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# Efficacy of limonene nano coatings on post-harvest shelf life of strawberries

Rajiv Dhital

*Southern Illinois University Carbondale*, [rajiv.dhital@siu.edu](mailto:rajiv.dhital@siu.edu)

Nathalie Becerra-Mora

*Southern Illinois University Carbondale*, [nathalie09@siu.edu](mailto:nathalie09@siu.edu)

Dennis G. Watson

*Southern Illinois University Carbondale*, [dwatson@siu.edu](mailto:dwatson@siu.edu)

Punit Kohli

*Southern Illinois University Carbondale*, [pkohli@chem.siu.edu](mailto:pkohli@chem.siu.edu)

Ruplal Choudhary

*Southern Illinois University Carbondale*, [choudhry@siu.edu](mailto:choudhry@siu.edu)

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1  
2 **EFFICACY OF LIMONENE NANO COATINGS ON POST-HARVEST SHELF LIFE OF**  
3 **STRAWBERRIES**

4  
5 **Rajiv Dhital<sup>1</sup>, Nathalie Becerra- Mora<sup>2</sup>, Dennis Watson<sup>1</sup>, Punit Kohli<sup>2</sup>, Ruplal Choudhary<sup>1</sup>**

6 <sup>1</sup> Department of Plant, Soil and Agricultural Systems, Southern Illinois University, Carbondale.

7 <sup>2</sup> Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale.

8 \*Corresponding Author: 1205 Lincoln Drive Room 176, Carbondale IL 62901, USA. Email:  
9 choudhry@siu.edu; [Phone: 618 453 6985](tel:6184536985)

10  
11 **ABSTRACT**

12 Strawberries are highly demanded fruits because of their color, nutritional values and appearance.  
13 The aim of this study was to develop and characterize alginate and limonene liposomes as edible  
14 coating materials and to determine their efficacy in shelf life extension and maintaining quality  
15 parameters of ‘Chandler’ strawberries. Alginate solution (1.5% w/v) and Limonene liposomes  
16 prepared from 80% lecithin and 20% PDA were used as edible coating materials. Fungal decay  
17 percentage, total yeast and mold counts, headspace atmosphere analysis, total soluble solids, pH,  
18 titratable acidity, total anthocyanin content and total phenolics were analyzed to assess fruit quality  
19 during 14 days at 4°C of storage. Days of storage was found to be significant in maintaining the  
20 quality of the strawberries. Among the coating types, limonene liposomes were found to be  
21 significantly more effective in maintaining the lower concentration of carbon dioxide (CO<sub>2</sub>), lower  
22 the change in pH (3.9), and had higher total anthocyanin (43.85) content during storage than those  
23 without a liposomal coating. Thus, limonene liposomes were found to be useful for extending the  
24 shelf life and maintaining quality of strawberry fruits.

25 **Keywords:**

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

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30

31 **1. Introduction**

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

40

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

47

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

56

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

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

65  
66 Limonene ((R)-(+)-para-Mentha-1,8-diene) is obtained from essential oil of citrus fruits i.e.  
67 orange, lemon, mandarin, lime, grapefruit (Moufida and Marzouk 2003). It is a colorless liquid  
68 hydrocarbon regarded as safe used largely by cosmetic, food and pharmaceutical industries and  
69 has Generally Recognized as Safe (GRAS) status by US Food and Drug Administration (EPA,  
70 1994). Limonene has a very strong antifungal property and is effective against food spoilage fungal  
71 species. It shows an antibacterial properties effective against pathogenic bacteria like;  
72 *Staphylococcus aureus*, *L.monocytogenes*, *Salmonella enterica* (Sharma and Tripathi 2008;  
73 Alonso-Gutierrez et al. 2013). Due to hydrophobic nature and tendency to degrade under oxidative  
74 conditions, limonene possess a challenge during its application as an edible coating material  
75 because of poor dispersion in water (Li and Chiang, 2012). To address this issue, low  
76 concentrations of limonene are used for dispersion in water, which in turn reduces its antimicrobial  
77 activity. The problem of hydrophobic nature and use of low concentrations of limonene limits its  
78 efficacy while using as a coating material. A new approach of encapsulation of phytochemicals in  
79 liposome that has both hydrophobic tails and hydrophilic heads through nanotechnology  
80 (Umagiliyage et al. 2017).

81  
82 Incorporation of antimicrobial compounds into edible films and coatings provides an innovative  
83 approach to improve microbial safety and shelf life of foods (Cagri et al. 2004). Some of the most  
84 commonly used antimicrobial agents in food are; benzoic acid, sodium benzoate, sorbic acid,  
85 potassium sorbate and propionic acid which can be incorporated into edible films and coatings  
86 (Cruz-Romero et al. 2013). Starch based edible coatings containing potassium sorbate applied on  
87 the surface of fresh strawberries reduced the microbial growth and extended the shelf life (Garcia  
88 et al. 1998). A bilayer edible coating made from plant based antimicrobial compounds, limonene  
89 and curcumin were applied in combination with methylcellulose (MC) for improving of post-  
90 harvest quality of fresh strawberries (Dhital et al. 2017). Edible films containing organic acids,

