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ORIGINAL PAPER

Utilization of chitosan-based coating enriched with *Syzygium aromaticum*, *Cinnamomum zeylanicum*, and *Thymus satureioides* essential oils mixture for strawberries with extended shelf life

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Abstract

The short postharvest life of strawberries is a major problem for both manufacturers and consumers. In the present work, chitosan (CS) and chitosan enriched with *S. aromaticum*, *C. zeylanicum*, and *T. satureioides* essential oils mixture (CS-EOs) were utilized as a coating for strawberries to extend their postharvest life. According to the obtained results, the coated fruits showed an acceptable total sensory quality (TSQ ≥ 5), were brighter and redder, and had maintained titratable acidity (TA), total soluble solids (TSS), and pH values during the 13 days of storage. Furthermore, coatings mitigated the decrease of weight and anthocyanin content by 2-folds on the 13th day and extended considerably the postharvest life of strawberries, especially in the case of the CS-EOs coating. Concerning the total phenolic level, the strawberries coated with CS-EOs exhibited greater values by 1.5-fold during storage time. Overall, these encouraging results suggest that the developed CS and CS-EOs coating could be applied in the food industry to extend the postharvest life and maintain the quality of the susceptible fruits.

Keywords Chitosan-based coating · Essential oils mixture · Strawberry

Introduction

The strawberry is considered one of the most consumed and popular fruits worldwide owing to its sensorial and nutritive qualities [3, 10, 16]. Nevertheless, this fruit is susceptible to microbial infections, water loss, and physiological and mechanical damage [20]. In addition, active and continuous physiological activities and metabolism reduce the ripening and senescence periods [4, 37]. Therefore, strawberry presents a short postharvest life.

As a result, multiple approaches such as controlled atmosphere storage, chemical treatments, radiation, and cold storage have been conducted to mitigate the deterioration and spoilage of postharvested strawberries [5, 7, 9, 15, 23, 51]. Nevertheless, several investigations have demonstrated the negative impact of these strategies on the fruit's nutritional quality or human health [12, 26, 42].

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With the rising requirement for fresh, healthy, and chemical-free foods, novel technologies such as natural and biodegradable coatings and packaging have been suggested to enhance food conservation and maintain quality [13, 54]. Contrary to coated foods, packaged foods can interact with the oxygen present in the package's headspace leading to spoilage by oxidation. Additionally, it has been reported that coatings improve the fruit's appearance [55]. In this context, applying biopolymers-based coatings in the food industry especially for fruits becomes a trend.

Generally, starch, alginate, gelatin, and chitosan (CS) were the most applied biopolymers [22, 46, 53, 59]. The latter is a linear polymer obtained by chitin deacetylation. Besides its abundance, CS is known for its biocompatibility, biodegradability, and biological activities [11, 47]. Nonetheless, the authors reported the attenuation of CS antimicrobial action following its application as a film or coating [60]. For that reason, the enrichment of the CS matrix with antimicrobial agents is needed to compensate for the regression in activity.

Essential oils (EOs) have been greatly studied for their excellent biological activities [56]. It has been verified that EOs with phenols and terpene alcohols as major constituents e.g. clove, cinnamon, and thyme EOs exhibit the strongest antimicrobial and antioxidant actions [29]. In addition, the synergism between these EOs was previously verified [30, 33]. However, no prior work in the literature has assessed the effect of clove, cinnamon, and thyme EOs mixture on CS coating for strawberries with extended shelf life and maintained quality.

In this paper, the physical and chemical characteristics, and nutritive quality of uncoated and coated (with CS and CS-EOs) strawberries were analyzed during 13 days of storage at 4 °C to emphasize the potential utilization of CS and CS-EOs coatings in the food industry.

Materials and methods

Materials

Shrimp chitosan C3646 with a deacetylation degree higher than 75% and molecular weight of 400 kDa was obtained from Sigma-Aldrich.

Syzygium aromaticum, *Cinnamomum zeylanicum*, and *Thymus satureioides* EOs were acquired from Nectarome Society (Marrakech). The chemical composition, refractive index, optical rotation, and density of the tested EOs were mentioned in our previous study [28].

The two fungal species (*Aspergillus* sp. and *Rhizopus* sp.) were isolated from contaminated strawberries using a scalpel. The contaminated fragments of the fruits were placed in Potato Dextrose Agar medium (PDA) supplemented with 25 mg/L of kanamycin and Amoxicillin-clavulanic acid for 1 week at 25 °C. Then, a sample of the developed fungal lawn was sub-cultured in a freshly prepared PDA medium. Finally, the fungal strains were morphologically identified using optical microscopy at different magnifications (Fig. 1).

