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Novel *Prosopis juliflora* leaf ethanolic extract coating for extending postharvest shelf-life of strawberries

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ABSTRACT

Strawberry (*Fragaria* x *ananassa*) is a rich source of nutrients, minerals, and antioxidants including vitamin C. Its susceptibility to mechanical injuries, dehydration, and fungal infections make its postharvest shelf-life very short. *Prosopis juliflora* water-soluble leaf ethanolic (PJ-WS-LE) extract previously described by our team has a strong antifungal activity. The present study investigates the potential effect of PJ-WS-LE extract coating on the extension of strawberries shelf-life, and the values of the extract alone and the extract embedded in 1% chitosan (edible coating) in delaying spoilage-related symptoms and in maintaining good storage quality parameters at 4 °C. PJ-WS-LE extract alone extended strawberries shelf-life at 4 °C by 2.32 times. Best storage quality-parameters were observed with strawberry samples coated with PJ-WS-LE extract embedded in 1% chitosan, this includes liked sensory characteristics, maintenance of firmness and total soluble solids levels, lower surrounding microbial count, lower percent weight loss and lower total antioxidant levels increase.

1. Introduction

Globally, it has been reported that 20% of the harvested vegetables and fruits are wasted every year by spoilage and around one third of the overall produced food is either wasted or lost (Leyva Salas et al., 2017; Mailafia, Okoh, Olabode, & Osanupin, 2017). Food and Agriculture Organization (FAO) of the United Nations stated that around 1.3 billion tons of food are wasted every year (FAO, 2017). Both human and animals have fruits as a major source of nutrients including vitamins. However, field contamination, contaminated harvesting tools, unsafe transportation and storage handling, and inappropriate display methods have led to the wastage of around 45% of the harvested fruits and vegetables around the world (Snyder & Worobo, 2018).

Fragaria x *ananassa* or strawberries are widely consumed raw berries which are popular in their processed forms such as jam and juices (Giampieri et al., 2012). Strawberries with their high nutritional quality are rich with antioxidants including vitamin C, folate and phenolic constitutes (Huang, Zhang, Liu, & Li, 2012). The delicate fruit has a very short postharvest life because it is susceptible to mechanical injuries, fast dehydration and fungal infection (Aday, Temizkan, Büyükcan, & Caner, 2013). A previous study of our team has estimated strawberry samples to have a home shelf-life between 1 and 3 days (I. Saleh & Al-Thani, 2019). Growth of mycotoxins-producing fungi on strawberries leads not only to the visible spoilage of fresh samples but also to the contamination of strawberry jam and juices with mycotoxins levels beyond the acceptable (Fernández-Cruz, Mansilla, & Tadeo, 2010).

Many of the spoilage control methods currently available are costly while others can be polluting (Garnier, Valence, & Mounier, 2017). In the case of strawberry, their most common spoiling fungi is *Botrytis cinerea* which causes postharvest grey mold decay (Ahmed, Shamki, & Reda, 2019). *B. cinerea* infection and other postharvest spoiling agents can occur during storage, they can also originate from the field before harvesting; In this case, they can remain latent until storage stages (De Simone et al., 2020). Infection signs are lacking, therefore, it is difficult to come up with an effective methods to predict risks (Shuping & Eloff, 2017). Preventive applications of synthetic fungicides during strawberry growing cycle is common to reduce losses in agriculture. The applied chemicals include but are not limited to Cyprodinil, Iprodione, Imidazoles, and Triazoles (Feliziani & Romanazzi, 2016).

Nowadays, the continued use of synthetic fungicides in field is facing challenges in consumer acceptance. Public awareness increased requests for organic fresh produces that are free of chemical residues, which in its turn increased research in the domains of biological control agents, natural anti-spoiling agents, and physical control methods (I. Saleh & Goktepe, 2019). Biological control agents include bacteria and yeast that are antagonistic to the spoiling pathogens (Díaz, Pereyra,

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Picón-Montenegro, Meinhardt, & Dib, 2020). Before commercializing such microorganisms, they must be proven not to have any phytotoxic effects. They must also be tested for the production of toxic secondary metabolites that can be harmful to the environment or to human health (Zhang, Li, Zhang, Chen, & Tian, 2020). Many "Generally Recognized as Safe" (GRAS) compounds have been tested for their anti-spoiling effect. According to the United States Food and Drug Administration, GRAS have antimicrobial properties beside their plant defense inducing characteristics (Kucharska, Sikora, Brzoza-Malczewska, & Owczarek, 2019). Among the natural compounds proposed, many plant extracts and essential oils have been reported to control postharvest diseases. One of the major concerns when applying natural products is their stability over time (Raveau, Fontaine, & Lounès-HadjSahraoui, 2020). Although physical spoilage control methods are usually costly and are not affordable in many of the agricultural areas, they are considered as a safe and effective option in many countries. Among the physical treatments that can extend strawberry shelf-life is a modified storage atmosphere (Feliziani & Romanazzi, 2016). In addition, the installation of ozone generators in strawberry cooling rooms is becoming common in the last few years (Panou, Akrida-Demertzi, Demertzis, & Riganakos, 2021).

A combination between an antimicrobial natural product and resistance inducers is among the explored options (Freimoser, Rueda-Mejia, Tilocca, & Migheli, 2019). Resistance inducers are activators of plant defense mechanisms. Among the resistance inducers, chitosan, benzothiadiazole and salicylic acid have previously been tested as coating material of strawberry samples (Feliziani & Romanazzi, 2016). Various treatments with chitosan have shown reduction in postharvest decay, since it acts as a physical barrier against mechanical injury and water loss (Romanazzi, Feliziani, Santini, & Landi, 2013). Combining the non-toxic biodegradable polymer of chitosan with an effective stable natural compound would increase the sensitive fruit shelf-life, maintain their quality characteristics, protect the environment, and decrease economical losses.

This study aims to test the recently characterized *Prosopis juliflora* water-soluble leaf ethanolic (PJ-WS-LE) extract, individually and in combination with chitosan, as a protective coating material for strawberry samples. *Prosopis juliflora* is an invasive species in the state of Qatar, chemical and mechanical invasion control are expensive and not recommended because of their possible adverse effects on the environment, nowadays the idea of control through utilization is recommended (Wei et al., 2018). Many countries use different parts of *P. juliflora* for different purposes. In India, the leaves of the tree are used for pharmaceutical purposes (Sharifi-Rad et al., 2019). The pods of *P. juliflora* are directly consumed in Argentina and Mexico (Pasiecznik et al., 2001; Vilela et al., 2009). In addition to many attempts to produce honey and other sugary food-products from the plant as being a high source of carbohydrates (da Silva, da Silva, JHC, & Costa, 2018). All of this imply the safety of usage of *P. juliflora* extracts. Although few studies reposted the plant's neurotoxicity, yet toxicity cases in cattle were accompanied by the over usage of the tree as a solo source of nutrients (da Silva et al., 2018). PJ-WS-LE extract has been proven as an effective, stable, natural compound against many spoiling agents including *B. cinerea* (Iman Saleh & Abu-Dieyeh, 2021). Being effective against the main strawberries spoiling agent and being free of toxic organic solvent, our hypothesis was that PJ-WS-LE extract would extend strawberries shelf-life and maintain their storage quality-parameters.

