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Research Paper

Edible coatings based on solid lipid nanoparticles containing essential oil to improve antimicrobial activity, shelf-life, and quality of strawberries

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ABSTRACT

Fungal rot is a major global health concern, particularly in delicate and soft-textured fruits such as strawberries. This study aimed to assess the effectiveness of coatings containing different levels of free essential oil of *Mentha* × *piperita* L. (MEO) and solid lipid nanoparticles carrying MEO (MEO-SLNs) on the microbial load, storage quality, and shelf life of strawberries (*Fragaria* × *ananassa* cv. Camarosa) for 20 days at 8 °C. The study evaluated several factors, including weight loss, firmness, color, ascorbic acid, phenolic content, antioxidant activity, microbial load, cellulase, catalase, polyphenol oxidase, and sensory properties at regular intervals. The results indicated that strawberries treated with MEO-SLN at a concentration of 0.2% exhibited the lowest weight loss (approximately 78%), the highest firmness (around 45%), the greatest antioxidant activity (about 98%), and the highest ascorbic acid content (approximately 41%) compared to the control group. This treatment also preserved the levels of total phenol and catalase activity, reduced the activity of cellulase and polyphenol oxidase, and delayed color development. Furthermore, strawberries treated with MEO-SLN at a concentration of 0.2% received higher ratings for texture firmness, freshness, taste, appearance, and overall acceptance, and showed a lower number of cfu (colony-forming units) on day 20 of storage. MEO-SLN treatments showed no signs of soft spoilage in artificially contaminated strawberries during storage. These findings suggest that the use of MEO-SLN coatings can effectively maintain the postharvest quality of strawberries, decrease spoilage, and extend the shelf life and nutritional value of the fruits during cold storage.

1. Introduction

Strawberry is a very popular fruit due to its attractive color, delicious taste, and high nutritional value, which is full of essential vitamins such as vitamin C, vitamin E, thiamin, riboflavin, niacin, and vitamin B6, as well as amino acids, minerals, phenolics, flavonoids, Carotenoids and antioxidant enzymes (Khodaei et al., 2021). However, strawberries are susceptible to spoilage due to their vulnerability to fungal decay, mechanical damage, and the texture of their softness (Ansarifar and Moradinezhad, 2022), resulting in a limited shelf life. One of the most common postharvest diseases affecting strawberries is soft rot, caused by various species of Rhizopus (Yan et al., 2021). This disease is typically controlled by the use of synthetic chemical products, which are frequently applied in an improper and excessive manner (Oliveira et al., 2019). Therefore, it is essential to explore alternative methods for preserving postharvest quality and extending the shelf life of strawberries.

Given the hazardous effects of synthetic fungicides, there is a growing trend toward using natural, safe, and environmentally friendly antifungals such as essential oils (EOs). The EO biological activities are quite extensive and include nutritional, medicinal, and antimicrobial properties (Kalleli et al., 2020; Perumal et al., 2021). *Mentha* × *piperita* L. (M), a member of the Lamiaceae family, has long been recognized for its economic significance (Camele et al., 2021). This versatile plant has been used for medicinal purposes, such as treating stomachaches, chest

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pains, irritable bowel syndrome, coughs, and colds. Its products are also commonly used as flavorings and fragrances in food and health products due to the presence of menthol (Vostinaru et al., 2020; Camele et al., 2021). Our previous study has shown that MEO mostly contains menthol, menthone, menthyl acetate, pulegone, and carvone (Vakili-Ghartavol et al., 2022). After conducting a thorough review of the literature, it was discovered that MEO has the potential to exhibit antimicrobial activity against a range of phyto- and food-borne pathogens (Camele et al., 2021). Additionally, previous studies on EOs have demonstrated antifungal effects in a dose-dependent manner (Boukhatem et al., 2020; Achimón et al., 2021). However, some studies have indicated that using these compounds during the post-harvest stage may lead to issues with fruit texture. For instance, the use of active packaging containing thymol/eugenol and thyme EO in fresh-cut lettuce (Viacava et al., 2018; Yuan et al., 2019), as well as a combination of thymol and potassium permanganate (an ethylene scavenger) in cherry tomatoes (Otoni et al., 2016; Álvarez-Hernández et al., 2021), resulted in decreased firmness of the fruits compared to the control. EOs also have the potential to exhibit cytotoxic properties when their concentration is increased (Yuan et al., 2019; Landi et al., 2021). Additionally, EOs possess certain characteristics, such as being volatile, hydrophobic, and aromatic (Reyes-Jurado et al., 2020), that affect their application.

Various methods of nanoencapsulation have been developed to address the challenges of using EOs. The use of nanoencapsulation has been found to have several benefits over free EOs, particularly in terms of preserving and extending the shelf-life of fruits. This has been demonstrated through the use of different types of nanocapsules on various fruits. Firstly, the small size of the nanocapsule system allows for a uniform distribution and gradual release of the EO on the surface of the fruit (Sotelo-Boyás et al., 2017). Secondly, as the particles decrease in size, their surface-to-volume ratio increases, resulting in more direct contact between the nanocapsule and the fruit surface. This facilitates the targeted delivery of EO, especially to areas with microorganisms (Granata et al., 2018). Lastly, the structure of the nanocapsule helps to preserve the EO and reduce its evaporation rate (Piña-Barrera et al., 2019). In this study, solid lipid nanoparticles (SLNs), a delivery system based on solid lipids, were used to overcome the challenges associated with using EOs. The use of this nanocapsule system offers several advantages, including loading drugs with low water solubility, achieving high drug loading, reducing particle size, increasing surface area, controlling drug release, enhancing drug stability, facilitating synthesis, ensuring high biocompatibility, and improving long-term drug stability (Sarhadi et al., 2020; Laein et al., 2022; Tavassolirajaee et al., 2022).

Although the use of nanocapsule coatings embedded with essential oils has been proposed as a novel method for controlling pathogenic microorganisms, improving quality, and extending the shelf life of fruits, there is currently no research investigating the impact of MEO-SLNs on microbial activity, storage quality, and shelf life of strawberries (*Fragaria* × *ananassa* cv. Camarosa). Therefore, the aim of this study is to evaluate the individual effects of free MEO, MEO-SLNs, and their different concentrations on preserving the quality and shelf-life of strawberries over a 20-day period. In addition, the physical, chemical, and sensory characteristics of the fruits will be analyzed to determine the influence of the treatments on fruit shelf-life. This research will contribute to the existing literature by providing insights into the effectiveness of these treatments as active packaging. Ultimately, these findings can be highly valuable for post-harvest management and food preservation practices.