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

93

94 Nano-technology has been extensively used to enhance the quality of fruits and vegetables (Yang  
95 et al; 2010). Encapsulation of antimicrobial compounds using the approaches of nanotechnology  
96 can address the problems of microbial degradation and hence improve the quality of fruits.  
97 Liposomes have been used in different fields of science and technology. In food applications,  
98 liposomes can be potentially used to improve the efficacy of antimicrobial compounds, delivery  
99 of nutrients and protection of sensitive ingredients in foods (Lasic,1993). Liposomes are used in  
100 the encapsulation of nutrients, proteins, enzymes, antimicrobial and flavors and their controlled  
101 release in food environment to delay the microbial spoilage and maintain the food quality  
102 (Makwana et al. 2015). Application of essential oils encapsulated in liposomes have shown to  
103 improve quality and extend the shelf life of fruits and vegetables (Alikhani-Koupaei, 2014). In our  
104 previous study (Dhital et al., 2017), limonene encapsulated in liposome were found to improve the  
105 post-harvest shelf life of strawberries. An improved antimicrobial activity of nano-encapsulated  
106 eugenol was reported by Shah et al. (2012b) against *E. coli* 0157:H7 and *Listeria monocytogenes*  
107 in bovine milk. Limonene encapsulated in nano-emulsion exhibited antimicrobial activities  
108 towards *Escherichia coli*, *Lactobacillus delbrueckii* and *Saccharomyces cerevisiae* (Donsi et al.  
109 2012). In previous studies, liposomes polydiacetylene were used to deliver and carry antibodies  
110 and drugs in mice. Results showed that injection up to 100 mg kg<sup>-1</sup> of polydiacetylene did not  
111 induce acute toxicity in the mice. (Gravel, et al., 2012).

112

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

122 and Nussinovitch, 1998). Alginate has wide range of application in industrial sectors due to their  
123 ability to retain water, film-forming, gelling, viscosifying and stabilizing properties. Their film  
124 forming properties make them useful in food processing industries. In addition, alginate coatings  
125 have shown good oxygen barrier properties (Conca and Yang, 1993) that eventually can retard  
126 lipid oxidation in fruits and vegetables (Kester and Fennema 1986). Alginate based coatings  
127 applied to fresh cut 'Fuji' apples showed that these coatings could carry antioxidants, which are  
128 responsible for the maintaining color of cut fruits during storage (Rojas-Grau et al. 2007 b)  
129 Mechanical injuries due to vibration can cause in a significant loss of fruits and vegetables. A  
130 study by Singh and Xu (1993) reported that about 80% of apples could be damaged by simulated  
131 transportation by truck. Damage due to vibration during the transportation was noted on different  
132 fruits and vegetables that include peaches, apricots, potatoes, tomatoes (Barchi et al. 2002). In  
133 strawberries, In-transit vibration causes skin abrasion and bruising which makes easier for  
134 microbes to enter inside the berries and cause degradation (Fischer et al. 1992).

135  
136 The goal of this study was to develop novel nanocoating treatments prepared from plant-based  
137 antimicrobials encapsulated in nano-liposomes. The objectives in this study were: (1) Preparation  
138 and characterization of the edible coating materials, (2) application of edible coating materials on  
139 strawberry fruits and analyze the quality parameter of strawberries treated with edible coatings  
140 during storage and 3) compare the efficacy of coating materials based on quality parameters.

141

## 142 **2. Materials and methods**

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

146

### 147 **2.1. Preparation of D- Limonene Liposomes**

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

153 film was dried overnight by placing the flask on a vacuum pump followed by film hydration by  
154 the addition of 50  $\mu$ M D- limonene prepared in Nano-pure water. The resulting solution then left  
155 sonicated for 20 minutes. Further, the solution was placed in a probe sonicator (VCX 500, Vibra-  
156 cell, Newtown, CT) at 76 °C for 15 minutes. The solution was then filtered through 0.45  $\mu$ m nylon  
157 fiber to remove the lipid aggregates. Thus, obtained liposome solution was collected and kept away  
158 from light at 4°C for 8 hours prior to use for experiments. Liposomes were polymerized by  
159 irradiation with a UV lamp emitting at 254 nm for approximately 2-5 min using a Pen Ray (UVGL-  
160 58, Minerallight, Upland, CA) UV source (4.5 mW/cm<sup>2</sup>) in air at 4°C. The polymerized liposome  
161 solution was dialyzed using a membrane Spectra/Por® Biotech Cellulose Ester (CE) membrane  
162 (MWCO: 100,000) for 48 hours changing the water every 4 hours. Thus obtained liposome  
163 solution was collected and stored at 4°C for further studies.

164

## 165 **2.2. Characterization of Liposomes**

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

171

## 172 **2.3. Alginate solution preparation**

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

176

### 177 **2.3.1. Characterization of alginate coatings**

#### 178 **2.3.1.1 Fluorescent imaging**

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

184 UV lamp source was used to excite the fluorescent molecules. A long pass band UV filter was  
185 used to select the excitation wavelengths. The emission spectrum was collected from 420 nm to  
186 500 nm.

187

### 188 **2.3.1.2 Scanning electron microscopy**

189 The SEM images of the alginate-coated berries were taken for the characterization of alginate  
190 coatings. Briefly, alginate coatings (1.5% w/v) were applied on the berries, followed by freeze-  
191 drying (-80 °C) for 24 hours. The freeze-dried samples were cut using a razor blade in small pieces  
192 (5 mm by 5 mm). They were sputter coated for 4 minutes with a layer of Ag-Pd using a DESK II,  
193 DENTON VACUUM sputter. The edges of the samples were grounded using a thin layer of silver  
194 paint (SPI, USA). The samples were imaged using a scanning electron microscope QUANTA FEG  
195 450 (FEI), the acceleration voltage was 5 kV using ETD detector at high vacuum.

196

## 197 **2.4. Application of coating materials**

198 The summary of application is represented in Figure 3.1. Briefly, berries were randomly selected  
199 and divided into three groups depending upon coating treatment types; limonene liposome,  
200 alginate and non-coated control. Each treatment was performed in triplicate and each replicate had  
201 20 berries. Berries were dipped in the solutions of 50 µM liposome solutions for 10 min. For  
202 Alginate coatings, 1.5 % alginate solution was cooled to room temperature and strawberries were  
203 dipped in the alginate solution for 3 minutes. The berries were then immersed in 5% w/v aqueous  
204 solution of CaCl<sub>2</sub> for 2 min. For the non-treated control samples, the berries were rinsed with sterile  
205 distilled water. All the treated berries were air dried at room temperature in an UV sterilized  
206 cabinet drier for 2 hours and packed in sterile clamshell box stored at 4° C.

