Chapter 3

Effect of Sodium Alginate Coating Enriched With Stevia Rebaudiana on Quality of Fresh-Cut Apples

Şeyda Öztürk[1]

Aslıhan Demirdöven[2]

Abstract

This study focused on determining the quality changes of fresh-cut apples coated with sodium alginate (SA) edible film and stevia combinations under modified atmosphere packaging (MAP) conditions. Cube-shaped pieces of apples were separated into three main groups: control (C, without coating), SA, and a combination of SA + stevia (SAS), and stored under passive-MAP (polypropylene-PP, 30 μm) conditions at 1 ± 2 °C for 3 days, and the effects of the film coatings on some quality properties of the apples were investigated. For this purpose, the polyphenol oxidase (PPO) activity and microbiological (total psychrophilic aerobic bacteria (TPAB), total mesophilic aerobic bacteria, and total yeast-mold capacity), total phenolic content (TPC), total antioxidant capacity (TAC), O_2%–CO_2%, and pH analyses were performed. As a result, it was determined that the stevia was effective in restricting enzyme activity and increasing the TPC and TAC. In addition, it was determined that the number of TPAB was <2 log cfu/g.

1. Introduction

Sodium alginate (SA), a polysaccharide, has begun to be evaluated in many areas, particularly in the food, pharmaceutical, and cosmetic industries because of its appropriate colloidal characteristics and adequacy to supply intense gels in aqueous solutions [1]. SA is also capable of forming a strong molecular chain and a good film [2]. Therefore, it can be used as an edible

1 Tokat Gaziosmanpaşa Üniversitesi Zile Meslek Yüksekokulu, ORCID: 0000-0001-7183-2478, drrseydaozturk@gmailcom

2 Tokat Gaziosmanpaşa Üniversitesi Mühendislik ve Mimarlık Fakültesi, ORCID: 0000-0003-1246-9132, aslihan.demirdoven@gop.edu.tr

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film or as an encapsulation agent to limit the dehydration of meat, fish, and fruit [3]. On the other hand, because it is non-toxic, biodegradable, biocompatible, and low-cost, it is seen as an important component in the production of edible film [4].

SA-based edible films improve the tissue, reduce water loss, and maintain physicochemical, microbiological, and antioxidant features in melon, papaya, and minimal processed apples [5,6,7]. SA-based films may also be a carrier of various additives, such as antioxidants, antimicrobials, and colorants [8].

Stevia extracts originating from South American have been used as a calorie-free, natural sweetener for many years in Japan, China, Korea, and Brazil [9]. Moreover, it was stated in the literature that stevia has antimicrobial and antioxidant properties, as Stevia contains a high amount of phenolic substances, vitamin C, carotenoids, and chlorophyll [10].

In this study, it was aimed to evaluate the changes in the enzyme polyphenol oxidase (PPO) activity and antioxidant properties, such as the total phenolic content (TPC) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation and microbiological quality of fresh-cut apples coated with SA edible film enriched with stevia (SAS) under passive-modified atmosphere packaging (MAP) storage at 1 °C for 3 days.

2. Material and methods

2.1. Material

Medium viscosity SA (2000 cP, 2%, Sigma, Germany), glycerol (Sigma, Germany), ascorbic acid (1.65 g/cm3, Tito, China), and dried stevia leaves, which were provided by a regional dealer (Tokat, Turkey), were used as the formulation materials. The packaging material used for the MAP was polypropylene (PP-30 μm). The film-applied apples (Malus domestica) were of the Amasya variety (Tokat, Turkey). The Amasya variety apple is the most important local apple cultivar in Turkey, and has high economic value.

