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The Use of *Opuntia ficus-indica* Mucilage Edible Coating to Extend the Shelf-Life of Packaged Strawberry and Red Raspberry

Master's Thesis

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Under the Supervision of Professor Maria Isabel Achaerandio

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Abstract

Rapid loss of quality and decay causes economic loss of strawberries and raspberries after harvest. The effects of an edible coating based on mucilage solution of *Opuntia ficus-indica* cladodes on the quality of packaged strawberries and raspberries were studied during their shelf-life. After treatment, decay index, weight loss, respiration rate, color characteristics, total soluble solids content, pH, total phenolics content, and antioxidant activity were recorded on 3, 7, 9, 11, 13, and 15 days of storage at 2 ± 2°C and were compared with uncoated fruit. Due to the sealed packaging used for the fruits, the weight loss and decay percentage were greatly reduced. Because of the total phenolic content available in the cactus mucilage, coated fruit had a higher total phenolic content compared to the control. Coating also reduced the decay index in raspberries, and coated raspberries did not show any decay incidents in 15 days of storage. The combination of the packaging and coating used proved effective in improving the post-harvest shelf-life of strawberries and raspberries up to 15 days in cold-storage.

Keywords: Fragaria × ananassa, Rubus idaeus, postharvest, packaging, fruit quality

Resumen

La pérdida acelerada de calidad y deterioro provoca pérdidas económicas en fresas y frambuesas después de la cosecha. Los efectos de un recubrimiento comestible a base de una solución de mucílagos de cladodios de *Opuntia ficus-indica* sobre la calidad de las fresas y frambuesas envasadas durante su vida útil fueron estudiados en este trabajo. Después del tratamiento, el índice de deterioro, la pérdida de peso, la intensidad respiratoria, las características del color, el contenido total de sólidos solubles, el pH, el contenido total de compuestos fenólicos y la actividad antioxidante se analizaron a los 3, 7, 9, 11, 13 y 15 días de almacenamiento a 2 ± 2°C y se compararon con frutas no recubiertas. Debido al envase termosellado utilizado para las frutas, la pérdida de peso y el porcentaje de deterioro se redujeron considerablemente. Debido al contenido en compuestos fenólicos totales disponible en el mucílago de cactus, la fruta recubierta tuvo un contenido en compuestos fenólicos totales más alto en comparación con el control. El recubrimiento también redujo el índice de deterioro en las frambuesas. Éstas no mostraron presencia de deterioro por presencia de crecimiento fúngico en 15 días de almacenamiento. La combinación del embalaje y el recubrimiento comestible utilizados resultó eficaz para mejorar la vida útil postcosecha de las fresas y frambuesas hasta 15 días en almacenado en frío.

Resum

La pèrdua accelerada de qualitat i deteriorament provoca pèrdues econòmiques en maduixes i gerds després de la collita. Els efectes d'un recobriment comestible a base d'una solució de mucílags de cladodis d'*Opuntia ficus-indica* sobre la qualitat de les maduixes i gerds envasades durant la seva vida útil van ser estudiats en aquest treball. Després del tractament, l'índex de deteriorament, la pèrdua de pes, la intensitat respiratòria, les característiques de la color, el contingut total de sòlids solubles, el pH, el contingut total de compostos fenòlics i l'activitat antioxidant es van analitzar als 3, 7 , 9, 11, 13 i 15 dies d'emmagatzematge a 2 ± 2°C i es van comparar amb fruites no recobertes. A causa de l'envàs termosegellat utilitzat per a les fruites, la pèrdua de pes i el percentatge de deteriorament es van reduir considerablement. Degut al contingut en compostos fenòlics totals disponible al mucílag de cactus, la fruita recoberta va tenir un contingut en compostos fenòlics totals més alt en comparació amb el control. El recobriment també va reduir l'índex de deteriorament en els gerds. Aquestes no van mostrar presència de deteriorament per presència de creixement fúngic en 15 dies d'emmagatzematge. La combinació de l'embalatge i el recobriment comestible utilitzats resultar eficaç per millorar la vida útil post collita de les maduixes i gerds fins a 15 dies en emmagatzemat en fred.

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1 Introduction

1.1 Strawberry, Raspberry, and Use of Edible coatings

Strawberries and raspberries are two of the most popular berries. They are characterized by unique and highly desirable taste and flavor and are rich in polyphenols and anthocyanin, vitamins, and amino acids (Campaniello et al. 2008; Rao et al. 2010). However, the fruits are highly perishable, resulting in a short harvest life due to mechanical injury, physiological deterioration, water loss, fungal decay, and high respiration rate (Vargas et al. 2006; Tezotto-Uliana et al. 2014). Customers do not buy the damaged berries, and fungally decayed strawberries cannot be sold to factories to make byproducts. Therefore, they can only be used to feed animals or to make composts. This causes economic losses to the fruit producers and food chain.

Traditionally various strategies such as the use of chemical fungicides have been used to control fungal infection. Nowadays, these methods have been banned in many countries because of their potentially harmful impact on the environment and human health (Aloui et al. 2014). Other techniques like microwave, heating, and ozonation have also been used to protect fresh fruits from decay, but their impact on the quality of the fruits may be considered a drawback.

Food packaging is one way of protecting the fruit from decay. Food packaging is used to protect food from chemical, biological, and physical external influences. Preferably this should be done in a cost-effective, environmentally friendly way (Marsh et al. 2007).

Packagings used for fruits and vegetables are usually polymeric because of their availability, low cost, and rapid production rate. In recent years the concern about the impact of those materials on the environment has led to consideration of biodegradable and environment-friendly materials, and the research on edible films and coatings for food packaging has increased (Aparicio-Fernández et al. 2018). Edible coatings are thin layers enrobed on various food to extend shelf-life. They can be consumed together with food without removal. Edible coatings are mostly tasteless, colorless, and odorless. They are made from natural compounds, which are biodegradable and edible in order to satisfy environmental concerns and respond to consumer demand (Pavlath et al. 2009).

Edible coatings were evident to extend the shelf-life storage by reducing weight loss, decreasing respiration and oxidative reaction rates; and by reducing or even avoiding physiological disorders of fresh produce (Quirós-Sauceda et al. 2014; Yan et al. 2019). Edible films and coatings afford numerous advantages over conventional non-edible polymeric packaging. They can reduce the complexity of the food package. Even if they are not consumed with the packaged product, they can reduce environmental pollution by virtue of their biodegradable nature (Del-Valle et al. 2005).

Edible coating has been used for centuries before the science behind it was understood. Wax is one example of edible coatings that have been used to create a shiny fruit surface to prevent moisture loss, and it is still used in the present day (Marsh et al. 2007). Fruits that have been coated with various edible coatings include Orange, Apple, Grapefruit, Cherry, Papaya, Lemon, Strawberry, Raspberry, Mango, Peach, Apricot, fresh-cut Apple, fresh-cut Peach, fresh-cut Pear, etc.

Edible coatings are made with a variety of natural substances such as polysaccharides, protein, lipids by the addition of surfactants and plasticizers (Lin et al. 2007). The edible coatings are mainly divided into three classes (Donhowe et al. 1993):

- Hydrocolloids, e.g., Polysaccharides, and some proteins
- Lipids, e.g., fatty acids, acryl glycerides and waxes
- Composites, e.g., protein/protein, polysaccharides/protein, lipid/polysaccharides

There are several methods to apply edible coating on the surface of fruits such as dipping, brushing, extrusion, spraying, solvent casting, etc. The dipping method is one of the easiest and most common ways to apply edible coating on fruits. In this method, the fruit is dipped in the coating solution and then dried.

