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Sodium alginate-coconut oil edible coating delayed postharvest senescence and enhanced antioxidant capacity of lemon (*Citrus latifolia*) fruit

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Abstract. *Truc NT, Nhung DTC. 2026. Sodium alginate-coconut oil edible coating delayed postharvest senescence and enhanced antioxidant capacity of lemon (*Citrus latifolia*) fruit. Asian J Agric 10 (1): g100156. https://doi.org/10.13057/asianjagric/g100156.* Postharvest quality deterioration of lemon fruit is closely associated with physiological metabolism and oxidative stress during storage. This study investigated the efficacy of Sodium Alginate (SA) and Coconut Oil (CO) coatings, applied individually or in combination, in regulating physiological metabolism, oxidative stress, and antioxidant defense responses of lemon fruit during ambient storage. Fruits were assigned to four treatments (Control, SA, CO, and SA-CO) using a completely randomized design with three replicates per treatment. Coated and uncoated fruits were stored at 25°C for 18 days, and key physiological (moisture loss, color), biochemical (vitamin C, total phenolics), reactive oxygen species (ROS), and antioxidant enzyme indicators were periodically evaluated. Relative to the control, the combined SA-CO coating markedly delayed deterioration. By day 18, SA-CO treated fruit showed weight loss of about 14%, which was roughly 2.5 percentage points lower than the control. Chlorophyll degradation was reduced, with SA-CO fruit retaining around 185 mg kg^{-1} chlorophyll, approximately 40 mg kg^{-1} higher than the control. Antioxidant compounds were better preserved, with vitamin C maintained at nearly 0.29 g kg^{-1} and total phenolics reaching about 0.15 g GAE kg^{-1}, both slightly higher than the control. The coating also improved antioxidant enzyme regulation. At the end of storage, SOD activity in SA-CO fruit was close to 29 U mg^{-1} protein, substantially lower than the control, whereas APX and CAT activities increased by about 12 U mg^{-1} and 25 U mg^{-1} protein, respectively. Correspondingly, reactive oxygen species decreased, with superoxide radicals recorded at around 1.5 mmol NO_2 kg^{-1} s^{-1} and hydrogen peroxide at about 0.8 μmol H_2O_2 kg^{-1}, both noticeably lower than the control. Overall, the combined SA-CO coating most effectively mitigated physiological degradation, improved antioxidant defense responses, and reduced oxidative stress, thereby supporting better retention of postharvest quality in lemon fruit stored under ambient conditions.

Keywords: Alginate coating, antioxidant enzymes, coconut oil, lemon fruit, reactive oxygen species

INTRODUCTION

Lemon fruit (*Citrus latifolia*) is widely cultivated worldwide because of its nutritional and functional value. It is rich in vitamin C, potassium, calcium, dietary fiber, essential oils, and bioactive phytochemicals, making it an important ingredient in beverages, condiments, and processed foods (Yahia 2011; Liaquat et al. 2021). However, lemon fruit is highly perishable after harvest due to moisture loss, peel de-greening caused by chlorophyll degradation, and fungal decay induced by *Penicillium digitatum* (Kaewsuksaeng et al. 2015; Perez et al. 2017). These deterioration factors rapidly reduce fruit quality and consumer acceptability, emphasizing the need for effective postharvest treatments to reduce water loss, delay peel color changes, and maintain overall quality.

Edible coatings based on polysaccharides, proteins, lipids, or their composites have been widely applied to fresh produce as environmentally friendly alternatives to conventional packaging. Such coatings can reduce moisture loss, delay physiological deterioration, suppress microbial decay, and maintain nutritional quality (Panahirad et al. 2021). Among these coating materials, Sodium Alginate (SA), a seaweed-derived polysaccharide, has shown promising effects in preserving the quality of fruits and vegetables, such as plums (Valero et al. 2013), mushrooms (Jiang 2013), kiwifruit (Liu et al. 2020), and wax apples (Duong et al. 2022). Nonetheless, alginate-based coatings are limited by their weak water-barrier capacity, which restricts their broader industrial application (Salama and Aziz 2021). To improve performance, SA coatings are commonly supplemented with natural essential oils that provide antimicrobial and antioxidant activity.

Coconut Oil (CO) is rich in saturated fatty acids, particularly triacylglycerols, and exhibits moisturizing, preservative, antimicrobial, and antioxidant properties (Ogbolu et al. 2007). Previous studies showed that CO-based coatings, especially when combined with beeswax, reduced water loss, respiration, peel yellowing, and pathogen infection, thereby maintaining firmness, ascorbic acid content and extending lemon storage life (Nasrin et al. 2020; Nasrin et al. 2023). Incorporating CO into SA matrices may further improve coating performance by enhancing hydrophobicity and reducing water vapor and

gas permeability. In polysaccharide-lipid composite systems, SA provides a film-forming semipermeable matrix, whereas CO contributes hydrophobic and antimicrobial functions, resulting in synergistic preservation effects (Valencia-Chamorro et al. 2011; Usman et al. 2025). However, the application of vegetable oils such as CO in polysaccharide-based coatings remains limited, and the interactions between edible oils and hydrophilic polymers, which strongly influence coating structure, stability, and barrier properties, are still not fully understood (Rhim et al. 2013; Usman et al. 2025). In addition, recent studies suggest that edible coatings can also modulate physiological and biochemical responses in fresh produce, functioning not only as passive barriers but also as active preservation systems (Usman et al. 2025). In particular, composite coatings may alleviate oxidative stress by regulating the balance between reactive oxygen species (ROS) and antioxidant defense systems, including enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), as well as non-enzymatic antioxidants such as ascorbic acid and phenolic compounds (Zhang et al. 2015). Supporting this perspective, recent work by Zandi and Tafti (2025) demonstrated that alginate-coconut oil composite coatings, especially when combined with natural antimicrobials, significantly improved quality attributes and extended the shelf life of Mazafati dates, confirming the practical effectiveness of this system.