2.2. Preparation of sodium alginate and sodium alginate-stevia film

For the production of the SA film, 1.25% (w/v) SA was dissolved in 45 °C of pure water. As a plasticizer, 10% (v/v) glycerol was used, and as antioxidant agent, 2% ascorbic acid was added to it. The formulation was mixed for 1 h at 45 °C and placed in an ultrasonic bath (Elmasonic S 100 (H), Elma, Germany), and then subjected to degassing for 30 min. For the production of the film in the SAS group, the concentration was added to the SA film formulation until reaching a volume of 2.5%. The extract was

obtained by adding 100 mL of distilled water at 100 °C to the dry leaves of 8.33 g of Stevia rebaudiana and maintaining it there for 30 min. This ratio was chosen according to that in previous studies [11].

2.3. Coating and packaging

Before processing, the apples were cleaned with tap water, dried, and cut into cube-shaped pieces with a laboratory knife (1.8 × 1.8 × 1.8 cm3). Then, the apples were quickly dipped into the film formulation without enzymatic browning for 30 min. The apple pieces were removed from the film, kept on perforated plates for 20 min, and then dried for 120 min in a drying oven (Memmert 100-800, Schwabach, Germany) at room temperature. Then, to ensure passive MAP conditions, the uncoated and coated samples were sealed via heat sealing (Packtech, impulse sealer FS 400 for PP/PE) by cutting them into appropriate sizes (20 × 30 cm^2), placing them into the packing material (PP), and storing them (Capri, CSS 501, 138 m3 ±2, Turkey) under passive MAP conditions (gas composition beginning at: 21% O2, 0.03% CO2, etc.) at 1 ± 1 °C and at 80%–90% relative humidity for 3 days. The analyses were carried out every 24 h.

2.4. Analysis methods

2.4.1. PPO activity

For the PPO activity, all of the samples were stocked at –80 °C until analysis. The apple samples (5 g) and cold distilled water (5 mL) were homogenized using ultraturax (IKA T18 Basic, Germany) for 1 min, and then the samples were centrifuged at 6000 rpm for 30 min at 4 °C (Hettich EBA 21, Germany). After centrifugation, the clear portion in the tubes was analyzed by filtration with double coarse filter paper. Activity measurement was performed using a spectrophotometer (PG T80 + UK) in 2.6-mL tubs, at a wavelength of 420 nm. For the substrate solution, 0.5 M of catechol solution was used as the model substrate. Next, 2000 μL of distilled water and 500 μL of substrate solutions were added into the cuvette (3 mL) in the blank preparation stage. For the sample measurement, 2000 μL of distilled water, 100 μL of substrate solution, and 500 μL of raw extract solution were added to another cuvette and the absorbance was measured at 420 nm. During the measurements (3 min), the absorbance values were recorded every 30 s. The 0.001 units of change that occurred in the absorbance per 1 mL of enzyme solution in 1 min was used as the activity unit [12].

2.4.2. Total phenolic content and total antioxidant capacity

The samples (20 g) were crumbled homogeneously in ultraturax and then mixed with methanol (20 mL, 70%). After this step, the samples were placed in an ultrasonic bath for 2 h and strained through filter paper. The prepared extract was used for the TAC and TPC analyses. The phenolic substance content analysis of the apples was conducted according to the spectrophotometric method defined by Franke et al. [13]. The TAC values were determined according to the spectrophotometric method that was presented by Re et al. [14]. The dilutions were considered for both analyses. Detailed information about the analysis procedures were provided by Karagöz and Demirdöven [15].

2.4.3. O2%–CO2% and pH value

The O2 and CO2 percentage of packages were measured using a Gaspace 2 (England) gas analyzer [16]. The pH values were measured via a pH-meter (WTW Inolab pH Level-1, Germany) [17].

