were preserved during storage (Moalemiyan et al., 2012).

CONNECTING STATEMENT TO CHAPTER III

Previous research showed that polysaccharides-based edible coating is effective in extending the post-harvest shelf life of fresh fruits and vegetables. Strawberry cut fruits are highly perishable due to the induced mechanical damages and increased respiration and transpiration rates. In this chapter, sodium alginate-based edible coating coupled with calcium chloride is evaluated to extend the shelf life of strawberry cut fruits stored at 4 ºC for up to two weeks.

CHAPTER III

THE EFFECT OF SODIUM ALGINATE-CALCIUM CHLORIDE EDIBLE COATING ON THE QUALITY PARAMETERS AND SHELF LIFE OF STRAWBERRY CUT FRUITS

ABSTRACT

Strawberry fruits have a short shelf life after harvesting due the physiological factors that enhance ripening such as respiration and transpiration. Sensory properties including color, texture, odor and flavor are the main factors that make fresh produces appealing to the consumers, and they change very rapidly upon harvest. For this reason, quality preservation is essential during post-harvest handling and storage of strawberries. Quality deterioration rates are higher in cut fruits due to the mechanical damages and the loss of the natural protective barriers, resulting in an increase in moisture loss, respiration rates and the deterioration of the sensory properties. The effect of sodium alginate-calcium chloride edible coating on quality preservation and shelf life extension of strawberry cut fruits stored at 4 ºC was studied. Control samples had mold growth initiated after one week of storage at 4 ºC, while the coated fruit samples had a mold free shelf life extension for up to 15 days. The sodium alginate-calcium chloride edible coating was effective in reducing respiration and transpiration rates and delayed the increase of the pH and soluble solids content. Furthermore, the coating reduced surface mold growth and preserved the sensory properties of the cut fruits such as color and texture.

1. Introduction

With the increased interest in ready to eat and nutritious snacks, cut fruits have become more popular and widely available in supermarkets, cafeterias, airlines catering, universities and schools. Cut fruits are highly perishable due to the mechanical damages caused by the cutting of fruits, which increases moisture loss, respiration rates and fruits' ripening. Quality deterioration such as oxidative browning and texture softening are also enhanced (Senturk Parreidt et al., 2018).

Berries including strawberries, blueberries, raspberries, blackberries and cranberries, are small fruits that are highly nutritive, and widely produced and consumed around the world. Berries are good sources of phytochemicals and they are introduced into many products such as yogurts, jams, jelly and cakes. Phytochemicals are also known by plants secondary metabolites. These are biologically active compounds that can be found in grains, fruits and vegetables, mainly in berries, grapes, apples, tomatoes and root vegetables. A clinical study confirmed that phytochemicals such as polyphenols, which are highly found in berries act as antioxidants and reduce the risk of oxidation induced chronic diseases such as cancer, diabetes and cardiovascular diseases (Heinonen, 2007). Anthocyanins belong to the flavonoids group and they are the polyphenolic compounds found in berries. Anthocyanins are the natural pigments responsible for the bright red, orange-red, purple, blue and black colors in fruits and vegetables (Jing & Giusti, 2011). The content and composition of anthocyanins in berries mainly depends on their cultivation and growth environment (Xu, 2012).

Strawberries are small red fruits that are highly produced and consumed. Based on the Food and Agriculture Organization (FAO, 2011) over 4.5 million tons of strawberries are harvested each year around the world, mainly in Spain, Egypt, USA and Mexico (F. FAO, 2011).

Strawberries contain 92% water, 7% carbohydrates, 0.6% proteins, 2% fibers, antioxidants, minerals such as potassium and manganese and vitamins such as vitamin C (ascorbic acid). Vitamin C content increases with fruit's maturity, for example green strawberries have only 20% of the ascorbic acid content that can be found in mature red fruits. Consuming 100g of strawberries per day can be enough to ensure our daily needs of vitamin C (40 mg to 90 mg of ascorbic acid per 100 g of strawberries). Furthermore, strawberries contain different types of organic acids including citric acid (accounting 90% of acid content) and malic acid which are considered as essential flavor components (Sharma et al., 2019).

Anthocyanins, carotenoids and chlorophyll are natural pigments found in strawberries. Main anthocyanins present in strawberries are pelargonidin-3-glucoside which provides the orange color and the cyanidin-3-glucoside responsible for the orange-red color. Anthocyanins also have some beneficial effects on human health acting as antioxidants and anti-inflammatory components. The change in the concentration of these pigments affects the color of the strawberry fruits during ripening. In fact, during ripening the anthocyanin content increases while the chlorophyll content decreases, which causes the change of the color from green to orange-red (Jing & Giusti, 2011). Sucrose, glucose and fructose are the main sugars found in strawberries making 99% of the total sugars in ripe fruits. The content of both glucose and fructose increases during ripening while the content of sucrose decreases with time. Studies showed that sugar content can be affected by both environmental factors and cultivation characteristics (Nunes, 2008).

1.1. Sodium Alginate and Calcium Chloride Coating Properties

Alginates are polysaccharides naturally produced by brown marine algae or seaweeds such as *Laminaria hyperborean* and *Macrocystis pyrifera*. Alginates can also be produced by

some bacteria such as *Azotobacter vinelandii,* that were first discovered in 1881. The different sources of alginates can have an impact on their physical and chemical properties, in addition to the coating thickness and viscosity. Alginates are considered as GRAS by the US Food and Drug Administration (FDA) and they are used as thickening agents and stabilizers. Also, they are approved food additive by the European Commission (EC) (Senturk Parreidt et al., 2018).

Alginate is made of linear copolymers of β-d-mannuronic acid and α-l-guluronic acid residues, connected to each other by glycosidic bonds. Different kinds of alginic salts are available with various characteristics. Sodium alginates (Figure 3.1) and potassium alginates are water soluble, while calcium alginates and alginic acids are water insoluble. Water soluble sodium alginate is commonly used in the making of edible films and coatings and should be mixed with divalent ions to reduce its water solubility. The addition of divalent ions such as calcium allows the formation of divalent salt bridges due to the binding of calcium ions between two chains, which provides rigid and dense gels (Senturk Parreidt et al., 2018).

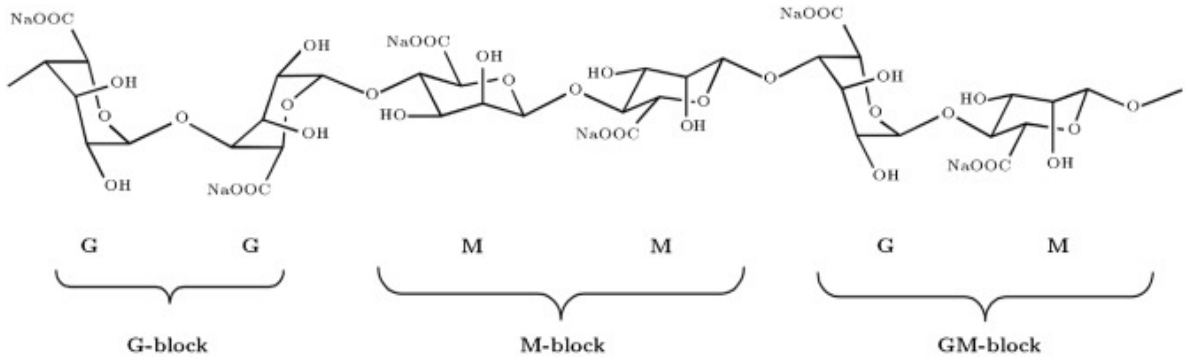

Figure 3.1: Sodium alginate composition (Daemi & Barikani, 2012)

The sources of calcium ions can also affect the gel formation. Calcium chloride salts ($CaCl_2$) allows the production of strong gels in comparison with the calcium lactate and calcium gluconate (Senturk Parreidt et al., 2018).

Also, $CaCl_2$ has the highest solubility among the calcium ions. Calcium chloride is a white salt that comes in three different forms which are anhydrous ($CaCl_2$), hydrated ($CaCl_2. 6H_2O$) with 49% water and partially dehydrated ($CaCl_2. H_2O$) with 14% water (Senturk Parreidt et al., 2018).

Two immediate cross-linking reactions are induced when the calcium chloride salts come in contact with the sodium alginate solution. The first reaction is the diffusion of the multivalent ions, which allows the creation of a linkage between the calcium ions and the carboxyl groups found in the alginate. This reaction allows the in-solubilization of the alginate film, while the second reaction is the dissolution of the alginate by the solution. Calcium chloride which is considered as GRAS by the FDA might affect the flavor of the food products and causes bitterness when used in high concentrations. While calcium lactate and calcium gluconate have no impact on the flavor of the fresh produces (Senturk Parreidt et al., 2018).

1.2. Applications of Edible Coatings on Strawberry Cut Fruits

Cut fruits and vegetables are wounded tissues with a shorter shelf life than the intact fruits due to the induced mechanical damages. The internal tissues in cut fruits are exposed to the external environment, which increases respiration and transpiration rates, oxidative browning and microbial growth (Yousuf et al., 2018). The soft texture of strawberries makes them more susceptible to mechanical damages and quality loss during post-harvest storage (Han et al.,2004).

A research study evaluated the effect of chitosan-based edible coating on the quality of strawberries and raspberries, which are highly perishable fruits. Control and coated strawberries and raspberries were stored at 2 ºC for 3 weeks at 88% RH, and for 6 months at -23 ºC. The chitosan-based edible coating delayed the change in fruits' color, pH, titratable acidity and

reduced weight loss and decay in refrigerated fruits. Another positive result was shown by reducing the percentage of drip loss in frozen fruits after thawing (Han et al.,2004).

Tomadoni et al. (2018) studied the effect of gellan-based edible coating with the incorporation of geraniol and pomegranate extract on the quality parameters of strawberry cut fruits stored at 5 ºC. After 7 days of storage, Gellan-geraniol based edible coating reduced microbial count in strawberry cut fruits, however bigger firmness loss in coated samples was observed when compared with control samples. Gellan-Pomegranate extract based edible coating showed no effect on the microbial growth in strawberry cut fruits (Tomadoni et al., 2018).

Garcia et al. (2012) studied the effect of cassava starch-based edible coating with or without potassium sorbate on the shelf life of strawberry cut fruits stored for 15 days at 5 ºC. A shelf life of 9 days for both uncoated and cassava starch-potassium sorbate coated sample was reported. Potassium sorbate did not inhibit the microbial growth. However, strawberry cut fruits coated only with cassava starch showed lower respiration and transpiration rates and a shelf life extension for up to 12 days. No significant effect on the soluble solids, titratable acidity, pH and colour of the strawberry cut fruits was observed (Garcia et al., 2012).

In spite of a lot of focus, application of edible coatings to cut fruits is still scarce. The reason for this lack is primarily associated with the difficulties in coating cut fruits and vegetables because of the exposure to the moister internal content of the product. Also, the respiration and transpiration rates are much higher in cut fruits, and edible films and coatings need a different consideration for application as coating materials. Moreover, from an economical point of view, strawberries are the main products benefiting from the coating applications due to their high perishability. It is for this reason; this thesis research is focussed on coating fresh fruits both in the fresh cut form (strawberries) as well as a semi-finished partially

pre-dehydrated cut fruit (pineapples) using sodium alginate-calcium chloride based edible coating.

1.3. Research Objective

The objective was to evaluate the effect of sodium alginate-calcium chloride edible coating on the quality parameters and shelf life extension of strawberry cut fruits stored for two weeks at 4 ºC.

1.4. Preliminary Experiments

Preliminary experiments using 16 different compositions of sodium alginates, calcium chloride and calcium lactate in different concentrations were used to coat strawberry cut fruits. The effects of essential oils such as pepper mint oil and lemon grass oil were also evaluated due to their role as antimicrobial agents. The addition of essential oils such as lemon grass extended the shelf life of strawberry cut fruits stored at 4 ºC for up to three weeks. However, quality deterioration including tissue softening, darkness and off- odours occurred only after one week of storage. Also, the effect of pectin-calcium lactate based edible coating and chitosan-calcium salts based edible coatings on the shelf life of strawberry cut fruits were evaluated. Surface mold growth was observed in pectin coated fruit samples after only 9 days of storage at 4 ºC, while surface mold growth was observed after 12 days of storage at 4 ºC in chitosan coated fruit samples. Based on the results obtained during preliminary experiments, a combination of 2% (w/w) sodium alginate and 2% (w/w) calcium chloride was considered as the best composition to be used in the coating of strawberry cut fruits. Low concentration of calcium chloride salts was preferred to be used since high concentrations might cause the development of bitter flavors (Senturk Parreidt et al., 2018).

2. Materials and methods

2.1. Samples Preparation

Strawberries were purchased fresh from the market and chosen based on similar degrees of maturity and stored at 4 ºC overnight until use the next day. Strawberries were washed with tap water to remove external impurities and cut into 1 cm thickness along their longitudinal axis. Cut fruits were drained for 10 min after washing and before coating (Gamboa-Santos & Campañone, 2018).

2.2. Coating Solutions Preparation

The methodology used by Gamboa-Santos and Campañone (2018) was used with some modifications. Calcium chloride salts were used instead of calcium lactate salts due to their effect as firming agents and their high-water solubility. Distilled water was used to prepare the coating solutions of 2% (w/w) sodium alginate (Sigma, Oakville, ON) and 2% (w/w) calcium chloride (Sigma, Oakville, ON). To prepare the sodium alginate solution, sodium alginate powder was added to distilled water and the beaker was placed on a magnetic stirring rod at 300 rpm with no heat until the sodium alginate powder was completely dissolved. To prepare the calcium chloride solution, calcium chloride salts were added in a volumetric flask with distilled water. The volumetric flask was shaken twice upside-down to dissolve the calcium chloride salts (Gamboa-Santos & Campañone, 2018).

2.3. Control Sample Preparation

For the preparation of control samples, strawberry cut fruits were only washed with tap water, cut into 1 cm thickness along the longitudinal axis, drained at room temperature and stored in plastic containers at 4ºC. Fruit samples were covered with polyvinyl chloride stretch film (PVC films) (Garcia et al., 2012).

2.4. Coating of Samples

Sodium alginate and calcium chloride solutions were poured into the plastic containers and the strawberry cut fruits were placed in a fabric mesh and dipped completely into the sodium alginate solution for 5 min, removed and drained for 1 min in a plastic mesh, dipped again this time in the calcium chloride solution for 5 min, removed, drained and left on a filter paper for 10 min at room temperature (22 ºC) to remove the surface excess of the coating solution (Gamboa-Santos & Campañone, 2018).

2.5. Experimental Procedure

The steps used in the experimental procedure for fruit preparation and coating are detailed in Figure 3.2.

2.6. Sample Storage

Different lots of samples were chosen based on similar shapes, sizes and thickness. Coated and control samples were stored in plastic containers and covered with polyvinyl chloride stretch film (PVC film). Small 4 to 5 holes were made in the PVC film (1-2 mm each) to maintain the atmospheric composition of air within the container (Figure 3.3). Plastic containers were stored in the refrigerator at 4 ºC for two weeks and tests were done on both control and coated samples every three days (days 0, 3, 6, 9, 12 and 15) with quality monitoring on a daily basis as detailed by Garcia et al. (2012).

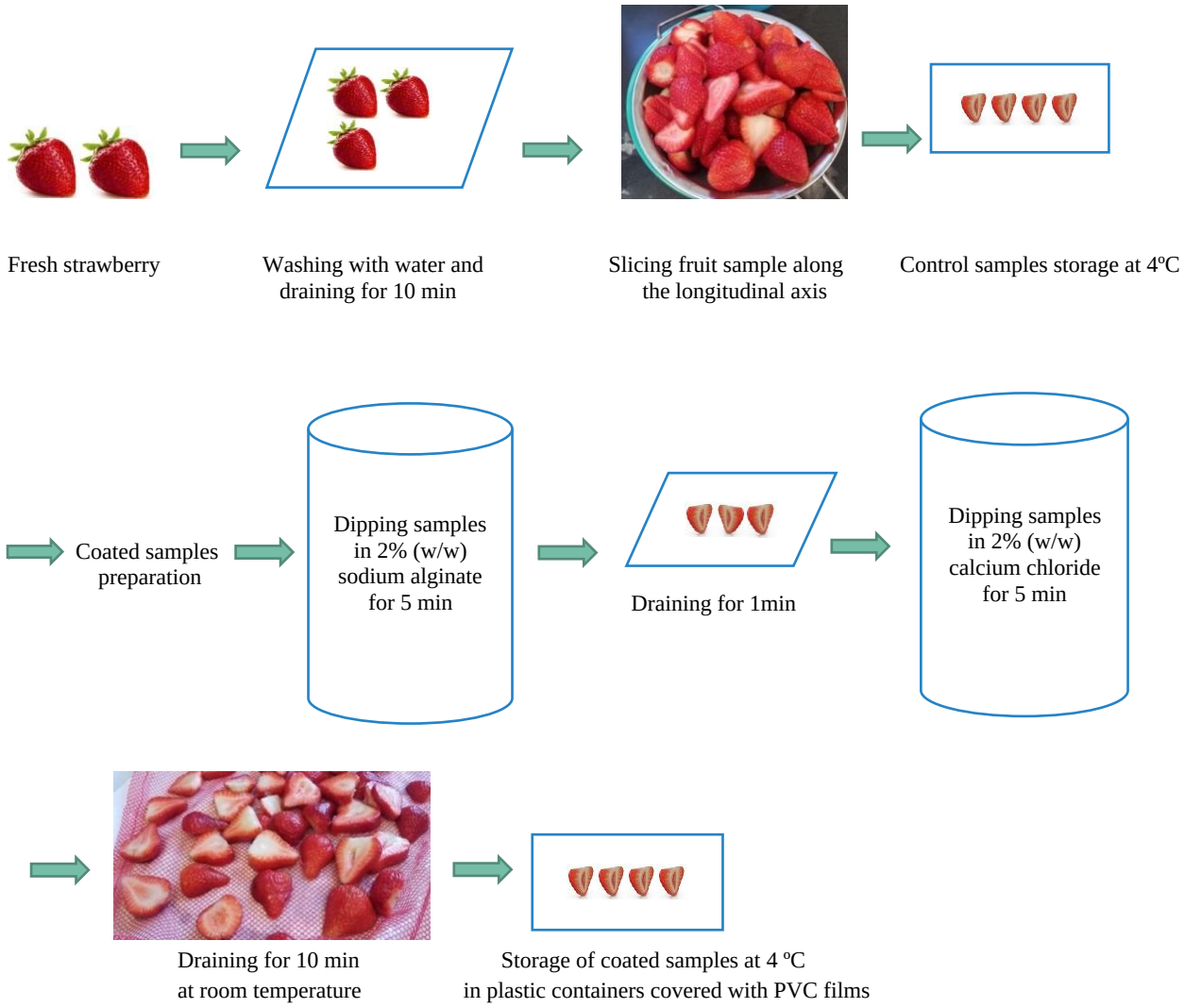

Figure 3.2: Objective 1 Experimental Procedure

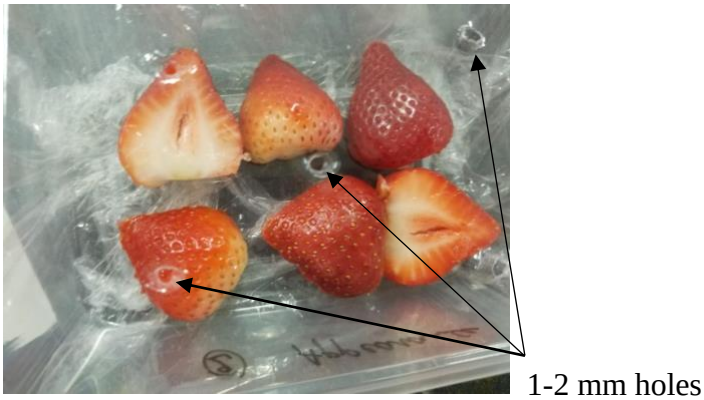

Figure 3.3: Sample storage in plastic containers covered with PVC films

3. Experimental Tests and Sample Analysis

3.1. Respiration

Respiration test was performed every three days during the two weeks of storage on both control and coated samples. Control and coated strawberry cut fruits (150 g) were placed for 2 h in an airtight Plexi-glass chamber (18 cm x 12 cm x 27 cm) at room temperature (22 ºC). The glass chamber is connected to a CO_2 sensor (ACR Systems Inc, St-Laurent, Quebec, Canada) that transfer the results to a data acquisition system (Smart Reader plus 7). The CO_2 concentration is collected every 1 min over a period of 2 h. Respiration rates are obtained from the regression slope of CO_2 concentrations versus time and evaluated as $mL.CO_2\ kg^{-1}\ h^{-1}$ (Maftoonazad, 2006). Both control and coated samples were discarded after every respiration test.