2. Materials and methods

2.1. Preparation of PJ-WS-LE extract

Fresh leaves of *P. juliflora* were collected and processed as previously describes by our team (Saleh and Abu-Dieyeh, 2021). Stock solution of 100 mg/ml of PJ-WS-LE extract was prepared and used to prepare the coating solutions.

2.2. Effect of PJ-WS-LE extract on strawberries shelf-life at room temperature and at refrigerator temperature

Sixty samples of fresh imported strawberries with no visual decay (Morocco) were divided into two groups (30 fruits each). A control group where samples were just kept in a sterile well-ventilated box at room temperature. A treated group where samples were first sprayed thoroughly with PJ-WS-LE extract solution, then left to dry in a sterile drainer before being moved to a sterile well ventilated box at room temperature (22 °C–24 °C) and normal room humidity (30%–40%). PJ-WS-LE extract solution was used at a concentration of 8 mg/ml which is double the highest *in-vitro* minimum inhibitory concentration (MIC) of the extract as shown in the team earlier study (Saleh and Abu-Dieyeh, 2021). The entire experiment was repeated twice.

Samples were monitored every 24 h to determine shelf-life, termination of shelf-life is the day at which a sample show fungal growth. Once rotten, a sample was removed from the box and the fungal contaminant was transferred with a sterile needle to a clean Potato Dextrose Agar (PDA) (Difco-USA) plate before discarding the sample. All PDA plates were incubated at 25 °C for 5 days then fungal species was morphologically and microscopically determined. Data was recorded to compare average shelf-life of control and experimental groups. Percentage of edible samples at 48h was also determined.

The same experimental setting was repeated on another 60 strawberry samples that were stored this time at 4 °C in the fridge. Experiment was terminated at day 10 and the firmness of the good remaining control and experimental samples was measured.

2.3. Sample preparation for long-term storage

One hundred forty fresh good-looking samples of imported strawberries (Driscoll-USA) were purchased and divided into four groups of 30 samples each. Two control (A and C) and two experimental (B and D) batches were prepared before being stored at 4 °C. Batches treatments were conducted as following:

- Batch A: untreated samples.
- Batch B: samples were thoroughly sprayed with 8 mg/ml PJ-WS-LE extract and left to air-dry.
- Batch C: samples were coated with 1% chitosan (CAS 9012-76-4, Himedia, India) by dipping them and leaving them to air-dry.
- Batch D: samples were dipped in 8 mg/ml PJ-WS-LE extract embedded in 1% chitosan and left to air-dry.

The experiment was organized in a completely randomized design, every five samples made a single replicate (one experimental unit) and were stored together in one sterile box, each treatment was performed in six replications and the entire experiment was repeated twice. The storage period of strawberry samples was 7 days which means that all quality-parameters were evaluated at day zero and then every 7 days until the end of the storage time (Mohammadi, Hashemi, & Hosseini, 2016).

2.4. Preparation of coating solutions

Chitosan coating solution was prepared at 1% concentration by dissolving chitosan powder in distilled water with 1% glacial acetic acid (IsoLab, Germany), the solution was stirred with a magnetic stirrer overnight. The pH of the solution was adjusted to 5.6 using 0.1M NAOH (Sigma-Aldrich, Germany). For PJ-WS-LE extract chitosan embedded coating solution, specific volume of the extract stock solution sterilized by filtration was added to achieve a final extract concentration of 8 mg/ml PJ-WS-LE extract in 1% chitosan (Chien, Sheu, & Lin, 2007).

2.5. Evaluation of sensory quality

The evaluation of the sensory quality was carried out on a 5-point scale for samples' taste, smell, color change and overall quality. The four attributes were evaluated using the withdrawn samples at every storage interval. Each sample was given a score using the following scale: 5 points for "extremely liked", 4 points for "liked", 3 points for "acceptable" 2 points for "disliked" and 1 point for "extremely disliked". Average score per batch per week was then calculated (Patel & Panigrahi, 2019).

2.6. Estimation of weight loss

All strawberry samples used in the trial had their weight taken at day zero. Weights of the remaining samples were measured at every storage interval. Total weight loss is the variation between primary and final weights of the sample during a certain storage interval. Average percent weight loss per batch was calculated (Patel & Panigrahi, 2019).

2.7. Estimation of total aerobic bacterial count

Strawberry samples withdrawn at every measurement interval were washed from the outside with 10 ml of sterile distilled water (SDW). Washing water was serially diluted (10^{-1} to10^{-3}) and spread on nutrient agar (NA, Scharlau) plates in duplicates for total aerobic bacterial count. Plates were incubated at 37 °C for 48h. Colony forming unit (CFU) per sample was calculated and average total aerobic bacterial count CFU per treatment batch was also calculated at each time interval (Foruzan Moradi, Aryou Emamifar, & Naser Ghaderi, 2019a).

2.8. Estimation of total fungal count

The washing water previously prepared was also spread on PDA plates for total mold and yeast count. PDA plates were incubated at 25 °C for 5 days. Mold CFU and yeast CFU per sample were calculated separately. Average mold CFU and yeast CFU per treatment batch was calculated at each time interval (Moradi, Emamifar, & Ghaderi, 2019a).

2.9. Evaluation of respiration rate

Strawberries samples of each treatment batch were placed in closed containers and the level of carbon dioxide produced was measured using carbon dioxide meter (OMEGA AMQ-102, UK). A reading of the CO_2 in ppm was taken every 2 s for around 50min. Respiration rate (ppm/s) was calculated using the slope of the trend line passing by the collected data, and weekly results were compared (Mohammadi et al., 2016).

2.10. Determination of samples firmness

Withdrawn samples of strawberries had their firmness measured using a penetrometer (Agriculture Solutions, USA). The probe of the penetrometer was inserted in two different locations in the middle of each fruit, and the average reading in Newton (N) was recorded. Average samples firmness (N) in each treatment batch was calculated at every measurement interval (Emamifar, Ghaderi, & Ghaderi, 2019a).