2.4.4. Microbial load

The sample (10 g) and peptone (Biomark, India) water (90 mL) were placed in a stomacher (IUL 707/470 Instruments, Spain), in a sterile stomacher bag. Then, the sample-peptone water mixture was homogenized at 200 rpm for 60 s (10–1). Decimal dilutions of peptone water (0.1%) were prepared from the obtained homogenate. The prepared dilutions were cultured busing the plate spread method on petri dishes that contained plate count agar (PCA, Orgamik, OM-HB250) to determine the total mesophilic aerobic bacteria (TMAB) and total psychrophilic aerobic bacteria (TPAB), and on petri dishes that contained potato dextrose agar (PDA, Orgamik, OM-HB265) to determine the total yeast-mold (TYM), and then incubated (TMAB; 2 days at 35 ± 2 °C, TPAB; 10 days at 6 ± 2 °C, TYM; 5 days at 25 ± 2 °C, Binder, BD23, Germany). At the end of the incubation, the results were stated as log cfu/g [18]-20].

2.4.5. Statistical analysis

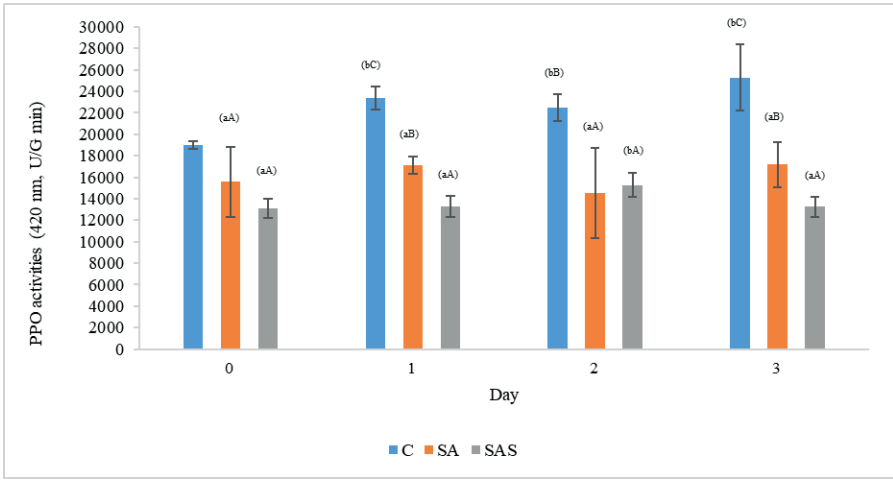
One-way ANOVA was applied to determine the differences between the samples and the effects of the storage time. SPSS Statistics for Windows 16.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. Data were evaluated using the Tukey multiple comparison test. $p < 0.05$ was accepted as statistically significant [21].

3. Results and discussion

3.1. PPO activity

The PPO activity results for the C, SA, and SAS samples are given in Figure 1. The data from the beginning and the end of the storage were 19.032 and 25.272 U/g min for the C samples; 15.600 and 17.192 U/g min for the SA samples; and 13.104 and 13.260 U/g min for the SAS samples, respectively. Considering the data during storage, the SA and SAS samples had lower enzyme activity ($p \leq 0.05$). In other words, the film coating suppressed the enzyme activity. This was thought to be due to the ascorbic acid, an antioxidant agent that was added to the film, and/or the film acting as an oxygen barrier.

In fruits and vegetables, browning occurs via mechanical damage, such as impact, cutting, peeling, and slicing. This reaction, called enzymatic browning, is mainly due to the relationship between phenols and PPO in the presence of oxygen, and is closely related to their concentration [22]. Olivas et al. [5] stated that the browning of apple slices was delayed by alginate coating and explained this protective effect by the presence of calcium chloride in some cases, which is defined as anti-browning agents and the coatings acting as a barrier against oxygen.



n = 4, (± standard deviation), $^{a, b} \leq 0.05$ *represents the differences in the samples between the storage days;* $^{A, B, C} \leq 0.05$ *represents the differences between the samples on the same storage day.*

Figure 1. PPO enzyme activity values of the coated (SA and SAS) and uncoated (C) fresh-cut apples

When the effect of the storage time on samples was examined, there were no statistical differences between the SA and SAS samples ($p > 0.05$). However, the PPO activity of the control sample increased significantly during the storage period ($p \leq 0.05$). Rojas-Graü et al. [23] and Oms-Oliu et al. [6] also made similar statements. They stated that alginate film coatings containing N-acetylcysteine and calcium chloride protected pear slices for 14 days and apple slices for 23 days in storage without enzymatic browning. Hui-Min et al. [24] mentioned that three types of edible coatings (carrageenan, carboxymethylcellulose, and SA) and their combinations prevented PPO activity in fresh peaches stored at 5 °C and reduced the degree of enzymatic browning.

