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Rajiv Dhital
Southern Illinois University Carbondale, rajiv.dhital@siu.edu

Nathalie Becerra-Mora
Southern Illinois University Carbondale, nathalie09@siu.edu

Dennis G. Watson
Southern Illinois University Carbondale, dwatson@siu.edu

Punit Kohli
Southern Illinois University Carbondale, pkohli@chem.siu.edu

Ruplal Choudhary
Southern Illinois University Carbondale, choudhry@siu.edu

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EFFICACY OF LIMONENE NANO COATINGS ON POST-HARVEST SHELF LIFE OF STRAWBERRIES

Rajiv Dhital[1], Nathalie Becerra- Mora[2], Dennis Watson[1], Punit Kohli[2], Ruplal Choudhary[1]

[1] Department of Plant, Soil and Agricultural Systems, Southern Illinois University, Carbondale.

[2] Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale.

*Corresponding Author: 1205 Lincoln Drive Room 176, Carbondale IL 62901, USA. Email: choudhry@siu.edu; Phone: 618 453 6985

ABSTRACT

Strawberries are highly demanded fruits because of their color, nutritional values and appearance. The aim of this study was to develop and characterize alginate and limonene liposomes as edible coating materials and to determine their efficacy in shelf life extension and maintaining quality parameters of 'Chandler' strawberries. Alginate solution (1.5% w/v) and Limonene liposomes prepared from 80% lecithin and 20% PDA were used as edible coating materials. Fungal decay percentage, total yeast and mold counts, headspace atmosphere analysis, total soluble solids, pH, titratable acidity, total anthocyanin content and total phenolics were analyzed to assess fruit quality during 14 days at 4ºC of storage. Days of storage was found to be significant in maintaining the quality of the strawberries. Among the coating types, limonene liposomes were found to be significantly more effective in maintaining the lower concentration of carbon dioxide (CO_2), lower the change in pH (3.9), and had higher total anthocyanin (43.85) content during storage than those without a liposomal coating. Thus, limonene liposomes were found to be useful for extending the shelf life and maintaining quality of strawberry fruits.

Keywords:

Strawberry, strawberry; edible coating; shelf life; limonene; liposome

1. Introduction

Consumption of fruits and vegetables have gained significant global attention in recent years. Fruits and vegetables are the source of nutrients such as proteins, vitamins, minerals, fibers, and phytochemicals that are essential to improve human nutrition and health (Li, 2008). The phytochemicals contribute to the normal functioning of the human body (Wettasinghe et al. 2002). The antioxidant compounds, such as ascorbic acid (AA), lycopene, β-carotene, and phenolics contribute to nutritional content of fruit and vegetables. These compounds are known to prevent oxidation caused by reactive oxygen species that lead to damage the cells and DNA, and cause some degenerative diseases (Hu and Jiang, 2007).

Strawberries (*Fragaria x ananassa),* with their characteristic appearance, color and nutritional values, are highly consumed fruits (Almenar et al. 2007). They are considered as a good source of nutrients, anthocyanins, flavonoids and phenolic compounds (Heinonen et al.1998). United states is the largest producer of strawberries in the world (Wu et al. 2012) and per capita consumption was 7.9 pounds in 2013 (USDA Report 2014). Of the total strawberry production, 81 percent comes from fresh market (NASS 2015).

Strawberries are one of the most susceptible foods prone to physical injuries and fungal spoilage (Park et al. 2005). These results in the change of several physiochemical properties, fungal growth that results in short shelf life and causes a significant postharvest loss. The goal of the research is to develop edible nano-coatings from plant based antimicrobials, which would maintain the postharvest quality of strawberries and extend their shelf life. In our preliminary studies, edible nano-coatings prepared by the nanoencapsulation of curcumin and limonene in liposomes when applied on the surface of strawberries were effective in extending the shelf life and maintain the quality (Dhital et al. 2017).

Application of plant based essential oils compounds as coating materials in foods have shown to prevent microbial growth and loss of nutrients; and increase the shelf life of foods (Salmieri & Lacroix, 2006). Essential oils of oregano, thyme, cinnamon, lemongrass and clove exhibit antimicrobial activity against strains of *E. coli* (Smith-Palmer et al. 1998; Hammer et al. 1999;

Friedman et al. 2002). Some plant essential oils and their components are responsible for increasing the sensory attributes of fruits and preventing the microbial growth. Terpene citral, a citrus essential oil is known to have antimicrobial properties and contribute for sensory properties of foods (Rodov et al. 1995).

Limonene ((R)-(+)-para-Mentha-1,8-diene) is obtained from essential oil of citrus fruits i.e. orange, lemon, mandarin, lime, grapefruit (Moufida and Marzouk 2003). It is a colorless liquid hydrocarbon regarded as safe used largely by cosmetic, food and pharmaceutical industries and has Generally Recognized as Safe (GRAS) status by US Food and Drug Administration (EPA, 1994). Limonene has a very strong antifungal property and is effective against food spoilage fungal species. It shows an antibacterial properties effective against pathogenic bacteria like; *Staphylococcus aureus, L.monocytogenes, Salmonella enterica* (Sharma and Tripathi 2008; Alonso-Gutierrez et al. 2013). Due to hydrophobic nature and tendency to degrade under oxidative conditions, limonene possess a challenge during its application as an edible coating material because of poor dispersion in water (Li and Chiang, 2012). To address this issue, low concentrations of limonene are used for dispersion in water, which in turn reduces its antimicrobial activity. The problem of hydrophobic nature and use of low concentrations of limonene limits its efficacy while using as a coating material. A new approach of encapsulation of phytochemicals in liposome that has both hydrophobic tails and hydrophilic heads through nanotechnology (Umagiliyage et al. 2017).

Incorporation of antimicrobial compounds into edible films and coatings provides an innovative approach to improve microbial safety and shelf life of foods (Cagri et al. 2004). Some of the most commonly used antimicrobial agents in food are; benzoic acid, sodium benzoate, sorbic acid, potassium sorbate and propionic acid which can be incorporated into edible films and coatings (Cruz-Romero et al. 2013). Starch based edible coatings containing potassium sorbate applied on the surface of fresh strawberries reduced the microbial growth and extended the shelf life (Garcia et al. 1998). A bilayer edible coating made from plant based antimicrobial compounds, limonene and curcumin were applied in combination with methylcellulose (MC) for improving of post-harvest quality of fresh strawberries (Dhital et al. 2017). Edible films containing organic acids,

protein and glycerol have shown to inhibit the growth of pathogenic organisms including *L. monocytogene*s, *S. gaminara* and *E. coli* 0157:H7 (Hettiarachchy and Satchithanandam, 2007).

Nano-technology has been extensively used to enhance the quality of fruits and vegetables (Yang et al; 2010). Encapsulation of antimicrobial compounds using the approaches of nanotechnology can address the problems of microbial degradation and hence improve the quality of fruits. Liposomes have been used in different fields of science and technology. In food applications, liposomes can be potentially used to improve the efficacy of antimicrobial compounds, delivery of nutrients and protection of sensitive ingredients in foods (Lasic,1993). Liposomes are used in the encapsulation of nutrients, proteins, enzymes, antimicrobial and flavors and their controlled release in food environment to delay the microbial spoilage and maintain the food quality (Makwana et al. 2015). Application of essential oils encapsulated in liposomes have shown to improve quality and extend the shelf life of fruits and vegetables (Alikhani-Koupaei, 2014). In our previous study (Dhital el al., 2017), limonene encapsulated in liposome were found to improve the post-harvest shelf life of strawberries. An improved antimicrobial activity of nano-encapsulated eugenol was reported by Shah et al. (2012b) against *E. coli* 0157:H7 and *Listeria monocytogenes* in bovine milk. Limonene encapsulated in nano-emulsion exhibited antimicrobial activities towards *Escherichia coli*, *Lactobacillus delbrueckii* and *Saccharomyces cerevisiae* (Donsi et al. 2012). In previous studies, liposomes polydiacetylene were used to deliver and carry antibodies and drugs in mice. Results showed that injection up to 100 mg kg^{-1} of polydiacetylene did not induce acute toxicity in the mice. (Gravel, et al., 2012).