2. Materials and methods

2.1. Coating material characterization

2.1.1. Essential oil coatings

Different concentrations of MEO (0.025% (MEO-0.025), 0.05% (MEO-0.05), 0.075% (MEO-0.075), 0.1% (MEO-0.1), and 0.2% (MEO-0.2)) were mixed with Polysorbate 80 (Sigma, Germany) as a surfactant using a Heidolph Reax Top mixer (Germany) at 150 g for 5 min. Distilled water was then added to the mixture to reach a total volume of 100%, and the resulting coating solutions containing MEO were obtained at room temperature. The control group was treated with distilled water (concentration of 0). In a previous study, Vakili-Ghartavol et al. (2022) identified and described the components present in MEO using gas chromatography-mass spectrometry.

2.1.2. MEO-SLN coatings

SLNs carrying MEO or without MEO were formulated using high-shear homogenization and ultrasound methods, as previously patented by Vakili-Ghartavol et al. (2023). Breifly, the lipid phase, containing 5% Glyceryl palmitostearate (Gattefossé, France) as a lipid and 2.5% Polysorbate 80 as a surfactant, and the aqueous phase, consisting of double-distilled water up to 100%, were each placed separately inside a Ben mai at a temperature of 70–75 °C. Once the lipid phase had melted, the MEO was added to prevent essential oil evaporation. The components of the lipid phase were then swiftly mixed, and the hot aqueous phase was added to the melted lipid phase in a Ben mai at the same temperature. The mixture was homogenized using a Diax 900 homogenizer (Heidolph, Germany) for 4.5 min. Subsequently, the resulting emulsion was ultrasonicated using a probe sonicator (Bransonic, USA) in 5 cycles, with each cycle consisting of 60 s of sonication separated by intervals of 15 s. After cooling the samples to room temperature, SLN solutions were obtained. Additionally, SLN formulations without MEO were prepared using the same methods.

2.2. Preparation of strawberry fruits

Strawberries (*Fragaria* × *ananassa* cv. Camarosa) were harvested from a greenhouse on April 25, 2021, at the stage of commercial maturity, when 75% of the fruit surface displayed a red color. They were carefully removed from the plant and immediately transported to the horticultural science laboratory. The selection of fruits was based on their uniformity in shape, size, color, and absence of external damage, pests, and diseases. The selected strawberries (a total of 1040 fruits) were then thoroughly washed to remove any surface contaminants. After that, they were immersed in a 1% sodium hypochlorite solution for 1 min to disinfect the surface and then rinsed with distilled water. Next, the fruits were immersed in a solution of *Rhizopus stolonifer* spores, containing 1×10^5 spores/ml, for 1 min to allow the spores to settle on the fruit surface. At this stage, the fruits were prepared for the application of coating treatments.

2.2.1. Application of coatings and storage

Various concentrations of free MEO and MEO-SLN formulations (0.025%, 0.05%, 0.075%, 0.1%, and 0.2%) were prepared according to sections 2-1-1 and 2-1-2. These concentrations were chosen based on the findings of our previous research and the cytotoxic effects of EOs. Our previous research showed that the mycelium growth of *Rhizopus stolonifer* was completely inhibited by 1000 ppm of MEO *in vitro* (Vakili-Ghartavol et al., 2022). Additionally, considering the cytotoxic properties of EOs (Yuan et al., 2019; Landi et al., 2021), we also tested higher and lower concentrations to determine their impact on the delicate and soft texture of strawberry fruits, and to determine the most effective concentration for preserving the quality of the fruits.

The prepared fruits were immediately placed in each coating solution for about 90 s, allowed for the draining of the excess coating solution, and then air-dried for 90 min at room temperature. finally, the samples were packaged in polyethylene pouches and stored at a temperature of 8 °C for 20 days. The appearance, biochemical, sensory, and microbial characteristics of fruits were examined at regular intervals of 5 days during the storage period.

2.3. Post-harvest quality parameters

2.3.1. Weight loss percentage

Weight loss was quantified using a digital scale with an accuracy of 0.001 g during storage by weighing the fruit at the beginning (W1) and at different sampling dates (W2), and calculated according to the equation outlined in Jahani et al. (2020).

$$Weight\ loss\ \% = \frac{W1 - W2}{W1} \times 100$$

2.3.2. Firmness

A digital fruit hardness tester was employed to measure the firmness of the fruit. This device was outfitted with a 3 mm-diameter cylinder probe, which displayed results in Newtons (N) (Ansarifar and Moradinezhad, 2022).

2.3.3. Color

The surface color of the fruit was determined using a HunterLab colorimeter. Measurements were taken at five separate points on the fruit's surface. The CIELAB color parameters (L*, a*, and b*, indicating Lightness, Green-Red, and Blue-Yellow, respectively) were measured on the first and last days of sampling (Day 20 for MEO-SLNs and Day 15 for MEO, Control, and SLN without MEO) to calculate Chroma ($\sqrt{a*^2 + b*^2}$, color intensity) and Hue (Arctanb*/a*, purity of color) (Al-Dairi et al., 2021).

2.3.4. Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used to measure the radical scavenging activities of treated fruits and controls (Piña-Barrera et al., 2019). In this process, 100 μL of each acidic methanol extract was mixed with 1900 μL of freshly prepared DPPH methanol solution (40 μg/mL) and left at room temperature in the dark for 20 min. The absorbances of these solutions were then measured at 517 nm. The percentage inhibition of free radicals (I %) was calculated using the following equation.

$$I\ \% = \left(\frac{A_{517\ DPPH} - A_{517\ sample\ with\ DPPH}}{A_{517\ DPPH}}\right) \times 100$$

where "$A_{517\ DPPH}$" is the absorbance of the free radical and "$A_{517\ sample\ with\ DPPH}$" is the absorbance of the sample with DPPH.

2.3.5. Total phenol content

Approximately 0.1 g of the fresh sample was powdered by grinding with liquid nitrogen in a mortar. Subsequently, approximately 2 mL of acidic methanol (1:99) was added and further ground before being centrifuged at 12000 g for 20 min at 4 °C. The supernatant was then stored in a refrigerator (Vakili-Ghartavol and Alizadeh-Salteh, 2016). This acidic methanol extract was used to measure the total phenol and antioxidant activities.

The total phenolic content was determined using the Folin–Ciocalteu method of Perumal et al. (2021) with minor modifications. Briefly, 50 μL of supernatant was mixed with 450 μL of distilled water and 2500 μL of 10 % Folin-Ciocalteau reagent. The mixture was then left to rest in a dark environment at room temperature for 6 min. Subsequently, 2000 μL of 7.5 % sodium carbonate was added and allowed to settle for another 90 min before the absorbance value was recorded at 760 nm. Gallic acid served as the standard and the results were recorded as gallic acid equivalent (GAE) per gram of fresh weight (mg GAE/g of fresh weight).