Table 1.1 shows different studies about edible coatings on various fruits using the dipping method.

Table 1.1: Effects of edible coating in fruits

Effect on Quality Parameter	Coating Material	Coating Method	Fruits	Whole /Fresh-cut	Duration of Storage and Temperature	Reference
Increased firmness and weight loss retention	Shellac, Gelatin, Persian Gum	Dip coating	Valencia oranges	Whole fruit	60 days at 5°C	Khorram et al. (2017)
	R-(+)-Limonen stabilized by *Ulva fasciata* polsaccharide	Dip coating	Strawberries	Whole fruit	8 days	Shao et al. (2018)
	Chitosan	Layer by layer	Strawberries	Whole fruit	8 days at 0°C	Yan et al. (2019)
	Gum Arabic, Calcium chloride	Dip coating	Mango	Whole fruit	28 days 25±2°C	Khaliq et al. (2015)
	Starchbased	Dip coating	Plum	Whole fruit	Different atmosphere	Basiak et al. (2019)
	Chitosan	Dip Coating	Mango	Whole fruit	16 days at 25°C	Jongsri et al. (2016)
Anti-browning and decreased decoloration	Aloe vera gel	Dip coating	Apple	Fresh-cut	6 days at 4±1°C	Supapvanich et al. (2016)
	Hydroxy propylmethyl cellulose	Dip coating	Grapes	Whole fruit	10 days, cold storage	Pastor et al. (2011)
	Starch	Dip coating	Banana	Whole fruit	12 days at 20±2°C	Thakur et al. (2019)
Increased phytonutrient and antioxidant	Chitosan nanoparticles	Dip coating	Grapes	Whole fruit	12 days at 25°C - 24 days at 12°C	Castelo Branco Melo et al. (2018)
	Chitosan	Dip coating	Orange	Whole fruit	120 days at 10±1°C	Gao et al. (2018)
	Carboxymethyl cellulose and *lactobacillus plantarum*	Dip coating	Strawberries	Whole fruit	16 days at 4°C	Khodaei et al. (2019)
	Chitosan	Dip coating	Blueberries	Whole fruit	4°C	Mannozzi et al. (2018)
	Chitosan	Dip coating	Mango	Whole fruit	14 days at 15±2°C	Zahedi et al. (2019)
	Aloe vera gel	Dip coating	Raspberry	Whole fruit	8 days at 4°C	Hassanpour (2015)
Increased antimicrobial activity	Antimicrobial Nanoemulsion	Dip coating	Fuji apple	Fresh-cut	16 days	Salvia-Trujillo et al. (2015)
	Thymol nanoemulsion	Dip coating	Strawberries	Whole fruit	5°C	Robledo et al. (2018)
	Salep solution enriched with grape seed extract	Dip coating	Strawberries	Whole fruit	20 days at 1°C	Emamifar et al. (2019)
	Malaysian stingless bee honey	Dip coating	Papaya	Whole fruit	10 days	Maringgal et al. (2019)
	Gelatin, Zein, Propolis ethanolic extract	Dip coating	Raspberry	Whole fruit	11 days at 5°C	Moreno et al. (2020)

These studies point to remarkable effects that edible coatings have on four different quality parameters of fruits: Firmness and weight loss retention, anti-browning and decoloration, phytonutrient and antioxidant and antimicrobial activity. In the studies listed, edible coating decreased the rate of weight loss and kept fruits firm for longer, slowed down the browning and decoloration, and maintained the glossiness of the fruit, preserved the phytonutrient content and antioxidant activity, and decrease the rate of infection (Maringgal et al. 2020).

As mentioned before, strawberries and raspberries are highly perishable. This is due to their high metabolic activity and susceptibility to growth of microbial molds and water loss and thereby shriveling and deterioration because of their thin

skin structure. Several studies, mentioned in Table 1.1, have tried to improve the shelf life of strawberries and raspberries. Shao et al. (2018) reported a reduction in weight loss and improved microbial stability in strawberries coated with R-(+)-Limonen stabilized by *Ulva fasciata* polysaccharide during 8 days of storage at 4 ± 1°C. In a study by Khodaei et al. (2019), strawberries coated with carboxymethyl cellulose and *Lactobacillus plantarum* had a reduced decay percentage and retained their ascorbic acid and total phenolic content during 16 days of storage at 4°C. A recent study used salep solution (water-soluble gum derived from tuberous wild orchid) and grape seed extract to coat strawberries. The coated strawberries preserved their physicochemical, sensorial, and microbial quality during cold storage (1°C). The coating formula with 1.5% salep solution + 3% grape seed extract increased the shelf-life of strawberry by limiting its microbial load up to 20 days (Emamifar et al. 2019). Hassanpour (2015) reported that raspberries coated with *Aloe vera* gel had higher antioxidant capacity, enzyme activity, and lower decay during 8 days at 4°C. In a very recent study by Moreno et al. (2020), raspberries coated with gelatin, propolis extract, and zein had significantly lower decay levels after 11 days at 5°C.

1.2 Cactus Mucilage

Cactus mucilage is a renewable, eco-friendly, cheap, and widely available carbohydrate in a cactus plant that can be used in biotechnological and industrial applications. The cactus plant covers large areas over the world, especially in Mexico, Tunisia, Brazil, and Ethiopia (Gheribi et al. 2019). Mucilage is stored in mucilaginous cells in chlorenchyma (external green cells) and parenchyma (internal cylinder of white cells), with parenchyma having more (Sepúlveda et al. 2007).

The mucilage from *Opuntia ficus-indica* is a complex polysaccharide of 33 to 55 sugar residues. As shown in Table 1.2, most studies have been focused on the mucilage extracted from the cladodes of *Opuntia ficus-indica* while other studies used its fruit pulps and fruit peels. It can also be seen that the most common way of extracting mucilage is by using water as a solvent for maceration followed by filtration and precipitation (Gheribi et al. 2019). Other techniques, such as mechanical pressing followed by precipitation, were also used.

Cactus mucilage, especially the mucilage of *Opuntia ficus-indica*, is used as an edible coating in various studies. In a study by Del-Valle et al. (2005), prickly pear cactus mucilage showed a protective effect on strawberry by retaining its firmness and maintaining its natural taste during 9 days of storage at 5 ± 0.5°C. Guava fruits coated by cactus mucilage had a delayed skin color development and increased firmness after 6 to 8 days at 27-28°C (Zegbe et al. 2015). Allegra et al. (2016) reported the efficacy of cactus mucilage on cut kiwifruit slices. The mucilage

Table 1.2: Cactus mucilage extraction methods (*Opuntia ficus-indica*)