We hypothesize that incorporating coconut oil into a SA matrix will synergistically enhance the coating's water and gas barrier properties, improve the regulation of oxidative stress-related pathways, and thereby reduce physiological degradation and maintain postharvest quality of lemon fruit more effectively than single-component coatings. To address this gap, the present study is the first to investigate SA-CO composite coating for lemon fruit. This work provides the first evidence that incorporating coconut oil into an alginate matrix can synergistically regulate both enzymatic and non-enzymatic antioxidant responses, mitigate oxidative stress, and improve postharvest physiological stability under ambient conditions. Therefore, this study aims to evaluate the effects of SA-CO coatings on the physiological changes, quality attributes, and antioxidant defense systems of lemon fruit during postharvest storage.

MATERIALS AND METHODS

Preparation of SA-CO coating film

The lemon fruit cv. "Num" (*C. latifolia*) were harvested from a farm in Binh Minh Town, Vinh Long Province, Vietnam, at the commercial maturity stage (105-115 days after fruit set) and transported to the laboratory within 45 minutes at an ambient temperature of 28±2°C. Fruits were selected based on uniformity in color and shape, absence of mechanical damage, and consistent weight. The fruits were washed with clean water to remove adhering dust and debris. Before the experiment, the fruits were surface-disinfected by immersion in a 0.1 g L^{-1} sodium hypochlorite solution for 5 minutes. Initial fruit quality parameters, including fruit weight (47.74±4.82 g/fruit), a* value (-10.26±0.86), total soluble solids (7.43±0.29°Bx), titratable acidity (7.45±0.48%), and vitamin C content (0.31±0.007 g kg^{-1}), were recorded before the application of treatments. Previous studies have indicated that a 1.5% SA-2.0% CO formulation is effective in reducing respiration rate, minimizing color changes and weight loss, and maintaining fruit quality (Truc et al. 2023). Therefore, this concentration was selected as the coating treatment for the present study.

The 1.5% SA-2.0% CO film was prepared following the procedure of Nguyen et al. (2026) with modifications. First, 1.5% (w/v) sodium alginate (General Purpose Grade, Fisher Chemical, UK) was dissolved in distilled water and stirred at 700 rpm for 1 hour at 60°C. Coconut oil (2.0%, v/v; Vietcoco, Vietnam) was then added and mixed using a magnetic stirrer at 700 rpm for 30 minutes to form a fine emulsion. Subsequently, 2% glycerol, 0.2% Tween-80, and 1.0% (w/v) calcium chloride were incorporated. Calcium chloride was included to act as a cross-linking agent to strengthen the alginate network, improve film integrity, and enhance coating adhesion on the fruit surface. The mixture was stirred for an additional hour at 700 rpm until a homogeneous and stable emulsion was obtained. No phase separation was observed during preparation or use. For comparison, the 2.0% CO solution was prepared following the same procedure as described above but without the addition of Sodium Alginate (SA). Similarly, the 1.5% SA solution was prepared using the same protocol but without the incorporation of Coconut Oil (CO). The coating solution was freshly prepared each day to ensure consistency and stability.

Lemon fruits treatment with SA-CO coating

Lemon fruit samples were divided into four treatment groups, each consisting of 108 fruits. Fruits were immersed for 1 minute in one of the following solutions: (i) distilled water (control), (ii) 1.5% SA solution, (iii) 2.0% CO solution, and (iv) 1.5% SA-2.0% CO solution. After treatment, the fruits were air-dried and then packed into 9 independent polyethylene bags per treatment (replicates), with 12 fruits per bag. Each polyethylene bag (22×18 cm) contained 32 perforations (Ø 0.6 cm) to allow gas exchange. All samples were stored at 25°C (RH = 60±5%) for 18 days. For sampling, fruits were randomly taken from each replicate bag at every 3-day interval until 18 days of storage.

Determination of physiological parameters in fruit samples

Weight loss

Weight loss of lemon fruit was determined for each fruit (n = 12) and expressed as the percentage reduction from its initial weight, following the method of Almenar et al. (2006).

Color change

The variations in lemon peel color were assessed every three days using a colorimeter (Hunter Lab, MH-C800

4500L, USA). Prior to measurement, the instrument was calibrated using a standard white calibration tile according to the manufacturer's instructions. For each fruit, color was measured at three equidistant points around the equatorial region. Each treatment involved twelve fruits (replicates), and the a* values were noted (Truc et al. 2021).

Total Soluble Solids (TSS) and Titratable Acidity (TA)

Fresh juice was extracted from lemon fruit and filtered through a clean cloth. For each treatment and time point, three independent replicate samples were prepared, and each replicate consisted of juice pooled from four fruits. Total Soluble Solids (TSS) were measured using a handheld refractometer (Atago, Japan) and expressed as a percentage. Titratable Acidity (TA) was determined by titrating with 0.1 N NaOH using phenolphthalein as the indicator, and TA was expressed as the percentage of citric acid (Kaewsuksaeng et al. 2015).

Determination of reactive oxygen species and antioxidant activity parameters in fruit samples

Hydrogen peroxide content

The hydrogen peroxide (H_2O_2) level was calculated in accordance with Wu et al. (2017) with minor modifications. After homogenizing fruit peel in 5% cold Trichloroacetic acid (TCA), the mixture was centrifuged at 10,000 × g for 10 minutes at 4°C. A 0.5 mL sample of the supernatant was combined with 4 mL of 5% TCA and 0.5 mL of the test solution (500 µM $(NH_4)_2Fe(SO_4)_2$, 50 mM H_2SO_4, 200 µM xylenol orange, and 200 mM sorbitol). Using an H_2O_2 standard curve, the ferric-xylenol orange complex was measured at 560 nm to determine the H_2O_2 levels following a 45-minute incubation period at room temperature. The results were given in mmol H_2O_2 per kilogram of fresh weight.

