



Determination of the Chemical Content and Antibacterial Activity of *Mentha spicata* Leaves and Evaluation of its Use in the Preservation of Some Foods

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ABSTRACT

Background: People have become interested in consuming high-quality, safe, natural foods with the increased spread of green consumerism worldwide.

Methods: Prepare a coating solution from leaves of spearmint *Mentha spicata* with glycerol, estimate the antimicrobial activity and application of this coated on apricots and study the effect on weight loss, soluble solids content, antioxidant activity and sensory evaluation.

Result: The qualitative detection of the bioactive compounds of the spearmint *M. spicata* leaves showed its continent included tannins, alkaloids, flavonoids, coumarins, terpenes, and saponins. The used concentrations of 12.5 and 25 mg mL⁻¹ from spearmint coating solution (SCS) showed a non-significant effect at ($P \leq 0.05$). In comparison, the 50 and 100 mg mL⁻¹ showed a significant effect at ($P \leq 0.05$) as antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus*. The weight loss of coated apricots after storage for 30 d at 4°C was 20.06, 18.52, 14.84 and 13.42%. Also, the soluble solids content was 17.3, 16.7, 14.1 and 13.9%. The antioxidant activity (Inhibition %) was increased to 30, 34, 39 and 47% using SCS of 12.5, 25, 50 and 100 mg mL⁻¹ as a coating material. The sensory evaluation found significant differences at ($P \leq 0.05$) between treatments for all characteristics. The coating kept the consumer's acceptance of consuming these fruits through characteristics of appearance, aroma and taste after storage for 30 d at 4°C.

Key words: Antibacterial activity, Antioxidant activity, Coating solution, Soluble solids content, Spearmint, Weight loss.

INTRODUCTION

Plants are a valuable source of biologically active substances that can be utilized in various fields such as agriculture, medicine, food and industry. These substances are harmless, non-toxic and have no side effects, making them safe and easily accessible. Researchers have explored the use of these substances in the food industry as antimicrobials and antioxidants to increase the shelf life of food (Alsoufi and Aziz, 2023; Karmakar and Roy, 2023; Bensehaila *et al.*, 2022). Spearmint (*Mentha* sp.) is a commonly used plant in the flavoring, pharmaceutical and cosmetic industries. Studies have shown that *Mentha* sp. has antimicrobial, antioxidant and other therapeutic properties due to having large numbers of bioactive compounds such as tannins, alkaloids, flavonoids, coumarins, terpenes and saponins (Pliego *et al.*, 2022). With the increase in "green consumerism," more people are interested in safe and natural foods. This has resulted in a higher demand for fresh and safe foods with high levels of freshness and quality (Ul Hasan *et al.*, 2021). Food producers use coating materials for coated fruits and vegetables to meet this demand and improve safety and quality assurance during marketing (Hazarika *et al.*, 2023; Tahir *et al.*, 2019).

Natural polymers like polysaccharides as a lone or by add other biological compound are commonly used in this industry due to their biocompatibility, biodegradability and

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ability to comply with biochemical modifications, making them easy to use in packaging to control changes in fruits and vegetables after harvesting, such as spoilage, weight loss and increased shelf life (Zare *et al.*, 2019; Ojha *et al.*, 2015) as in coated papaya with carnauba wax and *M. spicata* essential oil (Oliveira Filho *et al.*, 2023), bananas with corn starch and mint extract (Chithra *et al.*, 2022), apricots with chitosan and pomegranate (*Punica granatum*) extract (Gull *et al.*, 2021), pear with rosemary (*Salvia*

rosmarinus) extract and pullulan (Alsoufi and Aziz, 2021), apricots with *Fircus hirta* extract and basil seed mucilage (Nourozi and Sayyari, 2020), mango with banana starch (Hernández-Guerrero *et al.*, 2020), guava with chitosan (Nair *et al.*, 2018), guava with mint oil (Kabbashi *et al.*, 2017), mandarin with alginate and aloe vera gel incorporated with pomegranate peel (Chen *et al.*, 2016), peach with pullulan Al-Soufi (2015). Therefore, this study aims to increase the shelf life of apricots by using spearmint (*Mentha spicata* L.).

MATERIALS AND METHODS

Place of work

This study was conducted in the Laboratories of the Department of Product Evaluation and Service Performance at the Market Research and Consumer Protection Center, University of Baghdad, Baghdad, Iraq and the Department of Science, College of Basic Education at Mustansiriyah University, Baghdad, Iraq.