Alginate is a generic term for the salts and derivatives of alginic acid. Alginates are commercially produced from brown algae *Macrocystis pyrifera,, Laminaria hyberborea, Laminaria digitata, Ascophyllum nodosum, Laminaria japonica, Edonia maxima, Lessonia nigrescens, Durvillea Antarctica,* and *Sargassum* spp. (Draget, 2005). These compounds have good film-forming properties. The alginate films are typically uniform, transparent and water-soluble. Upon addition of calcium ions, alginate undergoes conformational changes resulting in the formation of calcium alginate (Moe, 1995). These compounds when applied as coating materials improved the quality of fruits and vegetables by reducing the shrinkage, moisture migration, oxidative rancidity, oil absorption , holding volatile compounds, improvement in sensory properties of products (Hershko

and Nussinovitch, 1998). Alginate has wide range of application in industrial sectors due to their ability to retain water, film-forming, gelling, viscosifying and stabilizing properties. Their film forming properties make them useful in food processing industries. In addition, alginate coatings have shown good oxygen barrier properties (Conca and Yang, 1993) that eventually can retard lipid oxidation in fruits and vegetables (Kester and Fennema 1986). Alginate based coatings applied to fresh cut 'Fuji' apples showed that these coatings could carry antioxidants, which are responsible for the maintaining color of cut fruits during storage (Rojas-Grau et al. 2007 b)

Mechanical injuries due to vibration can cause in a significant loss of fruits and vegetables. A study by Singh and Xu (1993) reported that about 80% of apples could be damaged by simulated transportation by truck. Damage due to vibration during the transportation was noted on different fruits and vegetables that include peaches, apricots, potatoes, tomatoes (Barchi et al. 2002). In strawberries, In-transit vibration causes skin abrasion and bruising which makes easier for microbes to enter inside the berries and cause degradation (Fischer et al. 1992).

The goal of this study was to develop novel nanocoating treatments prepared from plant-based antimicrobials encapsulated in nano-liposomes. The objectives in this study were: (1) Preparation and characterization of the edible coating materials, (2) application of edible coating materials on strawberry fruits and analyze the quality parameter of strawberries treated with edible coatings during storage and 3) compare the efficacy of coating materials based on quality parameters.

2. Materials and methods

Fresh strawberries of 'Chandler' variety purchased from local farms located in southern Illinois. Berries were visually inspected for bruises, visual fungal growth, and decay. Uniform sized berries were selected and stored at 4°C prior to coating application.

2.1. Preparation of D- Limonene Liposomes

Thin film dehydration method was used for the preparation of lipid film (Figure 1). Briefly, a mixture of soy-based lecithin and diacetylene (PDA; 10,12-Pentacosadiynoic acid) monomer with different weight ratios (100 % lecithin, 80% lecithin, 60 % and 50 % lecithin) was dissolved in 25 mL of dichloromethane in a 250ml round bottom flask. The solution was then subjected to rotary evaporation for 1 hour to evaporate the solvent and promote bilayer film formation. The resulting

film was dried overnight by placing the flask on a vacuum pump followed by film hydration by the addition of 50 µM D- limonene prepared in Nano-pure water. The resulting solution then left sonicated for 20 minutes. Further, the solution was placed in a probe sonicator (VCX 500, Vibra-cell, Newtown, CT) at 76 °C for 15 minutes. The solution was then filtered through 0.45 µm nylon fiber to remove the lipid aggregates. Thus, obtained liposome solution was collected and kept away from light at 4°C for 8 hours prior to use for experiments. Liposomes were polymerized by irradiation with a UV lamp emitting at 254 nm for approximately 2-5 min using a Pen Ray (UVGL-58, Minerallight, Upland, CA) UV source (4.5 mW/cm^2) in air at 4°C. The polymerized liposome solution was dialyzed using a membrane Spectra/Por® Biotech Cellulose Ester (CE) membrane (MWCO: 100,000) for 48 hours changing the water every 4 hours. Thus obtained liposome solution was collected and stored at 4°C for further studies.

2.2. Characterization of Liposomes

UV−vis absorption spectra of all the dialyzed non-polymerized and polymerized liposomes prepared with varying concentration of soy-based lecithin and PDA were recorded at room temperature using a PerkinElmer Lambda 25 (spectral slit width 1 nm) UV/vis spectrometer using a cuvette of 1 cm path length. Sterile distilled water used as blank and to calibrate the spectrometer at 400-800 nm.

2.3. Alginate solution preparation

Sodium alginate powder was dissolved in double distilled water to prepare alginate solution. Briefly, Sodium alginate was dissolved in 500 ml of water upon stirring at 70 °C for 2 hours on a hot plate to obtain a 1.5 % (w/v) solution.

2.3.1. Characterization of alginate coatings

2.3.1.1 Fluorescent imaging

In order to determine homogeneity of the coating, on strawberries using fluorescence microscopy,we labelled alginate with amino-pyrene. Strawberries were dipped in the solution of pyrene alginate and freeze- dried at -80 ºC for 24 hours. In order to check the homogeneity of the coating layer on strawberries, cross sectional slices of coated strawberries were made. A Leica inverted fluorescent microscope was used for fluorescent alginate coated strawberries imaging. A

UV lamp source was used to excite the fluorescent molecules. A long pass band UV filter was used to select the excitation wavelengths. The emission spectrum was collected from 420 nm to 500 nm.

2.3.1.2 Scanning electron microscopy

The SEM images of the alginate-coated berries were taken for the characterization of alginate coatings. Briefly, alginate coatings (1.5% w/v) were applied on the berries, followed by freeze-drying (-80 ºC) for 24 hours. The freeze-dried samples were cut using a razor blade in small pieces (5 mm by 5 mm). They were sputter coated for 4 minutes with a layer of Ag-Pd using a DESK II, DENTON VACUUM sputter. The edges of the samples were grounded using a thin layer of silver paint (SPI, USA). The samples were imaged using a scanning electron microscope QUANTA FEG 450 (FEI), the acceleration voltage was 5 kV using ETD detector at high vacuum.

2.4. Application of coating materials

The summary of application is represented in Figure 3.1. Briefly, berries were randomly selected and divided into three groups depending upon coating treatment types; limonene liposome, alginate and non-coated control. Each treatment was performed in triplicate and each replicate had 20 berries. Berries were dipped in the solutions of 50 µM liposome solutions for 10 min. For Alginate coatings, 1.5 % alginate solution was cooled to room temperature and strawberries were dipped in the alginate solution for 3 minutes. The berries were then immersed in 5% w/v aqueous solution of $CaCl_2$ for 2 min. For the non-treated control samples, the berries were rinsed with sterile distilled water. All the treated berries were air dried at room temperature in an UV sterilized cabinet drier for 2 hours and packed in sterile clamshell box stored at 4º C.