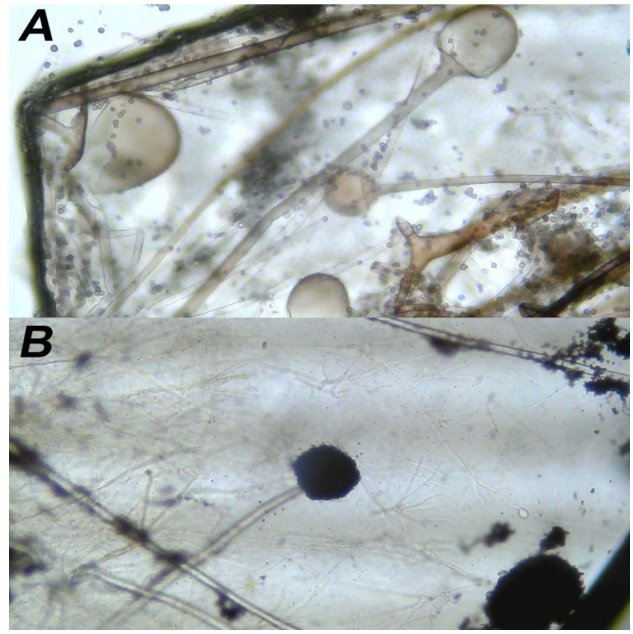

Fig. 1 Optical micrographs of the isolated fungi at ×100 magnification. **A** *Rhizopus* sp. and **B** *Aspergillus* sp

Preparation of coated strawberries

The utilized coating formulation was prepared by dissolving shrimp chitosan C3646 (Sigma-Aldrich) 2% w/v in acetic acid 1%. After homogenization, glycerol was added at 750 µL/g of chitosan. In the case of the formulation enriched with EOs, the three-EOs mixture (1/3 by volume for EO) and Tween 80 were added at 2% v/v of formulation and 0.2% v/v of EO, respectively.

The non-damaged and uniform strawberries cultivar Fortuna (22 ± 2 g) were acquired from a local market. Firstly, a washing process with NaClO 1% and sterile distilled water was performed for fruit disinfection. After drying, strawberries were separated into three groups of nine fruits (uncoated, coated with chitosan formulation enriched with EOs, and coated with chitosan formulation), and the quality was evaluated every four days for 13 days of storage at 4 °C in holed polyethylene zipper bags. The fruit coating was performed by dipping in the coating formulation for 30 s followed by oven-drying at 30 °C for 2 h.

Decay level

The visual assessment of decay was carried out for the inoculated strawberries.

Fungal growth conditions

Fresh cultures of *Rhizopus* sp. and *Aspergillus* sp. were inoculated into Potato Dextrose Broth (PDB) and incubated in an orbital shaker for 72 h at 25 °C. The fungal suspensions were adjusted to 10^7 spores/mL for subsequent tests.

Strawberries inoculation and determination of decay percentage

The prepared fungal solutions (10^7 spores/mL) of *Aspergillus* sp. and *Rhizopus* sp. were used for fruit inoculation. The uncoated and coated strawberries were sprayed with fungal suspensions and then stored in holed polyethylene zipper bags at 4 °C.

The decay percentage was determined as follows:

$$\text{Decay}(\%) = 100 \times (N2 \times 2 + N1 \times 1 + N0 \times 0)/(N \times 2),$$

where N, N2, N1, and N0 correspond to the total number of fruits, the number of fruits with a surface decay higher than 25%, the number of fruits with a surface decay lower than 25%, and the number of fruits with no decay, respectively [58].

Weight loss

The weight loss rate was measured using a high-precision balance (±0.0001 g) as follows:

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

pH and total soluble solids measurement

Strawberries of each group were homogenized and filtered. The pH of the obtained juice was determined using a pH meter (Eutech pH 700, USA), while the total soluble solids (TSS) parameter was measured using a Brix refractometer with ATC (Labart, India). For each test, three repetitions were performed.

Titratable acidity measurement

The titratable acidity (TA) was determined as described by Sangsuwan et al. [52]. Briefly, the strawberries of each group were blended with distilled water. The homogenized juice was titrated with NaOH 0.1 N to pH 8.1. TA was determined using the following formula:

$$TA = 100 \times \frac{90 \times \text{Volume added of NaOH} \times \text{NaOH normality}}{\text{Sample weight} \times 1000}$$

Surface color analysis

The Chroma meter (Konica Minolta Chroma meter CR-400, Japan) was utilized to express color parameters (L* = lightness; a* = red-green; b* = yellow-blue) of strawberries. A standard white plate provided by the manufacturer was used for the calibration of the Chroma meter. For each fruit, measurements were taken at three different points.