Raw material	Extraction	Yield of Extraction	Composition and Structure	Reference
Cladodes *Opuntia ficus-indica*	Homogenization in water with blender filtration centrifugation lyophilization resuspension in TCA solution dialyze against water additon of ethanol centrifugation lyophilization	1.124 mg/ml of tissue	Arabinose 67% Galactose 6% Xylose 20% Rhamnose 5%	Trachtenberg et al. (1981)
Cladodes *Opuntia ficus-indica*	Mechanical press	-	Two polysaccharidic entities: Linear β-(1-4)-galctose polymer and highly branched xyloarabinan	Di Lorenzo et al. (2017)
Cladodes *Opuntia ficus-indica*	Mechanical press of cladodes inner part Precipitation with ethanol	14%	Galactose 40% Arabinose 30% Xylose, rhamnose, glucose: Minor sugars NMR specific signals of arabinogalactan polysaccharide	Gheribi et al. (2018)
Cladodes *Opuntia ficus-indica*	Maceration in water, assisted with a microwave Precipitation with ethanol	8%	Arabinose Galactose Rhamnose Xylose Acide galacturonique	Felkai-Haddache et al. (2016)
Cladodes *Opuntia ficus-indica*	Maceration Centrifugation Decantation Percipitation with acetone Washing with isopropyl alcohol	-	Arabinose 44% Galactose 20% Rhamnose 7% Calacturonic acid 6% Xylose 22%	Medina-Torres et al. (2000)
Fruit peels *Opuntia ficus-indica*	Mechanical press Precipitation with ethanol	3%	Galactose 54% Arabinose 34% Xylose 10% Galacturonic acid 9% Backbone chain made of (1→4) linked β-D-Galp residues	Gheribi et al. (2019)
Fruit pulp *Opuntia ficus-indica*	Mixing in water Microwave-assisted extraction Filtration Freeze drying	-	Glucose 78% Arabinose 13% Xylose 5% Galactose 2% Mannose 2% Arabinoglucan structure	Salehi et al. (2019)
Fruit peels *Opuntia ficus-indica*	Maceration in water Precipitation with ethanol	4%	Arabinose 33% Galactose 23% Galacturonic acid 14% Arabinogalactan structure	Habibi et al. (2004)
Fruit pulp *Opuntia ficus-indica*	Blending with screw press Filtration, centrifugation Dialysis against water Precipiration with ethanol	3.8%	Uronic acid 23% Arabinose, rhanmnose, xylose, galactose Complex mixture of polysaccharides	Matsuhiro et al. (2006)
Cladodes *Opuntia ficus-indica*	Mix with water Decantation, centrifugation Supernatant mixed with ethanol	-		Peńa-Valdivia et al. (2012)

helped maintaining the firmness, ascorbic acid, pectin content, visual quality, and flavor score of the fruit during 12 days of storage at 5°C. In another study by the same author, coated figs retained their weight and firmness and maintained their visual appearance and brightness during 10 days of storage at 4 ± 0.5°C (Allegra et al. 2017). *Opuntia ficus-indica* mucilage obtained good results in improving the fruit's shelf-life. This natural material's availability and low-cost make it a great choice for us to study its effectiveness in improving the shelf-life of strawberries and raspberries.

2 Objective

This study aims to evaluate the benefits of coating highly perishable fruits such as strawberries and raspberries using edible coatings obtained from the mucilage of *Opuntia ficus-indica*. The following specific milestones have been set to be the objectives of this study:

- To extract the mucilage from *Opuntia ficus-indica* and characterize its antioxidant activity and total phenolic content.
- To measure the antioxidant activity, total soluble solids content, respiration rate, weight loss percentage, total phenolic content, and color characterization of strawberries and raspberries periodically for both coated and uncoated fruits.
- To evaluate the effect of packaging and edible coating produced from *Opuntia ficus-indica* mucilage on the shelf-life and specifically changes in antioxidant activity, changes in respiration rate, changes in color, changes in total soluble solids content, and changes in total phenolic of strawberries and raspberries during 15 days of storage at 2 ± 2 °C.

3 Materials and Methods

3.1 Equipment

- Büchner funnel and connection to vacuum system
- Centrifuge
- Digital balance
  - Model: Medifriger-BL
- Digital pH meter
  - Model: pH meter GLP22
  - Measuring range: -2...16
  - Resolution: 0.1/0.01/0.001
  - Measurement error: ≤ 0.005
- Digital refractometer
  - Model: ATAGO PR-101α
  - Accuracy: $\pm$0.1% Brix
  - Temperature compensation: 5° to 40°C
  - Brix range: 0.0 to 45.0% Brix
  - Callibration: Water only
  - Measurement time: 3 seconds
  - Sample volume: 0.1 ml or more
- Digital photo colorimeter
  - Model: Konica Minolta Chroma meters CR-400
  - Detector: Silicon photocells.
  - Display range: Y: 0.01% to 160.00%
  - Light source: pulsed xenon lamp

  - Measurement time: 1 second
  - Minimum measurment interval: 3 seconds
  - Measurement/Illumination area: Ø 8 mm / Ø 11 mm
- Digital thermometer
- Gas analyzer
  - Model: PBI Dansesor
  - Headspace gas analyzer for quality control of modified atmosphere package(MAP)
  - Measuring range:0-10 %
  - Resolution O_2: 0.001%, CO_2: 0.1%
  - Sensor accuracy at 1% O_2 and 20% CO_2: ±0.01% O_2, ±0.8% CO_2
- Hand blender
- Spectrophotometer
  - Model: Thermo Evolution 300 UV-Vis
- Thermosealing machine
  - Model: Ilpra Termosaldatrici
- Thermosealing microperforated film
  - Composition
    * PET: 12 μm
    * ADHESIVE: 2 μm
    * PP: 50 μm
  - Thickness: 64 μm
  - Oxygen transmission rate: $> 8100\text{ml}/m^2/24h/\text{bar}$
  - Water vapor transmission rate: $> 600g/m^2/24h/\text{bar}$
- Tray for food packaging
  - Material: PP/EVOH/PP
  - Oxygen transmission rate: $< 0.05\text{cm}^3/24h/\text{bar}$

3.2 Mucilage Preparation and Analysis

Opuntia ficus-indica cladodes were harvested from wild plants in Cubelles (Barcelona, Spain) during July 2020. The average physical dimentions of the selected cladodes were 140±15 mm long and 140±12 mm wide. The initial weight of the cladodes was 271±33 g. The thorns were then removed from the cladodes. Fresh cladodes were first washed with tap water to remove any adhering contaminants and then were washed with a cleaning solution of sodium hypochlorite and water (v/v ratio of 1:250) for 10 minutes. The initial pH of the cladodes was 9.90, which then got changed to 9.70 after washing. Cladodes were left at room temperature for 30 minutes to dry. After analyzing different methods of mucilage extraction, it seems that maceration with water, centrifugation, decantation, and precipitation with Ethanol does not require expensive and complex equipment and is easy to perform. Mucilage was extracted from cladodes according to the method of Peña-Valdivia et al. (2012) with some slight modifications. Fresh cladodes were cut into 5 cm × 5 cm sections. The cladodes pieces were mixed with water for 15 minutes at 90°C in 1:2 ratio (w/v). The extract was then decanted, centrifuged at 4600 × g for 10 minutes. The produced supernatant was mixed with ethanol 96% in 1:1 ratio (v/v) and then stored in the fridge at 4°C for one night. The mucilage was then separated from the supernatant by decantation.

The mucilage was then analyzed to measure its total phenolic content and antioxidant activity using the same method described in Section 3.11.

3.3 Fruits

Strawberries (*Fragaria × ananassa*) and raspberries (*Rubus idaeus*) were purchased from a local supermarket in Castelldefels, Barcelona. All fruits were immediately transferred to the laboratory in Campus del Baix Llobregat. From the batch, fruits with the same color and shape and without cues of any mechanical damage, defects, diseases, and fungal infections were then selected.

3.4 Coating, Packaging, and Storage Conditions

Figure 3.1 shows the experimental flow chart. Strawberries and raspberries each were randomly divided into 24 samples. 12 of which were treated with coating, and the other 12 remained untreated. 850g of strawberries were divided into two groups of approximately 425 g each, and 368 g of raspberries were divided into two groups of approximately 159 g. The strawberries and the raspberries in the first

group were treated by dipping for 15 minutes in the mucilage. The other groups remained untreated as control. The treated fruits were air-dried for 1 hour at room temperature. All groups were then divided into 6 groups of 30±1 g for strawberries and 12±1 g for raspberries and were placed in white PP-EVOH-PP plastic trays. This tray has an oxygen transmission rate of less than 0.05 $cm^3/24h/bar$, which makes it great for packaging fruits. The plastic trays were then sealed using a thermosealing machine with thermosealing microperforated film (12 μm PET, 2 μm ADHESIVE, 50 μm PP). They were stored at 2 ± 2°C. Fruits were sampled at 3, 7, 9, 11, 13, and 15 days of storage. Duplicate experiments were conducted for each fruit, and samples were analyzed in duplicate.