Ascorbic acid is an important antioxidant that is used in the food industry, which can be categorized as primary (chain-breaking) antioxidants and secondary (inhibitor) antioxidants. The primary antioxidants react with peroxyl radicals to prevent them from reacting with unsaturated lipid molecules. The secondary antioxidants delay the chain initiator reactions, and the effect is the binding of metal ions, oxygen capture, ultraviolet absorption, and inactivation of singlet oxygen. The secondary antioxidants have been reported as antioxidant synergists. Therefore, tocopherols (primary antioxidants), phospholipids (proton donors), ascorbic acid (oxygen traps), and flavonoids (primary antioxidants and metal chelates) are combined to provide a stronger protection [25]. However, the usage of ascorbic acid in this study may have had a synergistic effect by the presence of increased phenolic substances with stevia. It was also reported that stevia contains antioxidant and antimicrobial compounds, such as phenolics, vitamin C, carotenoids, and chlorophylls [10]. According to this study, it was observed that the films with the stevia extract decreased the enzymatic activity ($p \leq 0.05$). This may have been due to the antioxidant agents (phenolics) contained in the stevia, and the synergistic effect of the phenolics in stevia with the ascorbic acid.

3.2. Total phenolic substance

The TPC decreased in the C, SA, and SAS samples during storage ($p \leq 0.05$) because these compounds were exposed to oxygen and light as a result of cutting, and the polyphenolic enzyme activity, such as PPO, caused destruction [8]. In addition, this effect has also been seen in other studies using apples as material [26].

Generally, the phenolic content of the coated samples was higher than that in the C samples ($p \leq 0.05$). In a study on the effect of SA coating, it was

found that coated cherries exhibited higher TPC and TAC when compared to the uncoated samples [27]. Furthermore Rössle et al. [8] stated that the coating of apple slices with alginate generally provided better protection of polyphenolic compounds.

Table 1. Total phenolic substance and antioxidant capacity values of the coated (SA and SAS) and uncoated (C) fresh-cut apples

Samples	Total phenolic substance (GA mg/L)				Antioxidant capacity (μmol TE/g)			
	Storage time (day)				Storage time (day)			
	0	1	2	3	0	1	2	3
C	689.50 ± 50^{aA}	675.22 ± 76^{aA}	618.66 ± 64^{aA}	641.16 ± 103^{aA}	3.9201 ± 0.2^{bA}	3.5468 ± 0.2^{abA}	2.8134 ± 0.7^{aA}	3.1169 ± 0.2^{abA}
SA	2126.16 ± 255^{cB}	1522 ± 122^{abB}	12017 ± 285^{aB}	1678.66 ± 50^{bcB}	28.9909 ± 1.5^{bB}	17.3018 ± 3.5^{aB}	16.7734 ± 7^{aB}	14.2508 ± 2.9^{aB}
SAS	2544 ± 67^{bC}	2702 ± 254^{bC}	2209 ± 35^{aC}	2023 ± 102^{aC}	36.1588 ± 4.5^{bC}	24.8842 ± 7.5^{abB}	21.8409 ± 5^{abB}	19.1615 ± 6.7^{aB}

n = 4, (± standard deviation), $^{a, b}$ ≤ 0.05 represents the differences in the same column, $^{A, B, C}$ ≤ 0.05, respectively, on the same line.