## 207 **2.5 Fungal decay percentage**

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

211 
$$\text{Fungal decay \%} = (\text{the number of decayed fruits} / \text{total number of fruits}) \times 100$$

212



## 213 **2.6. Total Yeast and mold count**

214 Total yeast and mold count on the berry surface was performed by serial dilutions followed by  
215 spread plating over the surface of sterile DRBC plate method as recommended by the International  
216 Standard Organization (ISO 21527-1, 2008) with slight modifications. Briefly, berries from each  
217 treatment and untreated control were stirred (in 150 mL Erlenmeyer flask) at 150 rpm in 20 mL of  
218 0.1% (w/v) sterile peptone water for 30 min. The resulting suspension was then serially diluted  
219 from 1:10 to 1:10<sup>6</sup> dilutions. Then, 0.1 ml inoculum of each dilutions was used for plating and  
220 spread evenly over the plates. The plates were incubated at 25 °C for 5 days. Results were  
221 expressed as log colony forming units per ml (CFU/ml) based on average count of triplicate set.

222

## 223 **2.7. Headspace atmosphere analysis**

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

230

## 231 **2.8. Fruit weight loss**

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

236 
$$\text{Weight loss \%} = (\text{Initial weight} - \text{final weight} / \text{Initial weight}) \times 100$$

237

## 238 **2.9. Determination of Total soluble solids (TSSs), pH, Titratable acidity (TA)**

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

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

246

247

#### 248 **2.10. Analysis of total anthocyanin content**

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

255

#### 256 **2.11. Analysis of total Phenolic compounds content**

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

268

#### 269 **2.12. Statistical analysis**

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

277

### 278 **3. Result and discussion**

#### 279 **3.1. Characterization of coating materials**

##### 280 **3.1.1. Characterization of liposomes**

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

291

##### 292 **3.1.2. Characterization of alginate coatings**

###### 293 **3.1.2.1. Fluorescence imaging of fluorescent alginate coated berries**

294 The alginate concentration for this particular experiment was roughly ten times higher than the  
295 concentration of alginate originally used for the coating. As shown in the fluorescent micrographs  
296 (Figure 3), the coating extends along the berry and the thickness of the coatings was about 10  $\mu\text{m}$ .

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  $180 \text{ nm} \pm 40$ .

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  $\text{CO}_2$  concentration during the storage time ( $p < 0.05$ ) in both treated and non-  
318 treated strawberries (Figure 6). Concentration of  $\text{CO}_2$  at both 5<sup>th</sup> and 9<sup>th</sup> days of storage was  
319 significantly lower than that of 2<sup>nd</sup> day, but there was a significant increase observed in the  $\text{CO}_2$   
320 concentration after 9 days of storage. The increase in  $\text{CO}_2$  concentration at after 9 days can be  
321 related with the damage in fruits and fungal decay (Hernández-Muñoz et al. 2006).

322

323 A significant difference was observed in the concentration of CO<sub>2</sub> in liposome treated strawberries  
324 compared to those treated with alginate and non- treated control ( $p < 0.05$ ) (Figure 7). The liposome  
325 treated berries showed lower concentration of CO<sub>2</sub> up to 14 days of storage. These results provide  
326 an evidence to the antimicrobial characteristics of limonene against spoilage microbes in fruits  
327 during storage (Vu et al. 2011). The increased concentration CO<sub>2</sub> among alginate treated  
328 strawberries can be attributed to their lower gas exchange properties (Poverenov et al. 2014).  
329 Permeability of the edible coatings is one of the major factors which tend to effect the headspace  
330 composition of fruits and vegetables. If the coatings is not permeable enough, normal gases  
331 exchange is stopped which results in hypoxic conditions inside fruit tissue. This is indicated by  
332 generation of off- flavor and enhanced production of CO<sub>2</sub> (Baldwin et al. 1999; Han, 2005). The  
333 increase in the CO<sub>2</sub> composition in control and alginate treated berries can be attributed to the  
334 production of CO<sub>2</sub>, ethanol, organic acids produced by spoilage microbes (Jacxsens et al. 2003).

335

### 336 **3.3. Fruit weight loss**

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

346

### 347 **3.4. pH**

348 There was a significant difference in pH of the berries between 2<sup>nd</sup> and 5<sup>th</sup> days of storage in both  
349 treated and non-treated strawberries (Figure 9). The pH tend to rise significantly ( $p < 0.05$ ) from  
350 2<sup>nd</sup> to the 5<sup>th</sup> days of storage and there was a significant ( $p < 0.05$ ) decrease in pH in the 9<sup>th</sup> day  
351 compared to the 5<sup>th</sup> day, however the difference was not significant among 2<sup>nd</sup> day and 9<sup>th</sup> day.

352 Further, the pH of the berries increased on the 14<sup>th</sup> day but it was only significantly higher ( $p <$   
353  $0.05$ ) than 2<sup>nd</sup> day of storage. These results are in agreement with similar research conducted by  
354 Holcroft and Kader (1999) who observed increase in pH with the increase in storage days. The  
355 increase in pH during the storage can be related to the effects of respiration rates of fruits due to  
356 the increased level of oxygen (Zheng et al. 2007).

357

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

362

### 363 **3.5. Titratable acidity (TA)**

364 There was a non-significant difference between the coating materials. An increasing trend of TA  
365 was observed up to 9<sup>th</sup> days of storage among treated (Liposome and Alginate) and untreated  
366 strawberries (Figure 11). There was a significant ( $p < 0.05$ ) increase in the TA of the strawberries  
367 in 5<sup>th</sup> day of storage compared to the 2<sup>nd</sup> day. However, there was a not significant increase in the  
368 TA values in 9<sup>th</sup> days compared to 5<sup>th</sup> day of storage (Figure11). Further, there was a non-  
369 significant decrease in the values in the 14<sup>th</sup> days of storage. The decreased in TA content in the  
370 14<sup>th</sup> day of storage can be attributed to the loss of water from fruits (Hernandez-Munoz, Almenar  
371 et al. 2008) due to the respiration and microbial growth.