3.5 Measurement of Decay Percentage

Each sample was checked after 3, 7, 9, 11, 13, and 15 days of storage. The strawberries and raspberries were considered infected when a moldy brown spot was visually detected. The decay percentage of uncoated and coated fruits was calculated using Equation 3.1.

$$\text{Decay Percentage} = \frac{\text{Number of infected fruits}}{\text{Total number of initial fruits}} \cdot 100 \tag{3.1}$$

3.6 Measurement of Weight Loss

Both strawberries and raspberries were weighed after treatment and at each sampling day using a digital balance. The results were then recorded based on the percentage of the weight loss in comparison to the initial weight.

3.7 Measurement of Respiration Rate

A close system was used to measure the respiration rate. Strawberry samples (approximately 30 g each) and raspberry samples (approximately 12 g each) were placed in a 443 ml white plastic tray made airtight using a thermosealing machine. Level of O_2 and CO_2 of each sample were measured using a needle probe connected to a gas analyzer (PDI Biosensor). Respiration rate was then calculated using the Equation 3.2 (Saltveit n.d.).

$$\text{Respiration rate}(\frac{\text{ml}CO_2}{\text{kg} \cdot \text{h}}) = \frac{\Delta CO_2}{mt} \cdot V \tag{3.2}$$

Where V is the volume of the container (ml), t is the time at which the sampling is taking place (h), and m is the mass of fruit (kg).

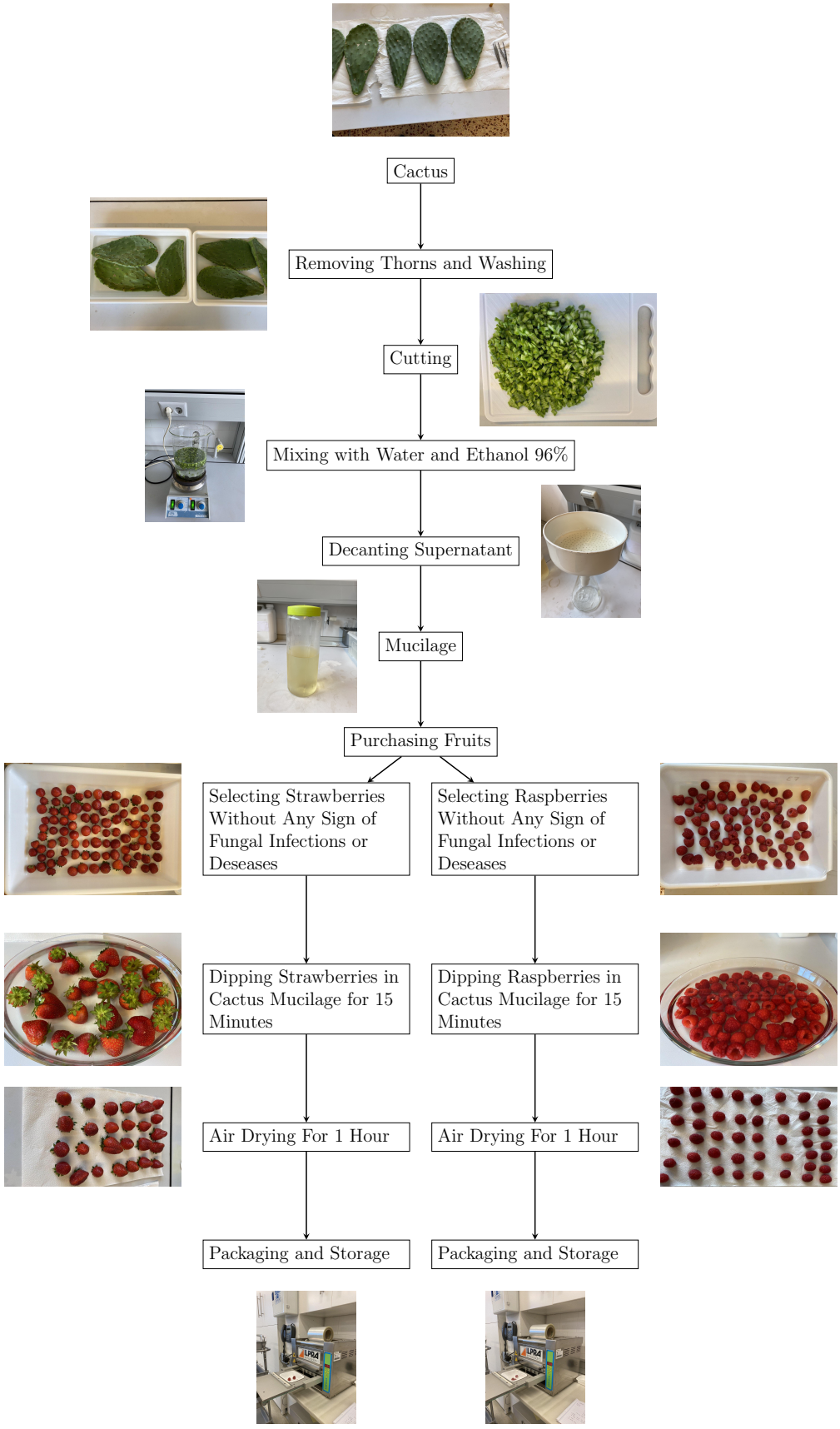


Figure 3.1: Mucilage Preparation and Coating Process

3.8 Color Characterization

The external color of all fruits from each tray was evaluated using a photo colorimeter configured in C.I.E.L.A.B scale Illuminant D65 10°viewing angle which provides L* (lightness), a* (position between red/magenta and green), b* (position between yellow and blue), and ΔE values. Three readings were taken at different sites on each fruit. Since the values of L* (lightness), C* (chroma), and H* (hue angle) are commonly used in the analysis of color in post-harvest treatment of fruits or vegetables (McGuire 1992). The chroma and hue angle are calculated using Equation 3.3 and 3.4 respectively.

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{3.3}$$

$$H^* = \arctan \frac{b^*}{a^*} \tag{3.4}$$

3.9 Measurement of pH and Total Soluble Solids

Total soluble solids (TSS) and pH of the strawberry and raspberry were measured on each sampling day. The Digital refractometer was cleaned and calibrated using distilled water. Fruits were homogenized using a hand blender, and the resulting homogenates were placed on the prism glass of the digital refractometer to measure °Brix. A digital pH meter was then calibrated and used to measure the pH of the homogenates.

3.10 Measurement of Total Phenolic Content And Antioxidant Activity

Strawberries and raspberries were blended and homogenized using a hand-blender. 5 g of the resulting strawberry homogenates and 2 g of the resulting raspberry homogenates were mixed with 15ml of distilled water in duplicate each. The mixtures were centrifuged 4500 × g for 10 m at 4°C. The resulting supernatant was removed, and the rest of the solution was diluted to 25 ml. Aqueous and methanolic extracts were obtained from the homogenates to analyze their total phenolic content and antioxidant activity.

The total phenolic content of the samples was measured on day 3 and day 13. The total phenolic was determined using the Folin–Ciocalteau colorimetric method (Waterhouse 2002):

- 20μl of homogenate, a galic acid calibration standard, and distilled water as blank were put into 1-cm, 2-ml plastic cuvettes.
- 1.58 ml water was added, followed by 100μl of FC reagent.
- The solution was mixed thoroughly and incubated for 8 minutes.
- 300μl of sodium carbonate solution was added and mixed.
- The solution was incubated for 2 hours at room temperature.
- Absorbance of the solution was then measured at 765nm.