Research period

The study was conducted from January 10, 2023, to December 10, 2023.

Spearmint and apricots

Fresh of all spearmint (*M. spicata*) leaves and apricots (*Prunus armeniaca* L.) fruit was obtained from the markets in Baghdad, washed with tap water to remove surface dirt, and dipped in the sodium hypochlorite solution 0.1% for 3 min to reduce probable microbial contamination. Then, they were rinsed with sterile deionized distilled water to remove traces of sodium hypochlorite solution and kept at 25°C until completely dried, then used in the coating experiment (Ul Hasan *et al.*, 2021).

Microorganism's strains

Many pure microorganism strains were obtained from the laboratories of the College of Science, Mustansiriyah University, Iraq, to conduct the necessary tests. They included *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus*. The morphological, cultural and biochemical characteristics were confirmed according to (Mac Faddin, 2000).

Extraction and detection of spearmint phytochemicals

The hot aqueous extract of spearmint was prepared by mixing 50 g of leaves with 100 mL of sterile deionized distilled water and put in a water bath at 100°C for 1 h, then cooling at room temperature and filtered (Whatman No.1). The filtrate extract was dried by freeze-dried and kept at 4°C until use, then detection phytochemicals: glycosides, alkaloids, terpenes, saponins, tannins, coumarins and flavonoids (Ullah *et al.*, 2011).

Preparation of coating solution

Spearmint coating solution (SCS) was prepared by dissolving dried spearmint extract (12.5, 25, 50 and

100 mg mL⁻¹) in sterile deionized distilled water, then adding 1% of glycerol (v v⁻¹) for the solution as a gelling agent to improve the viscosity and plasticity. In contrast, glycerol solution (GS) was prepared by mixing 1 mL of glycerol in 100 mL of sterile deionized distilled water. The solutions were sterilized through a pre-rinsed 0.22-μm filter and stored aliquots at 4°C until use (Ul Hasan *et al.*, 2021).

Estimation of antimicrobial activity of SCS

The Antimicrobial activity (inhibition %) was calculated according to the method of Alsoufi and Aziz (2021) by transferring 1 mL (10⁵ CFU mL⁻¹) of the microorganism strain to a Petri dish with nutrient agar, then distributing it by spreading and coating it with 1 mL of pre-sterilized SCS (12.5, 25, 50 and 100 mg mL⁻¹). Then, it was placed for 60 min in the laminar chamber under continuous sterile conditions till completely dry, then incubated at 37°C for 24 h; the control was sterile deionized distilled water. The growth of the microorganism strains was calculated at a rate of three replicates for each sample and the inhibition percentage was determined from the following equation:

Inhibition (%) =

$$\frac{\text{Number of colonies for sample}}{\text{Number of colonies for control}} \times 100$$

Estimation of weight loss

The apricots were coated by dipping them in the sterile deionized distilled water (control), GS (1%) and SCS (12.5, 25, 50 and 100 mg mL⁻¹) for 5 min, drying at room temperature and weighted (Gull *et al.*, 2021). The weight loss percentage while stored at 4°C for 30 d was calculated through the following equation as a method by Al-Soufi (2015):

Weight loss (%) =

$$\frac{\text{Weight after a storage period}}{\text{Initial weight}} \times 100$$

Estimation of total soluble solids content and antioxidant activity

The soluble solids content percentage and antioxidant activity (Inhibition %) during storage at 4°C for 30 d for apricots coated with sterile deionized distilled water (control), GS (1%) and SCS (12.5, 25, 50 and 100 mg mL⁻¹) was estimated by blending 10 g of fruit till homogenized. Then, it was filtered by muslin cloth to remove the fiber and get juice extract. The soluble solids content (%) was estimated in the juice using a KRUS HR900 manual hand-held refractometer (0-90% Brix/0.2% Brix) (Gull *et al.*, 2021), while the antioxidant activity (Inhibition %) was estimated according to radical scavenging method (Wani *et al.*, 2018) using 2,2-Diphenyl-1-picryl-hydrazil (DPPH) through mixing 0.1 mL of juice with 3.9 mL DPPH and stirring for 30 min to complete reaction. Then, it was filtered through filter paper (Whatman No.1) and absorbency was estimated at 517 nm using a UV-visible spectrophotometer.