2.5 Fungal decay percentage

Berries were visually evaluated for the presence of visible mold growth during the experiment. Any berry with visible growth was considered to be decayed. Fungal decay percentage was calculated by using the formula:

Fungal decay % = (the number of decayed fruits / total number of fruits) ×100

2.6. Total Yeast and mold count

Total yeast and mold count on the berry surface was performed by serial dilutions followed by spread plating over the surface of sterile DRBC plate method as recommended by the International Standard Organization (ISO 21527-1, 2008) with slight modifications. Briefly, berries from each treatment and untreated control were stirred (in 150 mL Erlenmeyer flask) at 150 rpm in 20 mL of 0.1% (w/v) sterile peptone water for 30 min. The resulting suspension was then serially diluted from 1:10 to $1:10^6$ dilutions. Then, 0.1 ml inoculum of each dilutions was used for plating and spread evenly over the plates. The plates were incubated at 25 ºC for 5 days. Results were expressed as log colony forming units per ml (CFU/ml) based on average count of triplicate set.

2.7. Headspace atmosphere analysis

In hermetically sealed 500 ml glass jars, each jar containing 5 berries coated with treatments were placed in and sealed. The jars were kept at 4ºC for 1 hour. Head space Carbon dioxide concentrations in the sealed jars were determined using an OXYBABY 6.0 gas analyzer (WITT-GASETECHNIK GmbH & Co KG, Witten, Germany) comprising an electro-chemical cell for oxygen analysis and an IR-absorption cell for carbon dioxide analysis. The experiment was performed in triplicate.

2.8. Fruit weight loss

Strawberries just after coating and air drying were weighed. Twenty berries corresponding to each coating treatment were used and the experiment was performed in triplicate. Weights of the berries were measured at 2, 5, 9 and 14 days after coatings. Weight loss was estimated as the percentage loss of initial weight.

$$\text{Weight loss \%} = (\text{Initial weight} - \text{final weight} / \text{Initial weight}) \times 100$$

2.9. Determination of Total soluble solids (TSSs), pH, Titratable acidity (TA)

TSS, TA and pH of strawberries was measured at different time intervals 2, 5, 9 and 14 days after coating treatment is done. Fruit from each coating treatments were crushed with the help of sterile mortar and pestle and juice will be collected. Sampling was done triplicate.

The TSSs of the resulting juice was measured at 20ºC by a Brix refractometer (r^2 mini, Reichert Analytical Instruments, Depew, NY). Similarly, pH of the juice was measured by a pH meter (Corning pH/ion analyzer 350). TA was determined by titrating the diluted juice (5ml juice diluted in 95ml distilled water) up to pH 8.2 using 0.1N NaOH.

2.10. Analysis of total anthocyanin content

Analysis of total anthocyanin content was performed at intervals of 2,5,9 and 14 days after coating treatment is done. Strawberry sample (2g) was crushed with 20 ml of methanol in 1% HCl with mortar and pestle. Then, the mixture was centrifuged at 1000×*g* for 20 min. The supernatant was collected and absorbance was noted at 530 nm. Absorbance readings was converted to milligrams of pelargonidin-3-glucoside per 100 g of fruit fresh weight, using a molar absorption coefficient of 36000 (Cordenunsi et al. 2003).

2.11. Analysis of total Phenolic compounds content

Fruit samples treated with different coatings and stored at different time intervals (2, 5, 9 and 14 days) were selected. Briefly, a 1.5 g strawberry sample grinded in a mortar and pestle was used and extracted with 20ml mixture of acetone, water and acetic acid (70:29.5:0.5 v/v). The samples were vortexed for 1 hour at room temperature for complete extraction, followed by centrifugation at 1640 g for 15 minutes at 20ºC. The supernatant was filtered and allowed to stand at room temperature for evaporation of solvent. The residue was then dissolved in distilled water to a volume of 20 ml. The experiment was done in triplicate. Total phenolic content of the extracted juice was determined by the use of Folin-Ciocalteu reagent as per the method of Slinkard and Singleton (1977). The standard calibration curve was prepared by using Gallic acid as a standard. The result was expressed as milligrams per liter of Gallic acid equivalents (GAE) per 100 gm fresh weight.

2.12. Statistical analysis

The tests conducted in triplicate for each sample and simple random sampling for each tests. Generalized linear mixed model analysis were carried out to determine the effect of coatings and days of storage on different quality parameters. The coating treatments were compared on the basis of fungal decay percentage, total yeasts and mold counts, weight loss, pH, total soluble solids content and titratable acidity, total phenolic content, and total anthocyanin content. Treatment means were separated using Fisher's protected least square mean separation at $P \leq 0.05$.Data were analyzed using SAS 9.4 version (SAS Institute, Inc., Cary, NC).

3. Result and discussion

3.1. Characterization of coating materials

3.1.1. Characterization of liposomes

Liposomes prepared with a mixture of different concentrations of lecithin and PDA were characterized by UV/Vis spectroscopy. Figure 2 shows the absorption spectrum of Lecithin: PDA nanovesicles. The yellow line corresponds to unpolymerized liposomes, the lack of absorption peaks demonstrates the absence of conjugation in PDA backbone. On the other hand, the blue line shows the absorption spectra of liposomes prepared from the mixture of 80% lecithin and 20% PDA after photo polymerization. From the absorption spectra after UV light irradiation, the peak present at 655 nm along with its narrow shoulder at 593 nm corresponds to π- π^* electronic transitions (Li et al. 2008; Day and Ringsdorf, 1978). The low absorbance value at lower wavelengths shows low scattering that indicates low polydispersity of nanovesicles (Tomaszewska et al. 2013).

3.1.2. Characterization of alginate coatings

3.1.2.1. Fluorescence imaging of fluorescent alginate coated berries

The alginate concentration for this particular experiment was roughly ten times higher than the concentration of alginate originally used for the coating. As shown in the fluorescent micrographs (Figure 3), the coating extends along the berry and the thickness of the coatings was about 10 µm.

297 The blue intense emission was due to the presence of amino-pyrene molecules chemically bound
298 to alginate polymer (Srivastava et al. 2009).

299

300 Alginate was labeled with amino-pyrene in order to image the presence and homogeneity of the
301 coating. Pyrene was selected due to the emission wavelength range and long lifetime excited state.
302 The emission intensity was recorded from 420 to 500 nm with a Nuance hyperspectral CCD
303 camera. Figure 4 corresponds to the fluorescent spectrum obtained from the fluorescence
304 micrograph (Fig. 3) on the amino-pyrene alginate coating. A maximum emission peak was
305 observed at 490 nm. As it is well known that pyrene emission wavelength shifts due to the presence
306 of stacking of pyrene molecules confirming the presence of self-associated pyrene excimer within
307 hydrophobic membrane of alginate coating (Uddin and Azam, 2013).

308

309 **3.1.2.2. Scanning Electron Microscopy of alginate coated berries**

310 Electron imaging (SEM) of strawberries coated with 1.5 % alginate was used to determine the
311 thickness of the coated layer. From the electron micrographs showed in Figure 5, we could
312 determine that the alginate layer was about 180 nm ± 40.

313

314 **3.2 Headspace atmosphere analysis**

315 The composition of gases present in the headspace atmosphere is dependent on the physiological
316 activity of the fruits and by the microbial metabolism (Poverenov et al. 2014). There was a
317 significant change in CO_2 concentration during the storage time ($p < 0.05$) in both treated and non-
318 treated strawberries (Figure 6). Concentration of CO_2 at both 5^{th} and 9^{th} days of storage was
319 significantly lower than that of 2^{nd} day, but there was a significant increase observed in the CO_2
320 concentration after 9 days of storage. The increase in CO_2 concentration at after 9 days can be
321 related with the damage in fruits and fungal decay (Hernández-Muñoz et al. 2006).