Superoxide radical content

Superoxide radical ($O_2^{-}\bullet$) production was measured following Elstner and Heupel (1976). Peel tissue was homogenized in 65 mM phosphate buffer (pH 7.8) containing 1 mM EDTA, 1% PVPP, and 0.3% Triton X-100, then centrifuged at 5,000 × g for 15 min at 4 °C. A 0.5 mL aliquot of the supernatant was mixed with 1.0 mL of 50 mM phosphate buffer (pH 7.8) and 0.5 mL of 10 mM hydroxylamine hydrochloride, and incubated in the dark for 20 min. Subsequently, 1 mL of the reaction mixture was combined with 1 mL of 19 mM *p*-aminobenzene sulfonic acid and 1 mL of 7 mM α-naphthylamine, followed by another 20 min dark incubation. The absorbance at 530 nm was compared with a sodium nitrite calibration curve to quantify nitrite formation. Because nitrite formation reflects the conversion of $O_2^{-}\bullet$ to nitrite, the resulting values represent a proxy for the superoxide production rate. Results were expressed as µmol NO_2 kg^{-1} FW min^{-1}.

Vitamin C content

Vitamin C content was determined following the method of Nguyen et al. (2021) with slight modifications. For each replicate, 0.5 g of flavedo tissue was homogenized in 10 mL of cold 5% metaphosphoric acid and subsequently filtered through Whatman No. 1 filter paper at 4°C. For the colorimetric reaction, 0.4 mL of the filtrate was mixed with 0.2 mL of 0.02% 2,6-dichloroindophenol, followed by the addition of 0.4 mL of 2% thiourea and 0.2 mL of 2% dinitrophenylhydrazine. The reaction mixture was incubated at 50°C for 1 hour in a water bath. After incubation, 1 mL of 85% sulfuric acid was added, and the mixture was allowed to stand for 30 minutes at room temperature. Absorbance was measured at 540 nm using a spectrophotometer (BioSpectrometer Basic D30, Germany). The results were calibrated using freshly prepared L-ascorbic acid standard solutions (0-100 µg mL^{-1}). A calibration curve (R^2>0.99) was established, and the data were expressed as grams of ascorbic acid per kilogram of fresh weight.

Total phenolic content

The crude extract was made using the procedure outlined by Ribeiro et al. (2008) with a few adjustments. The powdered lemon peel and 60% methanol (v/v) were combined, homogenized, and centrifuged at 15,000 × g for 20 minutes at 4°C. Using the Folin-Ciocalteu method described by Singleton et al. (1998), the total phenolic content of the supernatant was ascertained. 0.25 mL of Folin-Ciocalteu reagent was mixed with 0.05 mL of the extract to create the reaction mixture. Next, 0.75 mL of a 7.5% sodium carbonate solution and 2 mL of distilled water were added. The mixture was vortexed and incubated in a water bath at 40°C for 30 minutes. The absorbance was measured at 750 nm using a spectrophotometer (BioSpectrometer Basic D30, Germany). Quantification was performed using freshly prepared gallic acid standard solutions (0-1000 µg mL^{-1}) for calibration. The calibration curve (R^2>0.99) was constructed, and results were expressed as grams of Gallic Acid Equivalents (GAE) per kilogram of fresh weight.

Total chlorophyll content

For chlorophyll analysis, peel tissue was collected from approximately the outer 1.5 mm of the flavedo layer, ensuring that the white albedo tissue was not included. Chlorophyll was extracted by immersing 0.2 g of peel tissue in 10 mL N,N-dimethylformamide (DMF) at 4°C in the dark for 24 hours. The extract was then filtered through Whatman No. 1 filter paper. Absorbance was measured at 664 nm and 647 nm using a spectrophotometer (BioSpectrometer Basic D30, Germany), and chlorophyll content was calculated and expressed as mg kg^{-1} fresh weight according to Moran (1982).

SOD activity

In order to extract crude enzymes, peel tissue was homogenized in 65 mM phosphate buffer (pH 7.8) with 1% polyvinylpyrrolidone and 1 mM EDTA. This was followed by centrifugation at 15,000 × g for 20 minutes at 4°C. For analysis, the supernatant was utilized. Elstner and Heupel's (1976) indirect spectrophotometric approach was used to measure SOD activity. Phosphate buffer, xanthine oxidase (150 µg protein), xanthine, hydroxylamine hydrochloride, and crude enzyme extract were all included in the reaction

mixture, which was then incubated for 20 minutes at 25±2°C in the dark. After adding *p*-aminobenzenesulfonic acid and α-naphthylamine, color development was accomplished by incubating for 20 minutes in the dark. Absorbance was measured at 530 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit nitrite formation by 50% per minute per milligram of protein.

Catalase and ascorbate peroxidase activity

Crude enzyme extracts were made by blending of peel tissue with phosphate buffer solution that has a concentration of 100 mM and a pH of 7.5. The buffer also includes 1% polyvinylpyrrolidone and 1 mM EDTA. The homogenate was spun in a centrifuge at 15,000 × g for 20 minutes at a temperature of 4°C, and the liquid on top was used to test for catalase and ascorbate peroxidase, as described in Noctor et al. (2016).