Galic acid was used as a standard to prepare the standard calibration curve. The result was then expressed as mg GAE/kg FW, which is milligram of Galic acid equivalent per kilogram of fresh weight. Antioxidant activity of the fruits was measured in the sampling days (day 3 and day 13).

The antioxidant activity of the fruits were determined using the CUPRAC method (Apak et al. 2008):

- To prepare CUPRAC assay solution, $CuCl_2$ solution is made by dissolving 0.4262 g $CuCl_2 \cdot 2\,H_2O$ in water, and diluting to 250 ml. Ammonium acetate (NH_4Ac), is prepared by dissolving 19.27 g NH_4Ac in water and diluting to 250 ml. Neocuproine (Nc) solution is prepared daily by dissolving 0.039 g Nc in 96% ethanol and diluting to 26ml with ethanol. Trolox is prepared in 96% ethanol.
- To a test tube are added 1 ml each of Cu(II), Nc, and NH_4Ac, antioxidant sample solution and H_2O (1.1 - x) ml are added to initial mixture so as to make the final volume 4.1 ml.
- The tubes are stoppered, and after 1 h, the absorbance at 450 nm is recorded against a reagent blank.

Trolox was used as a standard to prepare the standard calibration curve. The result was then expressed as μmol TE/g FW, which is micromol of Trolox equivalent per gram of fresh weight.

3.11 Statistical Analysis

Data were analyzed by analysis of variance (ANOVA) with Minitab 19.2020.1 (Minitab, LLC). General linear model (GLM) procedures were performed for control and coating at different sampling times, and the significance of difference

between the groups was determined using Tukey's test ($p < 0.05$). Mean values are the average of duplicate analysis of duplicate samples of two independent experiments.

4 Results and Discussion

4.1 Mucilage Characteristics

Mucilage from *Opuntia ficus-indica* was analyzed for total phenolic content and antioxidant activity. Total phenolic content of the mucilage obtained using Folin-Ciocalteau is 492.96 ± 50 mg GAE / L mucilage (Calibration curve linear equation: $y = 0.0011x + 0.0004$; $R^2 = 0.9999$; $n = 6$). Antioxidant activity of the mucilage obtained using the CUPRAC method is $1421.82 \pm 139 \mu$mol TE / L mucilage (Calibration curve linear equation: $y = 0.0022x + 0.0189$; $R^2 = 0.9999$; $n = 6$) .

4.2 Decay Percentage

Table 4.1 shows both coated and uncoated strawberries did not show any sign of fungal infection in any sample for 15 days. On day 9, uncoated raspberries started to reveal signs of fungal infection. During the entire 15 days of storage, 17.28% of the uncoated raspberries were infected by molds. However, no sign of fungal infection was observed in coated raspberries during the 15 days of storage; therefore, coating raspberries with *Opuntia ficus-indica* mucilage decreased decay percentage significantly ($p < 0.05$). Robledo et al. (2018) reported that strawberries coated with thymol nanoemulsion, chitosan, and quinoa protein stored at approximately 5°C, had a significantly reduced decay percentage. By day 16, nearly all of the control strawberries were decayed, while about 40% of the ones coated were presented signs of mold infection. This was not the case for the strawberries coated with only chitosan and quinoa protein, and nearly all of them were decayed by day 16. Raspberries coated with *Aloe vera* gel had a reduced decay percentage in comparison to the control fruits which had a deterioration of about 22.54% after 8 days of storage at 4°C (Hassanpour 2015). In a study by Han et al. (2004), about 40% of control raspberries and more than 60% of control strawberries had signs of decay. Strawberries and raspberries coated with a chitosan-based coating showed a significant reduction in decay incidents. This effect was more pronounced in raspberries, where decay incidents remained very close to zero after 14 days of storage. Uncoated strawberries in a study by Tahir et al. (2018) revealed signs of fungal infection merely after the first 3 days of storage at 4°C. During the first 7 days of storage, 28% of the control strawberries were infected by molds while

Table 4.1: Decay percentage

Day	Strawberry		Raspberry	
	Uncoated	Coated	Uncoated	Coated
3	0.0%	0.0%	0.0%	0.0%
7	0.0%	0.0%	0.0%	0.0%
9	0.0%	0.0%	8.64%	0.0%
11	0.0%	0.0%	13.58%	0.0%
13	0.0%	0.0%	13.58%	0.0%
15	0.0%	0.0%	17.28%	0.0%

none of the strawberries coated with gum arabic (*Acacia senegal*) showed any signs of infection. We can see that the decay incidents in control fruits are relatively high in the studies mentioned than our results. This can be explained due to our sealed packaging, which limits the respiration rate and reduces fungal infections. Coating had a significant effect on the decay percentage in raspberries, which is in agreement with other studies on hydrocolloid-based coatings.

4.3 Weight Loss and Respiration Rate

Figure 4.1 shows that the weight loss percentage of raspberry increased during the storage period. This, however, was not the case for strawberry, and there was no significant increase during the storage period for either uncoated or coated samples. The weight loss percentage of strawberry at day 15 was $0.45 \pm 0.11\%$ for coated strawberries and $0.20 \pm 0.02\%$ for uncoated ones. The weight loss percentage of raspberry at day 15 was $1.83 \pm 0.52\%$ for coated raspberries and $1.06 \pm 0.18\%$ for uncoated ones. After 15 days of storage, the weight loss percentage is remarkably lower compared to other studies on hydrocolloid-based coated strawberries and raspberries. In Sogvar et al. (2016) experiments, control strawberries lost more than 10%, and more than 5% in the best *Aloe vera*-based coated sample after 12 days of storage. The strawberries were placed in a polystyrene box and were stored at 1 °C with 95% relative humidity. Similar results for strawberries and raspberries were observed by Han et al. (2004), where both strawberry and raspberry had a weight loss of more than 5% for both control and coated fruits with chitosan after 14 days. Emamifar et al. (2019) observed a weight loss percentage of at least 5% in strawberries after 12 days of storage in salep-based coated strawberries. Weight loss percentage observed by Khodaei et al. (2019) in strawberries coated with carboxymethyl cellulose (CMC) and packed using polyethylene terephthalate (PET) clamshell containers with low oxygen and moisture permeability stored at 4 °C for

12 days was more than 2%. The remarkable difference in the weight loss percentage between the mentioned studies and our study can be explained due to our use of sealed packaging. Sealed packaging reduces respiration and transpiration, and weight loss occurs mostly because of respiration and transpiration. Respiration causes a loss in carbon reserve, and transpiration causes water loss (Sogvar et al. 2016).

Figure 4.2 indicates that coating slightly increased the respiration rate in both strawberries and raspberries. This increase is significant in strawberries (Tukey, $p < 0.05$). The respiration rate remained nearly constant in coated and control strawberries. However, the respiration rate first increased and then decreased in raspberries. The increase in respiration rate is because of the higher amount of CO_2 available in the headspace atmosphere of the fruits. The polysaccharide content in *Opuntia ficus-indica* mucilage increases the content of CO_2 in the internal atmosphere of fruit. This was observed in a study by Allegra et al. (2016) where kiwifruits coated with *Opuntia ficus-indica* mucilage had an increased accumulation of CO_2.