322

A significant difference was observed in the concentration of CO_2 in liposome treated strawberries compared to those treated with alginate and non- treated control ($p< 0.05$) (Figure 7). The liposome treated berries showed lower concentration of CO_2 up to 14 days of storage. These results provide an evidence to the antimicrobial characteristics of limonene against spoilage microbes in fruits during storage (Vu et al. 2011). The increased concentration CO_2 among alginate treated strawberries can be attributed to their lower gas exchange properties (Poverenov et al. 2014). Permeability of the edible coatings is one of the major factors which tend to effect the headspace composition of fruits and vegetables. If the coatings is not permeable enough, normal gases exchange is stopped which results in hypoxic contditions inside fruit tisse. This is indicated by generation of off- flavor and enhanced production of CO_2 (Baldwin et al. 1999; Han, 2005). The increase in the CO_2 composition in control and alginate treated berries can be attributed to the production of CO_2, ethanol, organic acids produced by spoilage microbes (Jacxsens et al. 2003).

3.3. Fruit weight loss

The loss of weight in fruits is associated with respiration rate and evaporation of moisture through the skin. The rapid loss of water from the skin is one of the major factor that contributes to the perishability of strawberry fruits (Aharoni and Barkai-Golan, 1987). This leads to the dehydration of fruits and ultimately to shrinkage and deterioration. Edible coatings were found to prevent water transfer, protect the fruits skin from mechanical injuries resulting in delaying water loss (Ali et al.2011; Chien et al. 2007). In our study, no any significant difference was observed between the coating types. However, there was a significant difference noticed in between the days of storage in both treated and non-treated strawberries (Figure 8) with the highest weight loss observed in 14 days of storage.

3.4. pH

There was a significant difference in pH of the berries between 2^{nd} and 5^{th} days of storage in both treated and non-treated strawberries (Figure 9). The pH tend to rise significantly ($p < 0.05$) from 2^{nd} to the 5^{th} days of storage and there was a significant ($p < 0.05$) decrease in pH in the 9^{th} day compared to the 5^{th} day, however the difference was not significant among 2^{nd} day and 9^{th} day.

Further, the pH of the berries increased on the 14th day but it was only significantly higher ($p < 0.05$) than 2nd day of storage. These results are in agreement with similar research conducted by Holocroft and Kader (1999) who observed increase in pH with the increase in storage days. The increase in pH during the storage can be related to the effects of respiration rates of fruits due to the increased level of oxygen (Zheng et al. 2007).

Limonene liposome treated strawberries were found to have significantly lower pH values as compared to control ($p < 0.05$) (Figure 10.). Whereas, no significant differences were found between the liposome treated and alginate treated berries. Similarly, no any significant difference was observed among control and alginate treated berries.

3.5. Titratable acidity (TA)

There was a non-significant difference between the coating materials. An increasing trend of TA was observed up to 9th days of storage among treated (Liposome and Alginate) and untreated strawberries (Figure 11). There was a significant ($p < 0.05$) increase in the TA of the strawberries in 5th day of storage compared to the 2nd day. However, there was a not significant increase in the TA values in 9th days compared to 5th day of storage (Figure11). Further, there was a non-significant decrease in the values in the 14th days of storage. The decreased in TA content in the 14th day of storage can be attributed to the loss of water from fruits (Hernandez-Munoz, Almenar et al. 2008) due to the respiration and microbial growth.

3.6. Total soluble solids (TSS)

There was no significant differences in the TSS level observed between the coating types. The mean TSS value was tend to increase significantly (($p < 0.05$) from the 2nd days of storage to 5th days of storage (Figure 12), whereas significantly ($p < 0.05$) reduced in the 9th days of storage compared to 5th days. There was no significant change observed from 9th day of storage onwards.

3.7. Total Phenolic Content (TPC)

There was no significant difference on total phenolic content observed among the coating types on days of storage. However, there was an increasing trend in the TPC up to 14 days of storage among the treated and non-treated strawberries. There was a significant increase ($p < 0.05$) in the TPC content of strawberries from 2nd day to 5th day of storage but there was no significant increase from 5th day onwards to the 14th day of storage. (Figure 13). These results concurred with the findings by (Nunes et al. 2006). The increase in the phenolic content of strawberries during storage can be attributed to the accumulation of anthocyanins and the development of its dark red-brownish color (Nunes et al. 1995; Montero et al.1996).

3.8. Total anthocyanin content

Similarly, there was a significant increase in total anthocyanin content of the strawberries during storage with the highest values observed in the 14th days of storage (Figure 14). These findings are in agreement with the studies done by Jiang and Joyce (2003) and Ayala-Zavala et al. (2004). Anthocyanins are responsible for the characteristic red color of ripe strawberries (Timberlake & Bridle). They are biologically significant for their antioxidant properties (Wang et al.1996). A regulatory enzyme, phenylalanine ammonia-lyase is responsible for the biosynthesis of anthocyanin in fruits and vegetables (Martinez et al. 1996).

There was significant difference in total anthocyanin content of strawberries among the coating types (Figure 15). The liposome treated strawberries showed significantly higher ($p < 0.05$) amount of anthocyanin content compared to the alginate treated and control strawberries. Similarly. Alginate treated strawberries also had significantly higher anthocyanin content compared to control.

4. Conclusion

Limonene liposome was found to be an effective coating material for the shelf life extension and maintaining quality parameters of the strawberries. The result obtained in this study can be helpful to know that storage time significantly affects the quality of the treated and non- treated

strawberries. The study has shown the possibility of development and application of antimicrobial phytochemicals encapsulated in liposomes. The edible coatings prepared with limonene liposomes were effective in the preservation of post-harvest quality of strawberries. The strawberries coated with limonene liposomes were shown to have lower respiration rates compared to control and alginate coatings. Similarly, the strawberries coated with liposomes had significantly lower pH values (3.9) and higher anthocyanin contents (43.849). These results suggest that limonene liposomes can be effective in maintain post-harvest quality of strawberries.

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Figure Caption:

Figure 1: Flowchart for the coating treatments

Figure 2: Absorption spectrum of polymerized and non-polymerized 80% lecithin 20% Polydiacetylene liposomes

Figure 3: Fluorescence micrographs of strawberries coated with pyrene alginate (scale bar 100 μm). Blue emission comes from amino-pyrene excited state.

Figure 4: Emission spectrum of pyrene alginate coating. The emission corresponds to multiple points enclosed in Figure 3.

Figure 5: SEM images alginate coated strawberries (cross section). A) Uncoated strawberry (scale bar 5 μm). B). Alginate coated strawberry (scale bar 3 μm) C) Zoomed area showing alginate layer on the alginate coated strawberry.