The activity of the CAT enzyme was measured by tracking the drop in H_2O_2 absorbance at 240 nm over 90 seconds, using an extinction coefficient of 39.4 M^{-1} cm^{-1}. The reaction mixture contained 0.02 mL of 30% H2O2, 0.78 mL of 100 mM phosphate buffer (pH 7.5), and 0.2 mL of crude enzyme extract. CAT activity was calculated based on the rate of H_2O_2 decomposition and expressed as µmol H_2O_2 decomposed per minute per mg protein (µmol H_2O_2 min^{-1} mg^{-1} protein).

The activity of APX was measured by tracking how ascorbate was oxidized at a wavelength of 290 nm for 90 seconds, using an extinction coefficient of 2800 M^{-1} cm^{-1}. The reaction mixture contained 0.05 mL of enzyme extract, 0.05 mL of 10 mM ascorbate, and 0.89 mL of 0.1 M phosphate buffer (pH 7.5). The reaction was initiated by adding 0.01 mL of 10 mM H_2O_2 after which the decrease in absorbance was recorded immediately. APX activity was calculated based on the oxidation rate of ascorbate and expressed as µmol ascorbate oxidized per minute per mg protein (µmol ascorbate min^{-1} mg^{-1} protein).

Total protein content

The total protein concentration of the lemon peel samples was quantified using the Bradford method (Bradford 1976), with bovine serum albumin employed as the standard.

Antioxidant activity assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined following the method of Li et al. (2018) with minor modifications. Peel tissue was homogenized in cooled methanol and then centrifuged at 5,000 × g for 15 min at 4°C. A 0.15 mL aliquot of the extract was mixed with 1.85 mL of 120 µM DPPH solution in methanol. The mixture was vortexed and incubated in the dark for 30 minutes at room temperature. Absorbance was recorded at 525 nm using a spectrophotometer (BioSpectrometer Basic D30, Germany). A control sample was prepared using methanol instead of the extract. The percentage of DPPH inhibition was calculated as:

$$\%\text{DPPH inhibition} = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right) \times 100$$

Where, $\text{Abs}_{\text{control}}$ is the absorbance of the control and A_{sample} is the absorbance of the sample.

Statistical analysis

Experimental data were analyzed using SAS software (version 9.0; SAS Institute Inc., Cary, NC, USA) under a completely randomized design. For each sampling day, a one-way ANOVA was conducted to evaluate treatment effects. When significant differences were observed, treatment means were separated using the Least Significant Difference (LSD) test at a 95% confidence level. For destructive analyses, three biological replicates were used per treatment, with each replicate consisting of four fruits. For non-destructive measurements, including color and weight loss, a total of 12 fruits per treatment were used, with each fruit considered as one replicate.

RESULTS AND DISCUSSION

Effect of SA-CO coating on physiology of postharvest lemon fruit

Weight loss is a key indicator reflecting transpiration and respiratory activity during postharvest storage. As shown in Figure 1.A, weight loss increased progressively in all treatments, with clear differences among treatments. The control exhibited the highest weight loss, whereas the 1.5% SA-2.0% CO treatment significantly reduced weight loss ($P < 0.05$). This combined treatment was particularly effective in limiting weight loss during the later stages of storage, indicating improved maintenance of fruit physiological stability.

The a* value of the peel is an important color parameter reflecting changes associated with chlorophyll degradation and pigment development during postharvest storage. As shown in Figure 1.B, the a* value increased progressively over the storage period in all treatments, indicating a gradual color transition of the peel. Throughout storage, the control fruit exhibited a more rapid increase in a* values compared with the treated samples, particularly at later storage stages. In contrast, treatments with SA and CO significantly delayed the increase in a* values relative to the control ($P < 0.05$), indicating a slower progression of peel color change. Notably, the combined treatment of 1.5% SA-2.0% CO consistently maintained the lowest a* values throughout the storage period and differed significantly from both the control and single treatments, especially from day 9 to day 18 of storage. These results demonstrate that the combined application of SA and CO is more effective in retarding peel color development and preserving fruit external quality during postharvest storage (Table 1).

Total acidity is an important quality attribute reflecting organic acid metabolism and respiratory activity during postharvest storage. As shown in Figure 1.C, total acidity exhibited only slight fluctuations during storage and remained relatively stable across all treatments.

Throughout the storage period, no marked decline in total acidity was observed, indicating a slow rate of organic acid degradation. The control fruit showed a gradual increase in total acidity toward the later stages of storage. In contrast, fruits treated with SA and CO, either individually or in combination, maintained more stable acidity levels. Notably, the combined treatment of 1.5% SA-2.0% CO consistently maintained total acidity at levels comparable to or slightly higher than those of the control, with statistically significant differences observed at specific storage times ($P < 0.05$). This suggests that the combined treatment effectively delayed organic acid metabolism during prolonged storage. Overall, these results indicate that the combined application of SA and CO contributes to maintaining acidity stability, thereby preserving the internal quality of fruit during postharvest storage.

In lemon fruit, Total Soluble Solids (TSS) are commonly used as an indicator of changes in soluble carbohydrates and organic acid metabolism during postharvest storage. As shown in Figure 1.D, TSS values showed only slight fluctuations throughout the storage period across all treatments. At the early storage stage (days 0 to 3), no significant differences in TSS were observed among treatments. From day 6 onward, small but significant variations were detected, with control fruit generally exhibiting lower TSS values than treated fruit ($P < 0.05$). Treatments with SA or CO alone resulted in moderately higher TSS values at several sampling times. Overall, these results indicate that the combined application of SA and CO contributes to stabilizing soluble solids content and delaying metabolic changes in lemon fruit during postharvest storage.