2.11. pH measurement

Strawberries withdrawn at every measurement interval were blended into juice, which was filtered and had its pH measured using a digital pH meter (Jenway, UK). Average samples pH per treatment batch was calculated on a weekly basis. A pH 7 buffer solution was used to calibrate the pH meter before measurements (Naeem, Abbas, T, & Hasnain, 2019).

2.12. Total soluble solids (TSS) measurement

Strawberries juice samples previously prepared and homogenized had their TSS in brix measured. Two drops per sample's juice were focused on a refractometer (ANTAHI, New Zealand) and measurements were taken. Average samples TSS (%Brix) per treatment batch was calculated on a weekly basis. Distilled water was used to calibrate the refractometer before measurements (Moradi et al., 2019a).

2.13. DPPH radical scavenging assay

A 100 μl of each diluted strawberry juice samples was mixed with 1 ml of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (TCI, US) (100 mg/l) and incubated under dark conditions for 45 min at 37 °C. Samples were centrifuged and the supernatant change in color intensity was measured using a spectrophotometer (Jenway, UK) at 517 nm against methanol (Sigma Aldrich-Germany) as a blank. A 100 μl of methanol in 1 ml DPPH was used as a control. Percent radical scavenging activity of each samples was calculated using the below formula:

% radical scavenging activity = (absorbance of control-absorbance of sample) *100/ absorbance of control

Average % radical scavenging activity per treatment batch was calculated every week (Mohammadi et al., 2016).

2.14. Statistical analysis

Completely Randomized Design (CRD) was used as an experimental design. T-test at $P \leq 0.05$ was used to compare treated and non-treated samples in shelf-life experiments. Levene's test was used at a significance level of $P \leq 0.05$ to test variances equality assumption. The weekly percent weight change data were evaluated using one-way ANOVA followed by Tukey Post-Hoc test at $P \leq 0.05$ to separate the means among treatment levels. Bacteria, mold and yeast CFU levels of different treatment groups throughout the experiment time were compared using one-way ANOVA. Correlation matrix among percent change in weight, bacteria CFU, mold CFU and yeast CFU levels throughout the experiment was constructed using Pearson correlation test. Data were presented as average ± standard error of the Means (SEM). Statistical analysis were performed using SPSS (Ver. 27, SPSS Inc. Chicago, USA).

3. Results and discussion

3.1. Effect of PJ-WS-LE extract on strawberries shelf-life at room temperature and at 4 °C

The treated samples incubated at room temperature showed longer shelf-life than control samples. Calculated average shelf life of treated strawberries at room temperature was 72 h while none-treated fruits showed an average shelf-life of 49 h. T-test showed statistically highly significant difference between control and experimental samples shelf-life ($P = 0.007$). Percentage of edible samples after 48 h was also calculated, 62.5% of the treated samples were still good for consumption while only 33.3% of the non-treated samples were still edible within 48 h. Upon microscopic identification, three fungal species were behind the strawberry samples spoilage. *Botrytis cinerea* was isolated from 95.8% of the spoiled samples while *Cladosporium* sp. and *Rhizopus* sp. were isolated from 6.3% to 4.2% of the spoiled samples respectively.

Similar yet more significant results were observed with strawberry samples incubated at 4 °C. During the ten days of the experiment, the 17 control samples that rotten at different time slots showed an average shelf-life of 103h (4.3 days). On the other hand, all treated samples maintained appealing sensory qualities till the end of the experiment with a shelf-life of 240h (10 days). T-test comparing the overall shelf-life values at day 10 validate variances equality assumption and showed statistically highly significant difference between control and experimental samples shelf-life ($P = 0.001$). The percentage of edible samples at day 10 is 43.3% of the non-treated samples and 100% of the treated samples. Results showed that the crude extract was capable of totally preventing fungal growth on refrigerated strawberry samples for 10 days.

Upon microscopic identification, three fungal genera were behind the control strawberry samples spoilage. *Botrytis cinerea* was isolated from 70.6% of the spoiled samples (17 sample). While *Rhizopus* sp. and *Cladosporium* sp. were isolated from 17.6% to 5.9% of the spoiled samples respectively.

Average firmness of the 13 remaining control samples was 42.6N while average firmness of the 30 remaining treated samples was 44.5N. T-test showed no significant difference in the average firmness between treated and non-treated samples ($P = 0.511$).

Fig. 1 shows a comparative analysis of percent samples loss for fungal decay per day, it is clear that low temperature has extended strawberry samples storage-life (blue curves) compared to samples kept at room temperature. Addition of the PJ-WS-LE extract has significantly protected strawberries from rotting by adding two more days to life of some of the strawberry samples at room temperature, while samples in the fridge were totally protected during the 10 days of the experiments.

In-vivo analysis results showed that strawberry samples treated with PJ-WS-LE extract, both at room temperature and in the fridge had a slow-down effect on the rate of grey mold progression, which indicates that the active compounds in the crude extract were capable of preventing fungal growth on strawberry samples. Microscopic identification of strawberry spoiling agents showed a dominance of *Botrytis* sp., this indicates that 8 mg/ml of the PJ-WS-LE extract had a strong protective effect on strawberry, by significantly delaying fungal growth mainly *Botrytis* growth at both room temperature and at 4 °C. Results are in agreement with the effect of ultrasound treatment in extending strawberry shelf-life reported by Aday et al. (2013). A recent study also showed an increase in strawberry shelf-life of samples coated with an edible coatings based on basil seed gum (BSG) enriched with *Echinacea* extract (EE), samples coated with 3% BSG and 3% EE showed better quality characteristics when compared to control samples during the experiment (Foruzan Moradi, Aryou Emamifar, & Nasser Ghaderi, 2019b). The same researchers also showed a good efficacy of the edible coatings based on salep solution (SS) enriched with grape seed extract (GSE). At 1.5% SS and 3% GSE coating materials, samples showed a microbial load within the "good for consumption" range during the experiment (Emamifar, Ghaderi, & Ghaderi, 2019b). Comparison between shelf-life extension methods should take into consideration the feasibility of the treatment method. Considering the simple and cheap extraction method of PJ-WS-LE extract and its direct spraying in aqueous solution on fruit samples gives our results an advantage over what are previously reported with similar effectiveness in shelf-life increase.

3.2. Sensory quality of strawberry samples at long-term storage experiment

The change of sensory characteristics of strawberries stored at 4 °C was evaluated on a weekly basis. Fig. 2 represents the change of the score of fruits belonging to different treatment batches. As storage period passes, the overall sensory score of all strawberry batches decline. However, samples treated with 8 mg/ml of PJ-WS-LE extract (batch B) and samples coated with 8 mg/ml of PJ-WS-LE extract embedded in 1% chitosan conserved better scores during the three weeks of storage.