2.3.6. Ascorbic acid

The 2,6-dichlorophenolindophenol method was used to measure the ascorbic acid content of strawberry pulp (Perumal et al., 2021). Specifically, 5 g of the pulp was extracted with 5 mL of 1 % metaphosphoric acid and centrifuged at 3000 g for 10 min at 4 °C. Afterwards, 500 μL of this extract was mixed with 4500 μL of 0.05 mM 2,6-dichlorophenolindophenol and vigorously shaken for a few seconds. The absorbance of the resulting solution was then read at 515 nm and expressed in terms of milligrams per gram of fresh weight.

2.3.7. Enzyme activity

Approximately 0.2 g of strawberry fruit tissue was mixed with 0.02 M potassium phosphate buffer at pH 6.8 and 4 °C. The extract was then cold centrifuged at 15000 g for 20 min, and the supernatant was collected to measure enzyme activity (Atrash et al., 2018).

2.3.7.1. Cellulase activity. After incubating 50 μL of supernatant with 200 μL of 2.5 mg/mL cellulose in a phosphate buffer of pH 7 at 42 °C for 1 h, the reaction was then terminated by adding 750 μL of a 2:1 ethanol-acetone solution and then centrifuged at 4000 g for 15 min. The absorbance was measured at 550 nm (Zhang et al., 2018).

2.3.7.2. Polyphenol oxidase activity. A mixture of 100 μL of supernatant, 500 μL of 5 mM hydrogen peroxide, 500 μL of 2 % pyrocatechol, and 1900 μL of a 2 % M phosphate buffer at pH 6.1 was vortexed, and the absorbance was read at 410 nm (Atrash et al., 2018).

2.3.7.3. Catalase activity. The Catalase activity was performed using a modified version of the method outlined by Ali et al. (2005). The reaction mixture consisted of 20 μL of supernatant, 750 μL of 25 mM potassium phosphate buffer at pH 7, 750 μL of distilled water, and 750 μL of 10 mM hydrogen peroxide, which was vortexed. The absorbance changes at 240 nm over time were then measured to record the decomposition of hydrogen peroxide.

2.3.8. Antimicrobial assessment

Counting fungal colonies per milliliter juice (CFU mL^{-1}) was performed according to the method of Pietryczuk et al. (2018) on days 0, 5, 10, 15, and 20. For each count, 500 μL of fruit juice diluted to a ratio of 1:10 was distributed onto potato-glucose agar (PDA) and incubated at 25 ± 3 °C for 5 days. Afterwards, fungal colony count (CFU mL^{-1}) or the Log CFU mL^{-1} was recorded as the output.

2.3.9. Sensorial analysis

Sensory analysis was conducted by 10 trained panelists under normal conditions, as described by Viacava et al. (2018) with minor modifications. During testing, the taste, texture, appearance, freshness, and overall acceptability were rated on a 5-point scale: 5 (excellent; juicy and unchanged fruits), 4 (good; slight loss of freshness; less than 5 %), 3 (acceptable; considerable loss of freshness; between 15 and 20 %), 2 (weak; significant reduction in freshness; wrinkles on the fruit; 20–50 %), and 1 (very weak; withering or shriveling fruits with signs of rot; over 50 %). The acceptance threshold was 2.5; any score below this value for any evaluated sensory feature was considered to denote the end of shelf life.

2.3.10. Shelflife determine

The shelf-life of strawberry fruits was evaluated, as described by Tolasa et al. (2021), by counting the number of days post-harvest until they reached their final degree of ripeness and were still suitable for distribution. When 50 % of the samples showed signs of shriveling, decay, or softening that batch was at the end of its shelf-life.

2.3.11. Statistical analysis

The *in vivo* experiments conducted followed a completely randomized factorial design. The data was analyzed using Statistical Analysis System (SPSS) version 24.0 software. Means were compared using the Least Significant Difference (LSD) Test with a *P*-value of less than 0.05. Graphs were created using Microsoft Excel 2013.

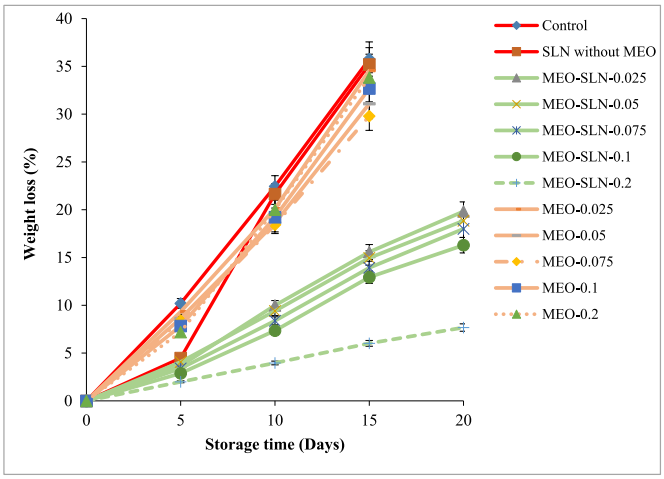

Fig. 1. The effect of different concentrations of free MEO and MEO-SLNs on the weight loss of strawberries inoculated with *R. stolonifer* during storage at 8 °C.

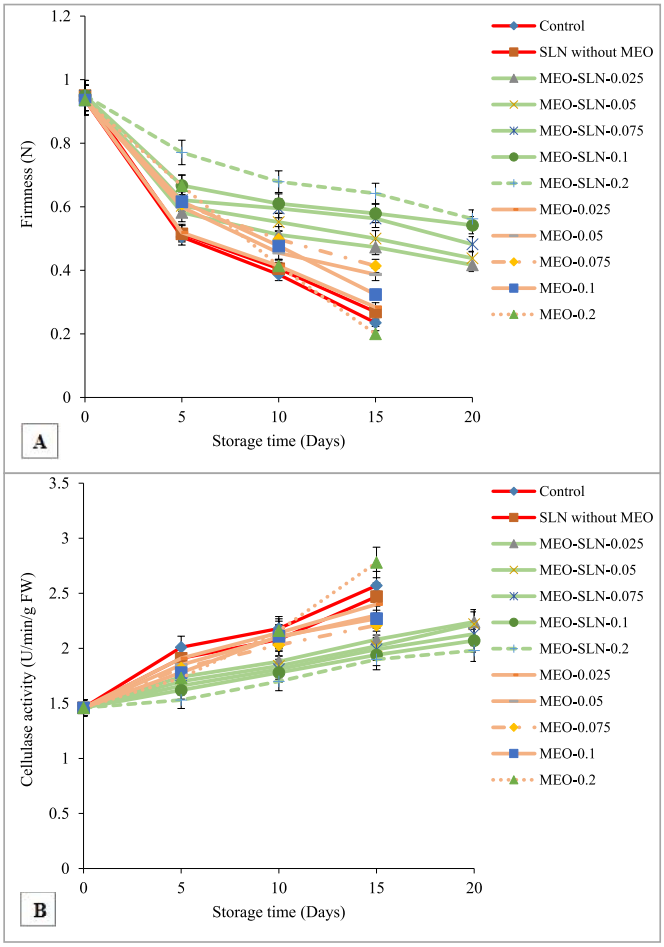

Fig. 2. The effect of different concentrations of free MEO and MEO-SLNs on A) Firmness and B) Cellulase activity of strawberries inoculated with *R. stolonifer* during storage at 8 °C.