Figure 6: Concentration of CO_2 on various days of storage in both treated and non-treated strawberries.LS-means with the same letter are not significantly different

Figure 7: Concentration of CO_2 in strawberries with various coatings up to 14 days of storage. LS-means with the same letter are not significantly different

Figure 8: Percentage weight loss on days of storage in both treated and non-treated strawberries . LS-means with the same letter are not significantly different

Figure 9: Mean pH on days of storage in both treated and non-treated strawberries. LS-means with the same letter are not significantly different

Figure 10: Mean pH on coating types. LS-means with the same letter are not significantly different

Figure 11: Mean Titratable acidity on days of storage in both treated and non-treated strawberries. LS-means with the same letter are not significantly different

Figure 12: Mean TSS on days of storage in both treated and non-treated strawberries. LS-means with the same letter are not significantly different

Figure 13: Mean TPC on days of storage in both treated and non-treated strawberries . LS-means with the same letter are not significantly different

Figure 14: Mean Total anthocyanin on days of storage. LS-means with the same letter are not significantly different

Figure 15: Mean Total anthocyanin on types of coatings. LS-means with the same letter are not significantly different

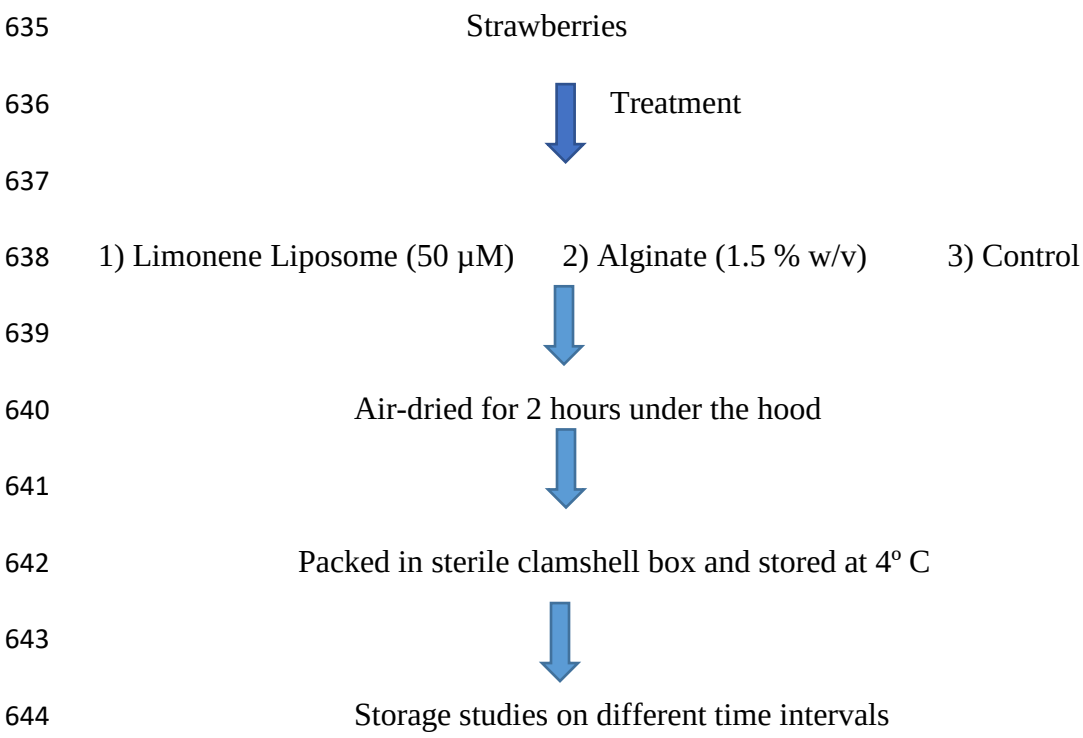
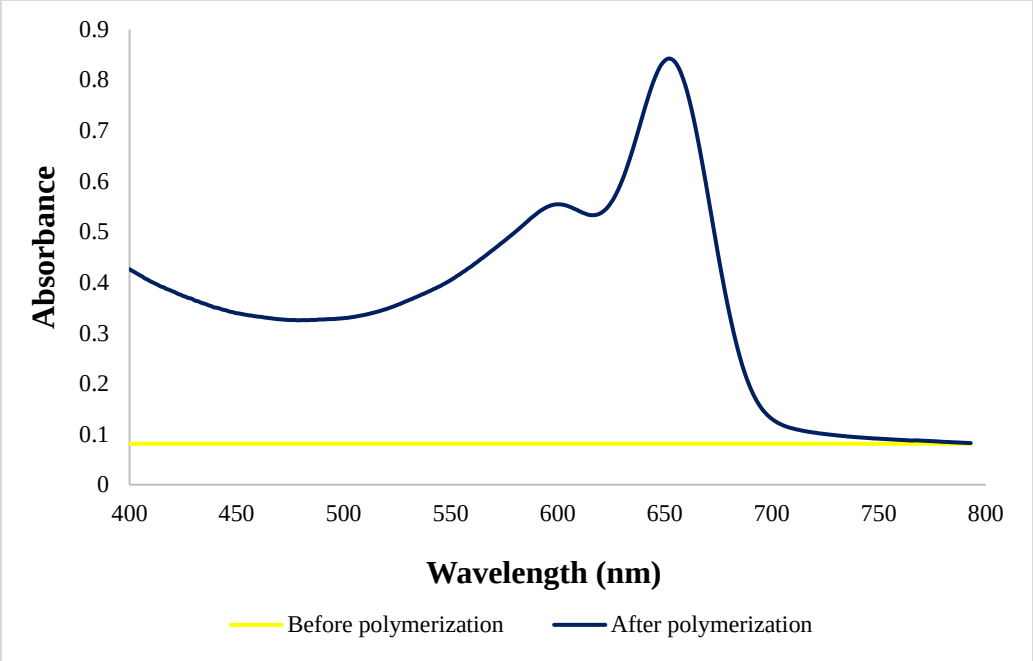


Figure 1: Flowchart for the coating treatments



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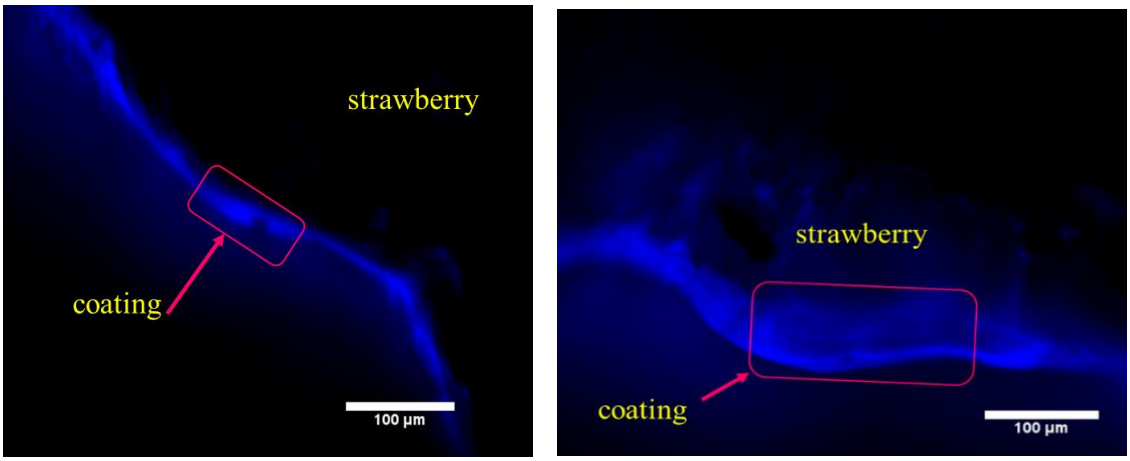