During preliminary experiments, fruit samples were returned to the refrigerator after the 2 h period of respiration test. Faster surface mold growth was observed on the surface of these samples. For this purpose, fruit samples were discarded after every respiration test during the two weeks of the experiment.

3.2. Transpiration

Using the standard method of AOAC 1994, The moisture loss was measured by the periodic weighing of fruit samples using a digital balance (Denver instrument, APX-323, NY, USA) to check the weight loss percentages with time. The difference between the initial weight on day 0, and the final weight measured every 3 days was considered as the total weight loss. The transpiration test was done in triplicates (Gol et al., 2013).

3.3. Color

The color characteristics of strawberry cut fruits were measured on the external red surface of the fruit samples using a calorimeter which is the tristimulus Minolta Chroma Meter (Minolta Corp, Ramsey, NJ) to determine the L value (lightness), the a* value (green-red chromaticity) and b* value (yellow-blue chromaticity). Calorimeter was calibrated using a white standard. Readings were done at room temperature on five to six samples of control and coated samples.

$$\Delta E = [(\Delta L^2 + \Delta a^2 + \Delta b^2)^{½}] \quad (3.1)$$

where ΔL, Δa* and Δb are the differences of L, a* and b* values, obtained on different test days in comparisons with the first day of storage.

Also, Chroma which is the color intensity and the hue angle were calculated using the following equations (Maftoonazad, 2006)

$$\text{Chroma} = (a^2 + b^2)^{½} \quad (3.2)$$

$$\text{Hue angle} = \tan^{-1}(b/a) \quad (3.3)$$

3.4. Texture

Seven to ten samples from each lot (control and coated) were subjected to a puncture test using TA. XT plus Texture Analyzer (Texture Technologies Corporation, Scarsdale, N.Y., U.S.A./ Stable Micro Systems, Godalming, Surrey, U.K.) fitted with a 25 mm diameter round tipped puncture probe with a speed of 10 mm/sec (Figure 3.4). The force deformation and firmness of the fruit samples were measured based on the force-deformation curve. Measurements are in Newton (N) (Maftoonazad, 2006).

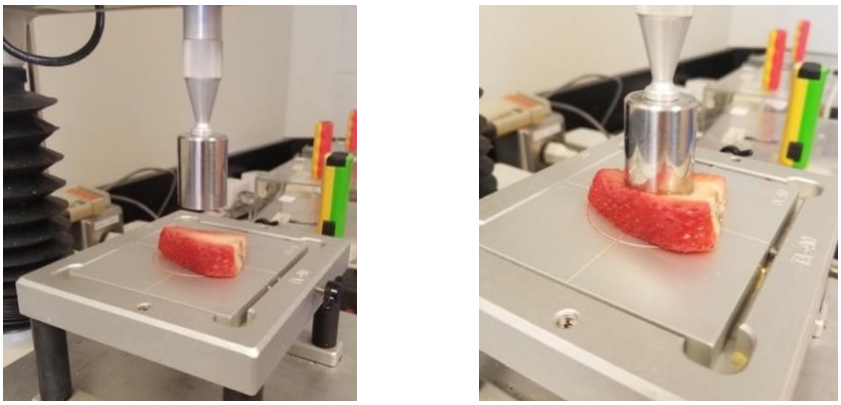

Figure 3.4: Puncture test on coated strawberry cut fruits

3.5. pH Measurement

The pH was measured with a standard calibrated pH meter (Brinkman Co., Mississauga, Ontario, Canada). pH Measurement was done by blending for 1 min, 50 g of strawberry cut fruits with 150 mL of distilled water. pH meter calibration was done with variable standard solutions at pH 7 and pH 10 (Maftoonazad, 2006).

3.6. Titratable Acidity

Titratable acidity was measured using the AOAC titrimetric method for fruits (AOAC, 1990) by titrating 10 mL of strawberry juice with 0.1 mol/L NaOH using phenolphthalein as an indicator. The tests were done in triplicates on coated and uncoated samples. The results are expressed in citric acid (%) (Maftoonazad, 2006).

$$\text{Titratable acidity (\%)} = \frac{V(NaOH)(0.1)(0.064)}{m} \times 100 \qquad (3.4)$$

where $V_{(NaOH)}$ is the mL of NaOH used during titration, (0.1) is the molarity of the NaOH solution used, (0.064) is the conversion factor for citric acid which is the main acid in strawberries and (m) is the mass of the strawberry samples used during the test (Velickova et al., 2013).

3.7. Total Soluble Solids (TSS)

Total soluble solids (TSS) were measured using a hand refractometer (ATAGO N1, Kirkland, DC, USA) and expressed in ºBrix scale. Samples were homogenised using a blender and few drops of strawberry filtrate were placed on the prism glass of the refractometer. Direct readings were taken. Tests were done in triplicates on both coated and control samples (Gol et al., 2013).

3.8. Yeasts and Molds Analysis

Microbial growth including yeasts and molds were analysed using the plate count method. 10 grams of different control and coated samples were transferred to a blender with 90 mL of saline peptone water (0.1 g peptone/100 mL water). After blending, a serial dilution was done and pour-plated onto Plate Count Agar (PCA). Plates were incubated for 5 days at 25 ºC for yeast and mold counts. Colony count was expressed as Log CFU/mL of strawberries. Tests were done every three days in triplicates on both control and coated samples during the two weeks of storage (Moreira et al., 2015).

3.9. Statistical Analysis

A statistical analysis system (Analysis ToolPak in Excel) was used to conduct One-way ANOVA at 95% level of confidence and 5% level of significance. The significance level used was ($p<0.05$). The effect of the edible coating on pH, moisture loss, respiration, TSS, Titratable acidity, firmness, color and microbiological count on strawberry cut fruits was evaluated (Maftoonazad, 2006).

4. Results and Discussion

During the storage of strawberry cut fruits at 4 ºC, tests were done every 3 days on both control and coated samples. Results were analysed to show the effect of edible coating on the shelf life and different quality parameters of strawberry cut fruits as detailed below:

4.1. Respiration

The respiration rates of control and coated samples were measured over the period of storage. Results are shown in (Figure 3.5). The amounts of CO_2 produced were measured every 1 min for 2 h using 150 g of both control and coated strawberry cut fruits. The results were taken during the first hour until the saturation of CO_2 (Velickova et al., 2013).

Based on the methodology, the respiration rates measured in (mL CO_2/kg.h) were higher in control samples than in coated fruit samples over the whole period of the experiment starting from day zero. Sodium alginate-calcium chloride edible coating played an effective role in decreasing the respiration rates in coated samples by reducing the amounts of CO_2 produced. On day zero the respiration rate in control samples was 104 mL/kg.h while it was 71.6 mL/kg.h in coated samples. Respiration rates decreased in control samples on day 3 to 81.3 mL/kg.h, while in coated samples it decreased to 64.7 mL/kg.h. On day 6 the respiration rates in control samples increased to 123.7 mL/kg.h and stayed stable until day 9 before it decreases to 68.5 mL/kg.h on day 15. In coated samples respiration rate increased to 99.6 mL/kg.h on day 9, before it decreases to 55.5 mL/kg.h on day 15 (Figure 3.5).

As can be observed in Figure 3.5. respiration rates in control and coated samples decreased with storage time starting from day 9, with a lower rate of respiration in coated samples. This behavior is observed in non-climacteric fruits such as grapes and strawberries that show a gradual decrease in the respiration rates during ripening (Thompson, 2016). While

climacteric fruits such mangoes and bananas show a rapid rise in the respiration rates during ripening (Gopala Rao, 2015)

Sodium alginate-calcium chloride edible coating reduced the respiration rates in coated strawberry cut fruits acting as a gas barrier, which caused a modification in the internal atmosphere and slowed down the respiration rates. One-way ANOVA statistical analysis showed a significant difference between the control and coated samples starting from day 6 ($p<0.05$).

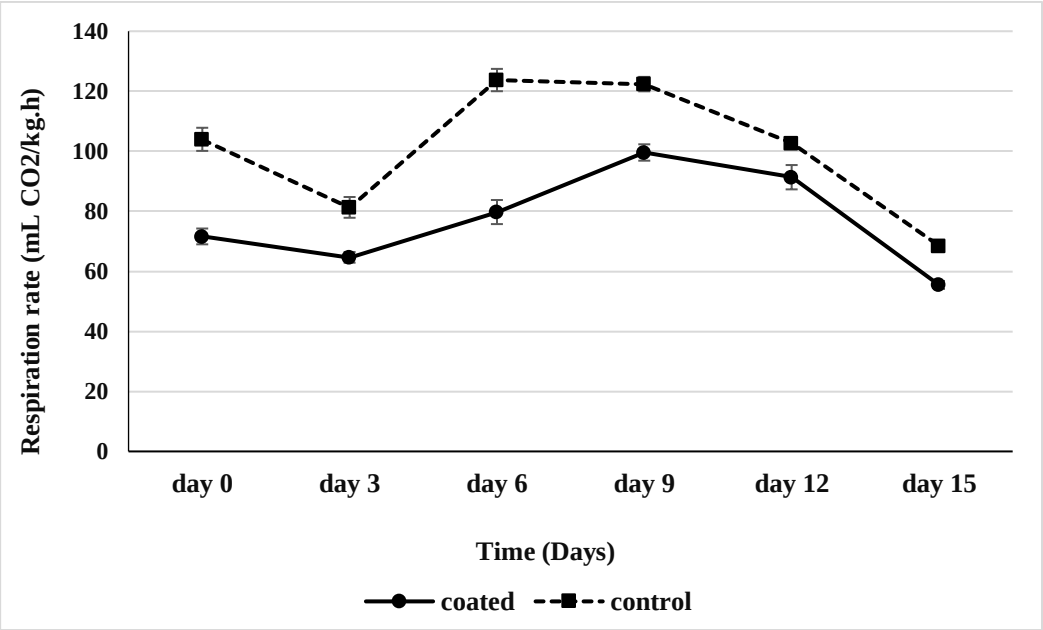

Figure 3.5: Respiration rate (mL CO_2/kg.h) vs time (days) in control and coated samples

Similar behavior was reported by Garcia et al. (2012) in cassava starch coated strawberries stored at 5 ºC for 15 days. High respiration rates were observed in treated and untreated samples on the first day of storage, followed by a significant reduction from day 1 to day 5. After 5 days of storage an increase in the respiration rates was noticed. The stress induced by fruit's cutting, handling and packaging causes an increase in the respiration rates, which explains the high values observed on day zero in both control and coated samples. Also, the

increase in the respiration rates after 5 days of storage can be explained by the fruit ripening and microbial growth (Garcia et al., 2012).

Other studies have showed that calcium dips can be effective in reducing respiration rates in addition to extending the shelf life of cut fruits. Lamikanra and Watson. (2004) reported a decrease in the respiration rates in cut cantaloupe fruits dipped in calcium salts solution and stored at 4 ºC.

Azarakhsh et al. (2012) also reported that alginate and gellan-based edible coating helped in reducing the respiration rates in pineapple cut fruits stored at 10 ºC. Also, it was found that Aloe Vera gel-based edible coating can reduce the respiration rate and microbial growth in sliced kiwifruits (Benitez et al., 2013). The edible coating act as an effective semi-permeable barrier to respiratory gases which creates a modified atmosphere surrounding the fresh produce and delays fruits ripening, maturation and decay (Gol et al., 2013).

4.2. Transpiration

The moisture loss (%) in control and coated samples over the 15 days of storage were measured and shown in Figure 3.6. Transpiration rates (expressed as moisture loss percentage) were measured in triplicates for fruit samples stored at 4₀ C. Based on the results of analysis during the 15 days of the experiment, the moisture loss percentages in coated samples were significantly lower ($p<0.05$) than in control samples. The weight loss in control samples after 3 days of storage was 5.2% while it was 4.0% in coated samples. Transpiration rates increased in both control and coated samples during the experiment. On day 15, moisture loss in control samples reached 13% while in coated samples it increased to 11%. Moisture loss in coated samples was reduced due to the water vapour barrier formed by the sodium alginate-calcium chloride edible coating on the fruit's surface (Figure 3.6). One-way ANOVA statistical analysis

showed that there is a significant difference in moisture loss among the control samples and among the coated samples starting from day zero ($p<0.05$). While, a significant difference in moisture loss between control and coated samples was observed starting from day 6 till day 15 ($p<0.05$).

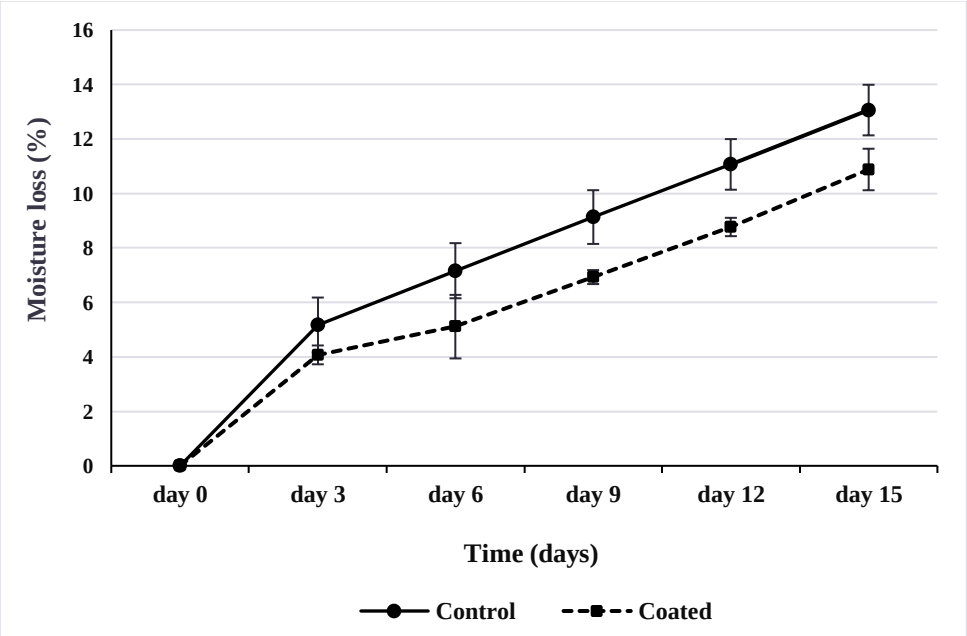

Figure 3.6: Moisture loss (%) vs time (days) in control and coated samples

Moisture loss is also related to quality loss and the reduction in weight and volume (shrinkage or shrivelling) of the fresh produce. Senturk Parreidt et al. (2018) also reported that the reduction of moisture loss in coated fruits during storage played an important role in the shelf life extension of the strawberry cut fruits. The thickness of the applied coating and the water vapour barrier are also essential factors that affect the mass transfer rates (Senturk Parreidt et al., 2018). Moisture loss was reported to be reduced when methylcellulose coating was applied on avocados (Maftoon Azad, 2006).

Strawberries coated with chitosan-calcium salts and stored at 2 ºC showed also a weight loss reduction during storage. Adding calcium salts into the coating matrix helped in reducing moisture loss (%) in the coated strawberries. Calcium salts can act as fillers and increase the counter ions interactions among the neighbouring molecules within the matrix, which decreased the hydrophilic tendency of the chitosan coating and its water vapor permeability (Han et al., 2004). Moisture loss has also been reported to be reduced in strawberries coated with hydroxypropyl methylcellulose and carboxymethyl cellulose (CMC) with the incorporation of 1% (w/w) chitosan and stored at 11ºC for 8 days (Gol et al., 2013).

4.3. Color Analysis

Color of control and coated strawberry cut fruits were evaluated over the 15 days of storage. The color of strawberry cut fruits was evaluated based on the Lightness (L value), green/red components (a* value) and Chroma (C value). The blue/yellow components (b* value) and the Hue angle (H value) didn't show any significant difference between the control and coated samples to be analysed (Maftoonazad, 2006).