3. Results and discussion

3.1. Weight loss

Strawberries are susceptible to weight loss due to their thin protective skin, which can lead to dehydration, wrinkling, spoilage, and a decrease in fruit quality (Dhital et al., 2018; Wani et al., 2021). According to the results, weight loss was observed in almost all fruits (Fig. 1). However, the fruits treated with MEO-SLNs experienced a lower value of weight loss compared to those treated with free MEO and the control group. Specifically, the strawberries treated with MEO-SLN-0.2 had the lowest weight loss on the 20th day of storage (7.67%), while the control group had the highest weight loss on the 15th day (35.76%). These findings are consistent with previous studies by Ansarifar and Moradinezhad (2022), who reported strawberries treated with Zein fiber film loaded with thyme EO demonstrated a significant decrease in weight loss compared to the control group. The weight loss change in the treated strawberries was likely a result of the application of the protective coatings.

3.2. Firmness

The firmness of fruit texture is a critical factor in assessing the quality and marketability of fruits, especially strawberries (Peralta-Ruiz et al., 2020). The results of this study showed that the treated fruits had significantly higher firmness compared to the control group (Fig. 2, a). Specifically, the MEO-SLN-0.2 treatment had the highest average fruit firmness on the 20th day of storage (0.562 N), while the control group had the lowest value on the 15th day (0.2 N). Our results were consistent with the findings of Wani et al. (2021), who observed that coated fruits experienced less firmness loss compared to the control group during a 12-day storage period, as a result of delayed maturation, reduced metabolic activities, and decreased fruit pulp degradation. The softening of the fruit may be attributed to ripening, the breakdown of cell wall components by microorganisms, or the activity of cell wall-degrading enzymes (Peralta-Ruiz et al., 2020).

3.3. Cellulase activity

The results of this study showed that the treated fruits had significantly lower cellulase activity compared to the control group (Fig. 2, b). Specifically, the MEO-SLN-0.2 treatment had the lowest average fruit cellulase activity on the 20th day of storage (1.98 U/min/g FW), while the MEO-0.2 had the highest value on the 15th day (2.78 U/min/g FW). The results of our study were consistent with those of previous studies by Wani et al. (2021) and Zhou et al. (2011) , who reported that cellulase activity decreased in strawberry and pear fruits treated with edible coatings compared to the control group. Other research has shown that fruits treated with essential oils (EOs) can reduce the activity of enzymes that break down cell walls during ripening, helping to maintain firmness during storage (Atrash et al., 2018). Our study further demonstrated that cellulase activity was higher in fruits exposed to a 0.2% concentration of MEO compared to the control. This could be due to the cytotoxic effects of a higher concentration of EO (Viacava et al., 2018).

3.4. Color

The red color of strawberries is due to the presence of anthocyanins, which is a qualitative feature effective in fruit marketing (Wani et al., 2021). Changes in the color parameters of strawberries coated with different materials are summarized in Table 1. There was a notable decrease in the L* value as storage time increased for all treatments, while a* and b* values showed an increase. The values of the color parameters such as L*, a*, b*, hue angle, and chroma in fruits treated with MEO-SLNs, particularly at concentrations higher than 0.05%, had minimal changes during storage. These results indicate that the MEO released from the SLN system helps preserve the color of the fruits. These results were consistent with the findings of Wani et al. (2021) and (Ansarifar and Moradinezhad, 2022), who asserted that postharvest treatments of strawberries extended the shelf-life of the fruit, leading to fruits with lighter skin color than control fruits.

Table 1
The effect of different concentrations of free MEO and MEO-SLNs on color parameters of strawberries inoculated with *R. stolonifer* during storage at 8 °C.

		%(wt/wt)	L*	a*	b*	Hue	Chroma
Control	First day	0	33.7 ± 0.33^{a}	11.08 ± 0.07^{k}	9.33 ± 0.12^{k}	14.09 ± 0.4^{a}	14.48 ± 0.07^{k}
	15 days	0	19.51 ± 1.1^{i}	35.74 ± 0.2^{a}	20.02 ± 0.03^{a}	29.25 ± 0.2^{fg}	40.97 ± 0.3^{a}
SLN MEO	without	0	22.21 ± 0.3^{h}	32.8 ± 0.6^{b}	19.41 ± 0.2^{b}	30.59 ± 0.7^{e}	38.14 ± 0.5^{b}
MEO alone	15 days	0.025	22 ± 0.43^{h}	31.74 ± 0.1^{c}	17.42 ± 0.5^{d}	28.75 ± 0.8^{gf}	36.21 ± 0.2^{c}
		0.05	23.76 ± 0.8^{g}	29.85 ± 0.8^{d}	16.96 ± 0.1^{de}	$29.6 \pm 0.6^{e.g}$	34.33 ± 0.7^{d}
		0.075	25.93 ± 0.9^{e}	28.61 ± 0.7^{e}	16.79 ± 0.3^{e}	30.41 ± 0.7^{e}	33.18 ± 0.6^{e}
		0.1	25.19 ± 0.3^{ef}	28.83 ± 1^{e}	16.83 ± 0.08^{e}	30.29 ± 0.7^{ef}	33.38 ± 0.9^{e}
		0.2	24.39 ± 2.1^{fg}	33.51 ± 0.7^{b}	18.45 ± 0.5^{c}	28.84 ± 0.5^{g}	38.26 ± 0.7^{b}
MEO-SLN	20 days	0.025	26.3 ± 0.8^{e}	24.4 ± 0.4^{f}	15.69 ± 0.5^{f}	32.73 ± 1.2^{d}	29.03 ± 0.2^{f}
		0.05	27.87 ± 0.5^{d}	22.43 ± 0.6^{g}	14.83 ± 0.1^{g}	33.48 ± 0.5^{cd}	26.89 ± 0.6^{g}
		0.075	29.11 ± 0.6^{c}	21.36 ± 0.5^{h}	14.2 ± 0.3^{h}	33.62 ± 0.4^{cd}	25.65 ± 0.6^{h}
		0.1	29.48 ± 0.3^{c}	19.11 ± 0.3^{i}	12.86 ± 0.3^{i}	33.93 ± 1^{bc}	24.04 ± 0.3^{i}
		0.2	31.11 ± 0.6^{b}	17.6 ± 1.2^{j}	12.22 ± 0.7^{j}	34.79 ± 0.5^{b}	21.43 ± 1.4^{j}

Data's in the table are reported as mean ± Standard Deviation (n = 5). Various letters between mean values within a column represent the statistical differences ($p < 0.01$), analyzed using LSD.