372

### 373 **3.6. Total soluble solids (TSS)**

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

378

### 379 **3.7. Total Phenolic Content (TPC)**

380 There was no significant difference on total phenolic content observed among the coating types on  
381 days of storage. However, there was an increasing trend in the TPC up to 14 days of storage among  
382 the treated and non-treated strawberries. There was a significant increase ( $p < 0.05$ ) in the TPC  
383 content of strawberries from 2<sup>nd</sup> day to 5<sup>th</sup> day of storage but there was no significant increase from  
384 5<sup>th</sup> day onwards to the 14<sup>th</sup> day of storage. (Figure 13). These results concurred with the findings  
385 by (Nunes et al. 2006). The increase in the phenolic content of strawberries during storage can be  
386 attributed to the accumulation of anthocyanins and the development of its dark red-brownish color  
387 (Nunes et al. 1995; Montero et al.1996).

388

### 389 **3.8. Total anthocyanin content**

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

397

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

403

## 404 **4. Conclusion**

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

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

415

#### 416 **Acknowledgement**

417

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426

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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  $\mu\text{m}$ ). 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  $\mu\text{m}$ ). B). Alginate coated strawberry (scale bar 3  $\mu\text{m}$ ) C) Zoomed area showing alginate layer on the alginate coated strawberry.

Figure 6: Concentration of  $\text{CO}_2$  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  $\text{CO}_2$  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

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

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

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

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

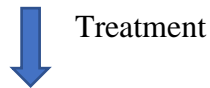
632 Figure 15: Mean Total anthocyanin on types of coatings. LS-means with the same letter are not  
633 significantly different

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635

Strawberries

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637

638 1) Limonene Liposome (50  $\mu$ M)    2) Alginate (1.5 % w/v)    3) Control

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640

Air-dried for 2 hours under the hood

641



642

Packed in sterile clamshell box and stored at 4° C

643

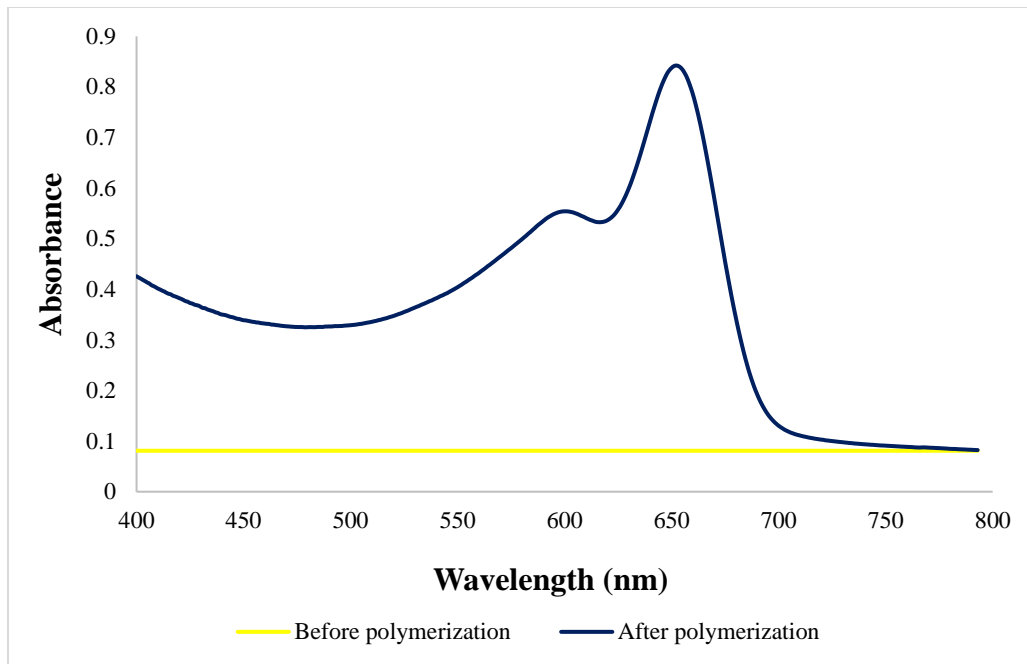


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Storage studies on different time intervals

645 Figure 1: Flowchart for the coating treatments

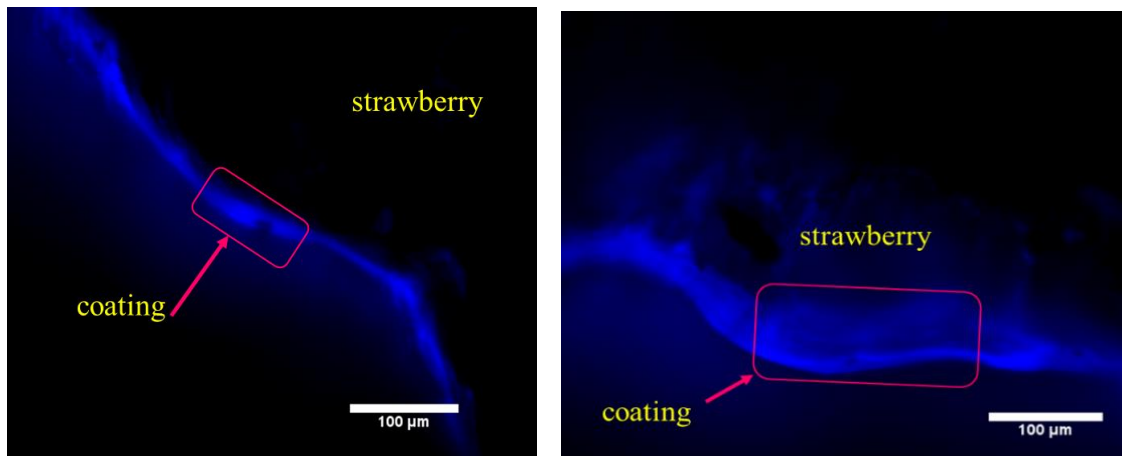
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648 Figure 2: Absorption spectrum of polymerized and non-polymerized 80% lecithin 20%  
 649 Polydiacetylene liposomes