4.3.1. L value

The decrease in L value is an indicator of surface darkening (González-Aguilar et al., 2009). On day zero both control and coated samples had similar values. L value in control samples showed a dramatic decrease starting from day 3. The L value declined from 31.6 on day zero to 20.8 on day 15.

In coated samples, the L value was almost stable until day 6. The L value decreased on day 9 and reached 26.7 on day 15.

The L value was higher in coated samples than in control samples during the whole period of the experiment, which indicates that the sodium alginate-calcium chloride edible coating prevented oxidative enzymatic browning (Figure 3.7). One-way ANOVA statistical analysis showed a significant difference in the L value between the control and sodium alginate-calcium chloride coated samples ($p<0.05$).

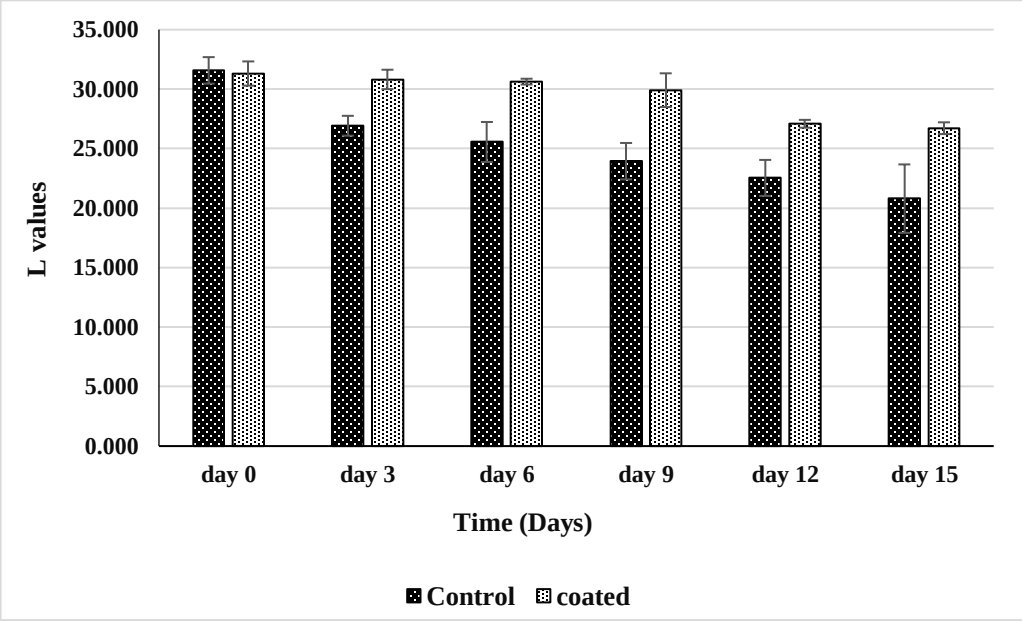

Figure 3.7: L values vs time (days) in control and coated samples

It has been reported that the L value decreased in uncoated cherry tomatoes, pectin coated cherry tomatoes and alginates coated cherry tomatoes stored at different temperatures (4 ºC and 12 ºC). However, L value was higher in coated samples than in control samples over the 21 days of storage (Narayanapurapu, 2012).

4.3.2. a* value

The a* value indicates the green/red components. On day zero, a* value was almost the same in control and coated samples with values of 32.4 and 32.6 respectively. In control samples the a* value slowly decreased during storage and reached 26.4 on day 15. The decrease in the

a*value in control samples might be a response to surface darkening and the formation of a red-brownish color. Also, surface mold growth negatively affected the redness of the fruit samples. The a* value in sodium alginate-calcium chloride coated samples was almost stable during the 15 days of the experiment. Coated samples didn't show a dramatic change in the surface color or tissue darkening (Figure 3.8). However, one-way ANOVA statistical analysis didn't show a significant difference between the control and sodium alginate-calcium chloride coated samples during storage ($p>0.05$).

It was also reported that the a* value increased slightly in sodium alginate coated cherry tomatoes and pectin coated cherry tomatoes stored at 4 ºC and 12 ºC. In control samples the a* value was higher due to the increased redness of the cherry tomatoes during the 21 days of storage (Narayanapurapu, 2012).

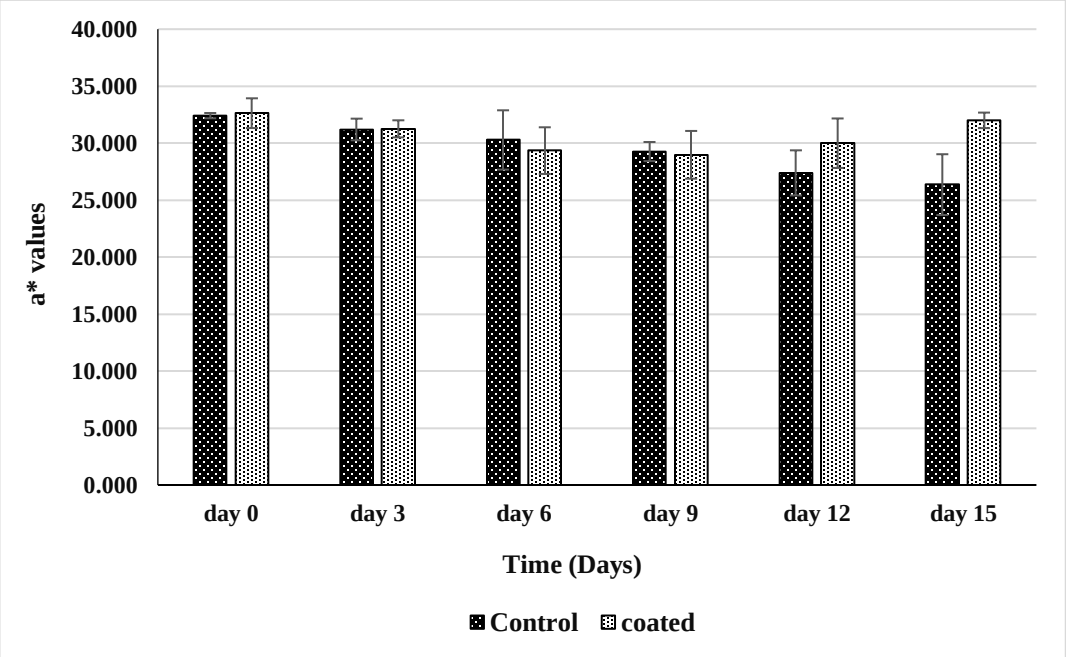

Figure 3.8: a* values vs time (days) in control and coated samples

4.3.3. C value

C value which is the Chroma of color was also measured. The C value in coated samples slightly decreased from 38.3 on day zero to 33.9 on day 9 and increased again on day 12 to finally reaches 36.2 on day 15. In control samples the C value gradually decreased from 37.8 on day zero to 29.9 on day 15. The C value was lower in control samples during the whole experiment. The difference between control and coated samples was mainly observed on day 12 and day 15 due to the maturation of fruits, surface browning and mold growth in control samples (Figure 3.9). However, one-way ANOVA statistical analysis didn't show a significant difference in the C values between control and sodium alginate-calcium chloride coated samples ($p>0.05$).

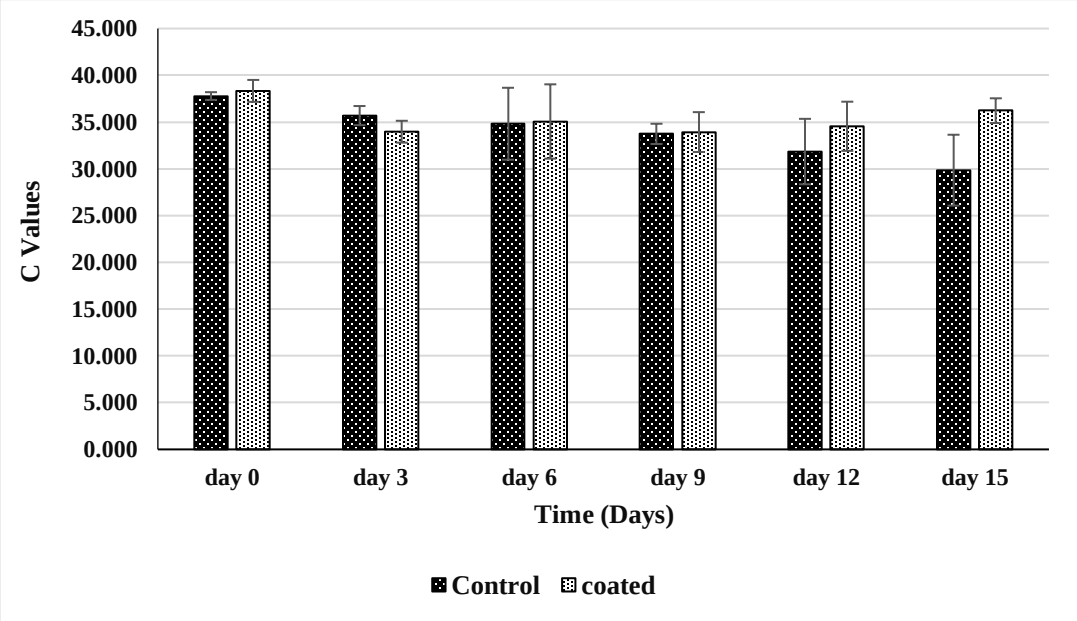

Figure 3.9: C value vs time (days) in control and coated samples

Collins and Perkins-Veazie (1993) also observed a decrease in the Chroma of the uncoated strawberries stored for 8 days at 1 ºC. During storage, the color of the fruits became

less vivid due to the development of a red-brownish color in the fully ripe strawberries. The edible coating maintained the Chroma of the color during storage (Gol et al., 2013).

4.4. Texture

Firmness of strawberry cut fruits was measured using 7 to 10 samples of control and coated samples. Results are shown in Figure 3.10. Firmness of coated strawberry cut fruits decreased during the 15 days of storage at 4 ºC. However, coated samples showed better results than control samples. A beneficial effect in the firmness retention was observed in coated samples during the 15 days of the experiment. Since day zero the texture of coated samples showed higher values than control samples due to the added calcium chloride salts that act as firming agents, as well as the lowered respiration and transpiration rates.

The firmness of control samples decreased from 57.3 N on day zero to 41.2 N on day 3, while in coated samples the firmness was almost stable during the first 6 days of storage. On day 6 firmness in control samples showed a dramatic decrease and declined to 11.8 N at the end of the experiment on day 15 with an extremely soft texture. In coated samples, firmness declined only to 36.6 N on day 15, thus remained firm.

Results showed the positive effect of the sodium alginate-calcium chloride edible coating on the texture of strawberry cut fruits, since a good texture was maintained over the whole period of the experiment. Values observed in coated fruits were three times higher than in control samples (Figure 3.10). One-way ANOVA statistical analysis showed a significant difference in the firmness among the control samples starting from day 0 till day 15 ($p<0.05$). However, among coated samples no significant difference was observed until day 9. Also, a significant difference in the firmness between control and sodium alginate-calcium chloride coated samples was observed starting from day zero till day 15 ($p<0.05$).

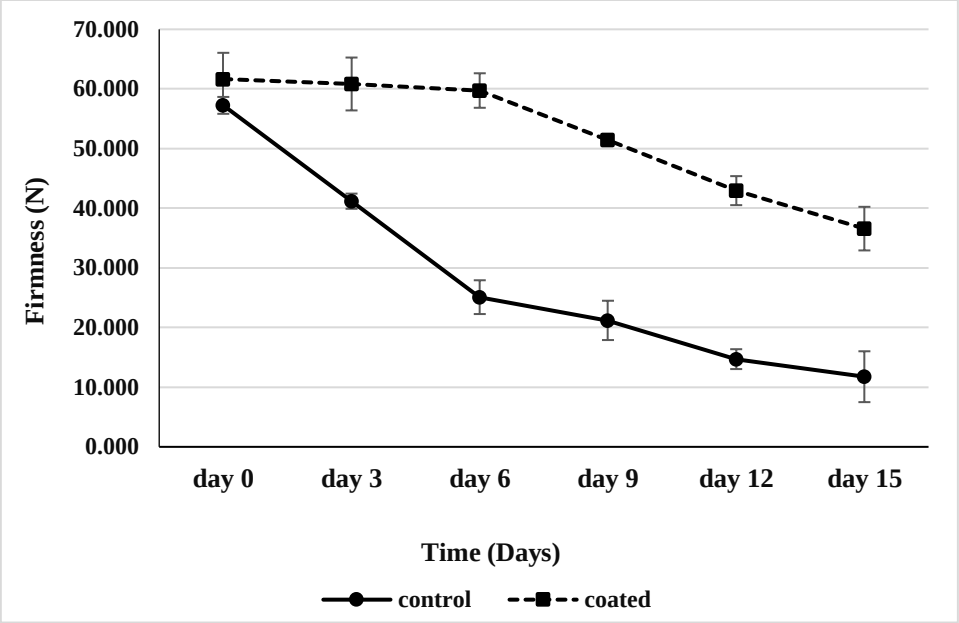

Figure 3.10: Firmness (N) versus time (days) in control and coated samples

Strawberry fruits have a fragile texture due to their particular structures, large cells and thin cell walls. The decline in the firmness of strawberries is an indicator of quality deterioration. Fruits become soft due to the biochemical changes in the cell turgidity and cell walls compositions. The changes are shown by a degradation of the middle lamella of the cortical parenchyma cells and a decrease in the pectin content (Velickova et al., 2013). Alginate-ascorbic acid coating have showed an improvement in the firmness of fresh cut papaya during storage (Tapia et al., 2008). Also, the addition of calcium chloride salts was reported to play an effective role in maintaining the firmness of the coated samples during storage acting as firming agents. Chitosan-oleic acid coating has been used to preserve the texture of strawberries (Vargas et al., 2008). Also chitosan coating was reported to maintain the firmness of fresh cut papaya stored at 5 ºC (González-Aguilar et al., 2009).

4.5. pH Measurement

The effect of edible coating on the pH of fruits is shown in Figure 3.11. A significant increase in the pH of the control samples over the period of storage was observed. The pH of the control samples was higher than the pH of coated sample during the 15 days of the experiment. On day zero the pH of control and coated samples was 3.6. The pH of control samples gradually increased and reached 3.8 on day 12, while in coated sample the pH was almost stable from day zero till day 12. The sodium alginate-calcium chloride edible coating reduced the pH of the coated samples in comparison with control samples. Also, it delayed the increase in the pH values during storage (Figure 3.11). One-way ANOVA statistical analysis showed a significant difference between the control and sodium alginate-calcium chloride coated samples during the 15 days of storage ($p<0.05$).

It has been reported that sodium alginate-based edible coating delayed the increase in the pH of the fruit samples, which delayed fruits' ripening and mold growth by maintaining the acidity of the fruits (Narayanapurapu, 2012). Moreover, the increase in the oxygen levels during respiration can also increase the pH of the strawberry cut fruits. Similar results were obtained in strawberries coated with Carboxymethylcellulose (CMC). A delay in the increase of the pH in coated strawberries was observed (Gol et al., 2013). Also, CMC-calcium chloride coating with the incorporation of ascorbic acid showed effective results in reducing the pH of fresh cut apples (Koushesh Saba & Sogvar, 2016).

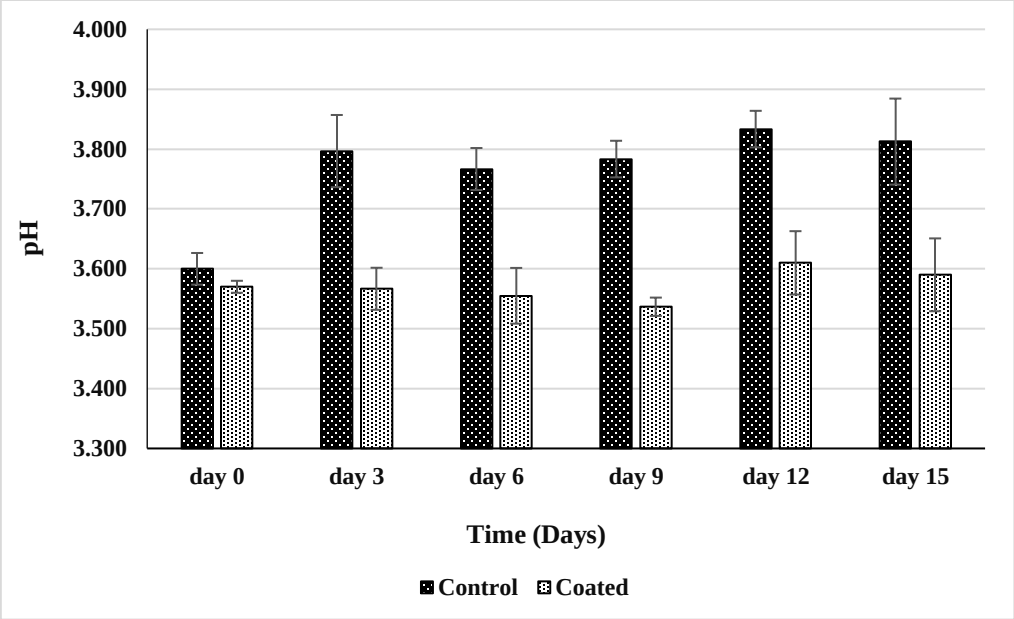

Figure 3.11: pH change vs time (days) in control and coated samples

4.6. Titratable Acidity

Titratable acidity was measured and expressed in citric acid (%). Based on the tests results, coated fruits have a higher citric acid (%) than uncoated samples. On day zero titratable acidity was almost the same in both control and coated samples. The acidity decreased at a slightly rate in coated samples during the 15 days of storage. On day 6, a significant decrease in TA was observed in control samples and the value reached 0.088%, while in coated samples it decreased to 0.091%. On day 9, TA decreased in coated samples to 0.081% and stayed almost stable till day 15. Also, in control samples TA declined on day 9 to 0.076% and stayed stable till day 15 with a value of 0.078% (Figure 3.12).

Coated fruits had higher TA (%) during the 15 days of the experiment. Sodium alginate-calcium chloride edible coating minimised the reduction of fruits' acidity (Figure 3.12). However, one-way ANOVA statistical analysis didn't show a significant difference in TA (%) between the control and coated samples ($p>0.05$).