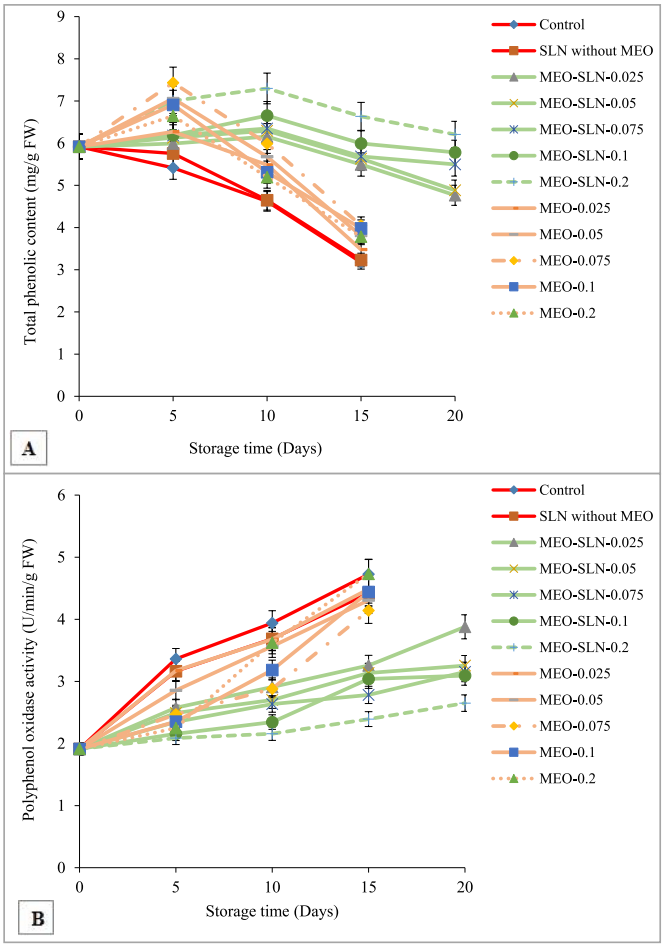

Fig. 3. The effect of different concentrations of free MEO and MEO-SLNs on A) Total phenolic content and B) Polyphenol oxidase activity of strawberries inoculated with *R. stolonifer* during storage at 8 °C.

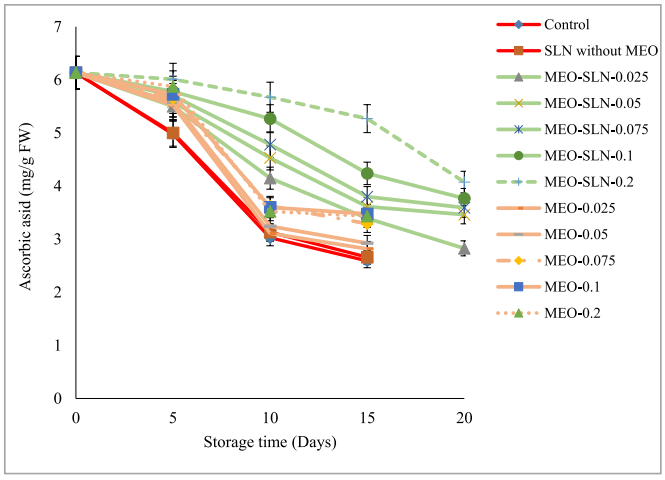

Fig. 4. The effect of different concentrations of free MEO and MEO-SLNs on the ascorbic asid content of strawberries inoculated with *R. stolonifer* during storage at 8 °C.

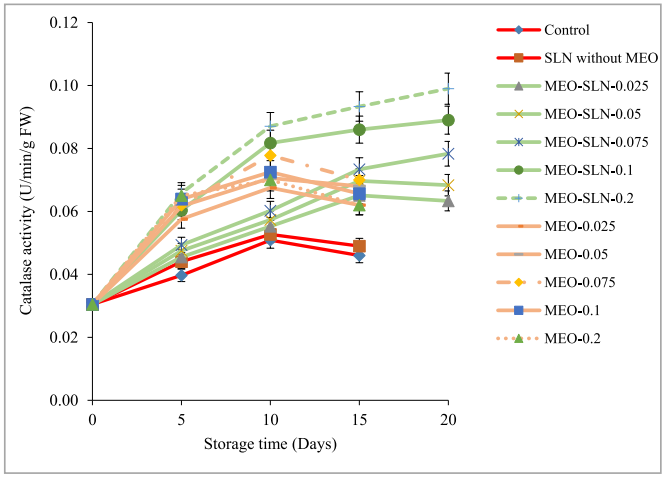

Fig. 5. The effect of different concentrations of free MEO and MEO-SLNs on the catalase activity of strawberries inoculated with *R. stolonifer* during storage at 8 °C.

3.5. Total phenol contents

The antioxidant properties of phenolic compounds in plants can protect cells under stressful conditions by eliminating ROS (Li et al., 2019). However, the results of a 20-day cold storage period revealed that the total phenolic content decreased heterogeneously in treated fruits. Specifically, the decrease was observed after the fifth day in fruits treated with free MEO and after the tenth day in fruits treated with

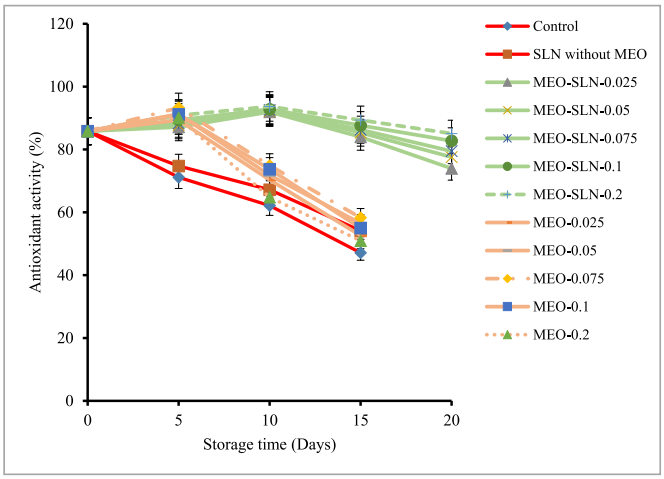

Fig. 6. The effect of different concentrations of free MEO and MEO-SLNs on the antioxidant activity of strawberries inoculated with *R. stolonifer* during storage at 8 °C.