During storage, the highest TPC (Table 1) was evaluated in the SAS samples ($p \leq 0.05$) because of the high phenolic content of stevia. However, the TPC values of the C, SA, and SAS samples at the beginning and at the end of storage were 689.50 and 641.16, 2126.16 and 1678.66, and 2544.67 and 2023.102 GA mg/L, respectively. The results showed that the film coating had a positive effect on the TPC ($p \leq 0.05$). In the literature, Oms-Oliu et al. [6] and Rössle et al. [8] stated that the addition of anti-browning agents to alginate-based coatings preserved the TPC and TAC better than in uncoated pears. Additionally, edible alginate coatings have been shown to have a positive impact in obtaining higher concentrations of total phenolics and antioxidant activity in control group cherries due to excessive maturation and aging processes [27].

3.3. Total antioxidant capacity

At the end of the storage, a decrease was observed in the TAC of the SA and SAS samples ($p \leq 0.05$) (Table 1). The coated samples (SA and SAS) had a high TAC when compared to the C samples and the difference between them was statistically significant ($p \leq 0.05$). This result was thought to be due to the addition of ascorbic acid. Similarly, Robles-Sánchez et al. [28]

stated that the rate of TAC of fresh cut mangoes coated with 2% alginate + 1% ascorbic acid was significantly higher than in the control mangos or those coated with only 2% alginate. They attributed these differences to the antioxidant properties of ascorbic acid. In another study, however, it was reported that antioxidant activity was high and did not change significantly during the experiments [29].

The storage-end TAC values of the C, SA, and SAS samples were 3.1169, 14.2508, and 19.1615 mM TE/g, respectively. According to these results, the coating increased the TAC ($p < 0.05$). Moreira et al. [30] added apple fiber and inulin to polysaccharide-based (alginate, pectin, and gellan gum) edible coating formulations (with the addition of ascorbic acid) and evaluated the effects of fresh apples on the quality characteristics and noted that a notable increase was seen in the antioxidant activity of the fresh apples immediately after the coating process.

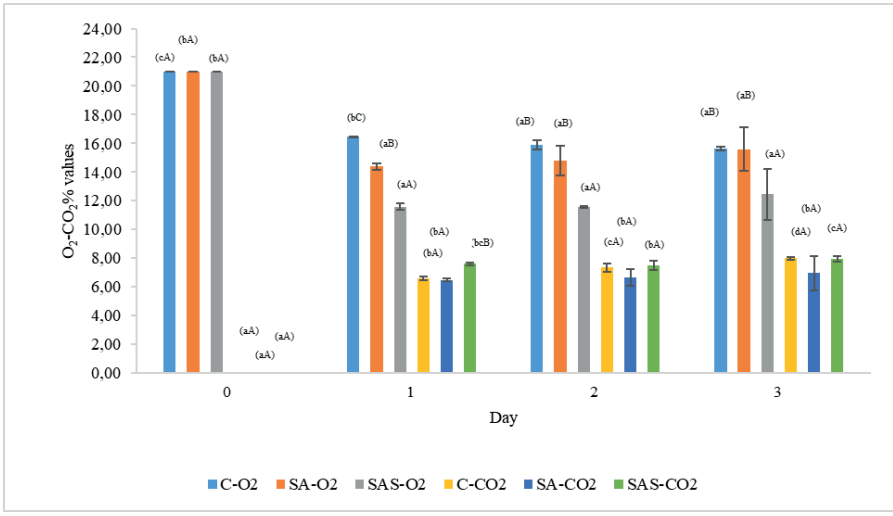
3.4. O_2% and CO_2% values

At the end of the storage, the O_2 concentrations (Figure 2) of all of the samples were decreased ($p \leq 0.05$) and the amount of CO_2 was increased in the packages ($p \leq 0.05$), as was expected, because fruits and vegetables consume oxygen and produce carbon dioxide while in respiration. During the storage period, the changes in the O_2% and CO_2% concentrations was highest between days 0 and 1 of storage in the packages. In other words, the maximum changes in respiration were seen between these days. In the following days, the increase in the amount of carbon dioxide and the decrease in the amount of oxygen slowed the respiration of the samples.