At the end of the three weeks of the experiment, samples coated with PJ-WS-LE extract alone and with PJ-WS-LE extract embedded in 1% chitosan conserved a liked sensory quality with sensory score averages of 4.4 and 4.3 respectively. Similar results has been recently described with BSG + EE coating and with SS + GSE coating (Emamifar et al., 2019b; Moradi, Emamifar, & Ghaderi, 2019b). This demonstrates the importance of edible coatings in maintaining good sensory quality parameters. Beside the feasibility of the extraction method, the choice of the edible coat to be adopted in preservation depends also on the availability of the natural product added to the coating material. Knowing as an invasive tree in many countries (including Qatar), *P. juliflora* is highly available and its utilization is encouraged to manage its widespread (Wei et al., 2018).

3.3. Weight loss of strawberry samples at long-term storage experiment

The effect of samples coating treatment on the weight loss was monitored over the course of three weeks. Percent change in weight was calculated for each treatment batch every week (Fig. 3). Weight loss increased with time, however, PJ-WS-LE extract showed efficacy in reducing weight loss independently and when embedded in chitosan.

Strawberries coated with 1% chitosan and 8 mg/ml of the PJ-WS-LE

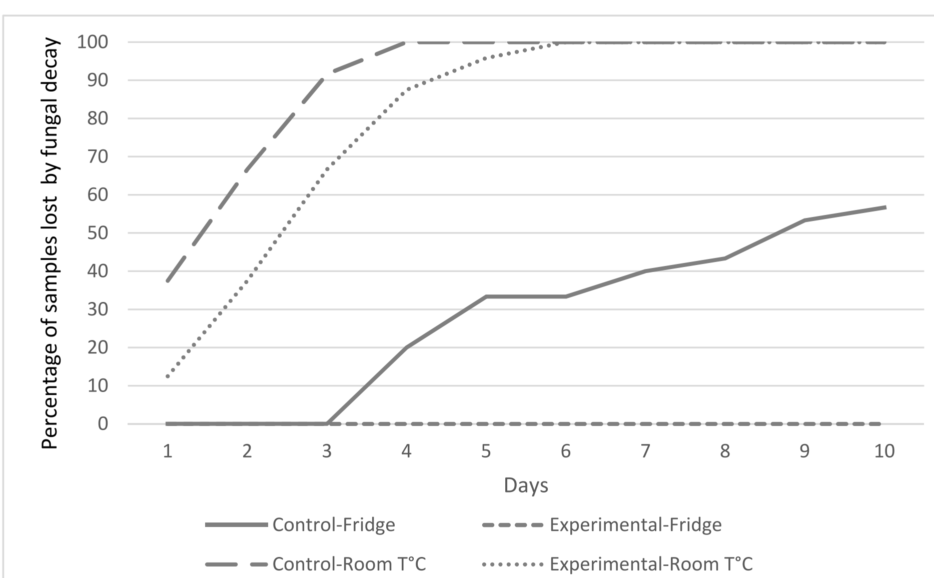

Fig. 1. Cumulative percent loss of strawberry samples stored at different conditions for 10 days. Experimental samples are samples treated with 8 mg/ml PJ-WS-LE extract.

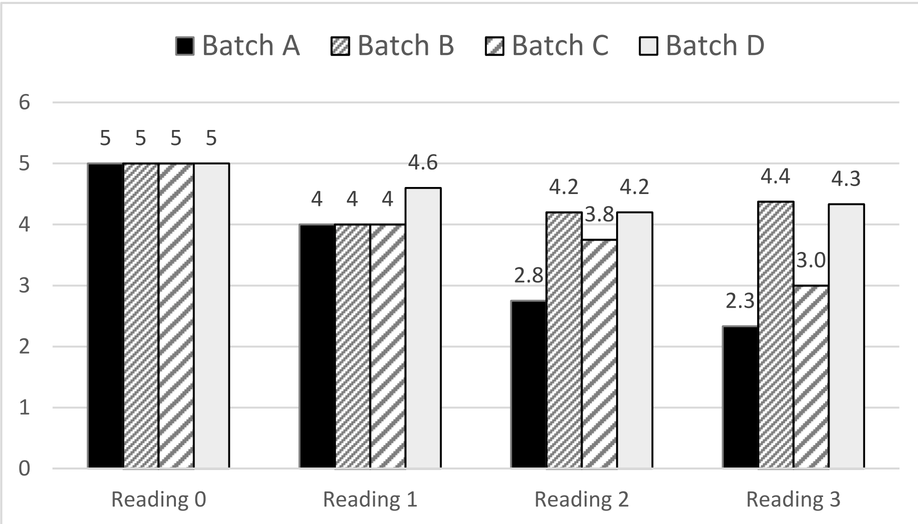

Fig. 2. Average sensory scores of strawberry samples stored at 4 °C and exposed to different treatments on a weekly basis. A: Negative control samples with no treatment; B: samples treated with 8 mg/ml PJ-WS-LE extract; C: samples coated with 1% chitosan; D: samples coated with 1% chitosan + 8 mg/ml PJ-WS-LE extract). Score from 1 to 5 are: 5 points for "extremely liked", 4 points for "liked", 3 points for "acceptable" 2 points for "disliked" and 1 point for "extremely disliked".

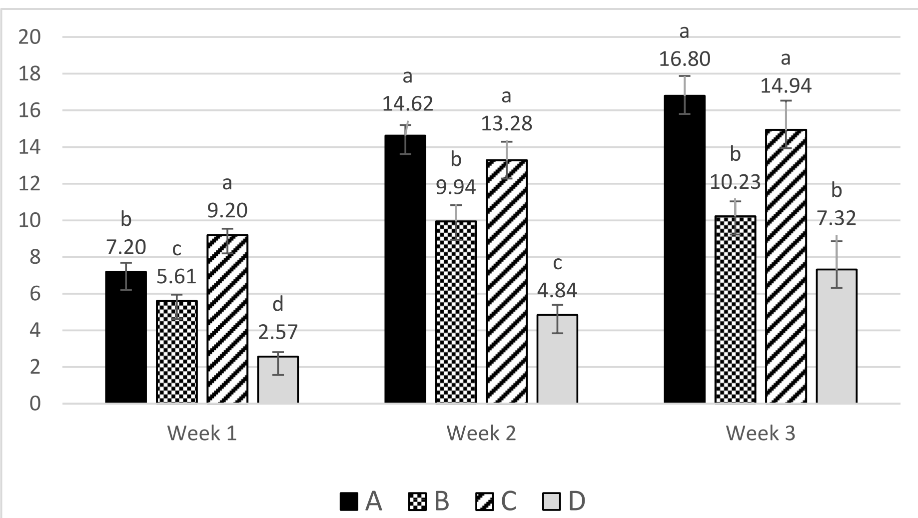

Fig. 3. Average percent change in weight of the overall strawberry samples stored at 4 °C and exposed to different treatments on a weekly basis ±SE. A: Negative control samples with no treatment; B: samples treated with 8 mg/ml PJ-WS-LE extract; C: samples coated with 1% chitosan; D: samples coated with 1% chitosan + 8 mg/ml PJ-WS-LE extract. [abc] Treatment columns with different letters have their values significantly different as shown by Tukey test at $P \leq 0.05$ for the data of each week.