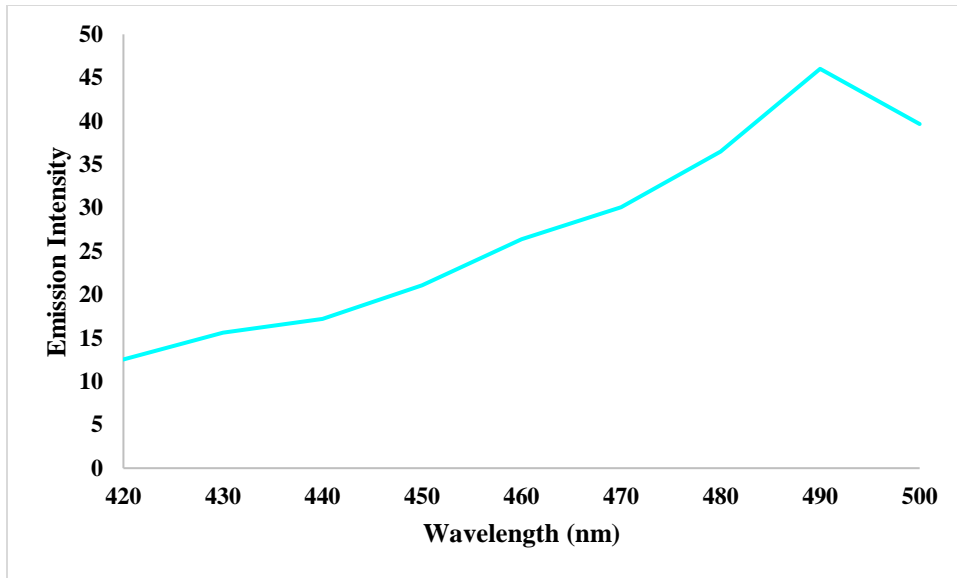
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652 Figure 3: Fluorescence micrographs of strawberries coated with pyrene alginate (scale bar 100  
 653 μm). Blue emission comes from amino-pyrene excited state.

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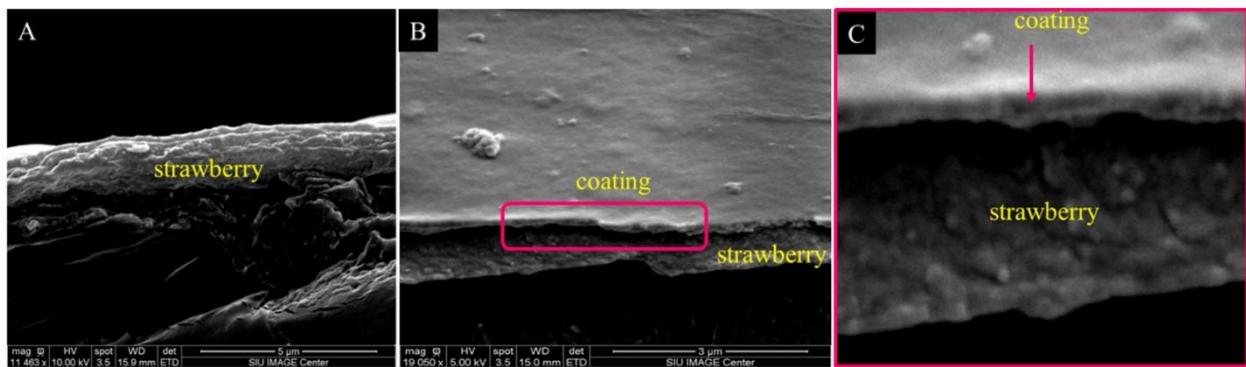
656 Figure 4: Emission spectrum of pyrene alginate coating. The emission corresponds to multiple  
 657 points enclosed in Figure 3.

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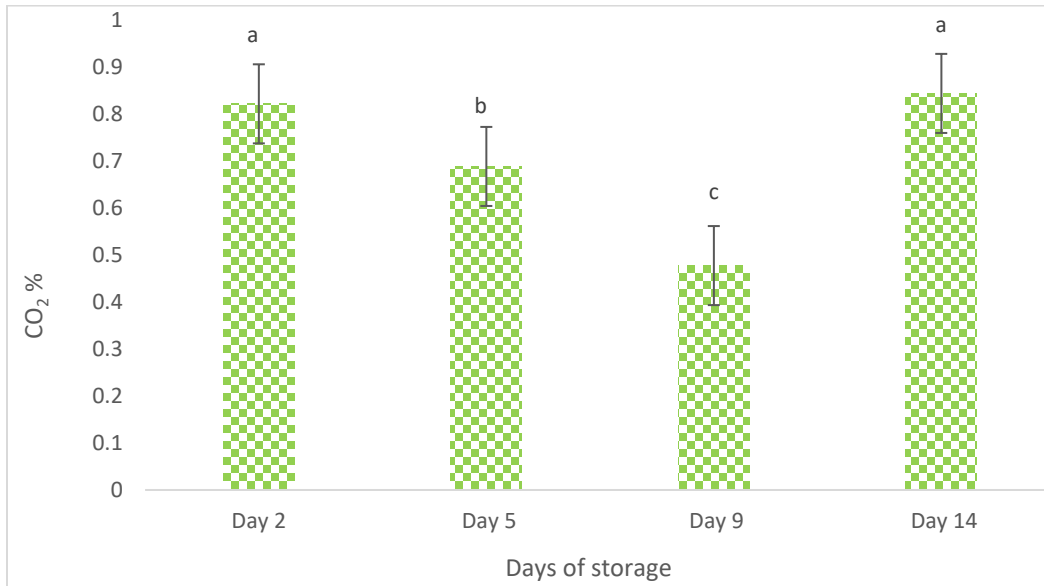
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663 Figure 5: SEM images alginated strawberries (cross section). A) Uncoated strawberry (scale  
 664 bar 5 μm). B). Alginated strawberry (scale bar 3 μm) C) Zoomed area showing alginated layer  
 665 on the alginated strawberry.