Effect of SA-CO coating on the reactive oxygen species and antioxidant activity of postharvest lemon fruit

Oxidative stress, characterized by the excessive accumulation of Reactive Oxygen Species (ROS) such as the superoxide radical ($O_2^{-}\bullet$), is considered one of the major causes of quality deterioration and reduced storage life in postharvest fruits. To elucidate the effects of different coating treatments on oxidative status, changes in $O_2^{-}\bullet$ content were monitored (Figure 2.A). $O_2^{-}\bullet$ levels generally increased during storage, with the control showing a markedly higher accumulation compared with coated fruits. The combined 1.5% SA-2.0% CO treatment consistently maintained the lowest $O_2^{-}\bullet$ levels, indicating a reduced oxidative burden and improved cellular stability.

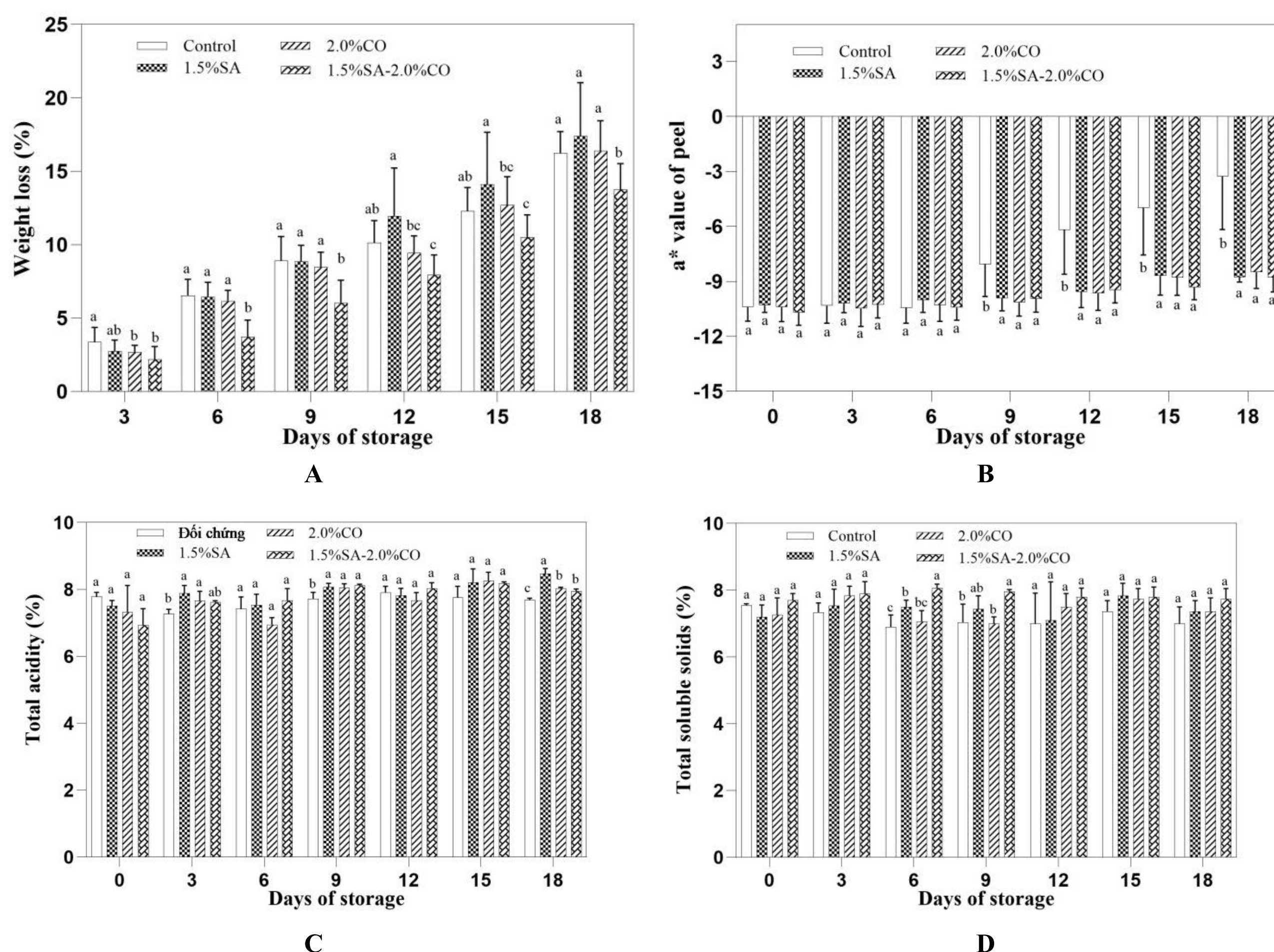


Figure 1. Effects of SA and CO treatments on postharvest physiological of lemon fruit during storage at 25°C for 18 d. A. Weight loss, B. Value of peel, C. Total acidity, D. Total souble solids. The vertical bars represent as mean ± standard deviation. Different letters above the bars showed significant differences between treatments using LSD test ($P < 0.05$)

Hydrogen peroxide (H_2O_2), another key ROS, was evaluated to further assess oxidative status (Figure 2.B). Although fluctuations were observed during storage, coated fruits maintained significantly lower H_2O_2 levels than the control, particularly under the combined SA-CO treatment. This suggests that the coatings effectively limited ROS accumulation, thereby mitigating oxidative damage.

Chlorophyll content, an indicator of senescence and visual quality, declined progressively during storage (Figure 2.C). The more rapid degradation observed in the control reflects accelerated senescence, whereas SA and CO treatments delayed chlorophyll loss, with the combined treatment showing the greatest retention. This indicates improved preservation of photosynthetic pigments and delayed senescence processes.

Phenolic compounds, which contribute to antioxidant defense, showed an overall increase during storage (Figure 2.D). The higher accumulation in coated fruits, especially under the combined treatment, suggests an enhanced non-enzymatic antioxidant response, likely contributing to improved resistance against oxidative stress.