Methanol was taken as blank for baseline correction. The antioxidant activity was calculated and expressed as inhibition (%) through the following equation:

Antioxidant activity (Inhibition%) =

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Sensory evaluation

Apricots were sensory evaluated after 30 d of storage at 4°C based on appearance (Visual characteristics of acceptability), aroma (touch and smell) and taste (sweet, sour, salt, bitter and umami) according to the method of Deng *et al.* (2005) through trained of 5 panelists (3 men and 2 women, aged 25-50 years) for this purpose. The same panelist evaluated all samples randomly to have less variability in white light individual rooms. Each panelist was guided to cleanse his/her mouth with distilled water, chew the apricot sample, score answers, and then cleanse again before evaluating the following sample. The panelist's answers scored on a 9-point scale [1: extremely poor; 3: poor; 5: acceptable (limit of marketability); 7: good; 9: excellent].

RESULTS AND DISCUSSION

Qualitative detection of spearmint phytochemicals

The results in (Table 1) show the qualitative detection of the bioactive compounds of the spearmint phytochemicals, which included tannins, alkaloids, flavonoids, coumarins, terpenes, saponins and glycosides.

Mentha sp. has many pharmacological activities; it has exhibited its potentiality as antimicrobial, antioxidant, anti-inflammatory, anticancer, analgesic, anti-intestinal parasites, antispasmodic, anti-bloating, anti-obesity, anti-diabetic and anti-emetic (Eftekhar *et al.*, 2021). These active proprieties have many bioactive constituents such as phenolic compounds, triterpenoids and steroids, flavonoids, carotenoids, α -tocopherols, ascorbic acid, monoterpenes, tannins, saponins and glycosides (Gull *et al.*, 2021).

Effect of SCS antimicrobial activity

The inhibition growth of *E. coli*, *P. aeruginosa*, *B. cereus*, and *S. aureus* was 66, 66, 68 and 67%, respectively, at a rate of 66.75% for 12.5 mg mL⁻¹ of SCS. It was 70, 69, 72 and 71%, respectively, at a rate of 70.50% for a 25 mg mL⁻¹ of SCS. It was 74, 72, 76 and 77 mg mL⁻¹, respectively, at a rate of 74.75% for 50 mg mL⁻¹ of SCS. It was 75, 73, 77 and 78 mg mL⁻¹, respectively, at a rate of 75.75% for 100 mg mL⁻¹ of SCS (Table 2).

The results show that the use concentration of 12.5 and 25 mg mL⁻¹ from SSC did not show a significant effect at ($P \leq 0.05$), while the concentrations of 50 and 100 mg mL⁻¹ showed a significant effect at ($P \leq 0.05$) as antimicrobial activity against microorganisms strain.

Table 1: Qualitative detection of the bioactive compounds in the spearmint (*Mentha spicata* L.).

Active compound	Indication
Tannins	+
Alkaloids	+
Flavonoids	+
Coumarins	+
Terpenes	+
Saponins	+
Glycosides	-

(+): Contain active compound.(-): Free of an active compound.

It is clear from the results that the G⁺ bacteria (*S. aureus* and *B. cereus*) were more sensitive to the SCS than the G⁻ bacteria (*P. aeruginosa* and *E. coli*). This is consistent with the scientific literature in this field, which states that most medicinal plants are more effective against G⁺ bacteria than G⁻ bacteria. The reason is the structural composition of the cell wall, as G⁺ bacteria lack a layer of outer membranes, which makes the permeability of substances entering the cell greater compared to G⁻ bacteria. Its inner wall is an internal barrier represented by lipopolysaccharides combined with multiple proteins, which can prevent the passage of many harmful substances into the cell (Eftekhar *et al.*, 2021).

The effect of the antimicrobial activity was mainly associated with bioactive compounds. Therefore, many studies have been on the antimicrobial activities of *M. spicata* extracts against a broad type of microorganisms such as *E. coli*, *Salmonella typhimurium*, *Proteus mirabilis*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Vibrio* spp., *Bacillus subtilis*, *S. aureus*, *Listeria monocytogenes*, *Mucor mucedo*, and *Aspergillus niger* (Eftekhar *et al.*, 2021).