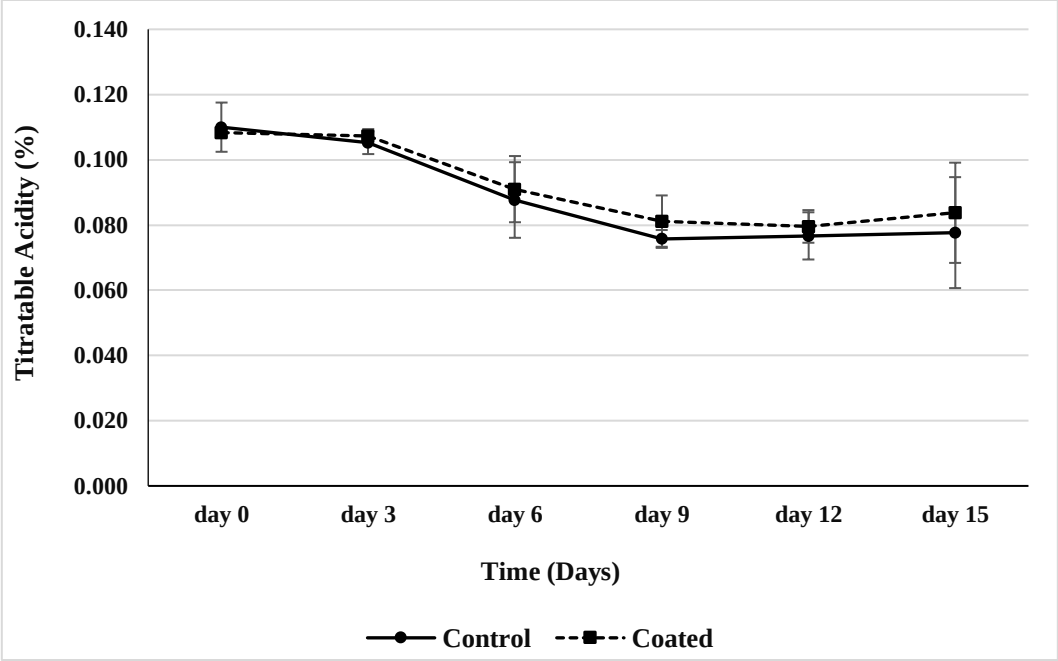

Figure 3.12: Titratable Acidity (%) vs time (days) in control and coated samples

Titratable acidity is related to the organic acid content in strawberries. Acidity decreases in the late stages of fruits' ripening, due to the use of the organic acids during respiration. Coating of strawberries with carboxymethylcellulose (CMC) and HPMC have showed a delay in the decrease in TA (%) in strawberries. The edible coating reduced the loss of ascorbic acid during the 16 days of storage, by reducing oxygen diffusion and respiration rates, which caused ascorbic acid retention (Gol et al., 2013). Slower rates of decreased acidity were also observed in pectin coated cherry tomatoes stored at different temperatures (Narayanapurapu, 2012). Moreover, methyl cellulose coated peaches showed a delayed reduction in the titratable acidity during storage (Maftoonazad, 2006).

4.7. Total Soluble Solids (TSS)

The total soluble solids content (ºBrix) is used as an indicator of fruit's maturity, and it is measured in fruits to study their maturation rates. Based on different studies, the total soluble

content (TSS) in fruits increases during storage. Edible coating can reduce the TSS content by delaying fruit's ripening (Narayanapurapu, 2012).

During the 15 days of storage, the TSS in control samples was higher than in coated samples, which suggests that sugars are synthesized in a slower rate due to the sodium alginate-calcium chloride edible coating. On day zero the values of TSS were 7.7 in control samples and 7.6 in coated samples. TSS in control samples increased to 8.3 on day 6, while in coated samples TSS remained almost stable from day 0 till day 6 with a value of 7.6. The edible coating reduced the rates of carbohydrates breakdown and delayed fruit's maturation (Figure 3.13). The decrease in the TSS content at the end of the experiment is an important indicator of the maturation of fruits since it can indicates fruits overripening (Yan et al., 2019). One-way ANOVA statistical analysis showed a significant difference between control and sodium alginate-calcium chloride coated samples during the experiment ($P<0.05$). However, no significant difference was observed among the control samples and among the coated samples during storage ($p>0.05$).

TSS content in strawberry fruits showed an increase in the values followed by a decrease, which represents the difference between commercially desired maturity and overripening. A decrease in the TSS content is observed in overripe fruits when compared with half ripen fruits (Yan et al., 2019). Similar behavior was detected on day 12 in control samples (Figure 3.13). Also, similar results have been reported in a study when TSS (%) in strawberries declined from 8.5 on day 4 to 5.7 after 8 days of storage (Gol et al., 2013)

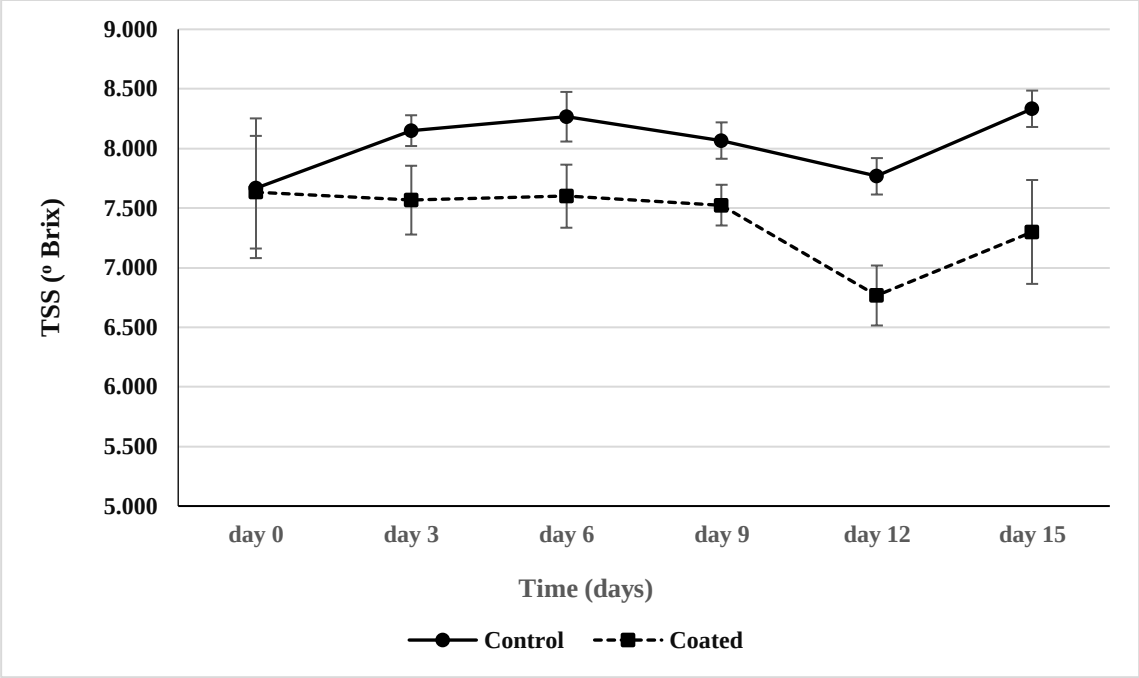

Figure 3.13: Total soluble solids (ºBrix) vs time (days) in control and coated samples

The increase in TSS content is related first to the increased moisture loss and the increased concentration of soluble solids. Moreover, it is related to the breakdown of complex carbohydrates into soluble solids due to the respiration and ripening of fruits. Starch is degraded rapidly into sugars such as sucrose, glucose, and fructose due to the activity of amylases, starch phosphorylase and 1,6-glucosidase enzymes (Narayanapurapu, 2012).

Carboxymethylcellulose (CMC), hydroxypropylmethyl cellulose (HPMC) and composites with chitosan (CH) have also been used to coat strawberries stored at 11 ºC. TSS content was lower in coated samples than in control samples. Cellulose derivatives reduced the TSS (%) in coated strawberries and delayed fruit's maturity (Gol et al., 2013). Also, it was reported that chitosan-beeswax coating delayed the increase in TSS content in strawberries stored at 20 ºC (Velickova et al., 2013).

4.8. Yeasts and Molds Analysis

A plate count analysis was performed every 3 days on control and sodium alginate-calcium chloride coated samples. On day zero, almost similar values were observed in control and coated samples. After only 3 days of storage the number of yeasts and molds in control samples increased to 5.9 log CFU/mL, while in coated samples it increased to 5.1 log CFU/mL. On day 6 minor mold growth was observed in control samples and the values increased to 6.15 log CFU/mL and reached 6.25 log CFU/mL on day 9. On day 12 the number of colonies for control samples was too high to count.

In coated samples the number of yeasts and molds slightly increased to 5.54 log CFU/mL on day 6 and reached 5.92 log CFU/mL on day 12. On day 15, minor mold growth was observed on the surface of the coated samples and the number of yeasts and molds has increased to 6.14 log CFU/mL (Figure 3.14). Sodium alginate-calcium chloride edible coating reduced mold growth in strawberry cut fruits. Mold growth reduction is also related to the citric acid retention and the delayed increase in the pH of the coated cut strawberries. One-way ANOVA statistical analysis showed a significant difference between the control and sodium alginate-calcium chloride coated samples ($p<0.05$).

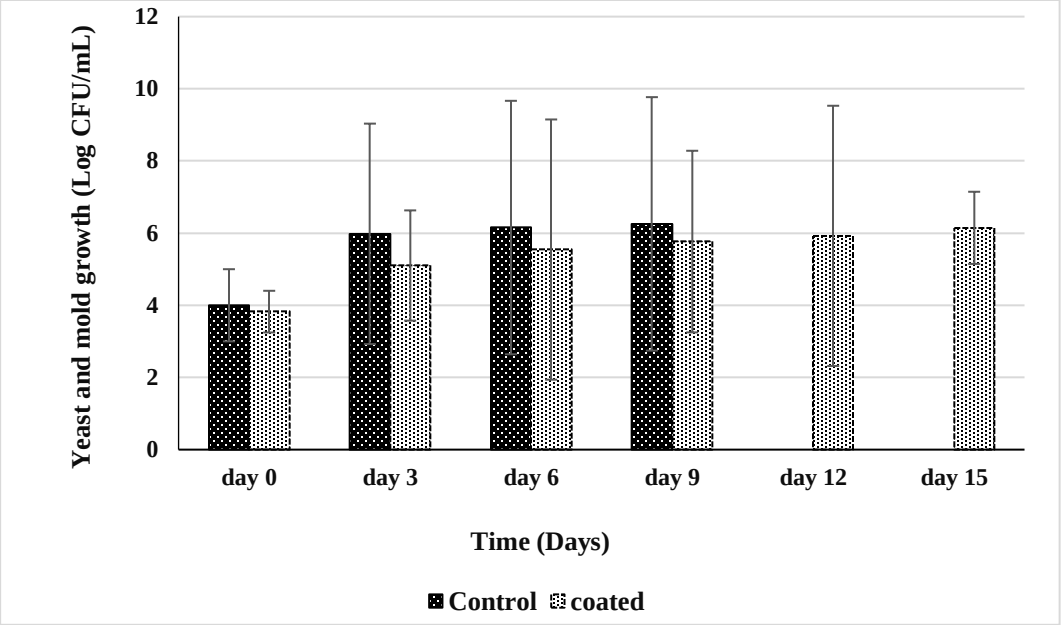

Figure 3.14: Yeast and mold growth (Log CFU/mL) vs time (days) in control and coated samples

Based on the Institute of Food Science and Technology (IFST), the number of yeasts and molds in coated samples during the 15 days of storage were within the acceptable limits of these microorganisms in fresh fruits which is 6 log CFU/mL (Khodaei & Hamidi-Esfahani, 2019).

It has been reported that chitosan based edible coating reduced the decay and mold growth in strawberries and raspberries stored at 2 ºC and 88% RH. Mold growth appeared on the surface of the control samples only after 5 days of storage while in coated samples mold growth was delayed (Han et al., 2004). Carboxymethyl cellulose (CMC) with the incorporation of probiotic *Lactobacillus plantarum* was also used to coat strawberries stored at 4 ºC for 15 days. A delay in yeasts and mold growth was observed in coated strawberries (Khodaei & Hamidi-Esfahani, 2019). In another study, chitosan coating reduced mold and yeast growth in fresh cut papaya and reduced mesophilic plates counts (González-Aguilar et al., 2009). Also, starch-based

coating with the incorporation of cold pressed seed oil (*Nigella sativa* oil) was reported to delay microbial growth in pomegranate arils (Oz & Ulukanli, 2012).

4.9. Appearance

Minor mold growth was observed on the surface of control samples on day 6 while in coated samples mold growth was observed only on day 15. Control samples stored at 4 ºC had an acceptable visual quality during the first 6 days of storage before the beginning of decay and mold growth. After 6 days of storage the color of the control samples became darker, with a minor appearance of mold growth on the surface. The color of the coated samples became a bit darker after 6 days with no mold growth (Figure 3.15). After 12 days of storage control samples had mold growth, very soft texture and browning while coated samples showed no mold growth and a slight darkening in the color (Figure 3.16). On day 15, control samples were completely grey with mold growth, and extremely soft with a watery texture. Coated samples showed a minor mold growth on the surface of some samples with tissue softening and color darkening (Figure 3.17).

The addition of calcium chloride salts helps in enhancing the texture of strawberry cut fruits since they can act as firming agents. Also, it helps in reducing mold growth, physiological disorders and oxidative browning (Soazo et al., 2015).

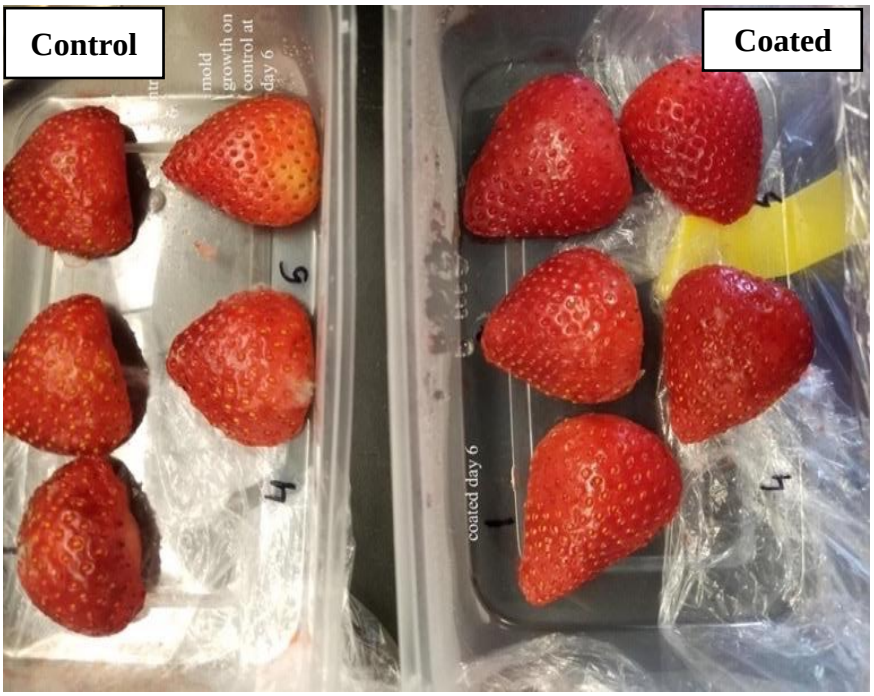

Figure 3.15: control and coated fruits on day 6

Figure 3.16: Control and coated fruits on day 12

Figure 3.17: Control and coated fruits on day 15

5. Conclusions

Minimally processed fruits and vegetables usually show uneven responses to edible coatings due the differences in their tissue structures, surface texture, turgidity and metabolic activity. However, a successful adhesion of the coating solution on the fruit surfaces can extend their shelf life and provide a fresh-like appearance (González-Aguilar et al., 2009).

Results demonstrated that the sodium alginate-calcium chloride edible coating extended the shelf life of strawberry cut fruits stored at 4 ºC for up to 15 days. The edible coating reduced transpiration and respiration rates acting as a protective barrier, which caused the reduction of mold growth and preserved the sensory properties of the cut strawberries. Also, sodium alginate-calcium chloride edible coating delayed the increase in the TSS content and the pH of the cut fruits through citric acid retention. The use of sodium alginate-based edible coating on cut fruits can help both consumers and producers reduce food losses and save money.

CONNECTING STATEMENT TO CHAPTER IV

In Chapter 3, sodium alginate-calcium chloride edible coating was evaluated for extending the shelf-life of strawberry cut fruits and the results indicated a full two-weeks of shelf life extension in coated samples in comparison with control samples under refrigerated storage conditions. In this chapter, the same concept is extended for another type of processed product, the dehydro-frozen pineapple slices. Problems associated with this product include discoloration, off flavor, and drip loss. The coating evaluation in this research is to prevent these changes since shelf-life extension is usually implicit in frozen preservation. Partial dehydration using microwave assisted osmotic dehydration under continuous flow medium spray condition (MWODS) technique was employed. The effects of MWODS technique and sodium alginate-calcium chloride edible coating on the quality parameters of frozen pineapple cut fruits are evaluated as detailed in this chapter.

CHAPTER IV

THE EFFECT OF MICROWAVE ASSISTED OSMOTIC DEHYDRATION AND SODIUM ALGINATE-CALCIUM CHLORIDE EDIBLE COATING ON FROZEN-THAWED PINEAPPLE CUT FRUITS

ABSTRACT

Osmotically dehydrated pineapple cut fruits were coated with sodium alginate-calcium chloride coating solutions and frozen stored at -20 ºC for 10 and 50 days. The effects of the microwave assisted osmotic dehydration under continuous flow medium spray condition (MWODS) and edible coating on the quality parameters of frozen pineapple cut fruits after thawing were evaluated. MWODS was used instead of the conventional osmotic pre-treatment prior to freezing to partially reduce moisture content in pineapple cut fruits without much solids gain. MWODS technique helps reducing treatment time and enhances the quality of the final produce by also reducing solids gain. As expected, higher moisture loss as well as reduced solid gain and processing time, all indicative of better quality, were obtained after the application of MWODS on the pineapple cut fruits in comparison with conventional osmotic drying.