Vitamin C content declined during storage but was better preserved in coated fruits (Figure 2.E). The higher retention observed in SA-CO-treated fruits indicates a protective effect against oxidative degradation, supporting the role of coatings in maintaining antioxidant quality.

The activities of antioxidant enzymes further supported these observations. The lower SOD activity observed in coated fruits, together with reduced ROS accumulation, suggests a decreased requirement for superoxide dismutation and reflects an overall reduction in oxidative stress. In contrast, higher APX and CAT activities in coated fruits (Figures 2.G and 2.H), particularly under the combined treatment, indicate enhanced enzymatic scavenging of H_2O_2 and improved redox regulation. These coordinated responses highlight a well-regulated antioxidant system, in which reduced ROS generation is accompanied by efficient downstream detoxification of H_2O_2 through APX and CAT, thereby maintaining cellular redox homeostasis.

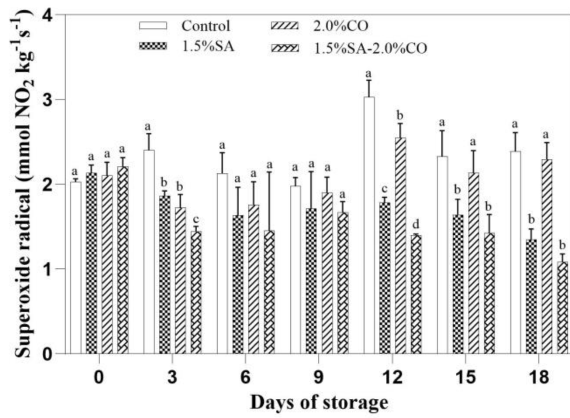
Antioxidant capacity, measured by DPPH radical scavenging activity (Figure 2.I), declined over time but remained higher in coated fruits. The SA-CO treatment maintained stronger antioxidant activity, indicating better protection against oxidative stress. Overall, SA-CO coatings enhance antioxidant systems, reduce ROS accumulation, delay senescence, and improve postharvest stability.

Discussion

During storage, chemical and biochemical reactions, along with physical changes and ROS accumulation, lead to deterioration in fruit quality. Edible coatings help to preserve the quality attributes of fruits by reducing moisture loss and gas exchange, protecting against damage, and maintaining visual appearance (Panahirad et al. 2021). Alginate-based coatings form semipermeable barriers that limit oxygen and carbon dioxide transfer, thereby lowering respiration and transpiration rates (Riva et al. 2020). In this study, 1.5% SA or 2.0% CO applied individually reduced weight loss to some extent, whereas their combined application was more effective. The 1.5% SA-2.0% CO treatment consistently showed the lowest weight loss from day 9, suggesting a synergistic effect. Comparable results have been reported in Mexican lime fruit coated with sodium alginate (1.0%) + pomegranate seed oil (0.05%) and in ber fruit treated with alginate-olive coatings, both of which effectively prevented weight loss (Rao et al. 2016; Rastegar et al. 2025). The improved performance is attributed to enhanced barrier properties that restrict oxygen diffusion and water vapor transfer (Duong et al. 2023). Overall, the combined 1.5% SA-2.0% CO coating effectively retards weight loss and maintains lemon fruit quality during storage.

Peel de-greening and chlorophyll degradation are key processes driving lemon senescence during storage, regulated by respiration, ethylene response, and oxidative metabolism (Hörtensteiner 2006). Herein, lemon fruits coated with SA or CO alone, and their combination significantly influenced a* values (Figure 1.B) and total chlorophyll content (Figure 2.C), indicating different physiological responses. SA coating forms a semipermeable barrier that modifies the internal atmosphere, reduces oxygen availability, and slows respiration, thereby delaying chlorophyll degradation (Arroyo et al. 2020; Riva et al. 2020). CO treatment also delayed peel de-greening, although its effect was weaker, likely due to lower film uniformity. The combined 1.5% SA-2.0% CO treatment was most effective, maintaining lower a* values and higher chlorophyll content throughout storage. This improved performance is attributed to the synergistic effect of polysaccharide-lipid coatings, which enhance gas and moisture barrier properties and better regulate senescence-related metabolism (Rojas-Graü et al. 2009; Rao et al. 2016). In contrast, control fruits showed a steady increase in a* values as chlorophyll degraded. A strong negative correlation between a* and chlorophyll content confirmed that peel color change is mainly driven by chlorophyll loss. This relationship was most evident in the control, while coated fruits with SA-CO, exhibited delayed yellowing and better color retention (Naeem et al. 2019).

The TSS in fruits may decrease while TA increases during storage, reducing quality and taste (Lin and Zhao 2007). Respiration converts organic acids into sugars, lowering TA and raising TSS (Amiri et al. 2017). Edible coatings help in maintaining TSS and TA by reducing respiration, thereby slowing biochemical changes and metabolite levels in coated fruits (Khaliq et al. 2016; Ebrahimi and Rastegar 2020). In the present work, 1.5% SA-2.0% CO maintained total acidity and stable TSS during storage by reducing respiration and weight loss (Figure 1.A). These coatings likely form a semipermeable barrier that limits oxygen movement, lowering respiration and preserving acidity and TSS in lemons stored at 25°C for 18 days. Previous research findings have reported that alginate coatings enriched with hexyl acetate oil in rose apples (Duong et al. 2023) and Mexican lime fruit (Mohammadi et al. 2024).