Weight loss

The results refer to significant differences at ($P \leq 0.05$) in the weight loss of all coated apricots treatments during storage for 30 d at 4°C (Fig 1). It was 23.19 and 21.82, 20.06, 18.52, 14.84 and 13.42% for sterile deionized distilled water, GS (1%), SCS 12.5, 25, 50 and 100 mg mL⁻¹, respectively.

The results show that the increase in concentration (mg mL⁻¹) of SCS for apricots led to a lower weight loss rate with significant differences at ($P \leq 0.05$) for all treatments. The lower weight loss in coated apricots by spearmint coating solution (SCS) indicates that this coating has contributed to improving barrier properties, therefore, reducing weight loss (Wang and Rhim, 2016). In this regard, Oliveira Filho *et al.* (2023) showed that the weight loss of coated papaya with carnauba wax with β -cyclodextrin and *M. spicata* essential oil was 7.5% compared to the control, which was 24.85% during storage for 15 d at 16°C. Also, Alsoufi and Aziz (2021) noticed that the weight loss of pear coated with rosemary and pullulan (1:1) was 0.21 and 6.7% at 10 and 21 d of storage at 4 and 25°C,

respectively. Kabbashi *et al.* (2017) observed that the weight loss of guava coated with mint oil was 9.5% after 9 d bench storage.

Fruit's freshness and shelf life are affected by moisture loss as it migrates through the fruit skin (epidermis) to the surrounding environment. Weight loss of fruits during storage was considered the main problem during the marketing process due to undesirable appearance, aroma, and taste changes that will lead to economic losses for workers in this field. So, coating is beneficial in ensuring food safety and quality and extending shelf life because it prevents spoilage and weight loss during storage (Alsoufi and Aziz, 2021).

Soluble solids content

The results (Fig 2) show that there was not a significant effect at ($P \leq 0.05$) for all treatments during storage for 15 d at 4°C, while 20, 25 and 30 d at 4°C showed a significant effect at ($P \leq 0.05$). The soluble solids content for apricots coated by control, GS and SCS at 12.5, 25, 50 and 100 mg mL⁻¹ were increased from 12.3% at zero time of storage to 20.4, 18.2, 17.3, 16.7, 14.1 and 13.9%, respectively, at 30 d of storage at 4°C.

The increased SCS concentration of apricots leads to a lower rate of soluble solids content, especially during 20, 25 and 30 d of storage at 4°C. These results were consistent with Gull *et al.* (2021), who observed that TSS content for coated apricots with chitosan and pomegranate peel extract was 25, 23 and 21% for control, chitosan, and chitosan+1.0% pomegranate peel extract, respectively after storage at 4°C for 30 d. Similar results were reported by Nourozi and Sayyari (2020) in apricots by enrichment of basil seed mucilage and *Fagonia cretica* extract to aloe vera gel. Ozturk *et al.* (2019) observed that the use of aloe vera and modified atmosphere packaging for coating cherry laurel (*Prunus lauracerasus*) leads to a lower soluble solids content level after 15 and 30 d of storage at 0°C compared to the control sample.

The increased soluble solids content is also attributed to reduced fruit water content, resulting in a higher concentration of soluble solids (Oliveira Filho *et al.*, 2023). Therefore, coating leads to less weight loss of fruit due to the reduction in respiration rate, contributes to delaying the ripening process in fruits and lowers the rate of increment in soluble solids content and ability to preserve firmness up to the end of storage. Therefore, measuring

Table 2: Effect of spearmint (*Mentha spicata* L.) coating solution (SCS) on growth inhibition of some microorganisms.

Microorganisms	Inhibition (%)			
	Spearmint coating solution (SCS) (mg mL ⁻¹)			
	12.5	25	50	100
<i>Escherichia coli</i>	66	70	74	75
<i>Pseudomonas aeruginosa</i>	66	69	72	73
<i>Bacillus cereus</i>	68	72	76	77
<i>Staphylococcus aureus</i>	67	71	77	78
Average (%)	66.75	70.50	74.75	75.75
LSD value	3.08 NS	4.02 NS	4.86*	4.79*

NS: Non Significant ($P \leq 0.05$); *Significant ($P \leq 0.05$).