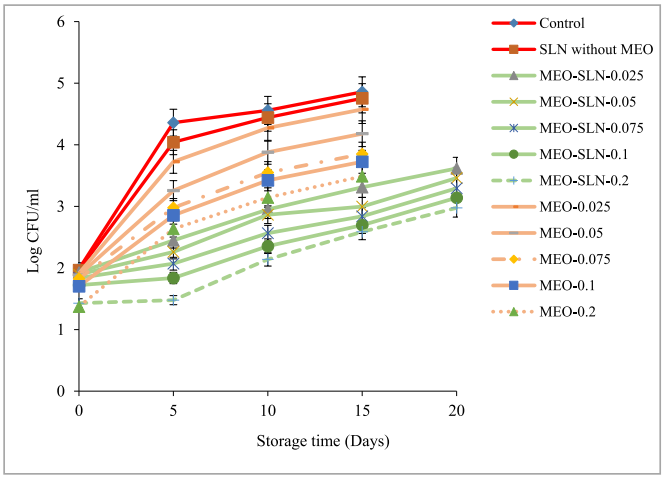

Fig. 7. The effect of different concentrations of free MEO and MEO-SLNs on microbial activity of strawberries inoculated with *R. stolonifer* during storage at 8 °C.

MEO-SLNs. In contrast, the decrease in fruits treated with SLN without MEO and the control group was gradual (Fig. 3, a). Interestingly, the highest total phenol content was observed on the 20th day in fruits treated with MEO-SLN-0.2 (6.2 mg/g FW), which was 1.9-fold higher than the control on the 15th day (3.17 mg/g FW). These results were consistent with the findings of Mohammadi et al. (2021), who reported that button mushrooms coated with a combination of Aloe vera gel and basil EO had an increased total phenolic content. These were likely attributed to the antioxidant properties of coating compounds such as EOs, which may create mild stress and stimulate the defense system of the plants, thus reducing the activity of polyphenol oxidase and preventing the degradation of phenols during storage (Atrash et al., 2018; Mohammadi et al., 2021).

3.6. Polyphenol oxidase activity

In this study, the results demonstrated that PPO activity generally increased over time (Fig. 3b). However, the PPO enzyme activity in the treated fruits (except for MEO-0.2) had a lower increase compared to the control. On the 20th day, the lowest level of PPO activity (2.64 units/min/gram FW) was observed for MEO-SLN-0.2, while on the 15th day, it registered the highest value of 4.7 units/min/gram FW for both MEO-0.2 and the control group. These results were consistent with the findings of Atrash et al. (2018), who reported that the PPO activity in lime fruits treated with savory EO was lower than that of the control group. Our results demonstrated that the treatment of MEO-SLNs in all concentrations and the treatment of MEO up to a concentration of 0.075% reduced the activity of PPO. It was likely that these edible coatings serve as a semi-permeable barrier for the exchange of gases, particularly oxygen, and safeguard the integrity of the cell membrane, thus reducing contact between phenols and PPO (Mohammadi et al., 2021).

3.7. Ascorbic acid

Ascorbic acid is a crucial nutrient for humans, serving as a water-soluble antioxidant that helps eliminate ROS and free radicals (Taheri et al., 2020). The findings of the study showed a significant decrease in ascorbic acid content over time in all samples ($P < 0.05$). However, this reduction was notably lower in fruits treated with MEO-SLNs compared to untreated control fruits (Fig. 4). The present findings are consistent with those of Jodhani and Nataraj (2019), who reported that edible coatings consisting of guar gum or a combination of guar gum and clove EO could help preserve the ascorbic acid content of strawberry fruits. The decrease in ascorbic acid levels may be attributed to the degradation of its molecules and the creation of dehydroascorbic acid through oxidation (Niazmand et al., 2021). These coatings served as semi-permeable barriers, regulating the flow of gases like oxygen and carbon dioxide, consequently decreasing oxidation processes (Wani et al., 2021).

3.8. Catalase activity

Catalase is a crucial enzyme responsible for maintaining the health of cells and tissues by converting hydrogen peroxide into water, thereby reducing the risk of cellular damage caused by ROS (Gebicka and Krych-Madej, 2019). The results indicated that the treated fruits exhibited higher catalase activity compared to the control fruits (Fig. 5). Specifically, for fruits treated with MEO, the highest catalase activity was observed on the 10th day of storage for MEO-0.075, followed by a decrease. However, for those treated with MEO-SLNs, there was a gradual increase in catalase activity as the concentration increased. Our results are consistent with the findings of Veloso et al. (2018); González-Locarno et al. (2020); and Perumal et al. (2021), who reported an increase in the activity of plant defense enzymes, such as superoxide dismutase, catalase, and peroxide dismutase, as well as the preservation of phenolic content in plants treated with EOs. The observed increase in catalase activity in fruit treated with nanocapsules can be attributed to the gradual release of EO from the nanocapsules (Piña-Barrera et al., 2019). In contrast, fruits treated with free MEO showed a decrease in catalase activity after the tenth day of storage, likely due to the evaporation of EO into the surrounding environment (Donsì et al., 2011).

3.9. Antioxidant activity

Antioxidant activity is a crucial factor in determining the quality of fruits and vegetables (Perumal et al., 2021), as it helps protect the human body from damage caused by ROS (Meitha et al., 2020). The results of the study showed that the antioxidant activity of strawberries decreased as the storage period increased for all packaging. Overall, the treated fruits had a higher DPPH radical scavenging capacity compared to the control (Fig. 6). This scavenging capacity for both MEO-treated and MEO-SLNS-treated fruits increased up to the fifth and fifteenth days, respectively. However, after that, their values started to decline. Therefore, both treatments were effective in scavenging DPPH free radicals, with MEO showing a short-term impact and MEO-SLNS having a longer-lasting impact. Our results are consistent with the findings of Wani et al. (2021), who reported that the use of edible coatings on