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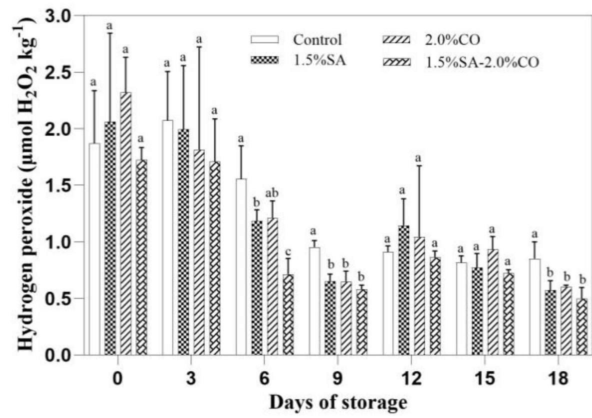

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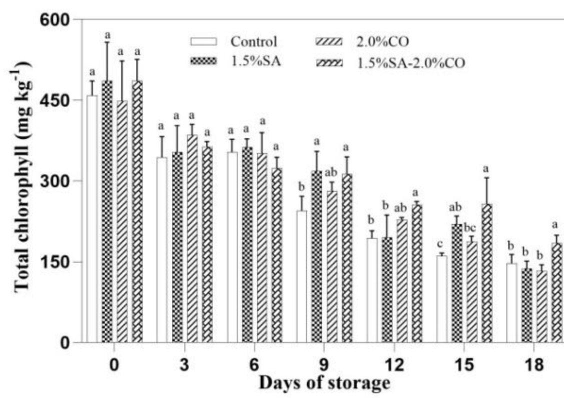

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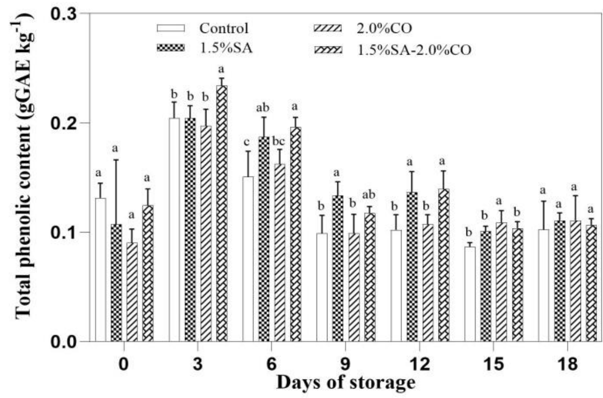

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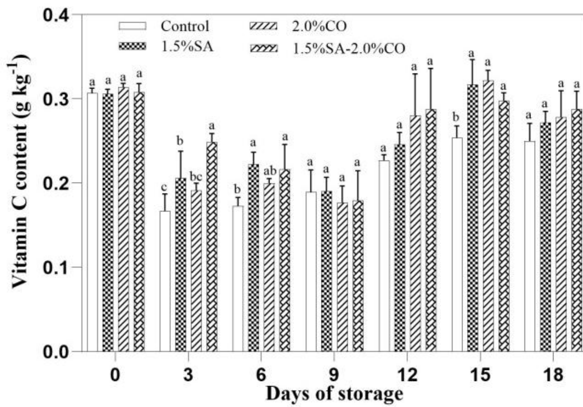

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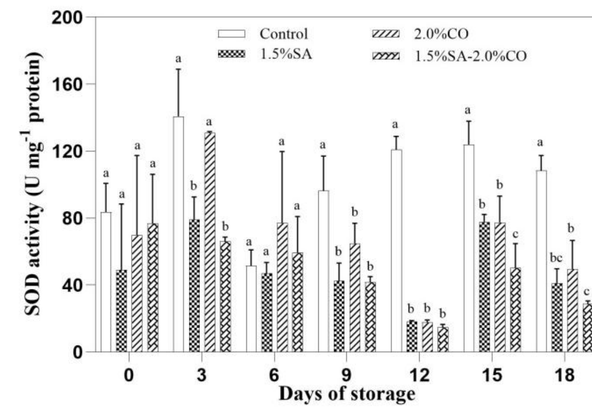

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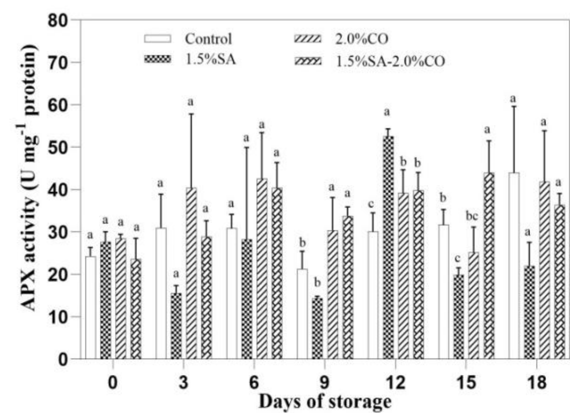

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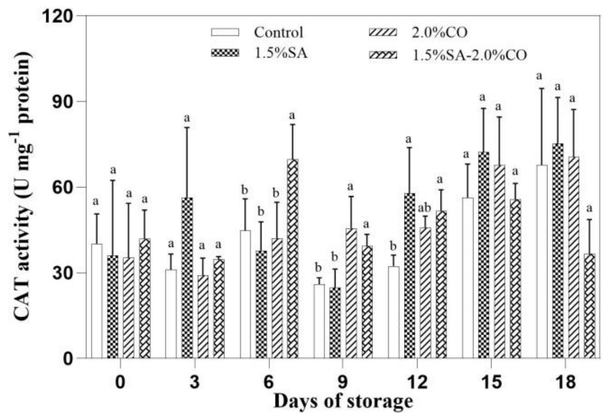