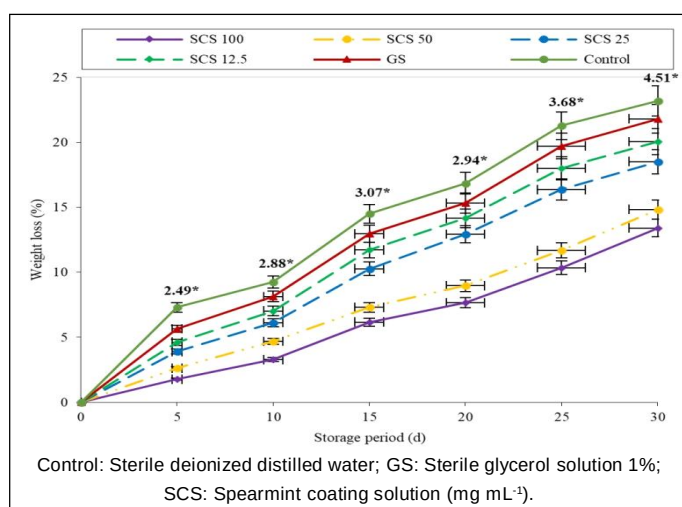


Fig 1: Weight loss (%) and LSD value [significant (*) at ($P \leq 0.05$)] of apricots coated with spearmint (*Mentha spicata* L.) coating solution (SCS) during storage for 30 d at 4°C.

total soluble solid content is essential for fruit ripening during storage (Gull *et al.*, 2021).

Antioxidant activity (Inhibition %)

The results refer to significant differences at ($P \leq 0.05$) in the antioxidant activity (inhibition %) of coated apricots with sterile deionized distilled water and GS (1%) was 13 and 19%, respectively. At the same time, the increase was continued for coated fruits by SCS at 12.5, 25, 50 and 100 mg mL^{-1} to be 30, 34, 39 and 47%, respectively, during 30 d of storage at 4°C (Fig 3).

The results show that the increased SCS concentration (mg mL^{-1}) of apricots leads to a higher rate of antioxidant activity (inhibition %) with significant differences at ($P \leq 0.05$) for all treatments. In this context, many researchers are referring to the contribution of

coatings to maintaining the antioxidant activity of fruits during storage, such as coating apricots with *F. hirta* extract and basil seed mucilage (Nourozi and Sayyari, 2020), guava with chitosan (Nair *et al.*, 2018) and mandarin with alginate and aloe vera gel incorporated with pomegranate peel (Chen *et al.*, 2016).

In general, the high significant value of the antioxidant activity of coated fruits was attributed to the effect of *Mentha* sp. extract, have shown antioxidant activities from effective quenchers of superoxide radicals due to its containing bioactive compounds such as phenolic acids, flavones, ascorbic acid, carotenoids and terpenes (Eftekhari *et al.*, 2021), or could be due to coating barrier properties, which modified internal atmosphere thus inhibiting oxidative destruction of antioxidant compounds (Gull *et al.*, 2021).

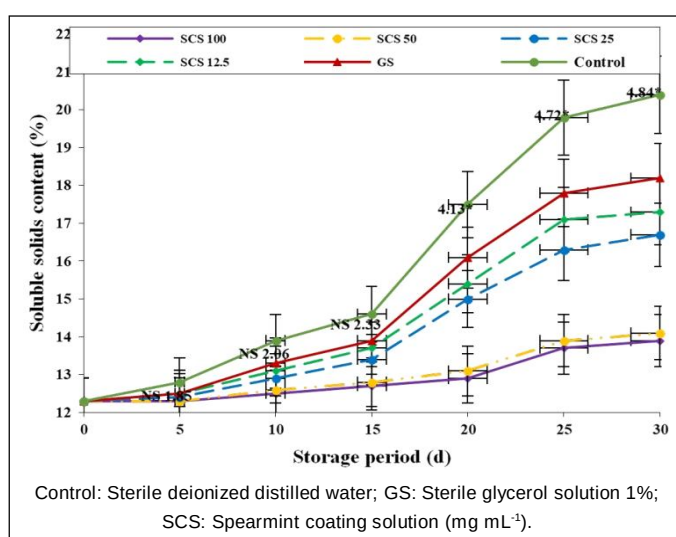


Fig 2: Soluble solids content (%) and LSD value [non-significant (NS) and significant (*) at ($P \leq 0.05$)] of apricots coated with spearmint (*Mentha spicata* L.) coating solution (SCS) during storage for 30 d at 4°C.