Furthermore, sodium alginate-calcium chloride edible coating significantly ($P<0.05$) reduced the drip loss in dehydro-frozen pineapple cut fruits after thawing and enhanced their sensory properties such as color and texture. The enhancement of the product texture also resulted from the calcium chloride treatment following the sodium alginate coating applied on the surface of the cut fruits due to its effect as a firming agent.

1. Introduction

Refrigerated storage is a short-term food preservation method, while freezing is a long-term preservation method. Freezing has been used successfully for decades to preserve a variety of different food products for weeks, months and even years. However, freezing changes several characteristics of the food products including their texture, appearance and other sensory attributes such as color, aroma and flavor. Microbial growth which is generally the most serious concern in food preservation is the least concern in freezing preservation since the water needed for the growth and activity of microorganisms is made completely unavailable due to their conversion into ice. So generally frozen foods are microbiologically safe. However, enzyme and chemical activities continue at a slow rate even under frozen storage conditions which can lead to off flavors and discoloration. The change in the overall quality of the food product sometimes negatively affects the consumer acceptance. Pre-treatments such as blanching (for vegetables), and short time osmotic dehydration (for fruits) can be used prior to freezing to inactivate the enzymes or reduced the available oxygen. Partial osmotic drying can also be used to reduce moisture content and enhance the quality of the frozen products after thawing (Rahman, 1999).

Pineapple is widely consumed around the world and it is the third most important tropical fruit after banana and citrus with a sweet and sour flavor. Pineapple is mainly produced in Thailand, Philippine, Brazil and Costa Rica where it is commonly sold fresh in the market. However, processed pineapples are very popular in the international trade in the form of canned slices, juice and frozen chunks. Frozen pineapple cut fruits are commonly available in the market due to their use in the production of smoothies (Mantilla, 2012).

Pineapple fruits are rich in phenolic compounds and minerals such as potassium, sodium, phosphorus, magnesium, sulphur, calcium and iodine (Singh et al., 2010). Also, they are good sources of vitamin C (22.4 ± 0.9 mg/ 100g of pineapple) and have a high moisture content reaching 87% of its initial weight (Mantilla, 2012). Pineapples are also excellent sources of bromelain enzyme that can be used in meat tenderization (Singh et al., 2010). Due to their high moisture content, a pre-treatment is required prior to freezing pineapples to prevent drip loss and minimise the losses of natural flavors, color and nutrients after thawing (Mantilla, 2012).

1.1. Osmotic Dehydration

Osmotic dehydration is a partial dehydration used as a pre-treatment to connective drying, pasteurization and freezing to enhance the product's quality. During osmotic dehydration fresh fruits or vegetables are dipped in a hypertonic solution, which is usually a solution concentrated with sugars, inorganic salts or mixed osmotic agents. Inorganic salts are calcium chloride and sodium chloride, while sugars include sucrose, lactose and maltodextrin. However, sucrose has been considered as the best dry substance to be used due to its effectiveness, convenience, and flavor desirability (Falade & Igbeka, 2007).

Osmotic dehydration is based on two simultaneous mass transfer phenomena. When the food produce is placed in a highly concentrated solution, water moves from the product where there is a high-water potential into the surrounding medium (Li, 2005). Water is accompanied with natural substances such as color pigments, vitamins and flavor components. In the opposite direction, solutes move from the concentrated solution into the food samples through their semi permeable biological membrane. Mass transfer during osmosis causes solid gain, moisture loss and weight reduction, however the final products are not considered yet as microbiologically stable. Furthermore, subsequent treatments are required (Agnelli et al., 2005). Moisture loss from

the fresh products can be up to 70% of the initial weight, but in most cases, it is between 30% to 50%. On the other hand, solid gain can reach 5 to 25% of the initial weight of the fresh produce depending on the method used. While the purpose of dehydration is to remove moisture and achieve an increase in the concentration of solids, this should be done through concentration of sugars already present rather than infusing sugar from the osmotic solution. Hence the driving force for osmotic dehydration is high rate of moisture loss and low solids gain. It is suggested that the moisture loss to solids gain ratio (ML/SG) be used as a criterion for optimization of the quality of osmotically dehydrated products. The concept of ML/SG was actually introduced as an index of product quality, with higher ML/SG promoting better quality (Shinde & Ramaswamy, 2019).

The temperature of the osmotic solution also plays an important role since the use of high temperatures can promote faster water movement, better mass transfer and higher rates of moisture loss (Li, 2005). After osmosis, the resulting product will have preserved color, texture, flavor and aroma and it should have a minimised risk of microbial growth due to the reduced water activity (Matuska et al., 2006).

1.2. Disadvantages of Osmotic Dehydration

Osmotic dehydration has a lot of beneficial effects on the sensory properties of fruits and vegetables when used as a pre-treatment for drying and freezing. However, osmotic dehydration has several disadvantages. This dehydration technique is both energy and time consuming, especially during the dehydration of high moisture fruits such as cranberries. Extensive solids gain and slow rates of water loss can also be experienced.

During osmotic dehydration, valuable solids and components leach out from the fruit's matrix into the solution, which negatively affects the sensory properties and nutritional content of fruits due to the loss of pigments and nutrients (Falade & Igbeka, 2007). The large solids gain

during osmosis can also form a concentrated solid layer below the external surface of the fresh produce. This solid layer reduces the driving force of water flow and creates a resistance to mass transfer, which causes a reduction in the dehydration rates. In addition, solid gain modifies the composition and the natural taste of the final produce, which explains the high sweetness of the osmotically dehydrated fruits (Singh et al., 2010).

1.3. Microwave assisted Osmotic Dehydration in Spray Mode (MWODS)

Conventional osmotic dehydration is time and energy consuming due to the slow rates of mass transfer (Li, 2005). Many technologies have been introduced to enhance the parameters of the conventional osmosis including vacuum, ultrasound, high pressure and microwave drying (Matuska, Lenart, & Lazarides, 2006). Microwave drying allows the diffusion of the microwave energy into the produce, which creates a fast and uniform heating within the product. Microwave assisted osmotic dehydration (MWOD) is designed by Li and Ramaswamy (2006) and it is an excellent alternative for the enhancement of conventional osmotic dehydration. This technique enhances mass transfer during osmosis and ameliorates the quality parameters of the final products (Li & Ramaswamy, 2006).

MWOD is designed by placing an osmotic dehydration chamber within the microwave cavity, and fruits are fully immersed in the osmotic solution, but the solution is continuously circulated in and out of the microwave system. Under immersion mode, microwave assisted osmotic dehydration is known as MWODI. The heat generated by the microwave (MW) field and absorbed by the water molecules in the syrup and fruit pieces creates an internal pressure gradient that enhances water movement to the surface of the produce. This is because there is more water inside the fruit than outside it in the osmotic solution. Moreover, the MW

enhances the out-flux of water / water vapour together with the external osmotic pressure enhances the dehydration process and reduces processing time (Li, 2005).

Using MWODI, less solutes will be gained by the fruits since the high-water outflow rate can cause a resistance for the inflow of the solids. As a result, this process was found to be very successful and the final product was reported to have more natural flavor, enhanced color, texture and nutritional properties (Li, 2005). This MWODI process was subsequently modified to MWODS which is microwave osmotic drying with a continuously moving osmotic solution in a spray mode rather than immersion mode (Azarpazhooh & Ramaswamy, 2010). The advantage of the MWODS system over the MWODI was that unlike the immersion system which completely covers the fruit pieces, this only showers the osmotic solution over the fruit pieces. Thus, in the MWODS system there is more opportunity for the microwave to reach the fruits and penetrate deeper into the fruit pieces, thereby facilitating a greater outflux for the water to move out of the fruit. This was first successfully applied to apples (Azarpazhooh & Ramaswamy, 2010) then extended to cranberries (Wray, 2015) and finally to mango (Shinde & Ramaswamy, 2019). All these systems favor better moisture loss, limit solids gain, provide a better ML/SG ration and a shortened treatment time.

1.4. Freezing Preservation

Freezing is the most widely used method for long-term food preservation, and it is commonly used by the food industry due the increased demand on non-seasonal products. Freezing is based on a change in the physical state through the conversion of water into ice, which allows the reduction of microbial growth and enzymatic activities during storage. It also delivers food products with a better maintained quality, good sensory properties and high safety degrees (Fennema & Powrie, 1964).

Freezing might have damaging effects on the quality of the food products and it is affected by the freezing rates, storage time and temperatures and thawing methods. Freezing rate has the major influence on the quality of the frozen products, since it determines the size and structure of the formed ice crystals. Also, it affects the cell dehydration and cell wall structures. Slow freezing forms large ice crystals in the food products and causes quality loss and tissue damage after thawing (Soazo et al., 2015). However, fast freezing forms small ice crystals and doesn't affect negatively the quality and sensory parameters of the frozen produces (Rahman, 1999).

1.5. Freezing Damage

Osmotic damage, solute-induced damage and structural damage are the three different types of cell damage that can be observed during freezing. Osmotic damage mainly occurs during slow cooling and it causes cell shrinkage, membrane damage and high drip loss during thawing. Osmotic damage is caused by the formation of ice crystals in the external cells after the movement of water from inside the cells (Rahman, 1999).

Solute-induced damage can be observed in slow and fast freezing and it is caused by the increased solutes concentrations during freezing which causes cellular damage. Solute-induced damage is mainly observed in products with high salt concentrations, for this purpose, cryoprotectants such as sugar can be added prior to freezing (Rahman, 1999).

Structural damage is caused by the formation of ice crystals within the cells, which allows the destruction of the cell membranes and organelles and releases the enzyme systems. Heat treatment such as blanching can be used prior to freezing to inactivate the enzymes and prevent the development of off flavors (Rahman, 1999).

1.6. Osmo-Dehydro-Freezing

In order to minimize the damaging effects of freezing on the quality parameters of the food products, partial removal of water through osmotic dehydration can be used as a pre-treatment (Rahman, 1999). This method in known by the osmo-dehydro-freezing and it enhances the quality characteristics of the frozen products such as their color, flavor and texture. Osmo-dehydro-freezing reduces the amount of free water in the fresh produce that can freeze later and cause cell wall rupture and tissue damage (Zhao et al., 2014).

Economic advantages can also be achieved through Osmo-dehydro-freezing. The partial removal of moisture from fresh produces prior to freezing allows the reduction of the weight and size of the food products. Also, it decreases storage loads which reduces packaging and distribution costs. Osmo-dehydro-freezing can also reduce energy use since less energy is required to freeze partially dehydrated products in comparison with fresh untreated products (Agnelli et al., 2005).

1.7. Research Objective

The objective of this research was to first partially reduce moisture content in pineapple cut fruits prior to freezing. Osmotic dehydration has been used as a pre-treatment for such purpose (Yan et al., 2019). In this research study, microwave assisted osmotic dehydration under continuous medium flow spray condition (MWODS) was used instead of the conventional osmosis to reduce processing time and solids gain. The secondary objective was to use sodium alginate-calcium chloride edible coating applied to the MWODS pre-treated pineapple cut fruits and freeze store at -20 ºC and evaluate the product quality following two storage periods (10 and 50 days). The final objective was to evaluate the effect of storage time, MWODS pre-treatment

and sodium alginate-calcium chloride edible coating on the quality parameters of the dehydro-frozen pineapple cut fruits.

2. Materials and Methods

2.1. Sample Preparation

Fresh pineapples were purchased from the market to prepare the fruit samples by cutting the pineapple fruits into 3 cm thickness slices in triangular shapes.

2.2. Conventional Osmotic Dehydration (CO)

Conventional osmotic dehydration was performed on pineapple cut fruits using the method of Ferrari et al. (2013) with few modifications. During CO, 40 ºBrix sucrose solution was used and the product / solution mass ratio was 1:10. Osmotic dehydration was carried with agitation (120 rpm) for 4 h at 40 ºC .

2.3. Microwave Assisted Osmotic Dehydration in Spray Mode (MWODS)

The schematic of the experimental setup is illustrated in Figure 4.1. The experimental setup was described before by Azarpazhooh and Ramaswamy (2010), Wray and Ramaswamy (2015) and Shinde and Ramaswamy (2019). MWODS setup consists of a commercial spray head (Waterpik, Model RPB-173C, 12.5 cm diameter, Waterpik Technology Inc., Markham, ON, Canada) placed on a custom made glass sample chamber (12.5cm diameter) and located inside a domestic microwave (Danby DMW1153BL 0.031 m^3, Guelph, ON, Canada) with a nominal power output of 1100 W at 2450 MHz. The fruit samples are placed in a nylon mesh on a porous acrylic plate “stage” inside the glass chamber. The stage allows the recycling of the dripped osmotic solution collected inside the glass chamber and the osmotic solution will then be pumped at the required flow rate using a peristaltic pump (Model 75211-30 Digital gear pump, Barnant Company, IN) through the spray head to rinse the fruit samples. The osmotic solution is

then collected and pumped through coils inside a steam-jacketed water bath (Model TDB/4 Groen division, Dover Corp, IL). The temperature of the osmotic solution is monitored using a pair of in-line Type-T thermocouples connected to the digital thermometer (Omega DP-462, Omega Technology, Laval, QC). The thermocouples are placed immediately before and after the microwave cavity to measure the temperature of the osmotic solution. The temperature of the water bath is set based on the required temperature of the osmotic solution.

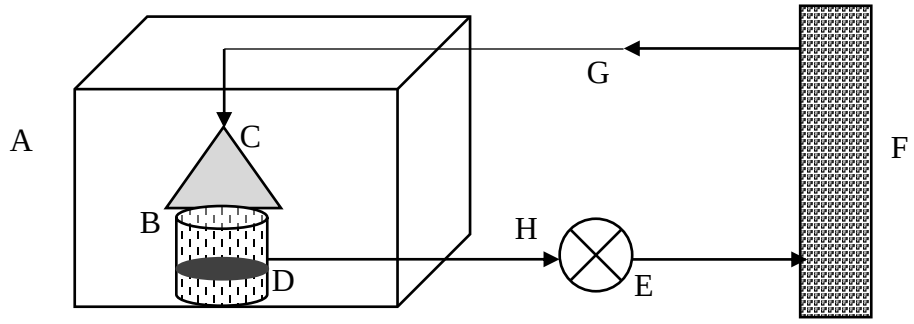

Figure 4.1: Schematic diagram of MWODS setup

A: microwave oven cavity, B: microwave transparent sample chamber, C: spray head, D: stage, E: digital gear pump, F: water bath (containing heat exchanging coils, not pictured), and G and H are thermocouple measuring points immediately before and after the solution enters and leaves the microwave cavity, respectively.

The experiment was carried on using a 40 ºBrix sucrose solution. Pineapple cut fruits were placed in a small plastic bag and transferred into the osmotic dehydration chamber inside the microwave. The sugar solution was sprayed on the fruit samples placed on the perforated acrylic plate inside the MW compatible container (100 g/run) in the osmotic chamber within the microwave, at 40 ºC for 10 minutes with a flow rate of 1500 mL/min. Temperature was

monitored, and the syrup circulation system was continuous from the bottom of the chamber up to the spray head. The experiment was carried for 10 min since it is a pre-treatment for freezing.

2.4. Coating Solutions Preparation

The methodology involves first the use of sodium alginate coating to infuse some alginate on to the surface of the fruits. Sodium alginate is water soluble and can be easily washed out and therefore a second dip in calcium chloride was used, which would replace the sodium ions by calcium ions resulting in texture forming calcium alginate. The methodology used by Gamboa-Santos & Campañone., (2018) was used with few modifications. Calcium chloride salts were used instead of calcium lactate salts due to their effect as firming agents. Distilled water was used to prepare the coating solutions of 2% (w/w) sodium alginate (Sigma, Oakville, ON) and 2% (w/w) calcium chloride (Sigma, Oakville, ON). To prepare the sodium alginate solution, sodium alginate powder was added to distilled water and the beaker was placed on a magnetic stirring rod at 300 rpm with no heat until the sodium alginate powder was completely dissolved. To prepare the calcium chloride solution, calcium chloride salts were added in a volumetric flask with distilled water. The volumetric flask was shaken twice upside-down to dissolve the calcium chloride salts.

Rodriguez, Garcia and Campañone., (2016) have also used the similar methodology but with different concentrations of the coating solutions. 1% (w/w) sodium alginate and 10% (w/w) calcium chloride were used as concentrations of the coating solutions. However, in this experiment, a low concentration of calcium chloride was used since high concentrations can cause the development of bitter flavors (Rodriguez et al., 2016).

2.5. Coating of Samples

To prepare the MWODS-alginate coated samples, the osmotically dehydrated pineapple cut fruits using MWODS technique were placed in a fabric mesh and dipped completely into the 2% (w/w) sodium alginate solution for 5 min. Fruit samples were removed and drained for 1 min in a plastic mesh and dipped again into the 2% (w/w) calcium chloride solution for 5 min. Samples were removed, drained and left on a filter paper for 30 minutes at room temperature.

Same procedure was used to prepare the CO-alginate coated samples. Osmotically dehydrated pineapple cut fruits using conventional osmotic dehydration (CO) were placed in a fabric mesh and dipped completely into the 2% (w/w) sodium alginate solution for 5 min. Fruit samples were removed and drained for 1 min in a plastic mesh and dipped again into the 2% (w/w) calcium chloride solution for 5 min. Samples were removed, drained and left on a filter paper for 30 minutes at room temperature (Gamboa-Santos & Campañone, 2018).

2.6. Freezing and Storage

Control and coated samples were stored in plastic containers, packaged in zip-sealed polyethylene bags and stored in the freezer at (-20 ± 2 ºC) for two different time periods (10 and 50 days) before thawing at room temperature (Han et al., 2004) .

2.7. MWODS Experimental Procedure

The entire experimental procedure including sample preparation, MWODS pre-treatment, coating solution preparations, coating, freezing and storage is shown schematically in Figure 4.2.