extract together had the lowest change in weight with an observable increase of this change from week to week. The extract alone also showed high effectiveness in protecting fruits from weight loss. Levene's test of the data of each week supported the hypothesis of equality of variances ($P = 0.08$), which allows the usage of one-way ANOVA, results shows the significant effect of the type of treatment on the percent weight change. Post-Hoc Tukey-test showed that the difference between the percent changes in weight of the strawberry batches exposed to the four different treatments are significantly different among eachothers in week 1 ($P = 0.001$). In week 2, there is no significant different between the percent change of weight of batches A and C ($P = 0.624$), however, batches B and D had significantly lower weight loss ($P = 0.001$). The same test was conducted for week 3, results categorized the treatment groups into two subsets (subset 1: batch A and C and subset 2: batches B and D). The two subsets have a high significant difference in their percent weight loss averages ($P = 0.002$), however the batches of each subset are not significantly different from eachothers, which indicates that towards the end of the experiment the extract alone and the extract embedded in 1% chitosan (batches B and D) were as effective in protecting fruits against weight loss. Final percent weight loss of fruits of batches B and D were 10.2% and 7.3% respectively compared to 16.8% weight loss in the control group (batch A).

8 mg/ml PJ-WS-LE extract had significantly decreased average weight loss in strawberries in both treatment groups B and D, which indicates that the extract can serve as a protective layer that help fruits to avoid water loss. There was a significant difference in weight loss between batches C and D. Therefore, PJ-WS-LE extract incorporated into the polysaccharide-based edible coating (chitosan) has significantly improved the water barrier properties of chitosan by reducing fruits surface evaporation rate. Similar results were seen with SS + GSE coating (Emamifar et al., 2019b) and with BSG + EE coating (Moradi et al., 2019b), which prove the importance of enriched edible coating in maintaining strawberries' weight. However, it is worth noting that strawberry is a sensitive fruit and that not all coating methods work for it, even those that show good results with other fruits. For example, Parreidt et al. have recently developed a new alginate-based coating that protects well cantaloupe samples from weight-loss, however, it has an adverse effect on strawberry samples by increasing their weight loss compared to the control samples (Parreidt, Lindner, Rothkopf, Schmid, & Müller, 2019). Such results highlight the importance of PJ-WS-LE extract as suitable coating material for soft fruits.

3.4. Respiration rate change in strawberry samples during the long-term storage experiment

Emitted CO_2 levels of samples of each treatment group were measured every 2 s for around an hour to give data that is used to plot the trend lines used for the slopes calculation. Weekly data was used to calculate weekly respiration rate for all treatment batches (Fig. 4).

Fig. 4 shows that the respiration rate of strawberry samples of all treatments represented by the carbon dioxide level decreases with time between week 0 and week 2 to show a slight increase towards week 3 in some samples. Week 0 results indicate that chitosan treatment slow down the respiration rate although carbon dioxide level is higher in the presence of the PJ-WS-LE extract (batch D). Plants of batch B coated with the extract alone showed the highest rate of respiration throughout the three weeks.

Respiration rate is an indicator of plants normal activity. In addition, levels of oxygen and carbon dioxide in packaged fruits and vegetables affect their metabolic activities. Results showed the highest respiration rate with samples coated with 8 mg/ml PJ-WS-LE extract alone. Batch D did not show similar results, this could be due to the stress caused by the layer of chitosan on the respiration rate by closing plants pores (Petriccione et al., 2015). Different protective treatments showed different effects on respiration rate of strawberry samples, a previous study assessed the effect of ultrasound treatment in increasing strawberry shelf-life and showed that treated samples had significantly lower respiration rates than the control. Samples from that study were stored in tightly closed containers which might have led to gases accumulation and therefore affected respiration rate (Aday et al., 2013).

3.5. Total aerobic bacterial, yeast and mold count

PJ-WS-LE extract in the strawberries coating material served as an antimicrobial barrier that kills surrounding bacteria, mold and yeast then slow down spoiling. From reading zero, samples coated for few hours showed much lower bacterial count than negative control samples and samples coated with chitosan alone. Effect is stronger in samples of batch D coated with the extract embedded in 1% chitosan.

Average aerobic bacterial count in CFU per gram of strawberry of each treatment batch was calculated between week 0 (day of treatment) and week 3 of storage at 4 °C (Fig. 5). The average CFU pattern of all treatments showed a decrease in CFU after one week of storage followed by an increase in week 2 to decrease again in week 3. Strawberry samples exposed to different treatment showed a consistent difference in their CFU throughout the weeks. Samples of batch B treated with 8 mg/ml of PJ-WS-LE extract showed a very low bacteria count at the day of treatment and in week 1 (302.8 CFU/g and 56 CFU/g respectively), the total number remain lower than the control group (batch A) by 64.3% and 35% in weeks 2 and 3 respectively. The lowest bacterial count was shown in samples treated with 8 mg/ml PJ-WS-LE extract stabilized in 1% chitosan to reach an average $<$ 10 CFU/g in week 3.

Average mold CFU (Fig. 6) and yeast CFU (data not shown) in of each treatment batch was calculated between week 0 (day of treatment) and week 3 of storage at 4 °C. The lowest yeast and mold counts were seen in batch D where CFU count reaches zero CFU/g mold and 292 CFU/g yeast in week 3. Batch B coated with 8 mg/ml of PJ-WS-LE extract alone showed lower mold and yeast count in the first three readings. However, in week 3 CFU of both mold and yeast dropped significantly in all batches to become equal in batches A, B and C, only experimental samples of batch D had zero mold count and a yeast count that is 38% lower than the control batch.

One-way ANOVA test conducted to evaluate the effect of treatment type on microbial count showed significance at $P \leq 0.05$ with the lowest CFU bacterial, mold and yeast counts for batch D ($P = 0.002$, $P = 0.001$ and $P = 0.001$ respectively).