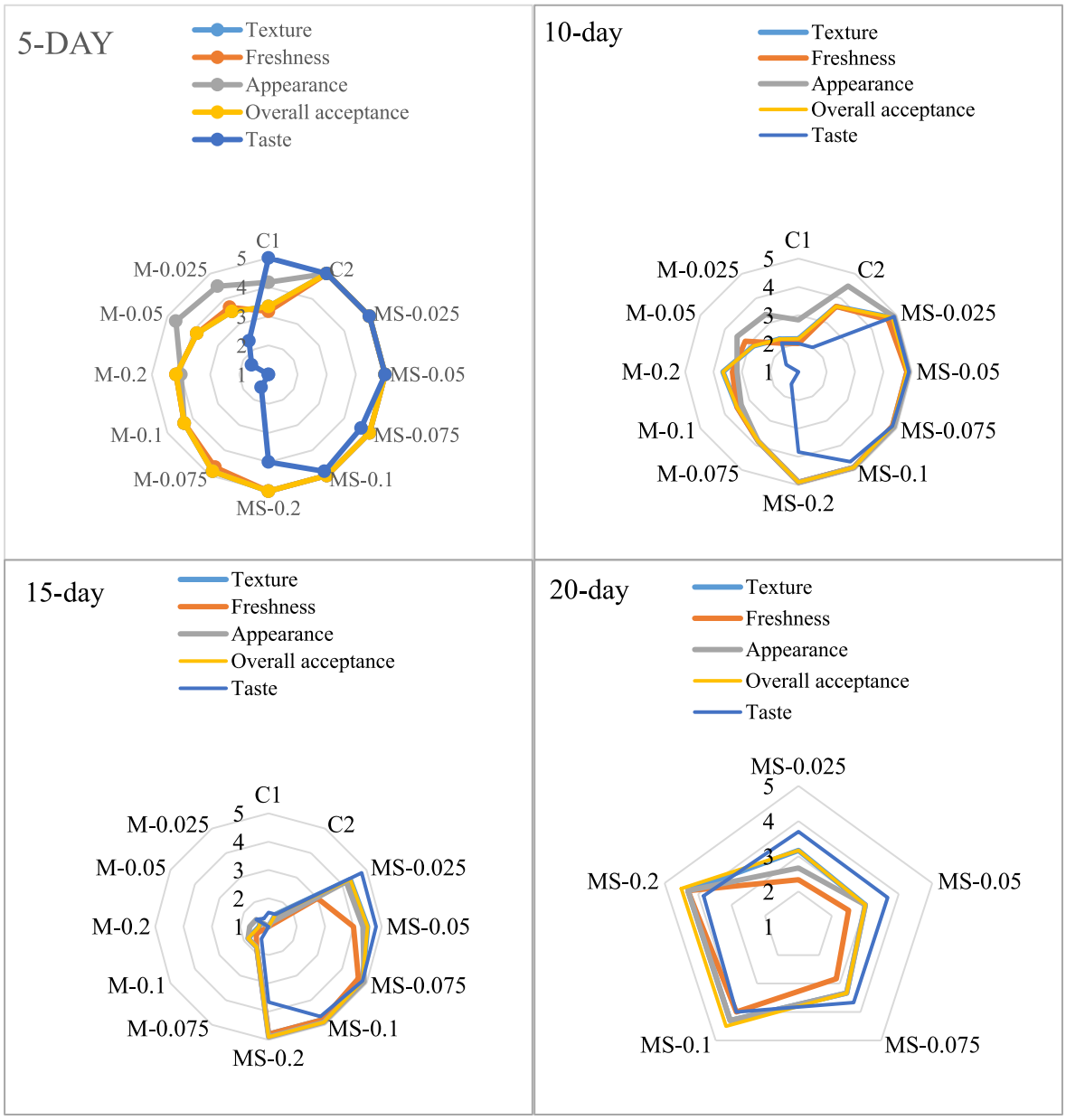

Fig. 8. The effect of different concentrations of free MEO (M) and MEO-SLNs (MS) on sensory attributes of strawberries inoculated with *R. stolonifer* during storage at 8 °C. C1 indicating Control and C2 indicating SLN without MEO.

strawberries resulted in higher antioxidant activity compared to control groups. Taheri et al. (2020) also reported that EOs have a powerful antioxidant action and reduce oxidative stress.

The DPPH radical scavenging capacity is dependent on the phenolic content concentration. A higher total phenolic content leads to greater antioxidant activity, which is influenced by the location and number of OH groups. The presence of OH groups results in hydrogen donation to free radicals, thereby enhancing the scavenging capacity (Niazmand et al., 2021). However, the DPPH radical scavenging capacity is not solely determined by the total phenolic content concentration. Other antioxidants, such as ascorbic acid, may also play a role (Hara et al., 2018). Therefore, the breakdown of these compounds into smaller molecules can decrease the DPPH radical scavenging capacity and antioxidant activity.

This difference in antioxidant activity was likely because the edible coatings acted as a barrier, forming a protective covering around the surface of the fruit and thus inhibiting oxidation by reducing oxygen penetration necessary for enzymatic oxidation of phenols (Mohammadi et al., 2021; Perumal et al., 2021). The findings of Piña-Barrera et al. (2019) showed that the stable release of EO from a nanocapsule system onto the surface of fruit could significantly increase its antioxidant activity.

3.10. antimicrobial assessment

Ripe strawberries are highly susceptible to fungal diseases and spoil quickly (Petrasch et al., 2019; Trinetta et al., 2020). The results showed that the fungal colony count ranged from 1.3 to 4.8 log cfu ml^{-1} (Fig. 7). The microbial load in fruits coated with MEO-SLNs was generally lower than in fruits coated with MEO. Additionally, it is important to note that fruits treated with MEO-SLNs did not exhibit any visible signs of spoilage (Fig. 9). On the 15th day, the fruits coated with SLN without MEO and the control group had the highest mean fungal colony counts (4.7 and 4.8 log cfu ml-1, respectively), while the fruits coated with MEO-SLN-0.2 on the 20th day had the lowest count (2.9 log cfu ml-1). The reduction in the number of logarithmic cycles of fungal microorganisms in treated