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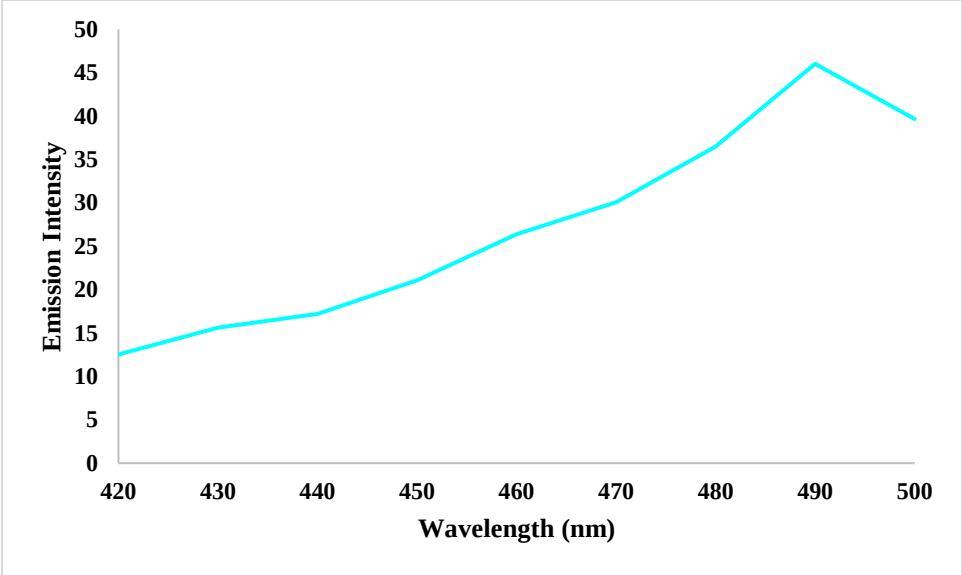

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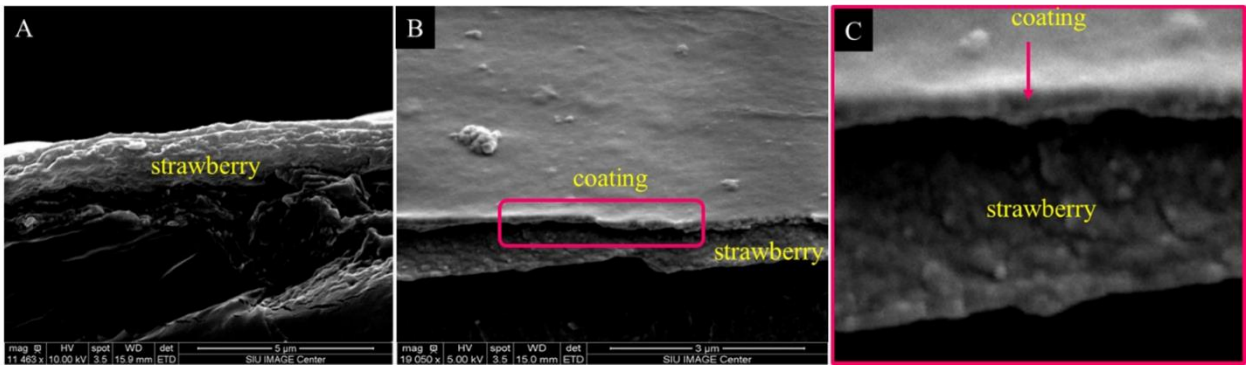

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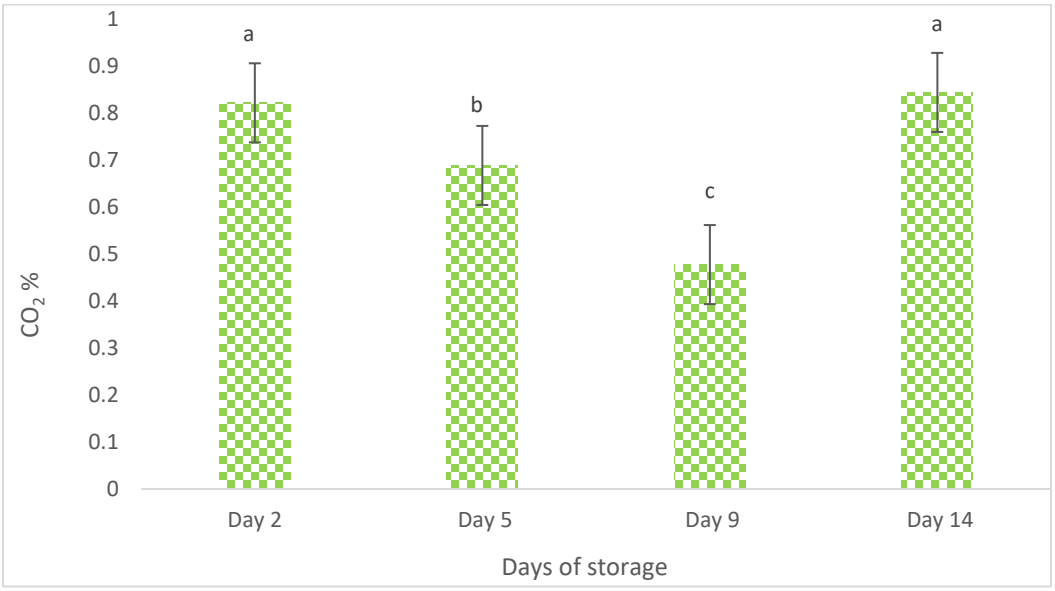


Figure 6: Concentration of CO_2 on various days of storage in both treated and non-treated strawberries.LS-means with the same letter are not significantly different

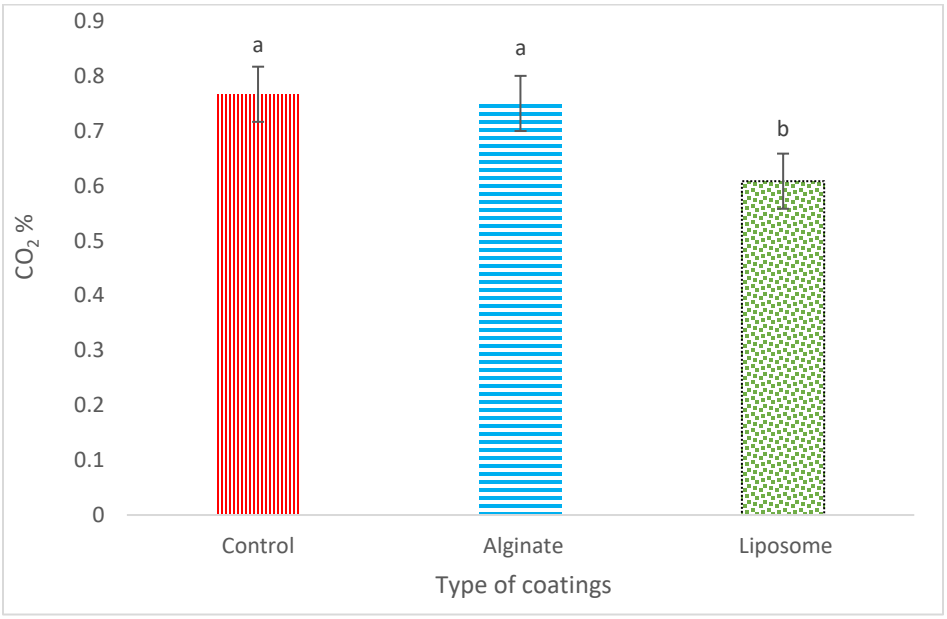


Figure 7: Concentration of CO_2 in strawberries with various coatings up to 14 days of storage. LS-means with the same letter are not significantly different