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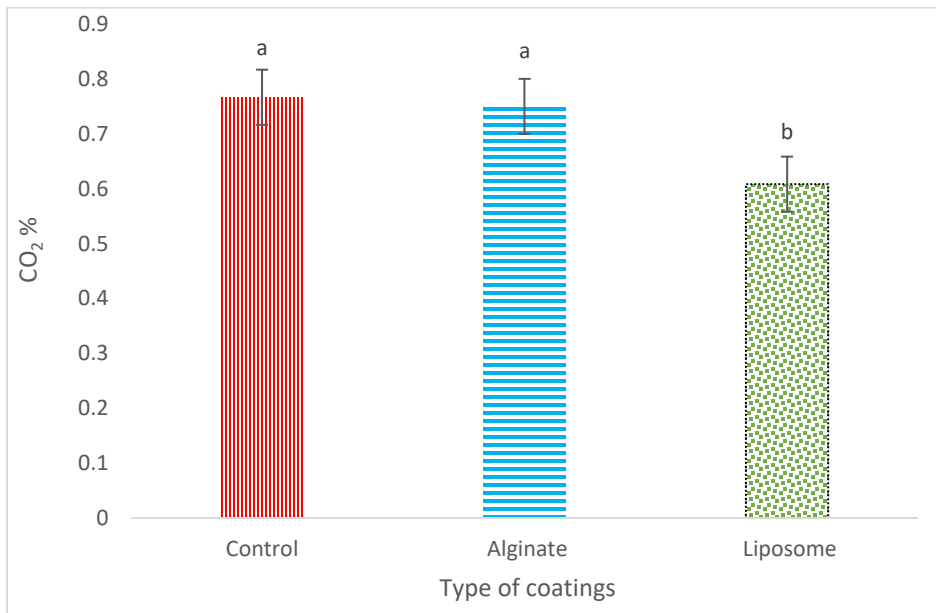
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669 Figure 6: Concentration of CO<sub>2</sub> on various days of storage in both treated and non-treated  
670 strawberries. LS-means with the same letter are not significantly different

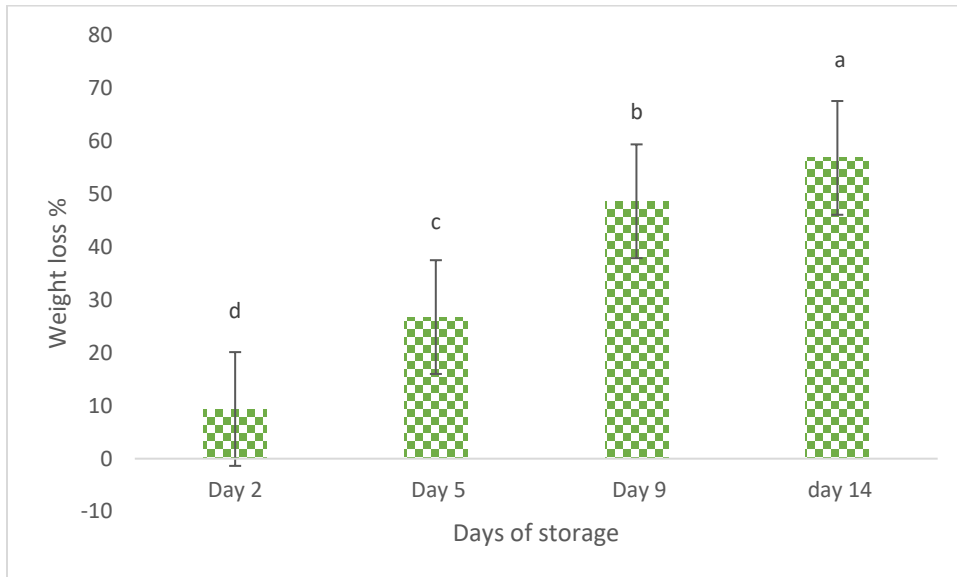
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673 Figure 7: Concentration of CO<sub>2</sub> in strawberries with various coatings up to 14 days of storage. LS-  
674 means with the same letter are not significantly different

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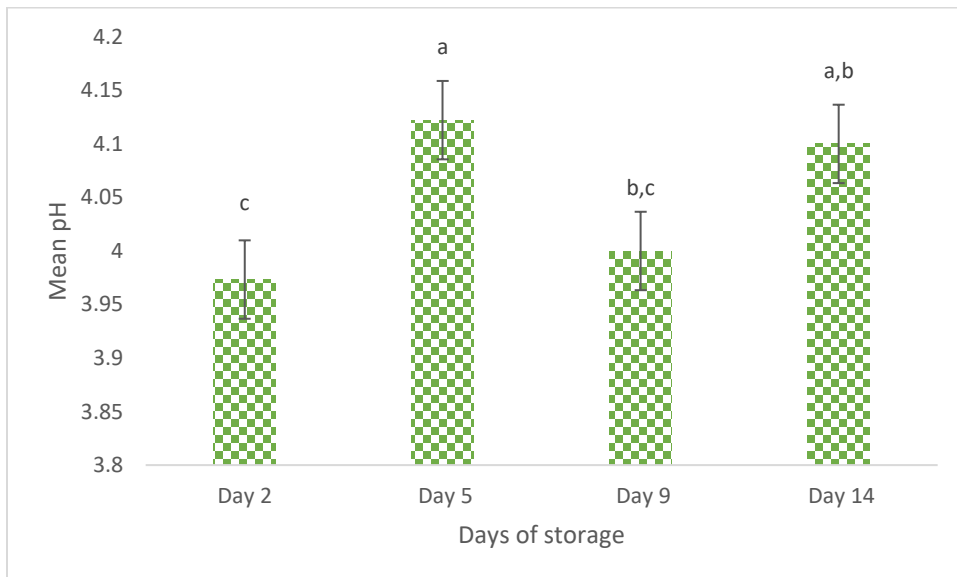


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677 Figure 8: Percentage weight loss on days of storage in both treated and non-treated strawberries .