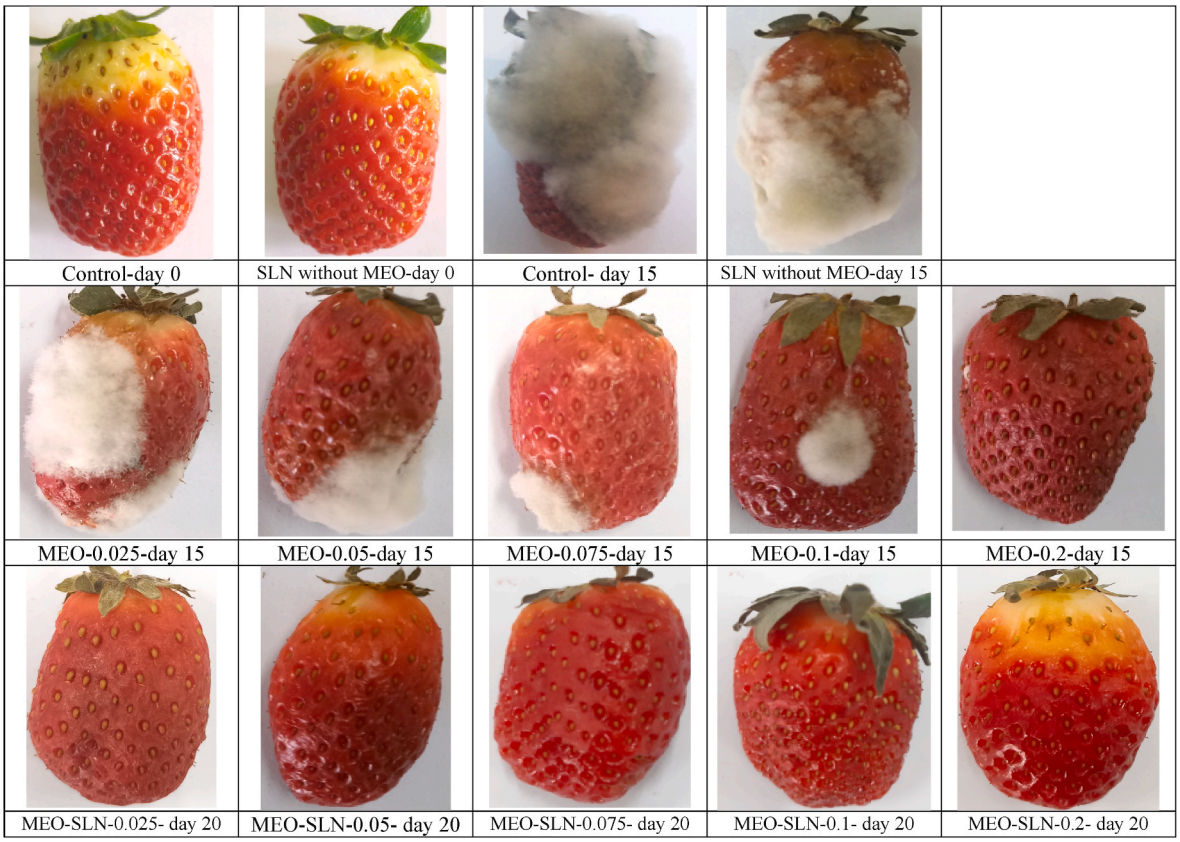

Fig. 9. The effect of different concentrations of free MEO and MEO-SLNs on microbial activity and shelf life of strawberries inoculated with *R. stolonifer*.

fruits compared with the control group could be attributed to the fungistatic effect of MEO (Vakili-Ghartavol et al., 2022) and the gradual release of EOs from the nanocapsule system (Mohammadi et al., 2021). This leads to a reduced microbial load on fruits treated with MEO-SLNs due to prolonged contact with the EOs (Peralta-Ruiz et al., 2020).

3.11. Sensory evaluation

The sensory characteristics, including appearance, texture, freshness, taste, and overall acceptance, of the treated fruits were examined to assess consumer satisfaction (Wen et al., 2017). The results indicated a significant decrease in the scores of the sensory attributes, from the first day to the end of the experiment, regardless of the treatments (Fig. 8). However, the most significant decrease in scores was observed in untreated control fruits, SLN without MEO, and fruits treated with free MEO, in comparison to fruits treated with SLN containing MEO. These fruits were removed from the experiment on the 15th day due to decay. After 20 days, the fruits treated with MEO-SLN at concentrations of 0.075, 0.1, and 0.2% showed a significant preservation of sensory properties. According to Ansarifar and Moradinezhad (2022) and Taheri et al. (2020), the incorporation of EOs and nanocapsules as active packaging had the potential to enhance the sensory qualities of strawberries. This was achieved through the effective delivery of MEO, suppression of microbial activity, increased bioavailability, the lipidic nature of EO and SLN formulations, a relatively uniform coating on fruits, and the odor reduction of EO in the nanocapsule system (Granata et al., 2018; Laein et al., 2022).

3.12. Shelflife determine

The shelf life of fruits can be determined by observing signs of rotting, shriveling, or softening. On the first day, the strawberries had a fresh appearance, firm texture, and pleasing taste. They were also colored on approximately 75% of their surface (Fig. 9). However, as time passed, the fruits showed varying degrees of shriveling and softening, regardless of the treatments. Unfortunately, by the 15th day, the fruits treated with free MEO, SLN without MEO, and the control group had spoiled and had to be discarded. This spoilage and softening may have been caused by the gradual disappearance of MEO due to evaporation from the surface of the fruit into the surrounding air (Perumal et al., 2022). Additionally, tissue damage at higher concentrations of MEO (0.1 and 0.2%) may have contributed to an increase in the activity of cell wall degrading enzymes, gas exchange, moisture loss, and the growth of microorganisms such as fungi, ultimately leading to the rotting of the fruits (Peralta-Ruiz et al., 2020; Álvarez-Hernández et al., 2021). On the contrary, fruits treated with MEO-SLNs at concentrations of 0.025 and 0.05% showed signs of shriveling and softening, while concentrations of 0.075, 0.1, and 0.2% were found to be suitable for distribution. These fruits showed no signs of microbial damage until the 20th day of storage. The delay in fruit ripening with MEO-SLN coatings, especially at concentrations of 0.075, 0.1, and 0.2%, can be attributed to several factors. Firstly, the sustained and gradual release of MEO from the nanocapsule system (Piña-Barrera et al., 2019) played a significant role. Additionally, the antimicrobial properties of the MEO's components, specifically menthol and menthone compounds (Vakili-Ghartavol et al., 2022), and the formation of a semi-permeable barrier by the lipidic nature of MEO and Percyrol present in SLN (Peralta-Ruiz et al., 2020; Perumal et al., 2021) helped prevent water loss and spoilage.

4. Conclusions

This study aimed to investigate the effects of different levels of MEO-SLN coatings, compared to free MEO, on the microbial load, storage quality, and shelf life of strawberries (*Fragaria* × *ananassa* cv. Camarosa) for 20 days at 8 °C. The results of this study showed that the quality of strawberries decreased during storage, such as the firmness of fruit

texture, total phenolic content, ascorbic acid, DPPH radical scavenging capacity, and sensory attributes as the storage period prolonged. Additionally, there was an increase in weight loss, cellulase activity, polyphenol oxidase activity, and microbial load during storage. However, strawberries treated with MEO-SLNs had better quality characteristics than those treated with MEO alone and the control group. Based on our results, we can conclude that the use of MEO alone did not have a significant impact on the quality of strawberries during the storage period. In other words, the bioactive compounds in the fruits treated with free MEO degraded at a higher rate, particularly at concentrations of 0.1 and 0.2%, resulting in tissue damage and loss of cell membrane integrity, as shown in Fig. 9. Furthermore, these fruits exhibited signs of microbial damage until day 15. However, encapsulating MEO in SLNs can greatly improve the preservation of strawberry quality, particularly at concentrations of 0.075, 0.1, and 0.2%. The fruits coated with MEO-SLN did not show any signs of microbial damage. The use of MEO-SLN coatings as fruit preservatives demonstrated stronger bioactive effects and acted as antioxidants, successfully preserving the nutritional content of strawberries. In summary, these edible coatings have the ability to inhibit microbial growth, enhance storage quality, and prolong the shelf life of perishable fruits, making them a safe and efficient alternative to harmful chemical additives for food preservation.

CRediT authorship contribution statement

Masoumeh Vakili-Ghartavol: Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Hossein Arouiee:** Writing – review & editing, Visualization, Supervision, Funding acquisition. **Shiva Golmohammadzadeh:** Writing – review & editing, Visualization, Supervision. **Mahboobeh Naseri:** Writing – review & editing, Visualization, Supervision. **Leila Bandian:** Visualization, Investigation.

Declaration of competing interest

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.

Data availability

The data that has been used is confidential.

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