Mold samples showing growth upon spread plate method were subjected to microscopic identification, the number of samples showing a certain fungi species was recorded regardless of the number of colonies per samples (data not shown). Our results showed that the dominant fungi in strawberry samples stored at 4 °C was *Cladosporium* sp. found in 60 samples of different treatment batches throughout the storage period followed by *Botrytis* sp. found in 35 samples.

Microorganisms surrounding fruits and vegetables increase in numbers during fruits ripening and play a role in fruits spoiling (Erkmenand & Bozoglu, 2016). The capability of the coating material to lower the surrounding microorganisms count could play a role in increasing plants shelf-life. PJ-WS-LE extract showed a high antibacterial activity from the first few hours after treatment. At the end of the three weeks, samples of batch B showed 64.3% lower aerobic bacterial count than the control group while samples of batch D showed zero bacterial CFU. As for fungi, batch D also showed the lowest final numbers with zero CFU/g mold count and yeast count that is 38% lower than the control batch A. The most encountered mold genus are *Cladosporium* and *Botrytis*, which proves, as shown in the *in-vitro* analysis, the extract efficacy against these two fungal types (Saleh and Abu-Dieyeh, 2021). SS + GSE and BSG + EE coating treatments of two recent studies showed that the coating materials acted as a retarding agent of

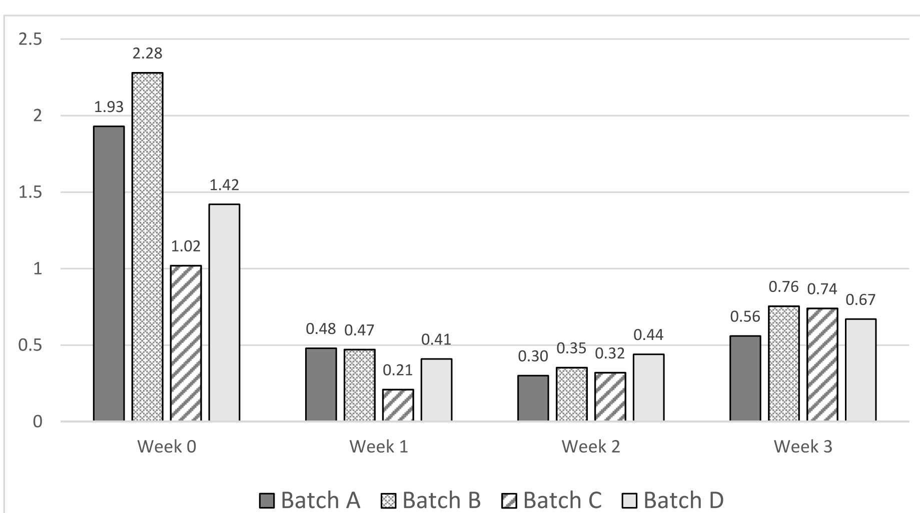

Fig. 4. Average carbon dioxide levels in ppm/s of strawberry samples belonging to the four treatment categories (A: negative control; B: sprayed with 8 mg/ml PJ-WS-LE extracts; C: coated with 1% chitosan; D: Coated with 8 mg/ml PJ-WS-LE extracts embedded in 1% chitosan) over the four weeks of the experiment.

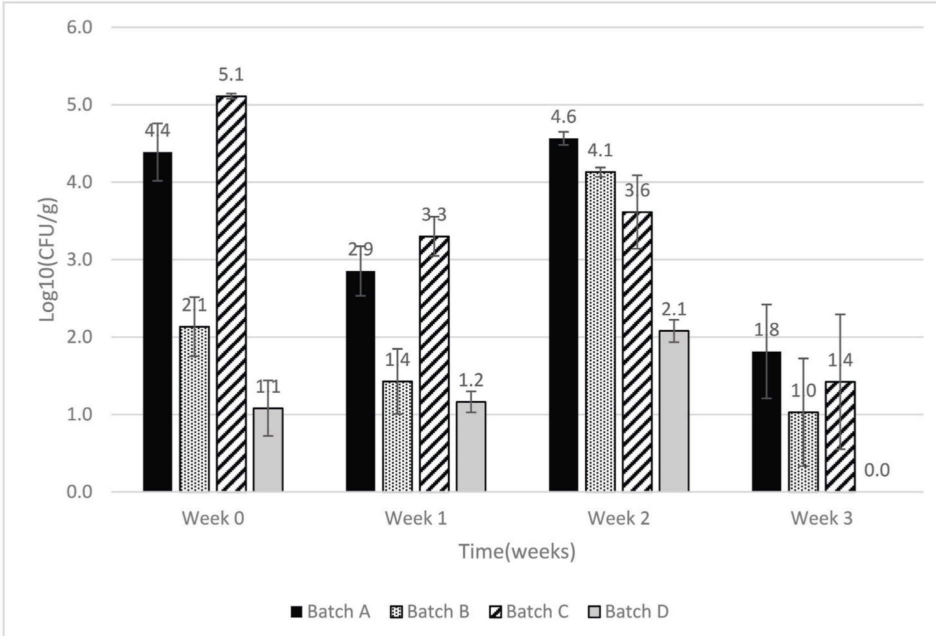

Fig. 5. Average aerobic surrounding bacterial CFU in Log_{10} CFU/g ±SE of strawberry represented in logarithmic scale of samples exposed to different treatment during three weeks of storage (A: negative control; B: sprayed with 8 mg/ml PJ-WS-LE extracts; C: coated with 1% chitosan; D: Coated with 8 mg/ml PJ-WS-LE extracts embedded in 1% chitosan).

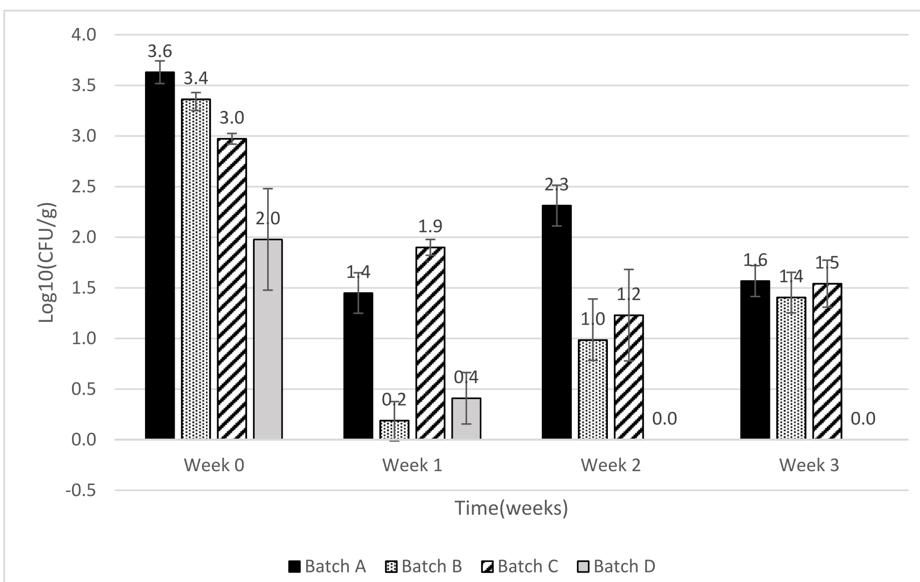

Fig. 6. Average surrounding mold spores CFU in Log_{10} CFU/g ±SE of strawberry represented in logarithmic scale of samples exposed to different treatment during three weeks of storage (A: negative control; B: sprayed with 8 mg/ml PJ-WS-LE extracts; C: coated with 1% chitosan; D: Coated with 8 mg/ml PJ-WS-LE extracts embedded in 1% chitosan).