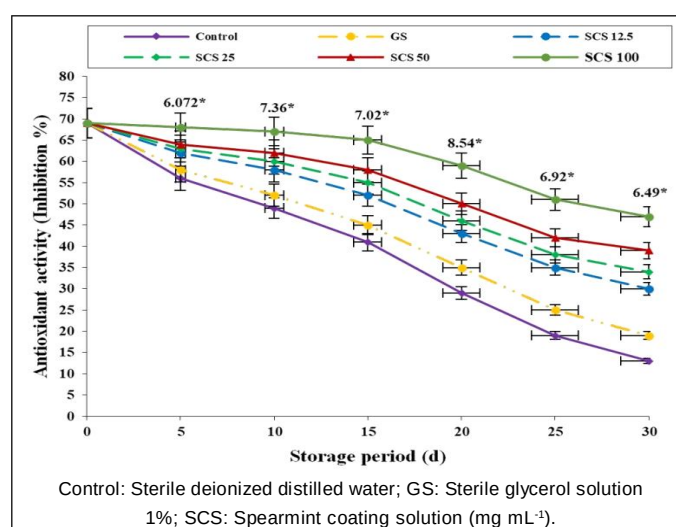


Fig 3: Antioxidant activity (Inhibition %) and LSD value [significant (*) differences at ($P \leq 0.05$)] of apricots coated with spearmint (*Mentha spicata* L.) coating solution (SCS) during storage for 30 d at 4°C.

Table 3: Sensory evaluation of apricots coated with spearmint (*Mentha spicata* L.) coating solution (SCS) after storage for 30 d at 4°C.

Treatment	Panelists answers scored		
	Appearance	Aroma	Taste
Control	4.3	6.1	5.3
GS	7.1	7.4	6.8
SCS 12.5	7.2	7.5	6.9
SCS 25	7.3	7.6	7
SCS 50	7.7	8.1	7.5
SCS 100	7.8	8.2	7.6
LSD value	1.962 *	1.527 *	1.488 *

Control: Sterile deionized distilled water. GS: Sterile glycerol solution 1%. SCS: Spearmint coating solution (mg mL⁻¹). * (P≤0.05).

Sensory evaluation

The results for sensory evaluation refer to significant differences at (P≤0.05) between treatments for all characteristics, where the point record by panelists for coated and un-coated apricots at zero time was 9, 9 and 8.6 for appearance, aroma, and taste characteristics, respectively. The coating of apricots with SCS ensured the consumer's acceptance of this fruit after storage for 30 d at 4°C. The panelist's appearance, aroma, and taste points were 7.8, 8.2 and 7.6 points for SCS 100 mg mL⁻¹ (Table 3). Sensory evaluation is considered an essential parameter for knowing the success of coating treatment through panelists' (consumers') answers for the general acceptability of fruits after the storage period. In this regard, Chithra *et al.* (2022) found a significant reduction in a physiological loss in weight and an extension of shelf life for bananas coated with corn starch and mint extract that could maintain their marketable acceptability for consumers up to 15 d of storage. Also, Gull *et al.* (2021) observed that the use of chitosan +1.0% pomegranate peel extract for coating apricots contributed to improvement in odor and overall acceptability score during storage at 4°C for 30 d., Kabbashi *et al.* (2017) noticed that the sensory quality for appearance, taste, and flavor of coated guava by mint oil was 84, 79 and 88%, respectively, after 9 d bench storage. Similarly, Gniewosz *et al.* (2014) showed that coating apples with pullulan films with incorporated meadowsweet flower extract demonstrated positive properties for quality during storage and contributed to decreased color changes and weight losses.

Using plant extracts with natural polymers to coat fruits to extend their shelf life is a promising technology that may improve the quality demanded by the consumer, thus increasing the turnover of business and sales (Alsoufi and Aziz, 2021). The preservative action of mint extract is due to the effect of phenolic compounds and antibacterial and antioxidant activities (Eftekhar *et al.*, 2021).

CONCLUSION

The study shows the possibility of using mint leaf extract (*Mentha spicata*) with glycerin in apricot coating to prolong shelf life, due to the high antimicrobial and antioxidant properties, reducing weight loss and maintaining soluble

solids content, appearance, aroma, and taste of this fruit during storage.

Conflict of Interest

The authors declare that there is no conflict of interest regarding this manuscript.

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