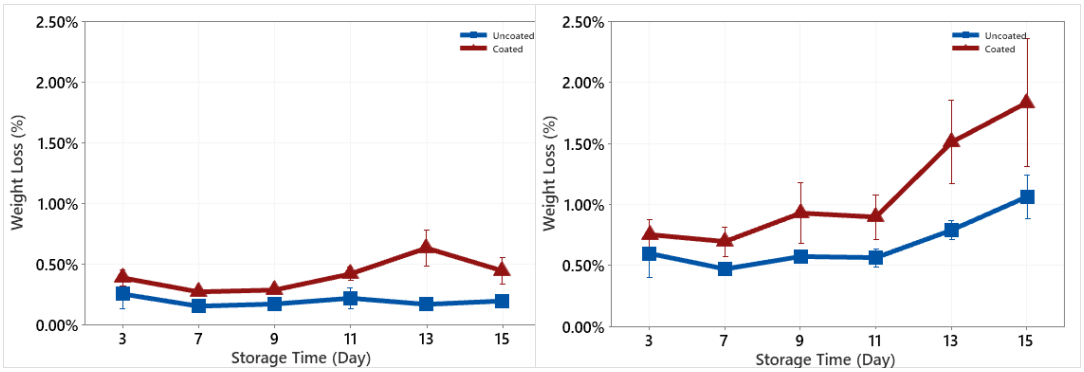
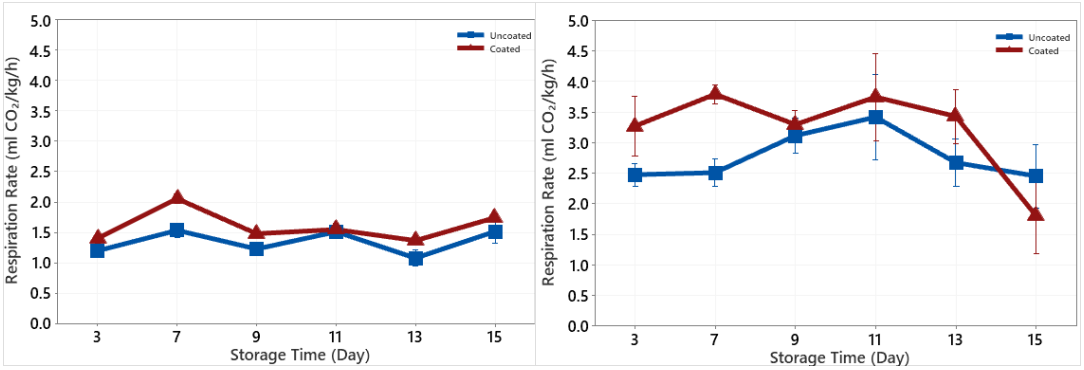


Figure 4.1: Weight loss percentage plot for strawberry and raspberry. (a) Strawberry. (b) Raspberry ($n = 4$). Data presented are the means and the bars indicate standard deviations. Mean values are the average of duplicate analysis of duplicate samples of two independent experiments.



(a) (b)

Figure 4.2: Respiration rate plot of strawberry and raspberry. (a) Strawberry. (b) Raspberry. Data presented are the means and the bars indicate standard deviations ($n = 4$). Mean values are the average of duplicate analysis of duplicate samples of two independent experiments. Respiration rate is measured in terms of CO_2 ($\mathrm{mlCO_2 \cdot kg^{-1} \cdot h^{-1}}$).

4.4 Color

Arguably one of the most important factors for consumers when buying raspberries and strawberries is the fruit's visual attributes. A key measurable visual attribute is the surface color. L*, C* and H* were used in the statistical analysis. The lightness of strawberries did not significantly change with coating ($p > 0.05$). However, in the raspberries, lightness significantly decreased with coating ($p < 0.05$) as seen in Figure. 4.4b. Similarly, chroma of strawberries did not significantly change with coating ($p > 0.05$). While chroma of raspberries decreased significantly due to coating ($p < 0.05$, Figure. 4.4d).

The hue angle in both coated and uncoated strawberries significantly decreased during the storage time ($p < 0.05$). This means that the strawberries got "redder" over time (Figure 4.4e). In contrast, the hue angle in both coated and uncoated raspberries significantly increased during the storage time ($p < 0.05$).

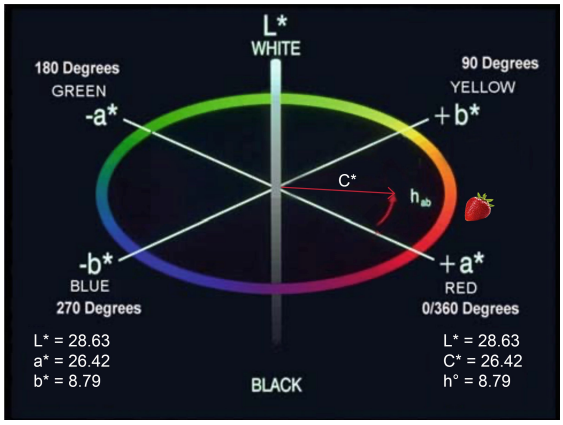


Figure 4.3: Representation of the color of the surface of the strawberry using L*, C*, and H*. This figure is adopted from Wrolstad et al. (2010).