microbial growth in fresh strawberries during cold storage (4 °C) by lowering microbial counts (Emamifar et al., 2019b; Moradi et al., 2019b). However, PJ-WS-LE extract showed the strongest antimicrobial efficacy with last total aerobic bacterial CFU and total mold CFU reaching zero in samples coated with chitosan enriched with 8 mg/ml PJ-WS-LE extract, a level that was not reached by any of the previously described extracts. Another recent study conducted by Rahimi et al. tested the effectiveness of different coating material on the total aerobic bacterial CFU count and total fungal CFU count in strawberry samples preserved at 4 °C for 12 days, the coating materials tested included *Aloe vera*, ascorbic acid, chitosan, starch, potassium sorbate and calcium chloride (Rahimi, Hanumaiah, Sahel, & Patil, 2019). Comparing their results to our two-weeks results shows that the control batches had comparable total aerobic bacterial count of 46 × 10^3 CFU/g and 39 × 10^3 CFU/g in Rahimi et al. study and in our study respectively. The 12 days old results of the total aerobic bacterial count in strawberries coated with their different coating material ranged between 7 × 10^3 CFU/g and 26 × 10^3 CFU/g, however, PJ-WS-LE extract embedded in chitosan showed a CFU of 15 × 10^1 CFU/g, which demonstrate the higher efficacy of our extract. Similar results were seen with the total fungal count where our fungal count at week 2 reaches zero CFU, which was not recorded with any of their tested coating material.

3.6. Effectiveness of PJ-WS-LE extract in maintaining strawberry samples weight

A correlation between the samples weight loss pattern and the change in microbial counts throughout the study would support our hypothesis stating that PJ-WS-LE extract acts as an antimicrobial barrier around the fruits and maintain their storage quality during the experiment including the lessening of weight loss. Pearson test results showed a significant correlation between bacterial, fungal and yeast counts patterns ($P = 0.001$). Most interestingly, the results shows a significant correlation between the percent weight change data and the total CFU counts (bacterial, mold and yeast) in the tested samples (Table 1).

Our teams' previous *in-vitro* analysis showed strong effectiveness of PJ-WS-LE extract against the most encountered fungi in this studies which are *Cladosporium* spp. and *Botrytis* spp. with MICs of 4 mg/ml and 1 mg/ml respectively. (Saleh and Abu-Dieyeh, 2021). Saleh and Abu-Dieyeh have also demonstrated the broad spectrum efficacy of PJ-WS-LE extract against some types of bacteria and fungi, which explains the correlation between mold, yeast and bacterial CFU change. Being a valuable source of phenolic compound gives *P. juliflora* extracts the capacity of initiating multiple antioxidative mechanisms which can act as anti-carcinogenic, anti-inflammatory, anti-allergic and antimicrobial agents. The antimicrobial activity of *P. juliflora* has been previously described by many older studies (de BritoDamasceno et al., 2018). Previous results, together with the significant correlation proven by Pearson test imply that the strong antimicrobial activity of the extract protects samples from fungal and bacterial rot and maintain their weights among other quality parameters.

3.7. Changes in strawberry samples physical and chemical properties during storage time (firmness, pH, TSS, antioxidant ability)

Samples were withdrawn from the trial every week to have their storage quality-parameters measured, Table 2 shows the variation in the average firmness, pH, total soluble solids and total antioxidants of samples with different treatments throughout the three weeks of storage at 4 °C.

Negative control batch of strawberry showed a 30% loss of their firmness throughout the storage period. However, all other batches showed stability in their firmness, which showed the effectiveness of the extract and the chitosan in conserving fruits quality. Note that one-way ANOVA test did not show significant difference in firmness among groups ($P = 0.434$). The results of strawberry juice pH of different batches throughout the weeks showed fluctuations in the numbers. However, when each batch results (all weeks results combined) were compared using one-way ANOVA, the overall results didn't show any significant difference in pH of strawberries of different batches ($P = 0.764$). Total soluble solids results showed an increase of TSS level of the control (batch A) samples during the three weeks of storage. Coated samples showed a slight insignificant decrease in their TSS levels. One-way ANOVA test for the overall results did not show significant TSS variation among the different groups ($P = 0.830$). DPPH radical scavenging activity of strawberry samples of the four treatment batches was measured on a weekly basis. The antioxidant activity increased in strawberry samples of all treatment groups with time. One-way ANOVA did not also show significant change among treatment batches ($P = 0.623$). However, the lowest increase was seen with strawberry samples of the coated batch D, which means that the PJ-WS-LE extract coat with 1% chitosan has successfully slowed down the antioxidant increase during fruits ripening.

Table 2
Average physical parameters ±SE of strawberry samples exposed to different treatments (A: negative control; B: sprayed with 8 mg/ml PJ-WS-LE extracts; C: coated with 1% chitosan; D: Coated with 8 mg/ml PJ-WS-LE extracts embedded in 1% chitosan) throughout three weeks.