Anthocyanin content

Among the beneficial compounds that strawberries contain, anthocyanin are considered very interesting due to their antioxidant, antimicrobial, and health-protective effects [21]. The content of these was determined as reported by [54]. Briefly, 5 g of strawberries were mixed with an absolute ethanol solution (40 mL) containing HCl (1.5 M). Next, distilled water was added to the filtered mixture to obtain a final volume of 100 mL. The absorbance was measured at 535 nm by a UV–VIS spectrophotometer (Jenway 6305 Spectrophotometer, USA). The anthocyanin level was expressed as mg of cyanidin 3-glucoside per kg of fruit fresh weight. For each test, three repetitions were performed.

Total phenolic content

The total phenolic level was measured following Côté et al. [6] procedure. Briefly, 4 g of strawberries were mixed with 5 mL of distilled water. Then, 50 mL ethanol was added to the mixture and stirred for 2 h. Afterward, Whatman No. 1 filter paper was utilized for filtration. To the filtrate, distilled water was added to obtain a final volume of 100 mL. Next, Folin–Ciocalteu reagent (2 mL) and 7% Sodium carbonate (1.5 mL) were added to the mixture. After incubation in the dark for 60 min, the absorbance was measured at 760 nm using a UV–VIS spectrophotometer. Gallic acid solutions were utilized for the construction of the calibration curve. The total phenolic level was expressed as mg of gallic acid per kg of fruit fresh weight. For each test, three repetitions were performed.

Sensory analysis

To avoid panelists health-risks, the sensory evaluation of strawberries was conducted on the 1st and 5th days of storage as described by Robledo et al. [48]. Briefly, fruits were analyzed by eight trained judges (four women and four men). The assessment was realized under ambient conditions at 50% ± 5 relative humidity and 25 ± 2 °C. The coated strawberries were washed before the test. The TSQ was determined according to the following parameters: aroma 15%, general appearance 20%, texture 20%, internal 10% and external 15% color, and taste 20% [57]. Scores between 1 and 9 were given [57]. Where 1, 3, 5, 7, and 9 refer to unusable, fair, acceptable, very good, and excellent, respectively [19, 25].

Statistical analysis

The obtained data were statistically analyzed By ANOVA in the SPSS program (IBM SPSS Statistics software version 25.0) using Tukey's test. Results were presented as mean ± standard deviation. Differences were presumed significant when ($P < 0.05$).