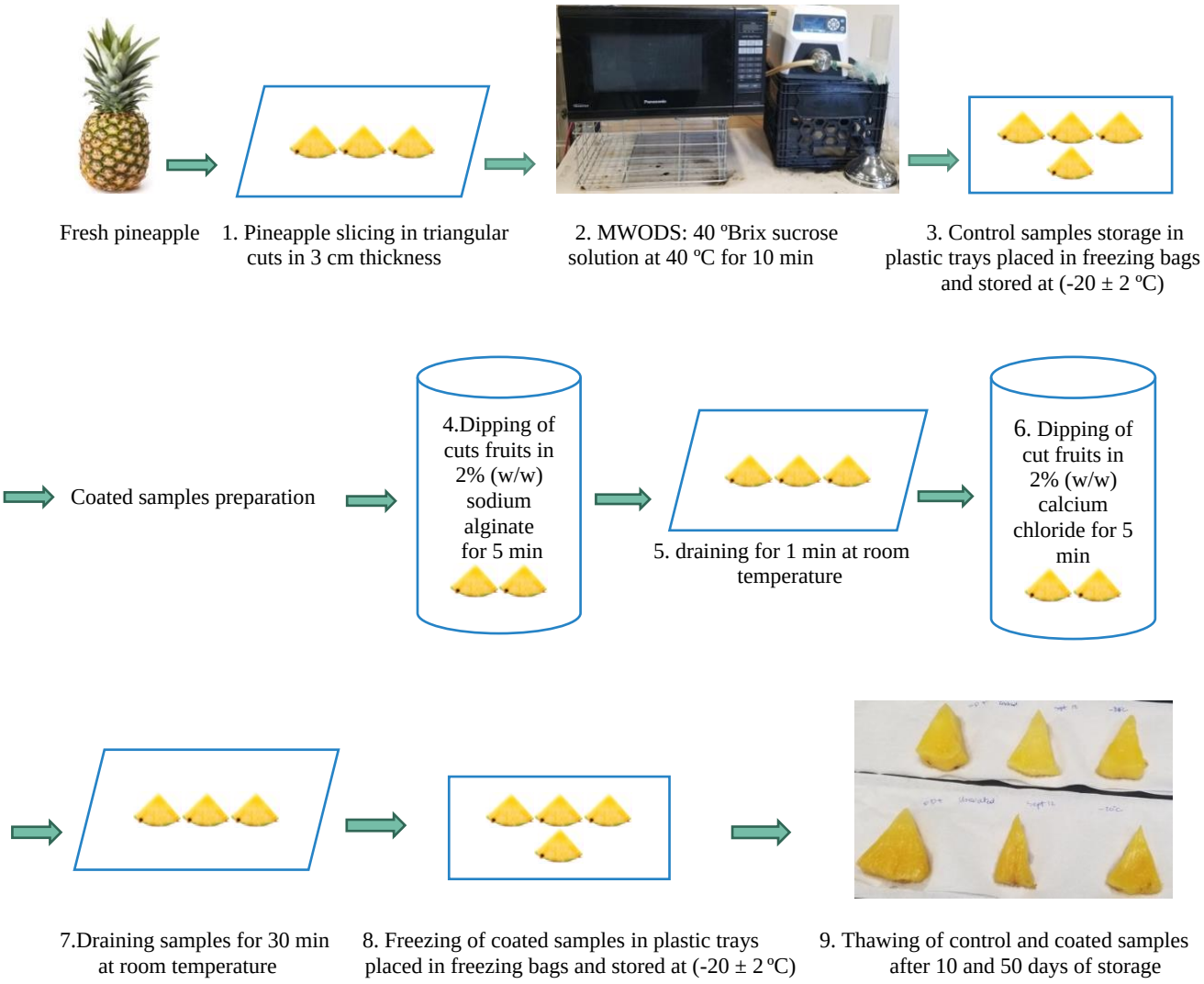

Figure 4.2: Experimental procedure for Objective 2

3. Experimental Tests

3.1. Weight Reduction, Moisture Loss and Solid Gain

A digital balance (Denver instrument, APX-323, NY, USA) was used to measure the weight of the pineapple fruit samples before and after osmotic dehydration (CO and MWODS) to calculate the weight reduction percentage after osmotic dehydration. Oven method was used to determine moisture content in both fresh and osmotically treated fruit samples using CO and

MWODS. Fruit samples were kept overnight in a hot air oven at 105 ºC. Following equations should be used to calculate Weight Reduction (%), Moisture Loss (%) and Solid Gain (%):

- $WR\ (\%) = 100\ \frac{(M0 - Mt)}{M0}$ (4.1)
- $SG\ (\%) = 100\ \frac{(Mt\ st - M0\ s0)}{M0}$ (4.2)
- $ML\ (\%) = 100\ \frac{(M0\ x0 - Mt\ xt)}{M0}$ (4.3)

where M_0 is the mass of the sample at time zero and M_t is the mass of the sample at time t. s_t is the solid fraction (dry matter) at time t and s_0 is the solid fraction at time 0. x_0 is the moisture fraction at time 0, while x_t is the moisture fraction at time t (Wray, 2015).

3.2. Ratio of Moisture Loss over Solids Gain

The ratio of ML/SG was used to describe osmotic dehydration efficiency and calculated with ML over SG (Azarpazhooh & Ramaswamy, 2010).

$$\text{Ratio} = \frac{ML}{SG} \quad (4.4)$$

3.3. Drip Loss

Drip loss is calculated by taking the weight of the thawed samples every 10 minutes for 1 hour at room temperature, until constant weight is reached. The difference between the weight measured at any time (t) and the initial weight of the sample was described as the drip loss (%) (Dermesonlouoglou et al., 2016).

$$\text{Drip Loss (\%)} = \frac{\text{Weight before thawing} - \text{weight after thawing}}{\text{Weight before thawing}} \times 100 \quad \text{(Zhao et al., 2014).} \quad (4.5)$$

3.4. Texture

Seven to ten samples from each lot (CO-coated, MWODS-coated and uncoated) are subjected to a puncture test. TA. XT plus Texture Analyzer (Texture Technologies Corporation, Scarsdale, N.Y., U.S.A./ Stable Micro Systems, Godalming, Surrey, U.K.) fitted with a 25 mm diameter round tipped puncture probe is used, with test speed of 10 mm/sec. Texture analysis is performed to determine the force deformation and firmness of the fruit samples based on a force-deformation curve with the measurements in Newton (N) (Maftoonazad, 2006).

3.5. Color

The color characteristics of pineapple cut fruits were measured using a calorimeter. tristimulus Minolta Chroma Meter (Minolta Corp, Ramsey, NJ) is used to determine the L value (lightness), a* (green-red chromaticity) and b* (yellow-blue chromaticity). Calorimeter is calibrated using a white standard. Readings are done at room temperature on four to five samples of pineapple cut fruits. Also, Chroma which is the color intensity and the hue angle will be used based on the values provided by the colorimeter or calculated using the following equations (Maftoonazad, 2006):

$$\text{Chroma} = (a^2 + b^2)^{½} \quad (4.6)$$

$$\text{Hue angle} = \tan^{-1}(b/a) \quad (4.7)$$

3.6. Statistical Analysis

A statistical analysis system was performed using (Analysis ToolPak in Excel). The analysis of variance was performed using One-way ANOVA and Two-way ANOVA at 95% level of confidence and 5% level of significance. The significance level used was ($p<0.05$) (Maftoonazad, 2006).

4. Results and Discussion

4.1. Weight Reduction

Weight reduction to achieve a residual moisture content of 20% or below is required for the drying of fresh produce to result in safety and stability and to protect the quality characteristics. However, prior to freezing, removal of 2% to 10% of the initial weight through osmo-dehydro-freezing can enhance the texture of the products after thawing (Zahoor & Mohammad, 2017).

Weight reduction (WR) was measured in the pineapple cut fruits after MWODS and after conventional osmotic dehydration (CO). WR in fruit samples using MWODS technique at 40 ºC and 40 ºBrix sucrose solution, was on average 14.8% after only 10 min of MWODS treatment. However, using conventional osmosis (CO), the WR reached ~14.4% only after almost 4 h of treatment of the pineapple cut fruits. MWODS was therefore very effective in reducing the treatment time (Figure 4.3). A slight increase in the WR was also observed in the osmotically dehydrated fruit samples using MWODS as a result of the application of sodium alginate-calcium chloride based edible coating.

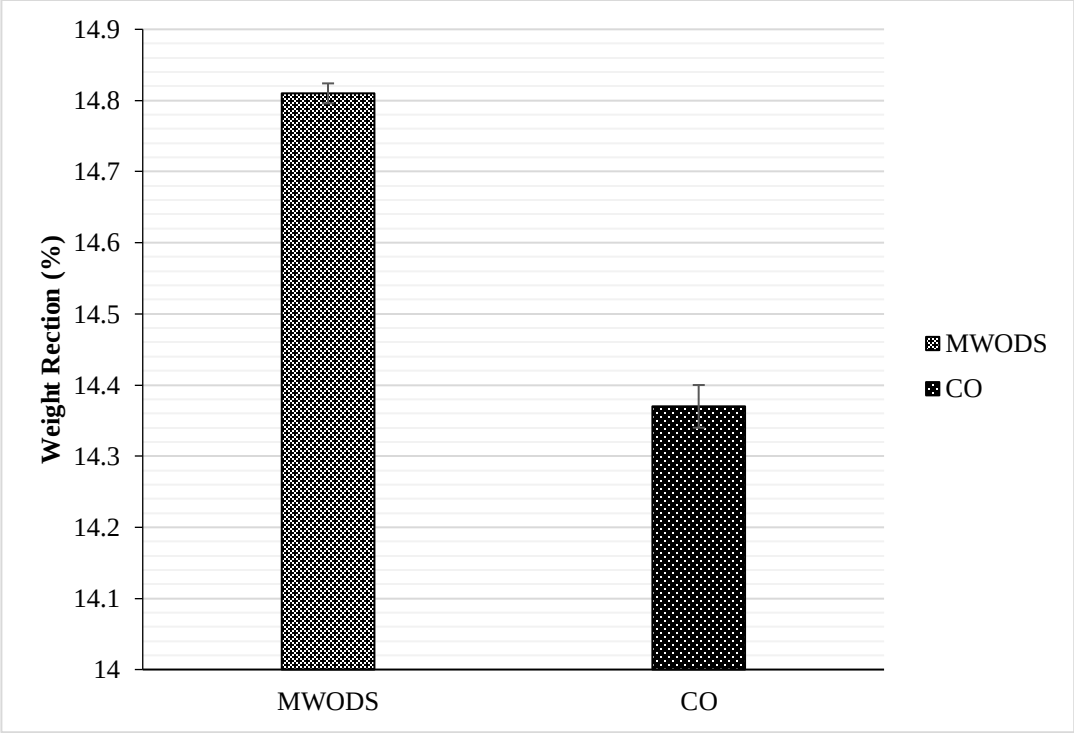

Figure 4.3: Weight reduction (%) in osmotically dehydrated samples using conventional osmotic drying (CO) and MWODS technique

Several previous works also reported long treatment times associated with conventional osmotic dehydration (CO) for achieving a significant degree of weight loss in fruits. Silva et al., (2014) reported that using conventional osmotic dehydration using 40% and 60% sucrose solutions at 27 ºC, the time required to reach a weight reduction of 24% to 40% of the initial sample weight was approximately 6 h of dehydration. Increasing the temperature and concentration of the sucrose solution can increase the weight reduction in the fruit samples (Li, 2005).

It was also observed that using microwave assisted osmotic dehydration under spray conditions (MWODS) at 40 ºC using 40 ºBrix sucrose solution, as used in this study, an average of 20 min was reported to be required to reach 20% weight reduction of the initial weight

of apple samples (Azarpazhooh & Ramaswamy, 2010). However, 210 min were required to achieve the same WR (%) under similar condition using conventional osmotic dehydration.

4.2. Moisture Loss

After 10 min of MWODS, moisture loss (ML) in pineapple cut fruits was 16.03%. After the subsequent dipping of the MWODS treated samples in the sodium alginate and calcium chloride coating solutions, ML increased as a result of the coating treatment. However, ML in pineapple cut fruits reached 19.1% in conventional osmotic dehydration but only after 4 h of treatment (Figure 4.4).

It was reported that in apple cylinders osmotically dehydrated using conventional osmosis (CO) and microwave osmotic dehydration by immersion (MWODI), higher ML (%) was obtained using MWODI in comparison with CO (Li & Ramaswamy, 2006) treated for the same time. Microwave treatment increased the speed of water transfer and diffusion through the tissues of the fresh fruits.

Li and Ramaswamy (2006), also found that under microwave osmotic dehydration by immersion (MWODI) at 40 ºC and 40 ºBrix, the moisture loss after 2 h reached 25%. However, a much higher moisture loss of 44% was obtained by Azarpazhooh and Ramaswamy (2010) in MWOD under spray mode (MWODS) due to better penetration of microwaves in the apple tissue using the spray mode as compared to the immersion mode. The spray mode (MWODS) also extended the moisture loss to 50% after 2 h of treatment. The enhanced microwave heating effect was reported to be behind the higher moisture loss in the microwave and medium circulation in the spray mode compared with the conventional osmosis (Azarpazhooh & Ramaswamy, 2010).

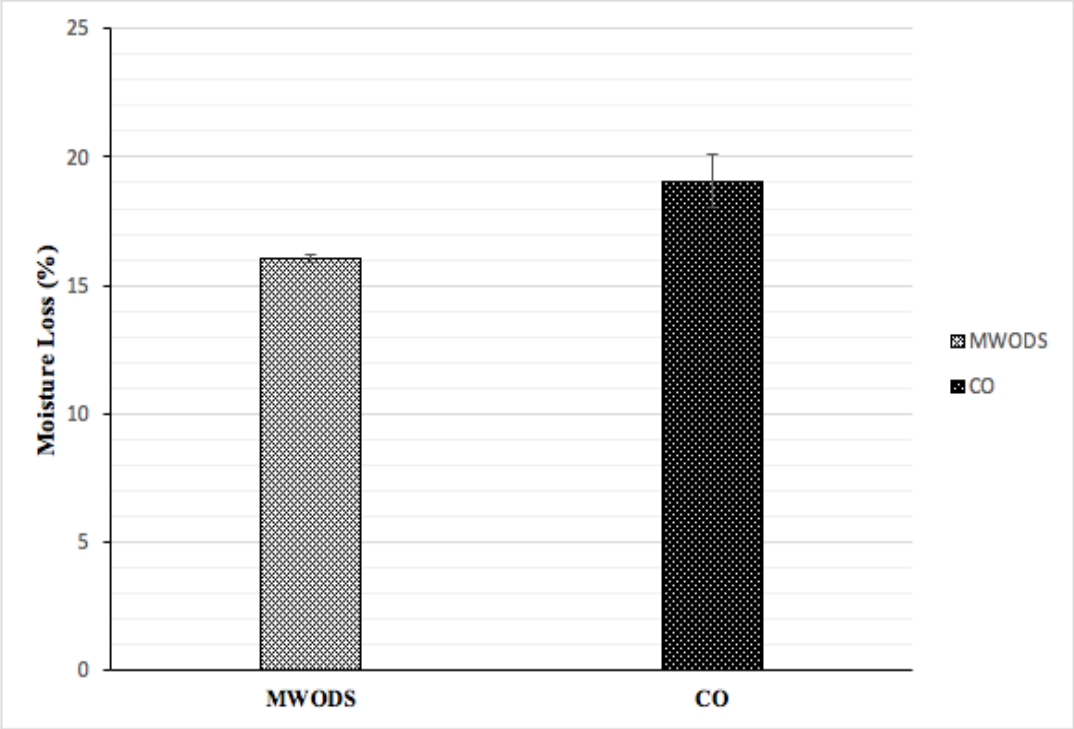

Figure 4.4: Moisture loss (%) in osmotically dehydrated samples using conventional osmotic drying (CO) and MWODS technique

It was observed that the coating of pineapple cut fruits after MWODS technique with sodium alginate and calcium chloride solutions caused a small increase in ML (%). This was caused by the precipitation of calcium chloride salts on the surface of pineapple tissue which resulted in an increase in the solids gain, resulting in a subsequent lowering of moisture content and constituting moisture loss. It also could have come from additional moisture loss facilitated by the different surface structure on the pineapple tissue due to calcium alginate deposits, which could alter the mass transfer properties. Similar results were observed in a study, where the effects of calcium salts on pineapple cut fruits during osmotic dehydration were evaluated. The addition of calcium lactate salts during the osmotic dehydration of pineapple cut fruits using 40% and 50% sucrose concentrations were reported to result in an increase in the ML (%) in the fruit samples (Silva et al., 2014).

4.3. Solid Gain

After 10 min of MWODS, the solid gain (SG) in the fruit samples was 2.5%. SG (%) in pineapple cut fruits was lower in the samples treated with MWODS in comparison with conventional osmosis (CO) where the SG reached 4.4%. Using one-way ANOVA statistical analysis, a significant difference in SG was observed between the samples treated with MWODS and the samples treated with CO ($p<0.05$). MWODS reduced SG which is a desirable effect (Figure 4.5).

According to Li., (2005) apple cylinders osmotically dehydrated for 3 h using 30 ºBrix sucrose solution at 50 ºC under MWODI, showed a decrease in the SG by 7.3% when compared with the conventional osmotic dehydration under the same conditions. The difference in the SG (%) is mainly contributed to the effect of the microwave energy in increasing the outflux of moisture diffusion which would retard the influx of solids infusion.

Using MWODS on apple fruits, solids gain was reported between 2.5 and 3% in the first 30 min of treatment and gradually increased to 3- 4.5% after 120 min of treatment. Also, higher solids gain was obtained using conventional osmosis in comparison with MWODS. They reported conditions favoring rapid moisture loss result in a simultaneous reduction in the solids gain. (Azarpazhooh & Ramaswamy, 2010).