Treatment Batch	Storage Week	Firmness (N)	pH	TSS (Bx)	DPPH RSA (%)
Batch A negative control samples	1	29.86 ± 3.5	3.61 ± 0.071	61.2 ± 3.1	14.16 ± 3.5
	2	26.24 ± 3.1	3.77 ± 0.052	69.0 ± 3.5	18.07 ± 4.5
	3	20.83 ± 2.1	3.73 ± 0.061	67.7 ± 3.2	32.90 ± 2.9
Batch B coated with 8 mg/ml PJ-WS-LE extract	1	29.53 ± 1.1	3.69 ± 0.033	61.2 ± 2.6	7.69 ± 4.7
	2	32.42 ± 3.2	3.66 ± 0.028	65.8 ± 4.4	17.42 ± 4.0
	3	30.59 ± 3.8	3.74 ± 0.037	63.0 ± 1.3	33.90 ± 4.2
Batch C coated with 1% chitosan	1	34.22 ± 4.4	3.65 ± 0.057	65.2 ± 5.9	11.63 ± 3.2
	2	35.10 ± 6	3.65 ± 0.042	63.2 ± 4.5	16.55 ± 3.9
	3	33.35 ± 2.9	3.67 ± 0.055	63.0 ± 4.6	38.46 ± 2.3
Batch D coated with 8 mg/ml PJ-WS-LE extract in 1% chitosan	1	31.6 ± 4.1	3.68 ± 0.038	65.2 ± 1.7	4.19 ± 2.2
	2	30.74 ± 6.2	3.74 ± 0.060	62.8 ± 1.9	18.50 ± 1.2
	3	32.47 ± 4.4	3.68 ± 0.039	64.3 ± 3.7	26.12 ± 3.9

Comparing the control (batch A) samples to coated samples showed that all three coating methods provide a protective layer that helped in maintaining firmness. Samples coated with chitosan (Batches C and D) showed a final firmness that is 36.7% higher than the final firmness of the control group (A) samples. Firmness is usually an indicator of the physical anatomy of the fruits' cells and tissues and it reflects cell wall strength and intercellular adhesion state, a low firmness is an indicator of destruction in the stability of the cell walls (Aday et al., 2013). SS + GSE and BSG + EE coating treatments showed maintenance of sample firmness with time compared to control (Emamifar et al., 2019b; Moradi et al., 2019b). However, an older study showed that the higher is the ultrasound power used to treat strawberry samples, the lower is the sample's firmness with time, which reflect possible treatment-induced damage (Aday et al., 2013). This highlights the importance of natural coating materials, which have minimum or no side effects of the nature of the fruits. PH levels did not change significantly throughout the experiment, a slight increase in the pH level was noticed with time and it could be due to degradation of organic acids inside the cells (Aday et al., 2013). Aday et al. also showed a slight increase of strawberry samples' pH with time with the highest increase in the control samples (Aday et al., 2013).

TSS stability in coated samples could be caused by the closure of minute pores of the strawberries' skin by the coating material which

Table 1
Correlation matrix between % change in weight of strawberry samples and their total CFU counts of mold, yeast, and bacteria.

	Mean	SD	% Weight Change	Mold CFU	Yeast CFU	Bacteria CFU
% Weight Change	9.83	4.44	1			
Mold CFU	53.96	99.10	0.469**	1		
Yeast CFU	8830.67	21193.79	0.308*	0.336*	1	
Bacteria CFU	6418.52	12467.89	0.501**	0.599**	0.595**	1

** p-value ≤ 0.01; * p-value ≤ 0.05.

decreases water loss (Patel & Panigrahi, 2019). Stability in strawberry treated samples TSS results is consistent with their average weight loss results that showed the highest weight loss with the control batch A, noting that weight loss is usually due to the loss of water content which consequently increases the total soluble solids concentration. Samples coated with SS + GSE in a recent study showed TSS increase with time, although TSS change was less in treated samples compared to control, but samples showed less stability compared to samples treated with PJ-WS-LE extract (Emamifar et al., 2019b). Similar results were seen with BSG + EE coating which lowered TSS increase with time but did not maintain it (Moradi et al., 2019b). Ultrasound, as a protective measure, showed a decrease in TSS with time, which indicates that the treatment, unlike PJ-WS-LE extract, has distorted the cell structure of the samples (Aday et al., 2013).

Fruits and vegetables juices are rich with antioxidants, which are responsible of the oxidation of free radicals to act as oxygen scavengers. DPPH free radical scavenging activity reflects the ability of the existing antioxidants to oxidize DPPH and therefore decolorize it (Patel & Panigrahi, 2019). As fruits mature, the antioxidants levels increase, sometimes as a mechanism of self-defense against ripening. The increased antioxidant contents is mainly due to the increase in lipophilic antioxidant compounds (Naeem et al., 2019). In this study, the antioxidant activity increased in strawberry samples of all treatment groups with time. However, the lowest increase was seen with strawberry samples of the coated batch D, which reflects the success of the treatment in slowing down fruits ripening.

4. Conclusion

The outcomes of the present study indicate that strawberries coated with 8 mg/ml PJ-WS-LE extract individually and strawberries coated with 8 mg/ml PJ-WS-LE extract embedded in 1% chitosan maintained liked sensory characteristics during the three weeks storage period at 4 °C. Both treatment batches have shown also significantly lower: weight loss percentage, total aerobic bacterial count, total yeast count and total mold count. The two batches treated with 8 mg/ml PJ-WS-LE maintained somehow their firmness and their total soluble solids levels during the storage period. Strawberry samples coated with 8 mg/ml PJ-WS-LE extract embedded in 1% chitosan showed the lowest increase in DPPH radical scavenging activity. PJ-WS-LE extract has beneficial effects in reducing the ripening-related symptoms and increases the storage shelf-life of strawberries at both room temperature and at 4 °C, noting that samples lasted much longer at lower temperature. PJ-WS-LE extract has been demonstrated as an effective antifungal agent against multiple spoiling agent, the *in-vitro* assays have now been supported by our *in-vivo* results showing promising results for a natural replacement of commonly used chemical fungicides. Showing the best storage quality-parameters, PJ-WS-LE- extract embedded in 1% chitosan is recommended as a coating material for strawberries in combination with low storage temperature to reach the best quality and the longest shelf-life possible. The antimicrobial effectiveness of PJ-WS-LE extract against the main strawberries spoiling agents opens also doors for the application of this natural product on strawberries in-field. Future studies will include the evaluation of the extract effectiveness on other crops beside evaluating the extract composition and toxicity, noting that preliminary cytotoxicity evaluation of PJ-WS-LE extract on normal HaCat keratinocytes cells did not show toxicity (data not shown).

CRediT authorship contribution statement

Iman Saleh: Methodology, Investigation, Formal analysis, Data curation, Writing – original draft. **Mohammed Abu-Dieyeh:** Conceptualization, Data curation, Visualization, Investigation, Supervision, Writing – review & editing.

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5. Abbreviations

PJ-WS-LE	*Prosopis juliflora* water-soluble leaf ethanolic
FAO	Food and Agriculture Organization
GRAS	Generally Recognized as Safe
SDW	sterile distilled water
NA	nutrient agar
CFU	Colony forming unit
PDA	Potato Dextrose Agar
N	Newton
TSS	Total Soluble Solids
DPPH	2,2-diphenyl-1-picrylhydrazyl
CRD	Completely Randomized Design
BSG	Basil Seed Gum
EE	*Echinacea* Extract
SS	Salep Solution
GSE	Grape Seed Extract

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2021.108641.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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