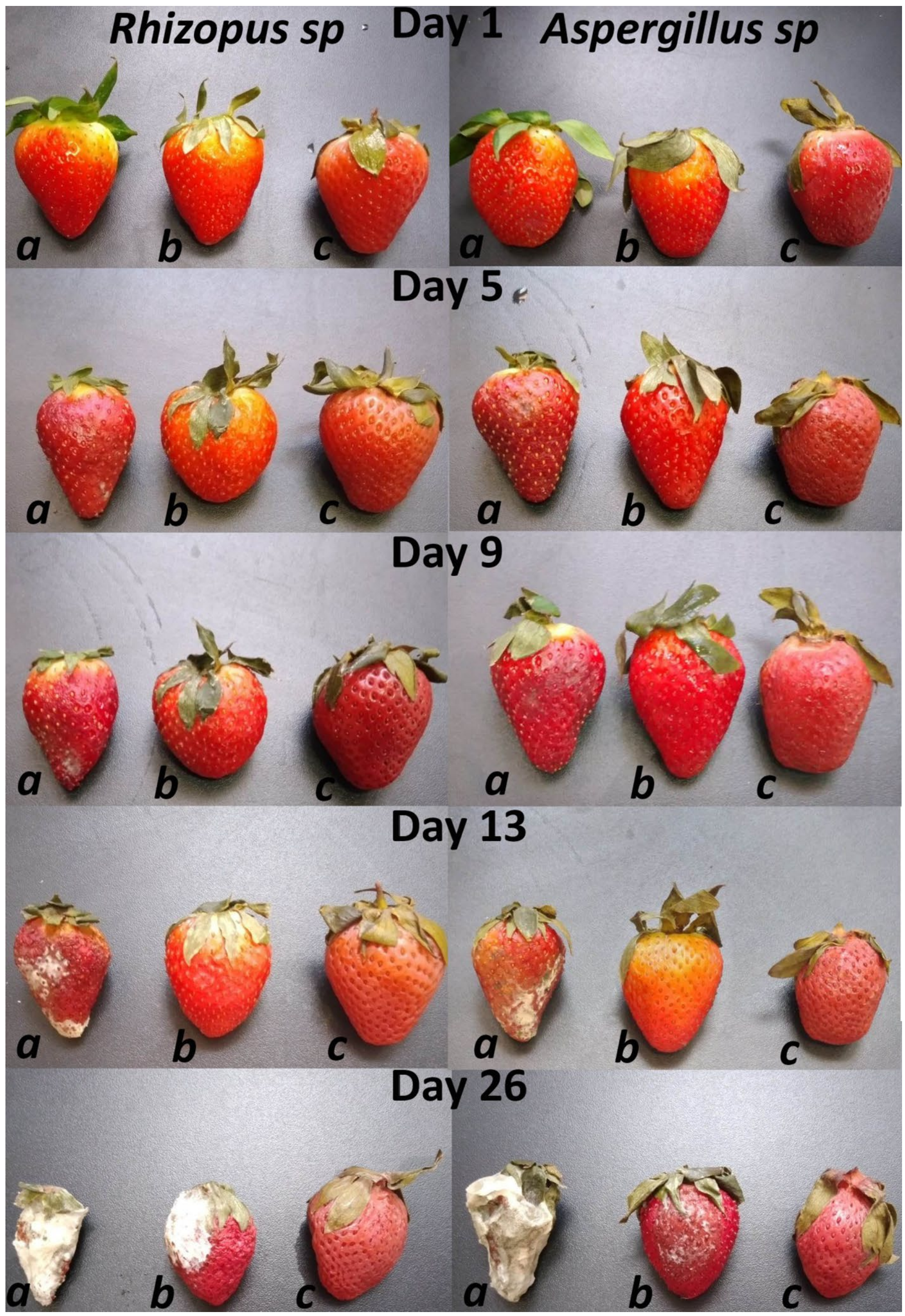

Fig. 2 Digital images of inoculated strawberries during storage time. **a** Control fruits, **b** fruits coated with chitosan, and **c** fruits coated with chitosan containing *C. zeylanicum*, *T. satureioides*, and *E. caryophyllus* EOs mixture

Table 1 Decay percentage of uncoated and coated strawberries following the inoculation with *Rhizopus* sp. and *Aspergillus* sp. and storage at 4 °C

	Decay (%)				
Fruits inoculated with *Rhizopus* sp.	Day 1	Day 5	Day 9	Day 13	Day 26
Uncoated fruits	0 ± 0.00^{a}	18.52 ± 3.20^{a}	42.59 ± 3.20^{a}	96.30 ± 6.41^{a}	100 ± 0.00^{a}
Fruits coated with CH	0 ± 0.00^{a}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{c}	74.07 ± 6.42^{b}
Fruits coated with CH-EOs	0 ± 0.00^{a}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{c}	0 ± 0.00^{d}
Fruits inoculated with ***Aspergillus*** sp.					
Uncoated fruits	0 ± 0.00^{a}	16.67 ± 0.00^{a}	38.89 ± 5.56^{a}	74.07 ± 6.42^{b}	100 ± 0.00^{a}
Fruits coated with CH	0 ± 0.00^{a}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{c}	61.11 ± 5.56^{c}
Fruits coated with CH-EOs	0 ± 0.00^{a}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{c}	0 ± 0.00^{d}

[a,b]Correspond to the percentage of decay groups in the same day independently on the fungal strain

Results

Determination of decay percentage

The evolution of uncoated and coated strawberries following the inoculation with *Rhizopus* sp. and *Aspergillus* sp. is presented in Fig. 2. Findings showed that control fruits started showing decay on the fifth day while the ones coated with chitosan only remained unspoiled until the 26th day. On the other hand, the strawberries coated with CH-EOs showed no decay even on the 26th day. These results are detailed in Table 1. According to the latter, the decay percentages were equal to 0% and 100% on the 26th day as regards the fruits coated with CH-EOs and the uncoated ones, respectively. On the other hand, the decay percentage was between 60% and 75% depending on the fungal strain in the case of the fruits coated with chitosan only.