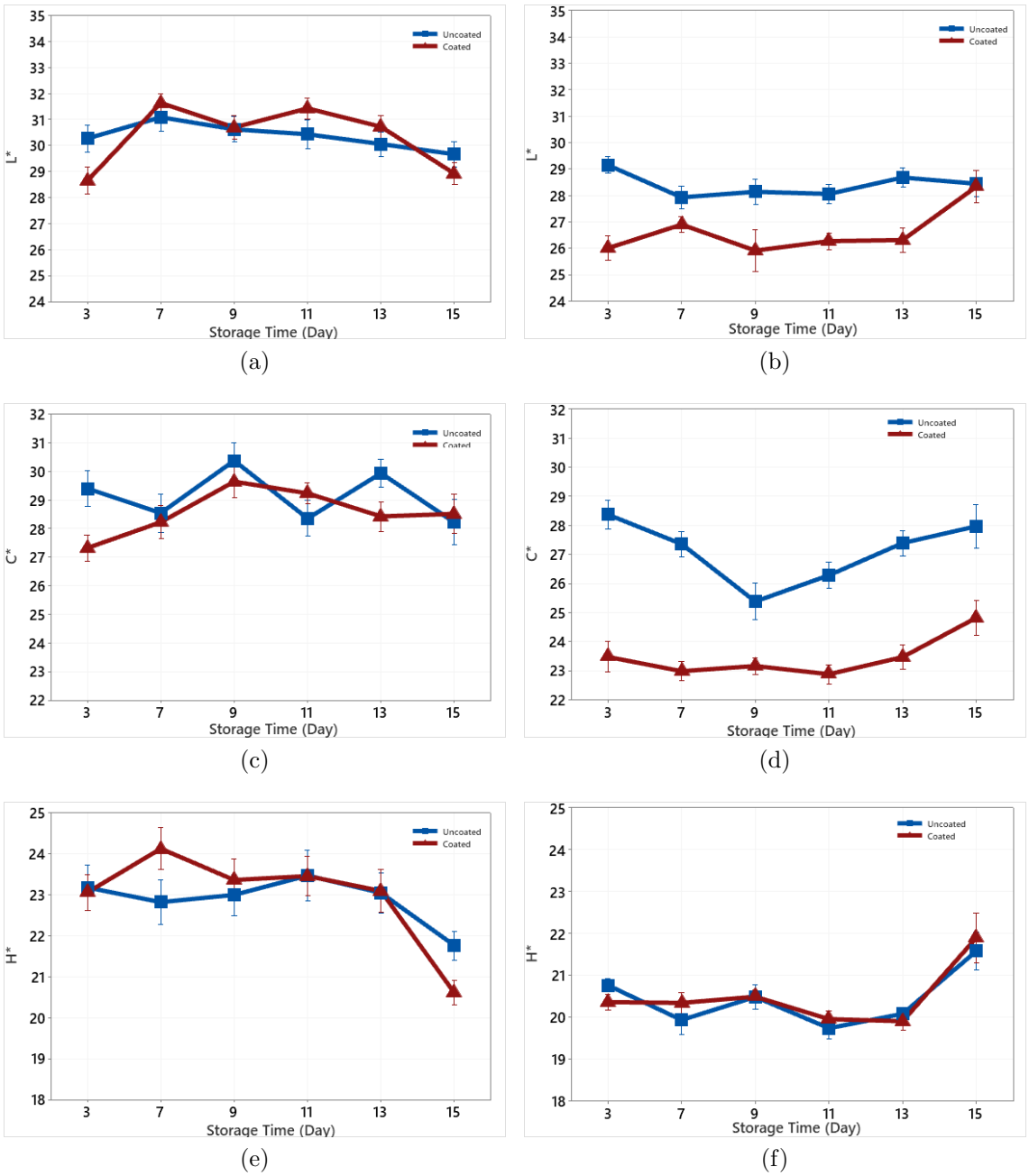


Figure 4.4: Color measurements plot for strawberries and raspberries. (a) Strawberry lightness. (b) Raspberry lightness. (c) Strawberry chroma. (d) Raspberry chroma. (e) Strawberry hue angle. (f) Raspberry hue angle. Data presented are the means and the bars indicate standard deviations ($27 \leq N \leq 42$). Mean values are the average of duplicate analysis of duplicate samples of two independent experiments.

4.5 pH and Total Soluble Solids Content

The pH of strawberry and raspberry increased during the storage for both coated and control samples (significant for raspberry, $p < 0.05$). There was no significant difference between the coated and control samples. Similar increasing trend for pH in strawberry has been reported by Sogvar et al. (2016), Khodaei et al. (2019), Shao et al. (2018), and Han et al. (2004) for coated and control samples.

Figure 4.5 shows that the total soluble solids content of strawberry and raspberry was higher in coated samples. This difference is significant in strawberry ($p < 0.05$) but not in raspberry ($p \approx 0.063$). Total soluble solids content in strawberry decreased during the storage for both coated and control samples. On the other hand, an initial increase and then a decrease was observed in raspberry. Similarly, Tahir et al. (2018) observed a higher soluble solids content in coated strawberries. The initial increase and the following decrease in the total soluble solids content of raspberry may reflect the process from commercial maturity to over-ripeness (Yan et al. 2019).

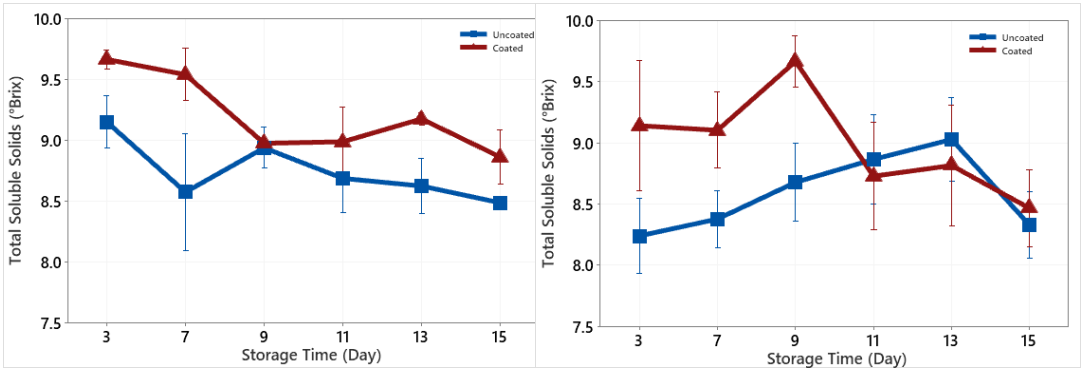
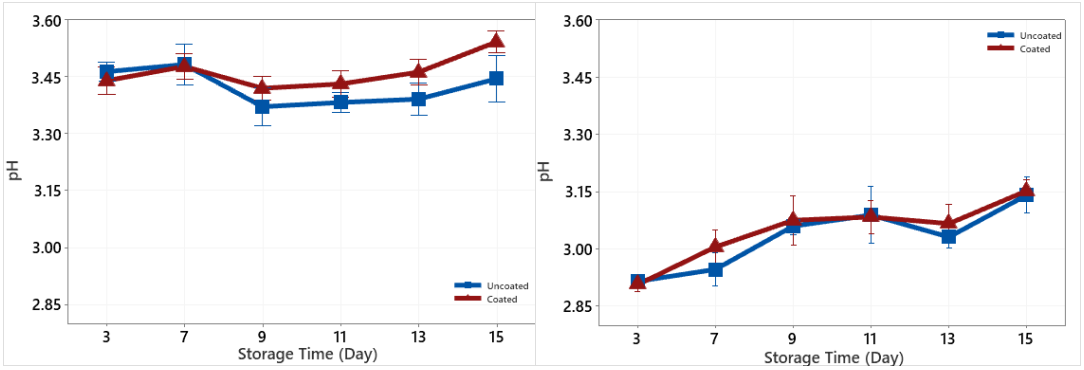


Figure 4.5: Total soluble solids content plot for strawberry and raspberry. (a) Strawberry. (b) Raspberry. Data presented are the means and the bars indicate standard deviations ($n = 4$).



(a) (b)

Figure 4.6: pH plot of raspberry and strawberry. (a) Strawberry. (b) Raspberry. Data presented are the means and the bars indicate standard deviations ($n = 4$). Mean values are the average of duplicate analysis of duplicate samples of two independent experiments.

4.6 Antioxidant Activity and Total Phenolic Content

As shown in Figure 4.8 antioxidant activity does not differ significantly in coated and control samples. Antioxidant activity increased in strawberry during the storage while it remained relatively stable in raspberry. A similar increase in antioxidant activity of strawberry during the first 6 days of storage was reported in Petriccione et al. (2015). Piljac-Žegarac et al. (2011) reported that both strawberry and raspberry had a more or less constant antioxidant activity in the cold without any treatment, which can show that both fruits have an inherent capacity to preserve antioxidant activity.

Figure 4.7 shows that the total phenolic content of coated samples is significantly ($p < 0.05$) higher than control samples for strawberry and raspberry in both methanol and aqueous extracts. These result are in agreement with several other studies about hydrocolloid-based coatings. Emamifar et al. (2019) and Sogvar et al. (2016) observed that polysaccharide-based coated strawberries had significantly higher total phenolic content. In another study by Chen et al. (2016), alginate-based coated Nanfeng mandarins had a notably higher total phenolic content compared to the control. This increase can be explained due to the total phenolic content available in the mucilage obtained from *Opuntia ficus-indica*.