678 LS-means with the same letter are not significantly different

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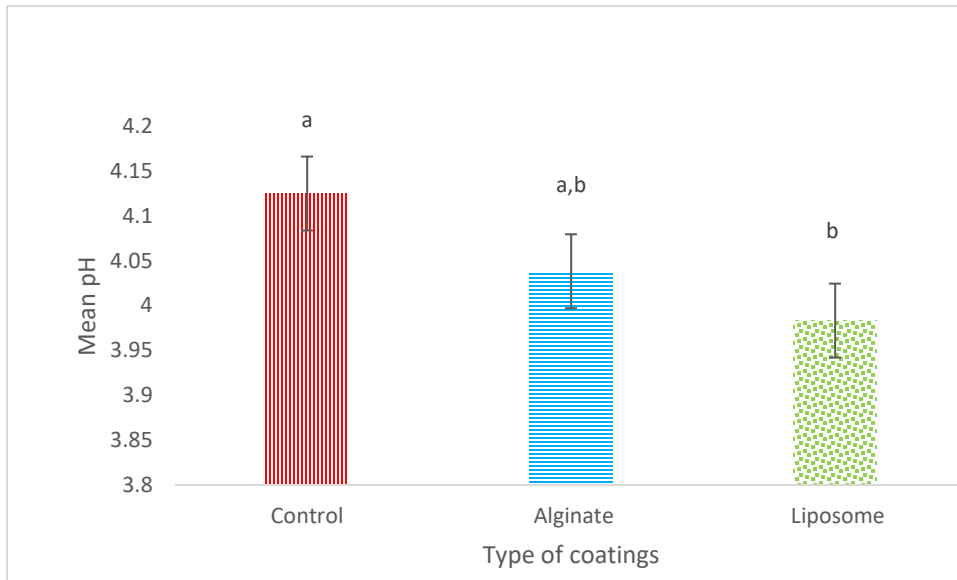
681 Figure 9: Mean pH on days of storage in both treated and non-treated strawberries. LS-means with

682 the same letter are not significantly different

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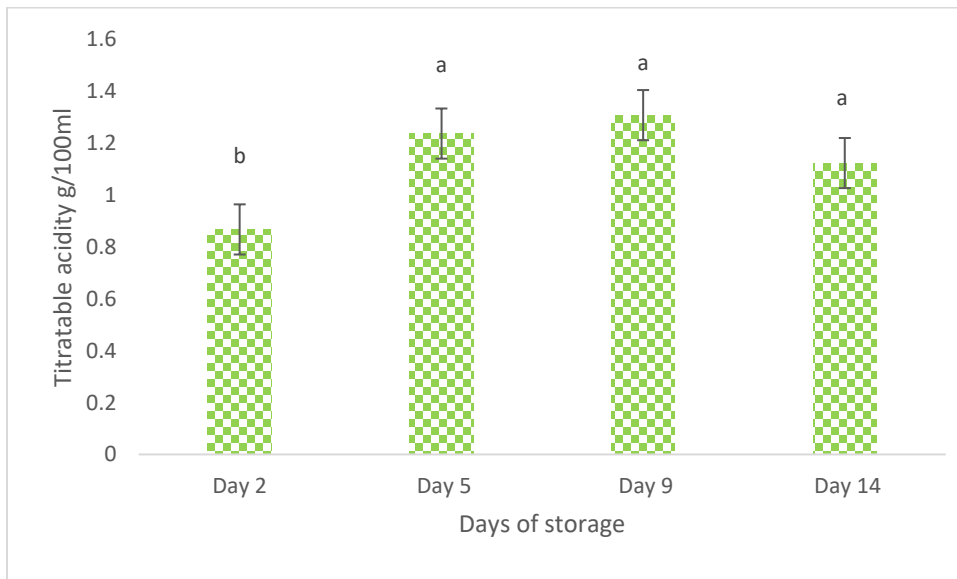
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687 Figure 10: Mean pH on coating types. LS-means with the same letter are not significantly different

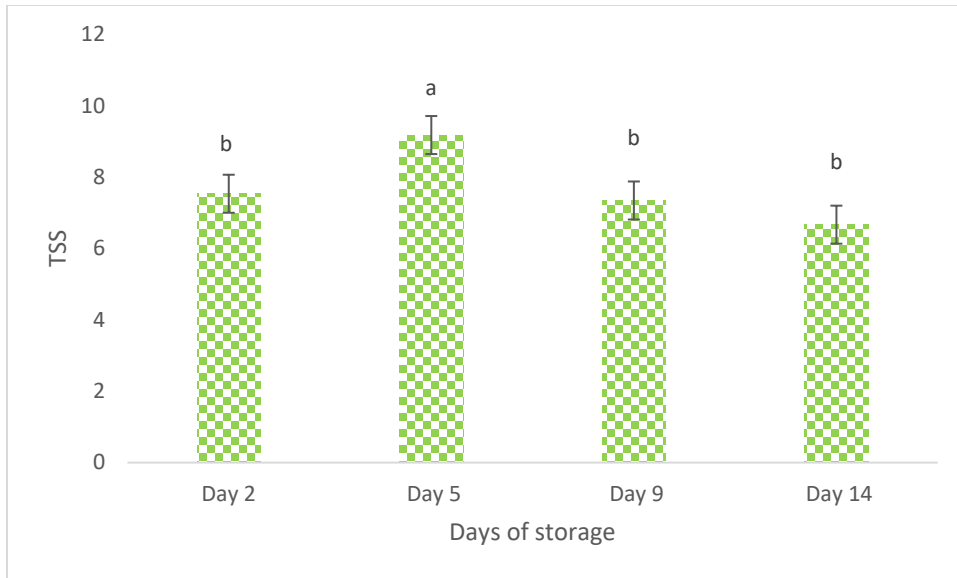
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690 Figure 11: Mean Titratable acidity on days of storage in both treated and non-treated strawberries.