Measurement of weight loss, pH, TSS, and TA

Figure 3 shows the progress of weight loss, pH, TSS, and TA parameters for the coated and uncoated strawberries during 13 days of storage at 4 °C. As regards the weight loss evolution (Fig. 3A), a considerable difference was observed between the coated and uncoated strawberries. For all fruits, the weight loss increased over time. On day 13, the control fruits showed 2-folds weight loss (around 40%) compared to the coated fruits (around 20%). In the case of the pH progress (Fig. 3B), control fruits showed the lowest pH value on the 1st day, followed by the ones coated with CS only then the ones coated with CS-EOs. Over time, a stabilization of the pH value around 3.5 was observed. Regarding the TSS parameter (Fig. 3C), the uncoated fruits showed lower TSS values compared to the coated fruits in the five first days. However, no significant difference in TSS values was noticed in the 9th and 13th days of storage. Contrary to the TSS parameter, the TA results showed higher values for the control fruits in the five first days (Fig. 3D).

Colorimetric measurement

The results of the colorimetric analysis are summarized in Table 2. Data revealed a reduction in L*, a*, and b* values with storage time ($P < 0.05$). Except for the b* parameter, the coated strawberries showed a high level of lightness and redness on the 13th day in comparison with the uncoated fruits ($P < 0.05$).

Calculation of anthocyanin and total phenolic contents

Figure 4 revealed a reduction in the anthocyanin and total phenolic content over time for all strawberry groups. Until day 9, both coated and uncoated strawberries showed equal values of anthocyanin content. However, a significant decrease in the anthocyanin level was noticed on day 13 in the case of the control fruits (Fig. 4A). As regards the total phenolic content, the fruits coated with CS-EOs showed greater values compared to the two other groups of fruits (Fig. 4B).

Sensory analysis of uncoated and coated strawberries

The TSQ of the uncoated and coated strawberries is summarized in Fig. 5. Findings revealed that both uncoated and coated fruits have acceptable TSQ after 1 and 5 days of storage ($TSQ \geq 5$). On the 1st day, control fruits showed higher TSQ values compared to the coated fruits, while no significant difference was noticed on the 5th day ($P < 0.05$).