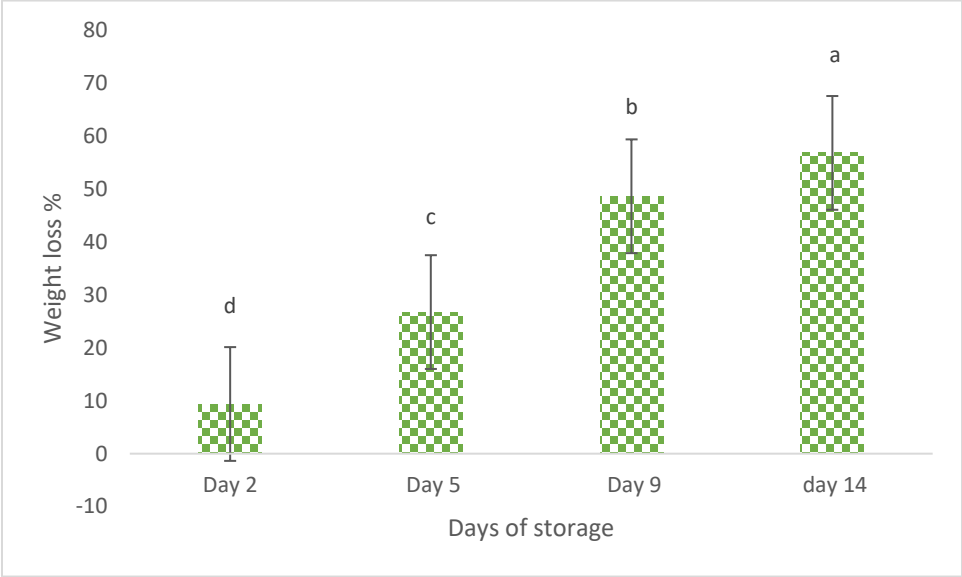


Figure 8: Percentage weight loss on days of storage in both treated and non-treated strawberries . LS-means with the same letter are not significantly different

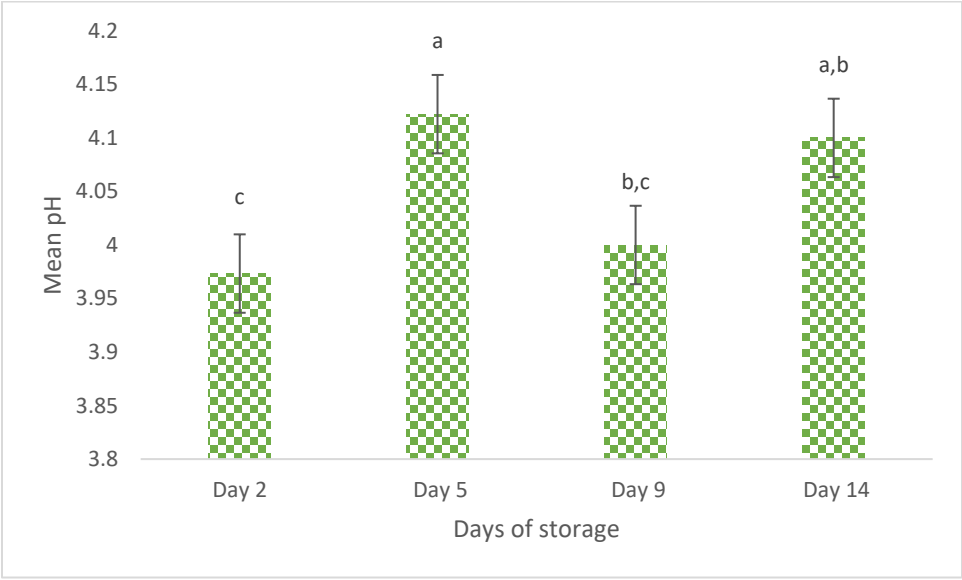


Figure 9: Mean pH on days of storage in both treated and non-treated strawberries. LS-means with the same letter are not significantly different

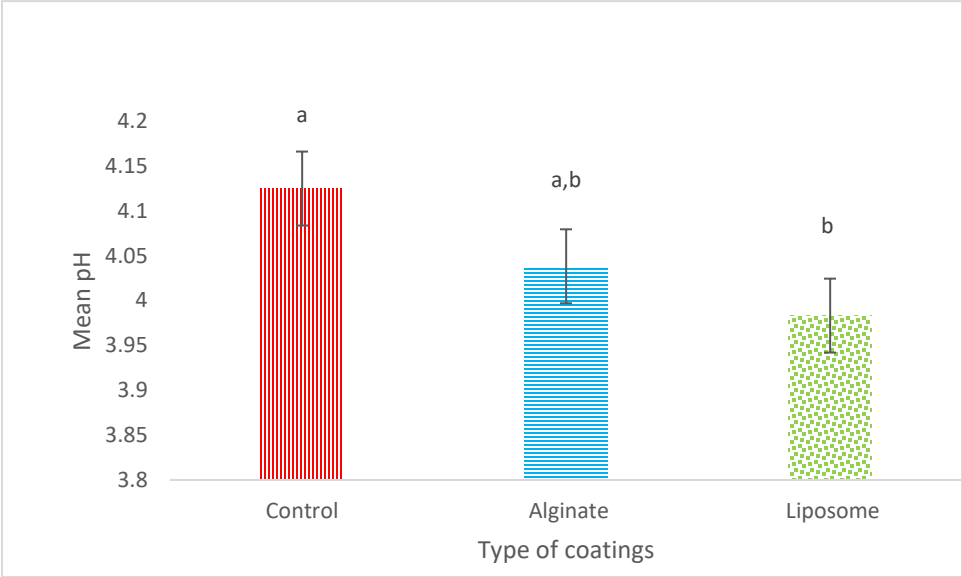


Figure 10: Mean pH on coating types. LS-means with the same letter are not significantly different

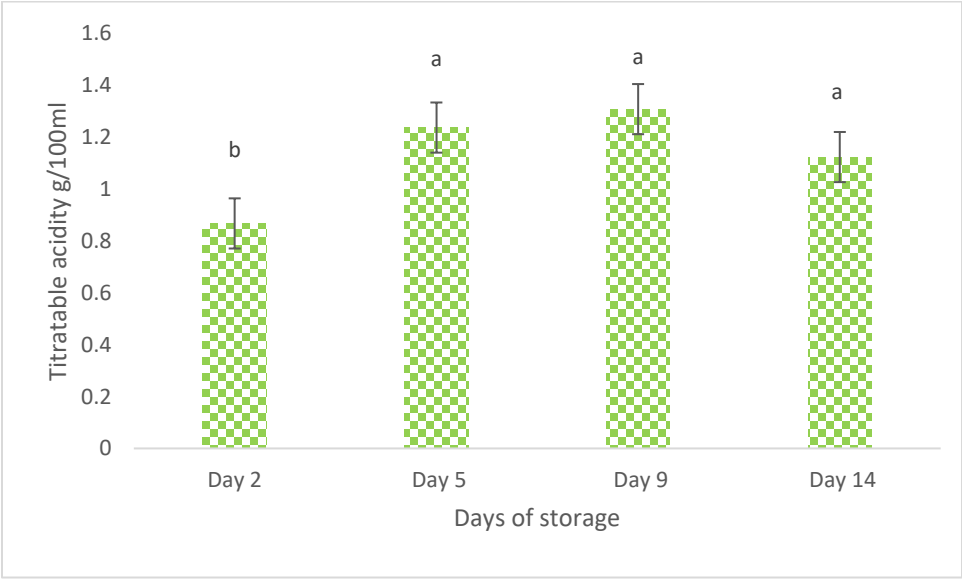
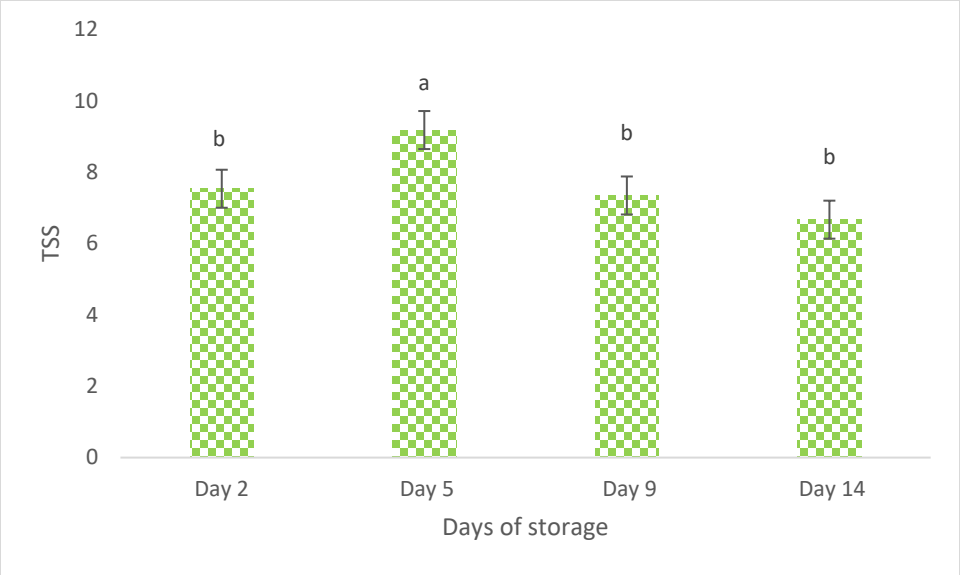