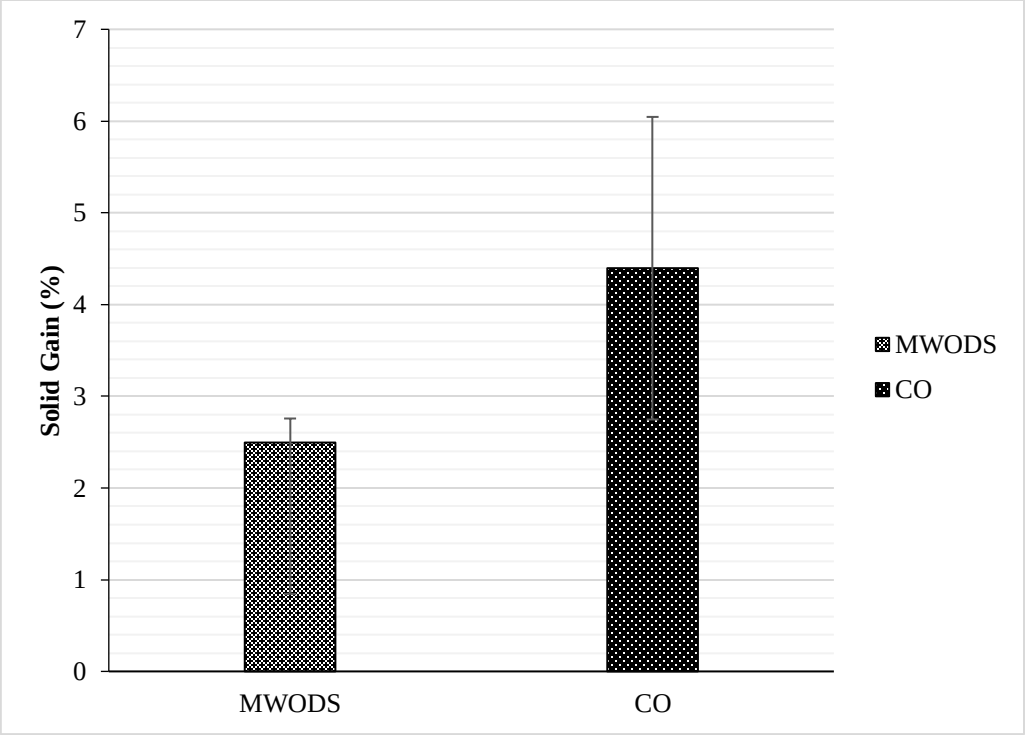

Figure 4.5: Solid gain (%) in osmotically dehydrated samples using conventional osmotic drying (CO) and MWODS technique

After the application of the sodium alginate-calcium chloride edible coating on the osmotically dehydrated samples using MWODS an increase in the SG was noticed, which is because of the absorption of the coating material. Using one-way ANOVA statistical analysis, a significant difference in SG (%) was observed between MWODS samples and MWODS-coated samples ($p<0.05$). The addition of calcium chloride after MWODS treatment which results in the formation of calcium alginate complex, explains the increase in the solid content in the coated samples.

Different behavior was observed by Matuska et al., (2006). The coating of fruits before osmotic dehydration reduced solids gain in the fruit samples. A decrease in SG (%) was observed in sodium alginate coated strawberries in comparison with uncoated samples after osmotic dehydration. Same effect was also observed after the addition of 0.6% (w/w) calcium

lactate to the sucrose solution during osmotic dehydration of apples. Solid gain reduction was reported to be caused by the decreased porosity of the fruit's cell walls after coating. However, in this research study an increase in the solid content was observed in MWODS-coated samples since sodium alginate-calcium chloride edible coating was applied after osmotic dehydration (Matuska et al., 2006).

The efficiency of the mass transfer in fruit samples also depends on the structure of their tissues. Pineapple cut fruits osmotically dehydrated for 2 h using 40% sucrose solution and calcium lactate salts, had higher solid gain than cut melons treated under the same conditions (Silva et al., 2014).

4.4. Ratio of Moisture Loss over Solids Gain (ML/SG Ratio)

The ratio of moisture loss to solids gain is an important indicator for optimization of the osmotic dehydration. ML/SG ratio was significantly higher in samples treated under MWODS in comparison with conventional osmotic dehydration (~6 vs ~4) (Figure 4.6). It is desirable to have a higher moisture loss and a lower solids gain. Similar behavior was observed in osmotically treated apple cylinders under MWODI and CO (Li and Ramaswamy, 2006), MWODS and CO with apple (Azarpazhooh & Ramaswamy, 2010), cranberry (Wray & Ramaswamy, 2015) and mango pieces (Shinde & Ramaswamy, 2019). Higher ML to SG ratio was reported to be indicative of better-quality dehydrated product.

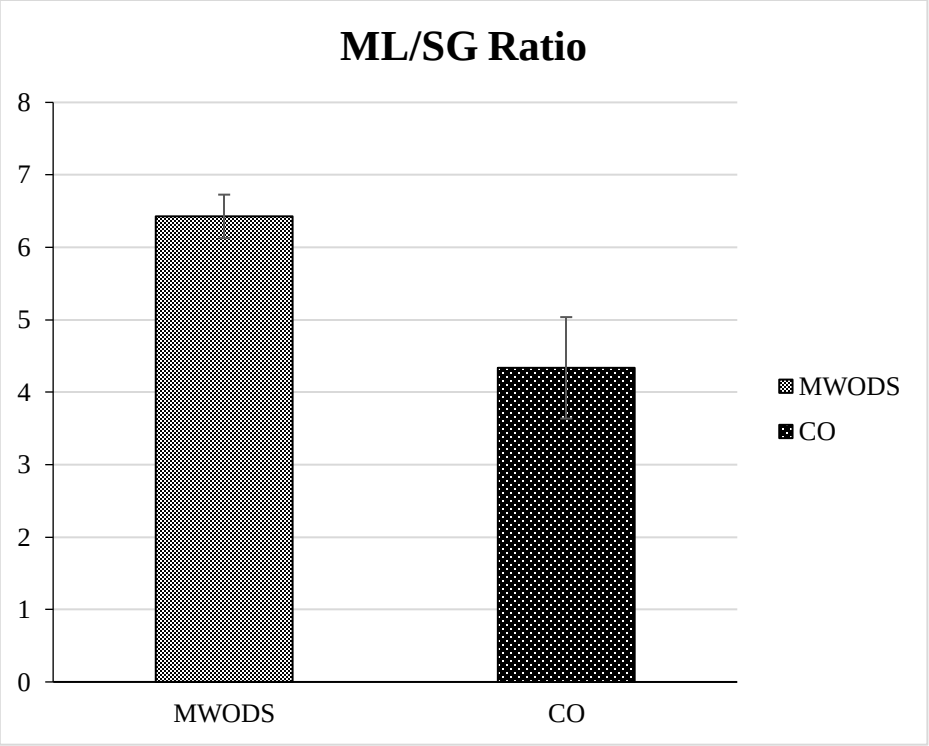

Figure 4.6: ML/SG Ration in osmotically dehydrated samples using conventional osmosis (CO) and MWODS technique

4.5. Drip Loss

Drip loss is mainly related to the formation of ice crystals within the cells of the fresh produce during freezing. The increase in the drip loss (%) indicates high recrystallization rates leading to the growth of ice crystals. Freezing storage can also be associated with multiple changes in fresh fruits due to their high moisture contents. The formation of ice crystals causes a rupture in the cell walls which induces mechanical damages and quality deterioration of the food products (Sirijariyawat & Charoenrein, 2012).

4.5.1. Effect of MWODS on Drip Loss

Osmotically dehydrated samples using conventional osmosis (CO) showed discoloration and extremely soft texture following 4 h of treatment, for this purpose samples treated with

conventional osmosis were not frozen stored. Only untreated samples (as control) and osmotically dehydrated samples under MWODS and MWODS-coated fruits were frozen at -20ºC.

Drip loss was measured every 10 min up to 60 min of thawing at room temperature of the untreated samples (control samples) and osmotically dehydrated samples under MWODS (MWODS samples and MWODS-coated samples) following storage at -20 ºC for 10 and 50 days. After 10 days of frozen storage at -20 ºC, untreated samples had a drip loss of 13.3%, while in MWODS samples the drip loss was 11.65%. Also, after 50 days of storage at -20 ºC, untreated samples showed a drip loss of 14.4% after 60 min of thawing at room temperature, while MWODS samples had a drip loss of 13.1%. Therefore, after 10 days and 50 days of storage, a decrease in drip loss (%) was observed in MWODS samples. MWODS treatment reduced the drip loss due to the reduction of moisture content prior to freezing (Table 4.1).

Similar results were reported when osmo-dehydro-freezing using different sugar solutions reduced the drip loss (%) in frozen rambutan after thawing (Lowithun & Charoenrein, 2009). Also, a reduction in drip loss was observed in frozen-thawed strawberries due to the osmotic dehydration treatment prior to freezing (Matuska et al., 2006). However, in the osmotically dehydrated strawberry cut fruits using sucrose solution and calcium chloride salts prior to freezing at - 40 ºC, Sirijariyawat & Charoenrein (2012) found no significant difference in the drip loss (%) after thawing at room temperature.

Using two-way ANOVA statistical analysis, a significant difference in drip loss (%) was measured among the untreated samples and MWODS samples at the two different times of storage ($p<0.05$). However, a significant difference in the drip loss (%) between the

untreated samples and MWODS samples was observed only between 0 and 30 minutes ($p>0.05$) after 10 days and after 50 days of storage.

Table 4.1: Drip loss (%) in untreated (control) and osmotically dehydrated samples using MWODS (MWODS samples) after 10 and 50 days of storage at -20 ºC

Thawing time (min)	Untreated (control) samples after 10 days	MWODS samples after 10 days	Untreated (control) samples after 50 days	MWODS samples after 50 days
0	0	0	0	0
10	2.03 ± 1.5	0.16 ± 0.1	1.37 ± 0.8	0.67 ± 0.2
20	5.98 ± 1.7	1.59 ± 0.2	4.87 ± 1.2	2.88 ± 0.4
30	8.33 ± 2.1	4.001 ± 0.7	7.73 ± 1.2	5.91 ± 1.3
40	9.63 ± 2.3	6.74 ± 0.3	9.87 ± 1.6	9.04 ± 1.1
50	11.45 ± 3.04	9.47 ± 0.9	11.32 ± 1.7	10.17 ± 1.2
60	13.26 ± 3.6	11.65 ± 1.07	14.36 ± 2.06	13.05± 0.3

4.5.2. Effect of the Edible Coating on Drip Loss

Drip loss was measured every 10 min up to 60 min of thawing at room temperature of the osmotically dehydrated samples under MWODS (MWODS-control) and sodium alginate-calcium chloride coated samples after MWODS treatment (MWODS-coated) following freezing storage at -20 ºC for 10 and 50 days. After 10 days of freezing at -20 ºC, MWODS-control samples showed a drip loss of 11.6% while in MWODS-coated samples the drip loss was 6.1%. Also, after 50 days of storage at -20 ºC, MWODS-control samples had a drip loss of 14.4% while MWODS-coated samples had a drip loss of 8.6%. The sodium alginate-calcium chloride edible coating therefore helped in reducing the drip loss in pineapple cut fruits by 47% after 10 days of freezing storage and 40% after 50 days of freezing storage thus acting as a natural barrier.

However, storage time negatively affected the drip loss in both control and coated samples, with 24% and 41% increase respectively in drip loss after 50 days of storage (Table

4.2). Clearly an effect of recrystallization phenomena that occurs in the frozen foods during storage led to the increase in the structural breakdown.

Based on the analysis of variance (ANOVA), a significant difference in drip loss (%) between MWODS-control samples and MWODS-coated samples was observed after 10 days and 50 days of storage ($p<0.05$).

Table 4.2: Drip loss (%) in MWODS-control samples and MWODS-coated samples after 10 and after 50 days of storage at -20 ºC

Thawing time (min)	MWODS-control samples after 10 days	MWODS-coated samples after 10 days	MWODS-control samples after 50 days	MWODS-coated samples after 50 days
0	0	0	0	0
10	0.16 ± 0.1	0.06 ± 0.6	0.67 ± 0.2	0.07 ± 0.1
20	1.59 ± 0.2	0.72 ± 0.6	2.88 ± 0.4	0.85 ± 0.1
30	4 ± 0.7	2.07 ± 0.1	5.91 ± 1.3	2.31 ± 0.2
40	6.74 ± 0.3	3.3 ± 0.1	9.34 ± 1.1	4.10 ± 0.1
50	9.47 ± 0.9	4.48 ± 1.06	11.27 ± 1.2	5.52 ± 0.9
60	11.65 ± 1.07	6.1 ± 1.6	14.36 ± 0.3	8.64 ± 1.1

Similar results were also observed in different studies. Sodium alginate based edible coating was reported to reduce the drip loss in the osmotically dehydrated and frozen strawberries after thawing (Matuska et al., 2006). Also, whey protein edible coating with and without the incorporation of beeswax, was reported to reduce drip loss (%) in frozen strawberries after thawing due to the function of the edible coating in the moisture transfer resistance (Soazo et al., 2015). In another study, chitosan-based edible coating was found to reduce drip loss in frozen strawberries after thawing (Zhao et al., 2014). The edible coating was able to reduce moisture loss from the fruit samples, survived the freezing conditions and low temperature storage and retained liquids.

During frozen storage, the development of extracellular ice crystals will cause cell separation in the middle lamella region, cell-wall rupture and cell shrinkage, which result in

quality loss, structure collapse, water dislocation and exudate production in addition to multiple deteriorative biochemical reactions. The deterioration of the structural integrity of the plant membranes will also prevent the retention of the hydrostatic pressure within the cells and causes drip loss and tissue softening (Rosenthal et al., 2018). Which explains the increase in drip loss (%) over time, after 50 days of storage in comparison with 10 days in untreated samples, MWODS samples and MWODS-coated samples (Table 4.1) and (Table 4.2).

The increasing trend of drip loss measurement is also in accordance with the decreasing trend of texture values (Dermesonlouoglou et al., 2016) as will be detailed in the next section.

4.6. Texture Analysis

To study the effect of MWODS technique and sodium alginate-calcium chloride edible coating on the texture of the pineapple cut fruits, texture analysis was performed before freezing and after thawing of the untreated samples (Fresh and Frozen thawed), osmotically dehydrated samples treated with MWODS (MWODS-unfrozen and MWODS-frozen thawed) and MWODS samples coated with sodium alginate and calcium chloride (MWODS-coated before freezing and MWODS-coated frozen thawed).

4.6.1. Effect of MWODS on Texture

Before freezing and after using MWODS treatment, a decrease in the firmness of the pineapple cut fruits from 61.1 (N) to 55.2 (N) was observed. This is the effect of MWODS treatment on the texture of pineapple fruit pieces. MWODS resulted in some texture degradation as most dehydration processes will do. The magnitude of this reduction is about 10% which is not unusual after osmotic dehydration treatment to remove as much as 40% moisture.

After freezing and thawing also the texture analysis was carried out. In this case, MWODS process improved the texture of the pineapple cut fruits since MWODS samples

showed better results than the untreated (control) frozen thawed samples (Figure 4.7). The firmness was 20% higher in MWODS samples compared with untreated (control) samples after 10 days of storage, and 14% higher after 50 days of storage.

However, storage time negatively affected the texture of all samples, since a decrease in the firmness was observed in both untreated control samples and MWODS samples after 10 and 50 days of frozen storage (Figure 4.7). ANOVA statistical analysis showed a significant difference between the untreated control and MWODS samples and among them before freezing and after thawing ($p<0.05$).

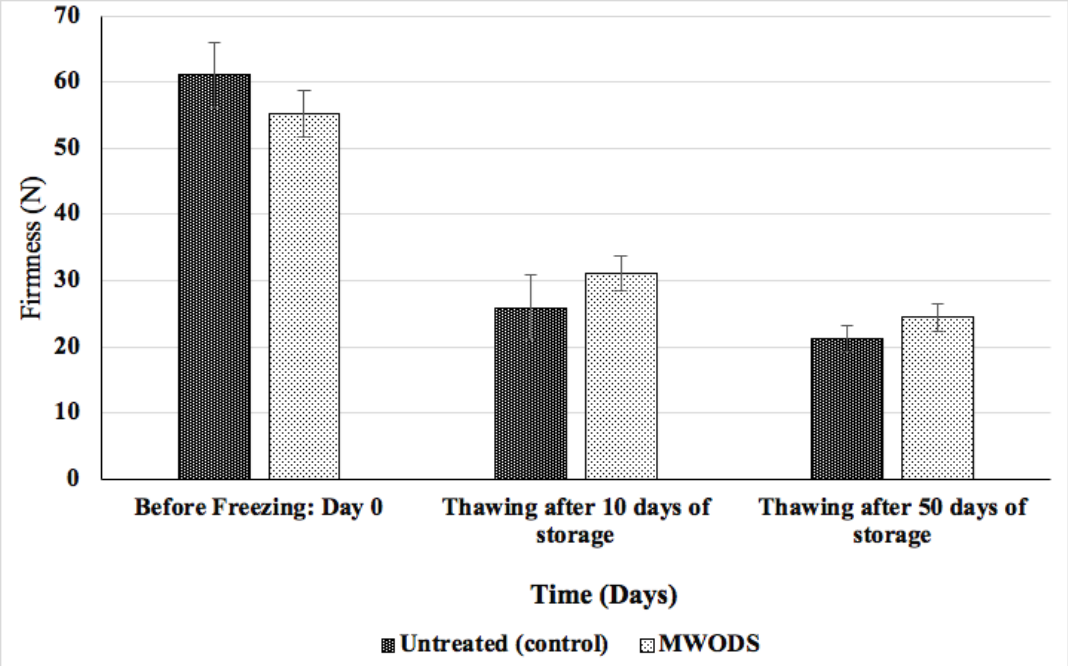

Figure 4.7: Firmness (N) of untreated fresh samples (control) and MWODS-samples prior to freezing and after thawing following 10 and 50 days of frozen storage

A decrease in the firmness of osmotically dehydrated strawberries was observed in general after thawing due to the formation of ice crystals during freezing. However, the values were better in osmotically dehydrated samples than in untreated fresh samples. The improvement

in the firmness of the pre-treated strawberries was caused by the protective effect on the cell's integrity induced by the osmotic dehydration process as reported by Dermesonlouoglou et al. (2016).

Fruits doesn't have the fibrous structure as vegetables to resist freezing damage, therefore fruits have a lower resistance to freezing process than vegetables. The texture damage in frozen-thawed fruit is mainly attributed to the semirigid nature of the damaged plant cells. Moreover, the formation of ice crystal during freezing causes undesirable changes in the texture of the fruits such as reduced crispness and turgor loss (Rosenthal et al., 2018).

4.6.2. Effect of the Edible Coating on Texture

Texture analysis was also performed after the thawing of the fruit samples to study the effects of sodium alginate-calcium chloride edible coating and storage time on the texture and quality parameters of the pineapple cut fruits.

After thawing, the firmness of the MWODS-control samples decreased to 31.2 (N) after 10 days of storage at -20 ºC and reached 24.4 (N) after 50 days of storage. In the MWODS-coated samples, the firmness decreased to 37.4 (N) after 10 days of storage at -20 ºC and reached 30 (N) after 50 days of storage. The edible coating therefore preserved the texture of the fruit samples and reduced the loss of firmness after thawing. As shown in Figure 4.8. The firmness was 16% higher in MWODS-coated samples compared with MWODS-control samples after 10 days of storage, and 18% higher after 50 days of storage.