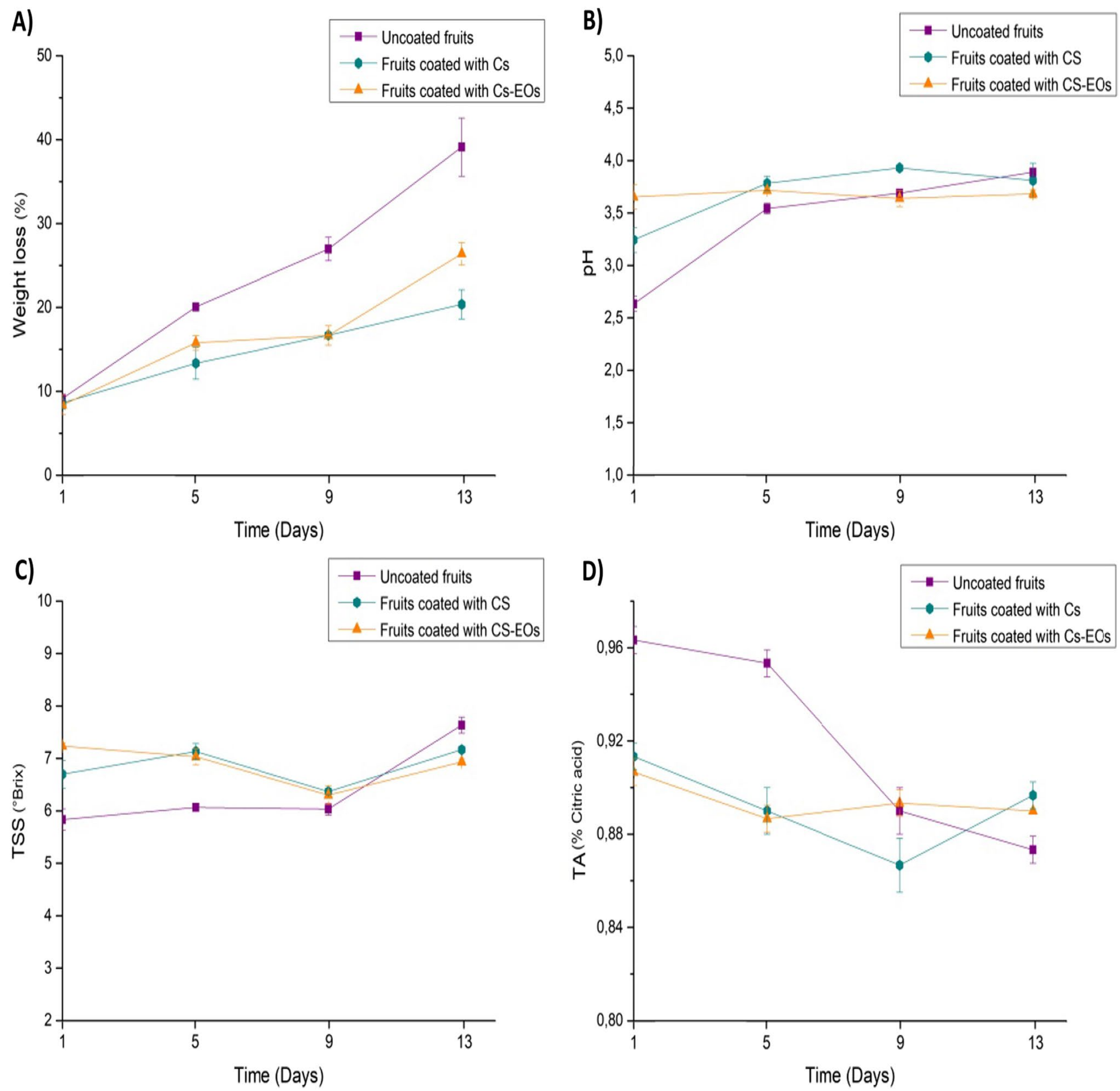

Fig. 3 Evolution of **A** weight loss, **B** pH, **C** TSS, and **D** TA parameters of the different groups of strawberries during the storage time at 4 °C

Discussion

The perishable and susceptible behavior of strawberries is a main concern [20]. The extension of strawberries' postharvest life by conserving the nutritive and sensorial qualities is important for both manufacturers and consumers [10]. It is recognized that EOs affect the sensorial quality of food [14]. However, the EOs synergism led to the use of low concentrations. Moreover, the utilization of chitosan as a coating mitigates this sensory impact by decreasing the diffusion rate of these EOs [39]. This led to a minimal modification in the TSQ during the first day of storage, which was verified in this work. Theoretically, coated fruits will exhibit higher TSQ than control fruits from one week of storage. Decay evolution is another fundamental parameter in quality determination. Findings revealed that CS

Table 2 Color parameters (L*, a*, and b*) of the coated and uncoated strawberries during the storage time at 4 °C

Days	Colors	Fruits		
		Uncoated fruits	Fruits coated with CS	Fruits coated with CS-EOs
Day 1	L*	$34.71 \pm 2.43^{a\alpha}$	$32.86 \pm 2.29^{ab\alpha}$	$34.43 \pm 2.20^{ab\alpha}$
	a*	$33.55 \pm 0.88^{a\beta}$	$33.25 \pm 0.51^{a\beta}$	$36.84 \pm 0.30^{a\alpha}$
	b*	$19.83 \pm 1.04^{a\alpha}$	$18.35 \pm 0.70^{a\alpha}$	$18.79 \pm 1.91^{a\alpha}$
Day 5	L*	$34.21 \pm 0.42^{a\alpha}$	$32.51 \pm 0.47^{a\beta}$	$35.90 \pm 1.88^{a\alpha}$
	a*	$30.87 \pm 0.77^{b\alpha}$	$30.16 \pm 0.85^{b\alpha}$	$30.61 \pm 0.94^{b\alpha}$
	b*	$19.10 \pm 2.56^{ab\alpha}$	$17.28 \pm 1.12^{ab\alpha}$	$17.63 \pm 0.44^{a\alpha}$
Day 9	L*	$30.45 \pm 0.98^{b\alpha}$	$30.09 \pm 0.85^{b\alpha}$	$33.14 \pm 2.70^{ab\alpha}$
	a*	$31.13 \pm 2.34^{ab\alpha}$	$31.38 \pm 2.01^{ab\alpha}$	$30.96 \pm 0.36^{b\alpha}$
	b*	$17.42 \pm 0.54^{b\alpha}$	$16.64 \pm 0.34^{b\alpha}$	$17.33 \pm 1.32^{a\alpha}$
Day 13	L*	$30.46 \pm 0.17^{b\beta}$	$31.63 \pm 0.64^{b\alpha}$	$32.05 \pm 0.47^{b\alpha}$
	a*	$25.34 \pm 2.00^{c\beta}$	$29.93 \pm 1.12^{b\alpha}$	$30.85 \pm 0.24^{b\alpha}$
	b*	$16.07 \pm 1.70^{b\alpha}$	$15.17 \pm 1.68^{b\alpha}$	$15.07 \pm 0.31^{b\alpha}$