Figure 11: Mean Titratable acidity on days of storage in both treated and non-treated strawberries. LS-means with the same letter are not significantly different



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693 Figure 12: Mean TSS on days of storage in both treated and non-treated strawberries. LS-means
694 with the same letter are not significantly different

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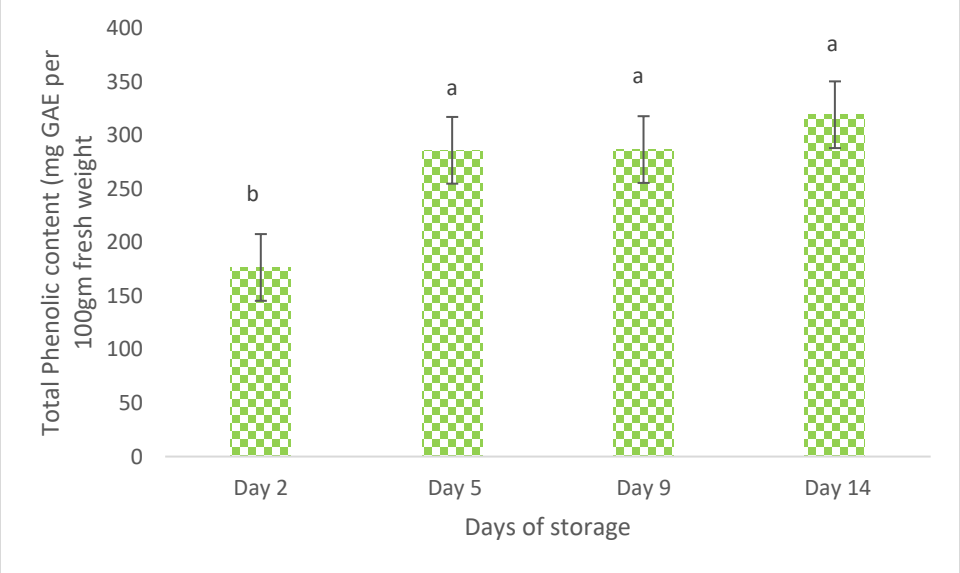

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697 Figure 13: Mean TPC on days of storage in both treated and non-treated strawberries . LS-means
698 with the same letter are not significantly different

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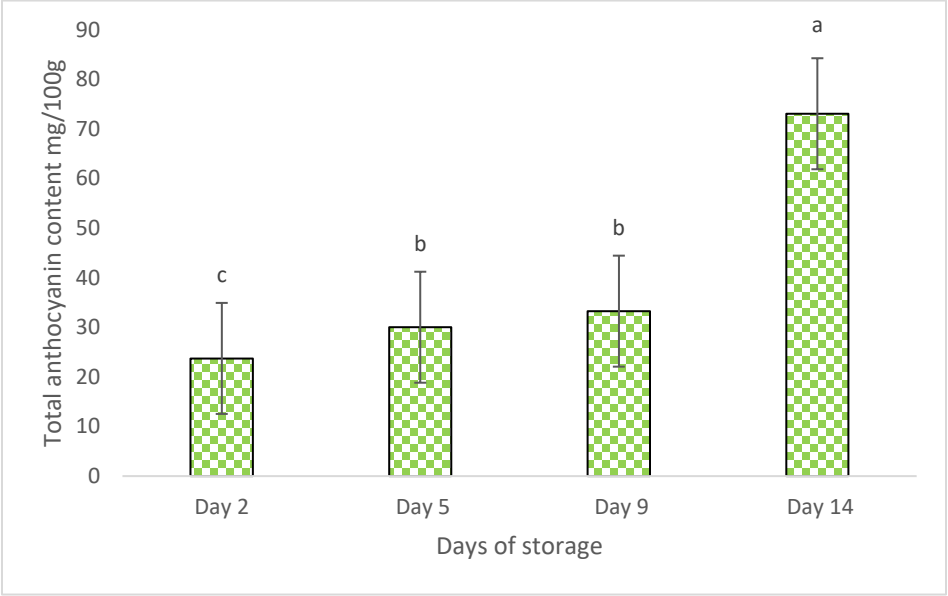

Figure 14: Mean Total anthocyanin on days of storage. LS-means with the same letter are not significantly different

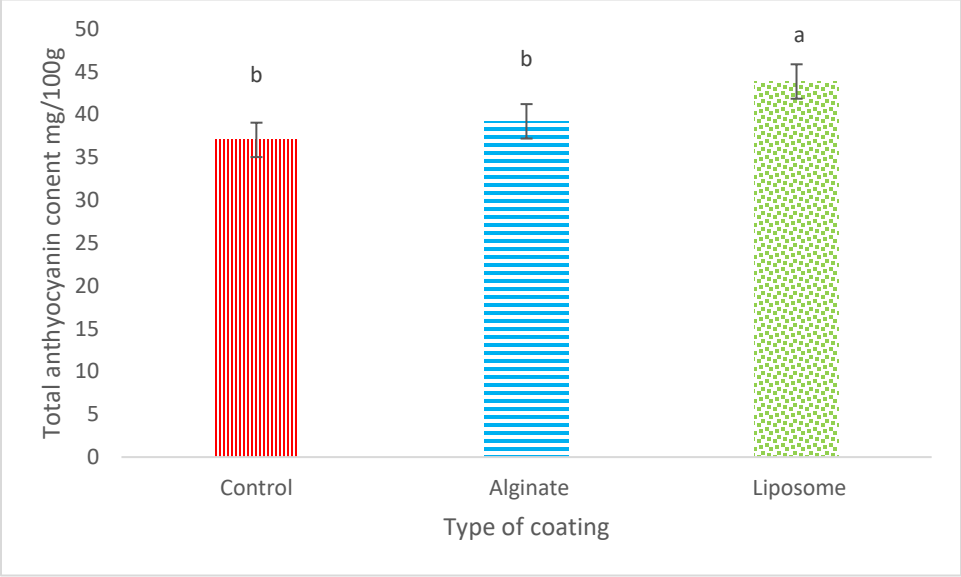

Figure 15: Mean Total anthocyanin on types of coatings. LS-means with the same letter are not significantly different