H

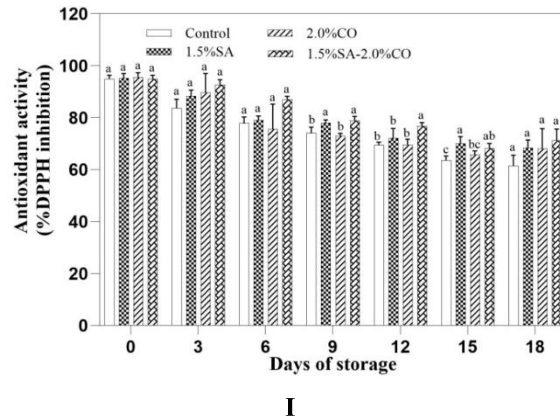

Figure 2. Effects of SA-CO coating on reactive oxygen species and antioxidant activity of postharvest lemon fruit during storage at 25°C. A. Superoxide radical, B. Hydrogen peroxide, C. Total chlorophyll, D. Total phenolic content, E. Vitamin C content, F. SOD activity, G. APX activity, H. CAT activity, I. Antioxidant activity. The vertical bars represent as mean ± standard deviation for triplicate samples. Different letters above the bars showed significant differences between treatments using LSD test ($P<0.05$)

Table 1. Effects of SA-CO coating on the appearance of postharvest lemon fruit during storage. Images were captured under standardized lighting conditions with a fixed distance and scale

Treatments	Day 0	Day 18
Control		
1.5% SA		
2.0%CO		
1.5%SA-2.0%CO		

Total Phenolic Content (TPC) and vitamin C are key non-enzymatic antioxidants that protect against ROS-induced oxidative stress (Blokhina et al. 2003). Fruit antioxidant capacity is closely associated with their levels. Edible coatings (SA, CO, and SA-CO) significantly influenced TPC (Figure 2.D) and vitamin C (Figure 2.E) in lemons, with the combined 1.5% SA-2.0% CO treatment consistently maintaining higher levels than single coatings or the control. This effect is attributed to improved barrier properties that reduce oxygen permeability and moisture loss, thereby limiting weight loss (Figure 1.A) and slowing phenolic oxidation. In contrast, single coatings provided only partial protection. The simultaneous preservation of TPC and vitamin C indicates effective mitigation of oxidative stress, and TPC may serve as an indirect indicator of antioxidant retention (Aljabary 2024). Phenolic compounds detoxify ROS by donating hydrogen and decomposing peroxides (Rice-Evans et al. 1996). Higher TPC levels have also been observed in coated fruits such as guava, pomegranate, and lemon (Gonz and Mart 2004; Ehteshami et al. 2019; Naeem et al. 2019), supporting the increased antioxidant activity (Figure 2.I). Related findings have been reported for oil-enriched coatings in lime fruit (Naeem et al. 2019; Rastegar et al. 2025).

During storage, fruits generate Reactive Oxygen Species (ROS), including superoxide, hydrogen peroxide, and hydroxyl radicals, which induce oxidative damage and accelerate senescence (Karlia et al. 2020). Edible coatings can enhance antioxidant enzyme activities, such as SOD, CAT and APX, thereby reducing ROS accumulation and preserving fruit quality (Huang et al. 2019). A negative correlation between ROS levels and chlorophyll retention underscores the main role of oxidative stress in postharvest senescence. Excess ROS, particularly $O_2^{-}\bullet$ and H_2O_2, promote chlorophyll degradation by disrupting chloroplast membranes and activating chlorophyll-degrading enzymes, leading to the loss of green color (Apel and Hirt 2004; Hörtensteiner 2006). In the present study, SOD, CAT, and APX activities were higher in fruits treated with 1.5% SA-2.0% CO than in the control (Figures 2.F-2H). It also maintained higher a* values (Figure 1.B) and total chlorophyll content (Figure 2.C) compared with other treatments. Enhanced ROS-scavenging capacity likely preserved chloroplast integrity and delayed chlorophyll degradation, resulting in improved pigment retention, consistent with previous findings on oil-based coatings (Kumarihami et al. 2022; Rastegar et al. 2025). Moreover, the 1.5% SA-2.0% CO coating strengthened both enzymatic (SOD, CAT, APX) and non-enzymatic (TPC and vitamin C) antioxidant systems, thereby improving ROS detoxification and limiting oxidative damage. These enzymes convert reactive species into water, thereby protecting cellular structures and delaying senescence (Apel and Hirt 2004). Similar effects have been reported for composite lipid-based coatings, which enhance antioxidant capacity and delay oxidative processes in fruits such as lime, rose apple, and strawberry (Parreidt et al. 2019; Duong et al. 2022; Mohammadi et al. 2024). In addition, alginate-based coatings have been shown to preserve total phenolics and vitamin C while increasing SOD, CAT, and APX activities in mango and plum (Bal 2019; Tarabih 2020). Overall, the 1.5% SA-2.0% CO coating effectively enhanced antioxidant defense systems by integrating enzymatic and non-enzymatic components, thereby reducing oxidative stress and maintaining fruit quality during storage.

In conclusion, this study shows that combining SA and CO into a single edible coating can effectively maintain several key quality attributes of lemon fruit during ambient storage. The SA-CO coating formed a functional semipermeable barrier that reduced moisture loss, slowed peel degreening, and helped preserve the measured physicochemical properties throughout storage. In addition, the coating strengthened the antioxidant defense response of the fruit. Coated lemons exhibited lower SOD activity, higher APX and CAT activities, and better retention of vitamin C and total phenolics, which corresponded with reduced hydrogen peroxide and superoxide radical accumulation. Among the tested formulations, the 1.5% SA-2.0% CO coating consistently delivered the strongest protective effects, highlighting the benefit of integrating hydrophilic and hydrophobic components within a single film matrix. Future studies should focus on evaluating the performance of SA-CO coatings under commercial storage and supply chain conditions, including large-scale application feasibility, sensory acceptance, and microbial stability. Such investigations will help determine the practical applicability of this coating strategy in postharvest handling and distribution systems.

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