$^{a,\,b}$The color parameter groups for the same group of strawberries while $^{\alpha,\beta}$ indicate the color parameter groups in the same day

and CS-EOs-based coatings preserved strawberries for 13 days and 26 days, respectively. Nevertheless, the estimated postharvest life of this fruit is less than five days at 4 °C [17, 25, 40]. Generally, decay results from the microbial infection of the fruits [54]. The fruit-microorganisms contact is inhibited by CS and CS-EOs coatings. The proposed modes of action were the formation of a thick layer that acts as a barrier, the bactericidal and fungicidal effects of the chitosan soluble fraction and EOs [29, 32], and the anti-adhesion potency of the CS-EOs formulation [30, 31, 33, 34]. We have demonstrated in our prior work that CS-film enriched with the combination of *C. zeylanicum*, *T. satureioides*, and *E. caryophyllus* EOs totally inhibits the adhesion of *Rhizopus* sp. and *Aspergillus* sp. while there is a fungal adhesion on the CS-film without EOs [36]. These findings could explain the contamination of the fruits coated with CS only on the 26th day. The inhibition of strawberry decay through the application of the CS and CS-EOs coating was accompanied by significant maintenance of the fruit's lightness and redness. The difference between the coated and uncoated fruits concerning these two parameters was noticed on day 13. In fact, the decrease in lightness can

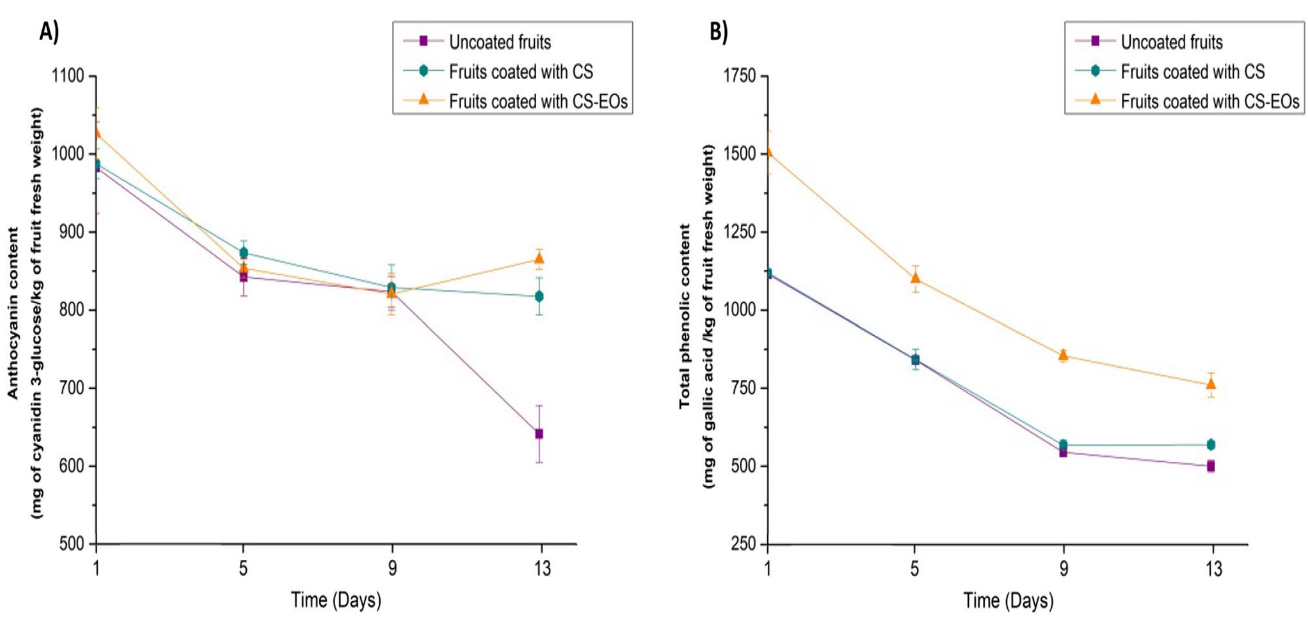

Fig. 4 Evolution of **A** anthocyanin and **B** total phenolic contents of the different groups of strawberries during the storage time at 4 °C