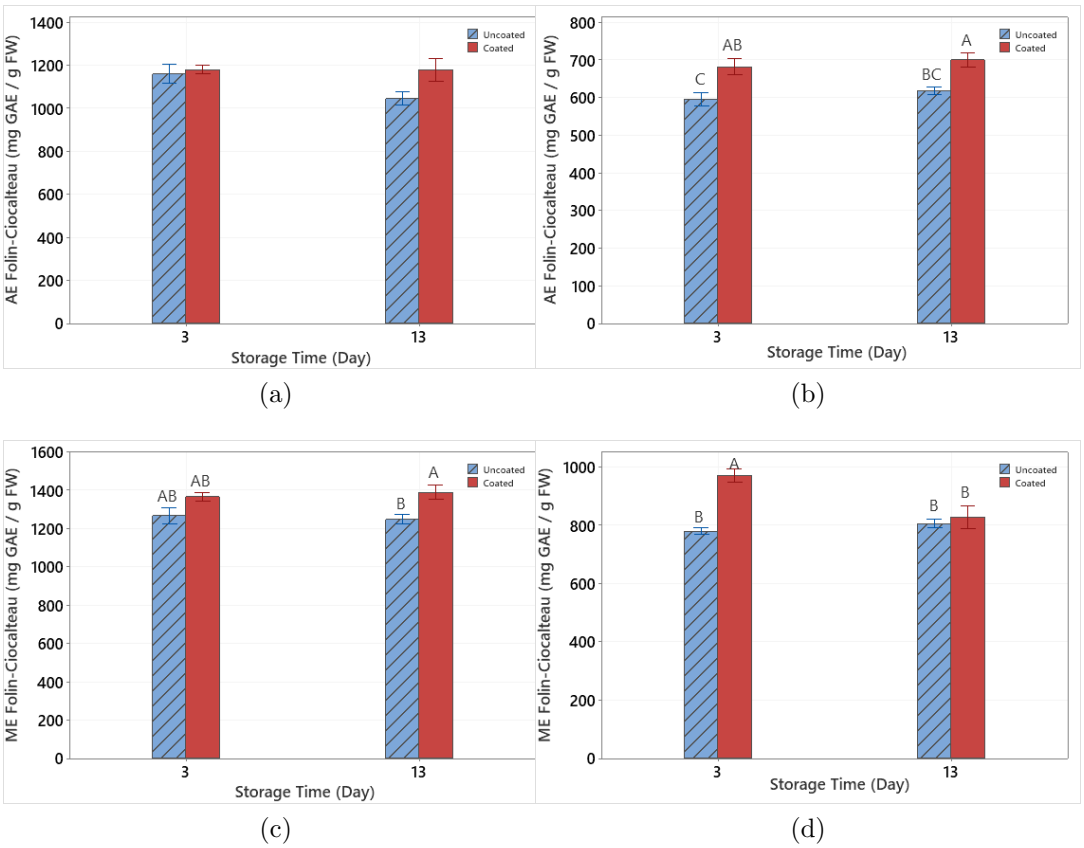


Figure 4.7: Total phenolic content plot of raspberry and strawberry. (a) Strawberry aqueous extract total phenolic content. (b) Raspberry aqueous extract total phenolic content. (c) Strawberry methanol extract total phenolic content. (d) Raspberry methanol extract total phenolic content. Data presented are the mean values, and the bars indicate standard deviations ($n = 16$). Mean values are the average of duplicate analysis of duplicate samples of two independent experiments. Bars with no shared letters are significantly different ($p < 0.05$). Total phenolic content is measure in terms of mg GAE/kg FW.

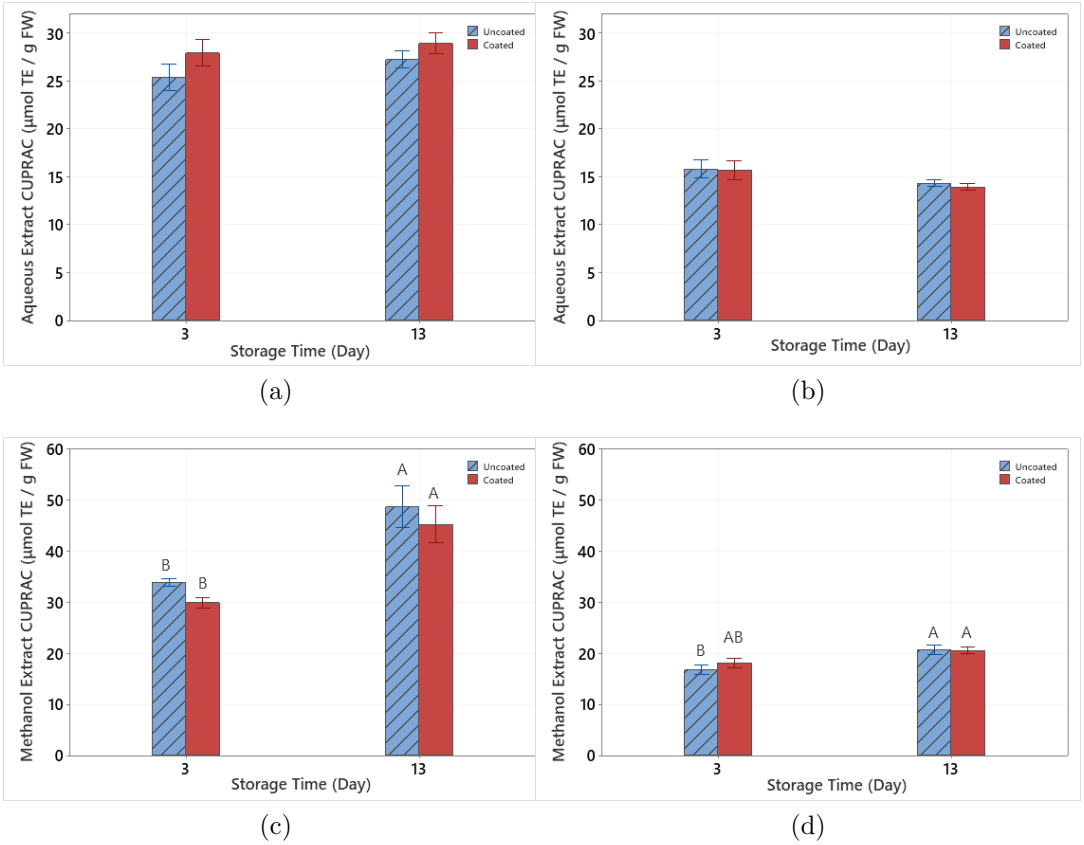


Figure 4.8: Antioxidant activity of raspberry and strawberry. (a) Strawberry aqueous extract antioxidant activity. (b) Raspberry aqueous extract antioxidant activity. (c) Strawberry methanol extract antioxidant activity. (d) Raspberry methanol extract antioxidant activity. Data presented are the means and the bars indicate standard deviations ($n = 16$). Mean values are the average of duplicate analysis of duplicate samples of two independent experiments. Bars with no shared letters are significantly different ($p < 0.05$). Antioxidant is measured in terms of μmol TE/g FW.

5 Conclusions

From the results of our experiments we can conculde:

1. The mucilage from *Opuntia ficus-indica* was extracted and analyzed. The antioxidant activity was $1421.82 \pm 139\mu$mol TE / L mucilage and the total phenolic content was 492.96 ± 50 mg GAE / L mucilage.

2. The sealed packaging used reduced the decay percentage and weight loss of fruits drastically compared to other studies that did not use sealed packaging.

3. Fruits coated with cactus mucilage retained more of their total phenolic content compared to uncoated fruits, which can be due to the total phenolic content of the mucilage.

4. Coated raspberries did not show any sign of fungal infection, while 17.28% of uncoated raspberries had fungal infection by the end of the experiment.

5. The combination of sealed packaging and coating improved the shelf-life of strawberry and red raspberry remarkably by maintaining the total phenolic content, delaying fungal decay, and reducing weight loss during 15 days of storage at 2 ± 2°C.

In this study, we observed that sealed packaging and *Opuntia ficus-indica* mucilage coating extended the shelf-life of strawberry and red raspberry. We can further explore this topic by incorporating other eco-friendly materials in our coating solution to extend the shelf-life of various highly-perishable fruits.

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