However, storage time negatively affected the texture of the samples, since a decrease in the firmness was observed in the MWODS-control samples and MWODS-coated samples after 50 days of storage (Figure 4.8). ANOVA statistical analysis showed a significant difference

between the MWODS-control samples and MWODS-coated samples and among them before freezing and after thawing ($p<0.05$).

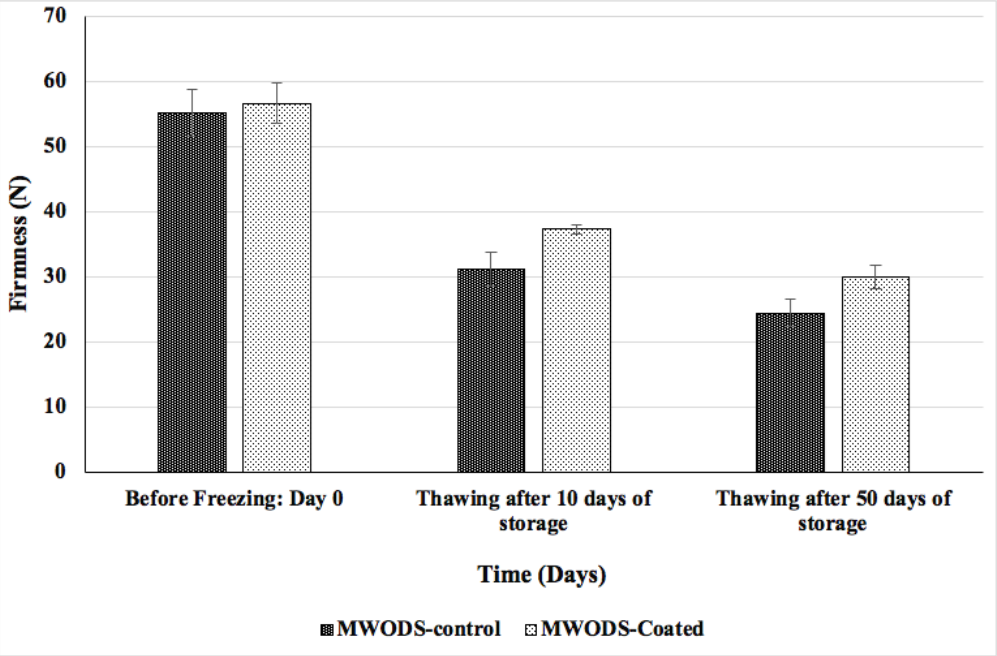

Figure 4.8: Firmness (N) of MWODS-control samples and MWODS-coated samples prior to freezing and after thawing

An irreversible loss in produce's firmness happens during freezing due the formation of ice crystals and cell lysis. A decrease in the firmness of strawberries coated with whey protein after thawing was observed by Soazo et al. (2015). However, Han et al. (2004) showed the beneficial effect of chitosan coating with and without the incorporation of calcium salts on the texture preservation of strawberries after thawing. The addition of calcium chloride salts to the chitosan coating showed better results due to their effect as firming agents (Han et al., 2004). Calcium chloride salts can also interact with the pectic acids found in the plant's cell walls and form calcium pectate that can maintain the structure of the fruits (Soazo et al., 2015). It was also reported that an improvement in the texture of frozen-thawed strawberries was observed after the use of chitosan coating with the incorporation of calcium chloride salts (Zhao et al., 2014).

4.7. Color Analysis

The color parameters of pineapple cut fruits before and after various treatments were measured. The Lightness (L value), green/red component (a* value), blue/yellow components (b* value) were primarily evaluated and Chroma (C value) and Hue angle (H value) were calculated. The a* value and Hue angle (H value) didn't show any significant difference between MWODS-control samples and MWODS-coated samples.

4.7.1. L Value

After the treatment of the pineapple cut fruits with MWODS, L value decreased from 62 in the fresh control fruit pieces to 42 in MWODS samples. MWODS process caused some surface dullness or darkening resulting in a decrease in the lightness value of the pineapple cut fruits (Table 4.3).

After freezing and thawing, the L values in MWODS-control samples (uncoated) and MWODS-coated samples stored at -20 ºC for 10 days and 50 days were also measured to study the effect of the sodium alginate-calcium chloride edible coating on color changes in frozen pineapple pieces.

Storage time further affected the lightness of the uncoated samples, since the L values decreased after 10 and 50 days of storage. However, in MWODS-coated samples the L value was almost the same after 10 and 50 days of storage (Table 4.3). The sodium alginate-calcium chloride edible coating therefore helped to maintain the surface lightness of the fruit samples during freezing and prevented surface darkening. The L value in MWODS-control samples was lower than in MWODS-coated samples after 10 and 50 days of storage. After 10 and 50 days of storage, the L values were respectively 25% to 38% lower in MWODS-control samples compared with MWODS-coated samples.

ANOVA statistical analysis showed a significant difference in the L value between the untreated samples (control) and MWODS samples prior to freezing ($p<0.05$). Also, after thawing a significant difference was observed between the MWODS-control samples and MWODS-coated samples following 10 and 50 days of storage ($p<0.05$). However, there was no significant difference among the MWODS-coated samples between the two storage time periods, 10 and 50 days ($p>0.05$).

Table 4.3: L values in MWODS-control and MWODS-coated samples prior to freezing and after thawing

	Prior to Freezing		**Thawing After 10 days of storage at -20 ºC**		**Thawing After 50 days of storage at -20 ºC**	
	Untreated samples	MWODS treated samples	MWODS-control samples	MWODS-coated samples	MWODS-control samples	MWODS-coated samples
L value	61.95 ± 1.4	42.01 ± 0.7	31.52 ± 1.04	41.59± 0.75	25.83 ± 2	41.2 ± 1.34

Silva et al. (2014) reported that osmotically pretreated frozen pineapples showed a decrease in the L value after thawing, and a decrease in the luminosity was observed in osmotically dehydrated samples in comparison with fresh samples.

4.7.2. b* Value

The b* value which is the green/yellow indicator was measured in both MWODS-control samples (uncoated) and MWODS-coated samples. After MWODS, b* value remained almost the same in untreated and control samples. The values slightly decreased from 35 in untreated samples to 33.2 in MWODS samples. MWODS didn't show a negative effect on the b*value of the pineapple cut fruits (Table 4.4).

After thawing, the b*value was measured in uncoated control and coated samples to study the effect of the sodium alginate-calcium chloride edible coating. The b*value decreased in both after thawing. However, in b* values in MWODS-control samples the values were lower than in MWODS-coated samples. Also, the frozen storage time negatively affected the yellow color of the pineapple cut fruits although the coated samples showed better results (Table 4.4). The sodium alginate-calcium chloride edible coating preserved the yellowness of the fruit samples during freezing and storage for 10 and 50 days at -20 ºC. One-way ANOVA statistical analysis showed a significant difference in the b* value between the MWODS samples and MWODS-coated samples after thawing following the two different storage periods ($p<0.05$). However, no significant difference was observed between the untreated samples and MWODS samples prior to freezing ($p>0.05$).

Table 4.4: b* values in MWODS-control and MWODS-coated samples prior to freezing and after thawing.

	Prior to Freezing		**Thawing After 10 days of storage at -20 ºC**		**Thawing After 50 days of storage at -20 ºC**	
	Untreated samples	MWODS treated samples	MWODS-control samples	MWODS-coated samples	MWODS-control samples	MWODS-coated samples
b* value	34.91 ± 1.2	33.19 ± 1.6	25.04 ± 1.04	27.02 ± 0.73	21.99 ± 1.7	25.59 ± 3.1

Dermesonlouoglou et al. (2016) showed that after the thawing of osmotically pretreated strawberry fruits stored at low temperatures (above -12 ºC) and very low freezing temperatures (below -12 ºC) a retention in the color was observed in the osmotically dehydrated samples when compared with the untreated samples prior to freezing.

4.7.3. C Value

Before freezing, a slight decrease in the Chroma (C value) was observed in osmotically dehydrated pineapple cut fruits using the MWODS method. The C value slightly decreased from 35 in untreated samples to 33.2 in MWODS, which means that MWODS did not affect negatively the color Chroma of in pineapple cut fruits prior to freezing (Table 4.5).

After thawing, C value was measured in MWODS-control samples (uncoated) and MWODS-coated samples to study the effect of the sodium alginate-calcium chloride edible coating on the color Chroma of pineapple cut fruits. After thawing, C value decreased in both control and coated samples. However, lower values were obtained in MWODS samples. Also, the increase in storage time negatively affected the color Chroma in both MWODS-control samples and MWODS-coated samples (Table 4.5). The sodium alginate-calcium chloride edible coating somewhat reduced the loss of color chromaticity during storage.

Table 4.5: C values in MWODS-control samples and MWODS-coated samples prior to freezing and after thawing

	Prior to Freezing		**Thawing After 10 days of storage at -20 ºC**		**Thawing After 50 days of storage at -20 ºC**	
	Untreated samples	MWODS treated samples	MWODS-control samples	MWODS-coated samples	MWODS-control samples	MWODS-coated samples
C value	34.95 ± 1.2	33.21 ± 1.6	25.31 ± 1.05	27.44 ± 0.72	22.29 ± 1.7	25.65 3.1

Using one-way ANOVA statistical analysis, no significant difference in the C value between untreated and MWODS samples prior to freezing was observed ($p>0.05$). However, a

significant difference in the C value between MWODS-control samples and MWODS-coated samples was observed after thawing ($p<0.05$).

Based on the literature, an increase in the C value is normally observed in osmotically pretreated pineapples prior to freezing. The increase in the sugar concentrations caused by the higher moisture loss, which also increases the concentration of the color pigments in the plant's tissue might resulted in an increase in the chromaticity values. Such results have been observed using papaya and guava by Silva et al. (2014).

5. Appearance

Thawing of pineapple cut fruits after 10 days of frozen storage at -20 ºC caused a change in the appearance and surface darkening in uncoated samples. The MWODS-coated samples showed no surface browning, and the appearance of coated pineapple cut fruits was fully maintained after thawing (Figure 4.9). Similar results were observed after thawing following 50 days of storage at -20 ºC (Figure 4.10). The sodium alginate-calcium chloride edible coating maintained the appearance of the pineapple cut fruits and prevented surface darkening.

Figure 4.9: The appearance of MWODS-control samples and MWODS-coated samples after thawing for 1 h at room temperature following 10 days of storage at -20 ºC

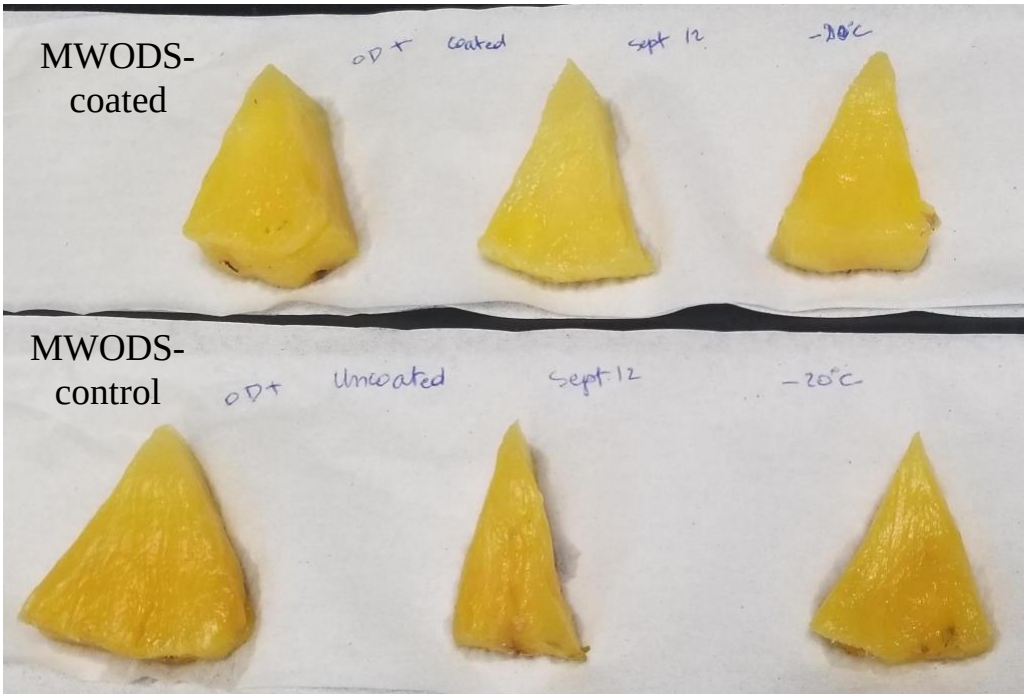

Figure 4.10: The appearance of MWODS-control samples and MWODS-coated samples after thawing for 1 h at room temperature following 50 days of storage at -20 ºC

6. Conclusions

Microwave assisted osmotic dehydration under spray condition (MWODS) technique enhanced the mass transfer parameters of the conventional osmotic dehydration by reducing processing time and solid gain (%) and increasing the moisture loss as also observed by other researchers who used it primarily for the purpose of osmotic dehydration (Azarpazhooh & Ramaswamy, 2010). In this study, MWODS was used as a pre-treatment to reduce moisture content in pineapple cut fruits prior to freezing, which enhanced the sensory properties of the final products and reduces drip loss after thawing which has also been observed by Zhao et al. (2014) with conventional osmotic drying. In comparison with the conventional osmotic dehydration, a significant enhancement of moisture loss and a significant reduction in the treatment time and solid gain (%) was observed in pineapple cut fruits treated with MWODS. This combination resulting almost doubling the ML/SG ratio, a parameter that has been closely related to the quality of osmotically dehydrated products. Also, an enhancement in the texture and a reduction in drip loss after the thawing of pineapple cut fruits was clearly observed.

Furthermore, the special sodium alginate-calcium chloride edible coating applied on the osmotically dehydration pineapple cut fruits after MWODS technique helped to significantly reduced the drip loss and enhanced the sensory properties after thawing. Based on the results analysis, the sodium alginate-calcium chloride coating was recommended as an excellent pre-treatment for enhancing the quality parameters of frozen pineapple cut fruits by preventing surface darkening, reduced drip loss and maintained the tissue.

CHAPTER V

GENERAL SUMMARY AND FUTURE RESEARCH

1. General Summary

In this research study, the effect of sodium alginate-calcium chloride edible coating on fresh and frozen cut fruits was studied. Sodium alginate-calcium chloride based edible coating was applied on strawberry cut fruits stored at 4 ºC. The edible coating acted as a natural barrier and reduced respiration and transpiration rates in cut fruits, which delayed mold growth in coated samples for up to two weeks, while in control samples mold growth appeared after only one week of storage at 4 ºC. The sodium alginate-calcium chloride coating also reduced the pH and the total soluble solids (TSS) content in the cut strawberries. TSS is an indicator of fruits maturity and the increase in TSS (%) during storage indicates fruits ripening. Moreover, the preservation of the fruit's color and the prevention of surface darkening and tissue softening are additional advantages of the sodium alginate-calcium chloride coating. The incorporation of the calcium chloride salts played in essential role in the maintenance of the produce's texture due to their effect as a firming agent.

The effect of sodium alginate-calcium chloride coating on frozen-thawed pineapple cut fruits was also studied. Osmo-dehydro-freezing is the osmotic dehydration treatment prior to freezing to reduce moisture content in the fresh produces. Beside the economic advantages and the decreased energy use, this method improves the texture and appearance of the final products. Osmo-dehydro-freezing decreases the content of free water in the fresh produce, that will freeze later and cause cell wall rupture and tissue damage.

Instead of using conventional osmotic dehydration, continuous flow medium Microwave Assisted Osmotic Dehydration under spray condition (MWODS) technique was used in this

research. MWODS technique allows the creation of osmotic dehydration under microwave field, which reduced processing time. The final products will also have enhanced sensory properties and more natural flavors due to the reduced solids gain. Based on the results' analysis, MWODS technique reduced processing time and solid gain (%) in pineapple cut fruits when compared with conventional osmosis. Also, it preserved the texture of the fruit samples and reduced drip loss after thawing.

Furthermore, sodium alginate-calcium chloride edible coating reduced drip loss in frozen-thawed pineapple cut fruits stored at -20 ºC for 10 and 50 days. Also, the edible coating preserved the color, the appearance and the texture of pineapple cut fruits after thawing.

2. Suggestions for Future Research Studies

Based on the positive results obtained during this research study, we conclude that the sodium alginate-calcium chloride edible coating can extend the shelf life of fresh produces and enhance their sensory properties, which reduces food losses and helps both producers and consumers save money. For future studies, different ideas can be realized to improve the functionality of the edible films and coatings. Edible coatings act as carriers for bioactive components such as vitamins and minerals that can be added during manufacturing depending on their chemical compositions and surface properties, which can be very beneficial and enhances the nutritional contents of the food products.

Essential oils were added during the preliminary experiments due to their effects as antimicrobial agents. Both lemon grass and pepper mint oils showed beneficial effects in extending the shelf life of strawberry cut fruits and preventing mold growth for up to three weeks at 4 ºC. However, sensory properties such as produce's texture, odor and color were negatively

affected. In an upcoming research new methods of essential oils application on the fruit's surface can be applied without negatively affecting the sensory properties of the fruits can be studied.

In another preliminary experiment, the effect of edible coating on apple cut fruits to prevent oxidative browning during storage was studied. The apple cut fruits were dipped in ascorbic acid that acts as an antioxidant and dipped after in the edible coating solutions. Two different lots of fruit samples were prepared. Sodium alginate-calcium chloride edible coating was applied on the first lot, while pectin-calcium lactate edible coating was applied on the second lot. After few hours of refrigerated storage, the control uncoated samples became dark, while coated samples showed an oxidative browning after over 10 days of storage. However, this beneficial effect was also observed in samples treated only with the antioxidant. After four weeks of storage at 4 ºC, apple cut fruits treated with ascorbic acid and coated with sodium alginate-calcium chloride had a slight enhancement in the texture and appearance in comparison with the samples treated only with ascorbic acid. For an upcoming research idea, the production of an edible coating with different kinds of antioxidants can be produced to prevent the oxidative browning in cut apples and avocados.

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