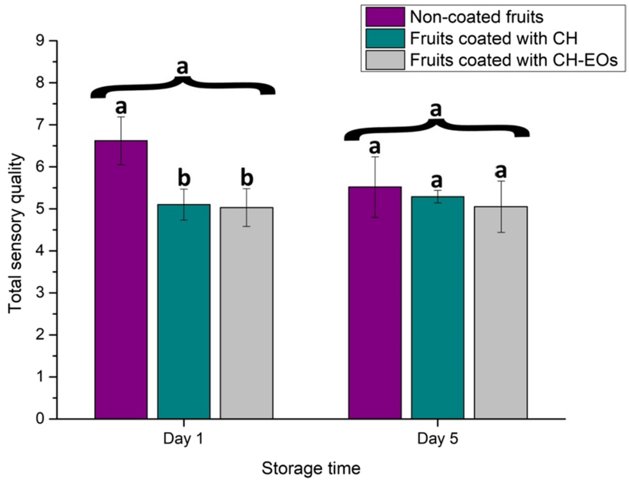

Fig. 5 Total sensory quality of uncoated fruits, fruits coated with chitosan, and fruits coated with chitosan containing *C. zeylanicum*, *T. satureioides*, and *E. caryophyllus* EOs mixture after 1 and 5 days of storage

be attributed to the formation of brown and dark tissues following inappropriate storage conditions and microbial infection [43]. On the other hand, the drop in the red color can be explained by the reduction in the anthocyanin content as a result of the hydrolytic enzymes' action [38, 43]. That was verified by the anthocyanin assay in the present work. Slightly lower values were found by Morsy et al. [27], where the anthocyanin content in strawberry nectar was around 800 mg of cyanidin 3-glucoside per kg of fruit fresh weight on day 1 and kept decreasing over time. While higher values were found by da Silva Simão et al. [8]. This difference might be related to the analyzed part of the fruit. The assessment of the weight loss showed a drop in this parameter considering the coated fruits. Several investigations have studied the water vapor transmission of CS and CS-EOs layers [2, 35, 45]. In fact, chitosan forms a semi-permeable barrier that reduces water loss and respiration, thus mitigating fruit weight loss [44]. TSS, TA, and pH are related parameters that directly affect the fruit flavor and consequently the consumer acceptability [1, 18, 25]. Findings showed that the TA and pH values were inversely correlated. In other words, higher concentrations of citric acid led to lower pH values. According to the obtained data, TA values during the first days of storage were lower as regards the coated strawberries. That might be related to the drying condition in the coating process. It has been claimed that the generation of organic acids decreases when temperature increases [50]. However, the three parameters slightly varied during the storage time as regards the coated fruits. This could be related to the deceleration of the ripening and respiration processes caused by CS coating [49]. Among the bioactive compounds present in strawberries, phenolic constituents are at the top of the consumer's requirements due to their positive effect on human health notably the anti-inflammatory, antioxidant, and anti-aging activities [24, 41]. Findings showed that the total phenolic level in the first day of storage was around 1100 mg of gallic acid per kg of fruit fresh weight considering the uncoated strawberries and the ones coated with CS only. Analogous results were found by Shankar et al. [54]. The greater values noticed in the case of the fruits coated with CS-EOs can be attributed to the chemical constituents of the utilized EOs e.g. eugenol, thymol, carvacrol, and chavicol [28].

Ultimately, the coating of strawberries with CS and CS-EOs not only extends their postharvest life but also mitigates the weight loss, and maintains the visual appearance and the nutritional quality.

Conclusion

In summary, a novel postharvest procedure using chitosan and essential oils mixture was developed for the conservation of the strawberries' quality. The coated fruits showed acceptable TSQ, stable TSS, pH, and TA values, and a minor decrease in the lightness, redness, weight, and anthocyanin content during the storage time. Additionally, fruits coated with CS enriched with thyme, clove, and cinnamon EOs mixture were more resistant and richer as regards the total phenolic content. These encouraging findings emphasize the eventual use of CS and especially CS-EOs as a coating for susceptible fruits.

Author contributions Anouar Mouhoub: Conceptualization, methodology, software, validation, formal analysis, data curation, writing——original draft, Writing—review and editing. Amine Guendouz: Conceptualization, methodology, validation, formal analysis, data curation, writing—review and editing, Supervision. Zainab El Alaoui-Talibi: Conceptualization, validation, formal analysis. Saad Ibnsouda Koraichi: Conceptualization, validation, supervision. Cédric Delattre: Conceptualization, validation, formal analysis, supervision. Cherkaoui El Modafar: Conceptualization, methodology, validation, resources, formal analysis, supervision. All authors have approved the final version of the manuscript.

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Declarations

Competing intersts The authors proclaim no conflicts of interest.

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