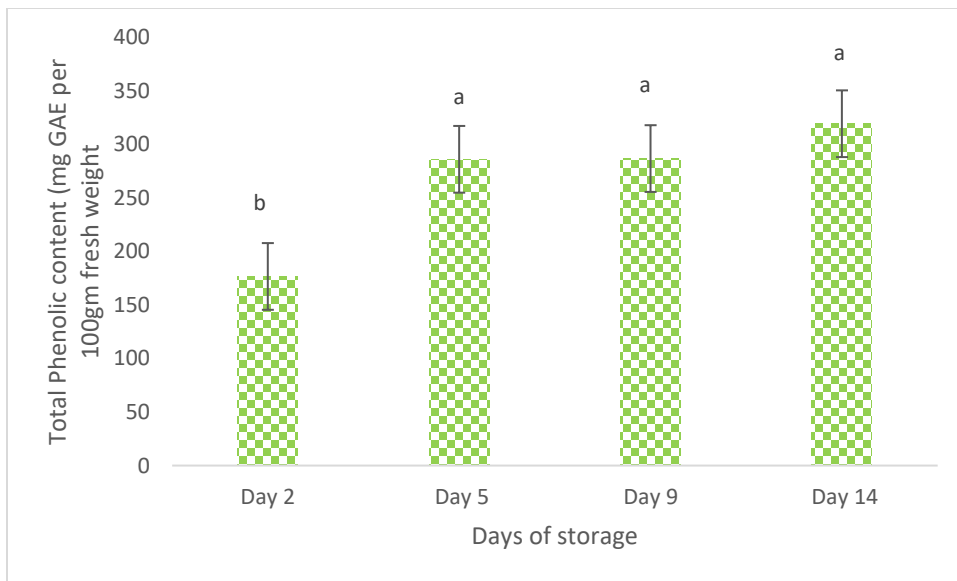
691 LS-means with the same letter are not significantly different



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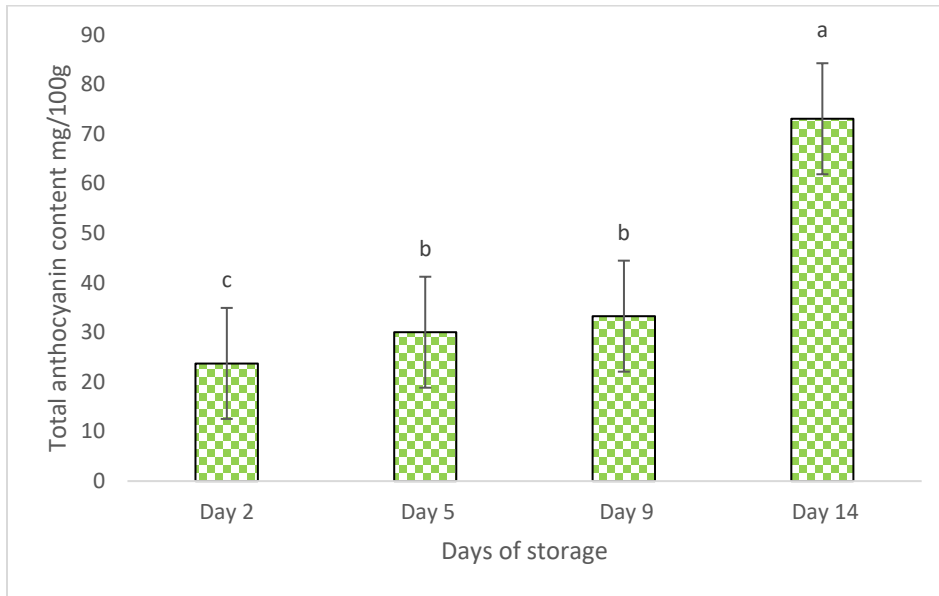
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697 Figure 13: Mean TPC on days of storage in both treated and non-treated strawberries . LS-means  
 698 with the same letter are not significantly different

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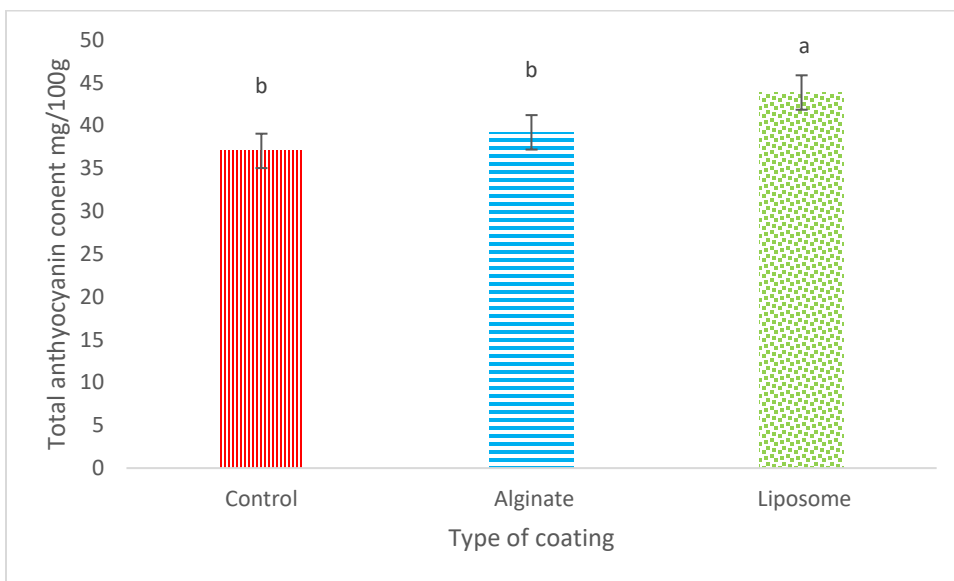
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703 Figure 14: Mean Total anthocyanin on days of storage. LS-means with the same letter are not  
704 significantly different

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706

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

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