The CO_2 ratio of the C samples was higher than the SA samples at the end of the storage, but there were no statistically significant differences between the SAS and C samples ($p > 0.05$). Similar results were published for chitosan- and alginate-based edible coatings, which decreased the respiration rate of minimally processed mango and papaya [31],[7].

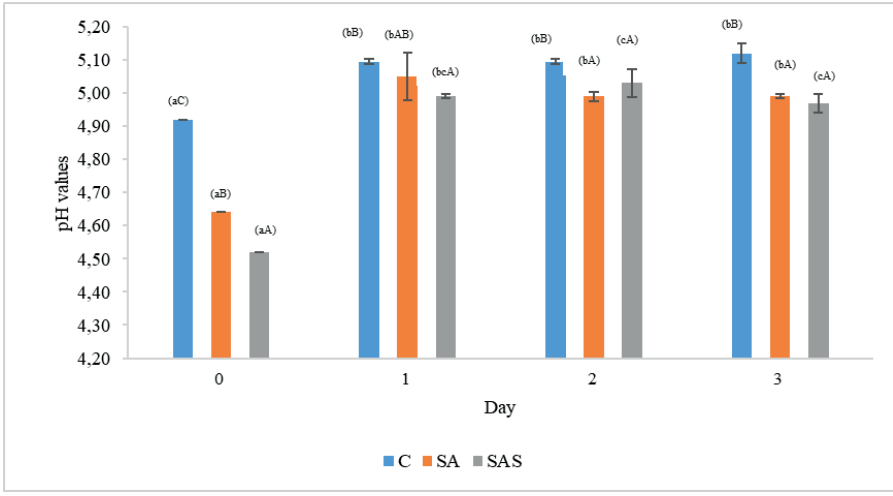
3.5. pH value

On the third day of the storage, an increase was observed in the average pH (Figure 3) of the film-coated and C samples. However, this increase was not statistically significant in the C samples, but it is significant in the film-coated samples ($p \leq 0.05$). In fact, Maftoonazad et al. [32] reported that at any time during storage, their control samples had higher pH values than the coated peaches and it was identical to the results of previous studies [33].



n = 2, (± standard deviation) while the O_2%–CO_2% data were evaluated among themselves, $^{a, b} \leq 0.05$ represents the differences in the samples between the storage days; $^{A, B, C} \leq 0.05$ represents the differences between the samples on the same storage day.

Figure 2. O_2%–CO_2% values of the coated (SA + SAS) and uncoated (C) fresh-cut apples



n = 4, (± standard deviation), $^{a, b} \leq 0.05$ represents the differences in the samples between the storage days; $^{A, B, C} \leq 0.05$ represents the differences between the samples on the same storage day.

Figure. 3. pH values of the coated (SA + SAS) and uncoated (C) fresh-cut apples

There are other studies in the literature indicating that the increase in the pH values in control samples was higher than that of the coated fruit [34]. The coated samples (SA and SAS) had lower pH values than the C samples ($p \leq 0.05$) because of the ascorbic acid content of the film. Moreover, it was seen that the SA and SAS samples had a higher rate of change than the C samples on day 1.

3.6. Microbiological analysis results

The TMAB counts of the C, SA, and SAS samples at the beginning of storage were under 2 log cfu/g (Table 2). In the SA and SAS samples, the total mesophilic aerobic bacteria count was determined on day 1 and in the C samples on day 2. However, at the final stage of storage, there were no statistical differences between the total mesophilic aerobic bacteria counts of the three samples ($p > 0.05$). This result was similar with the study of Guerreiro et al. [29]. The aerobic mesophilic microorganism count was determined as 3.81 log cfu/g for the control samples, 3.74 log cfu/g for the 1% SA + 0.3% citral samples, and no difference was stated between them. However, Moreira et al. [30] stated that gellan gum films applied on fresh-cut apples had an impact on reducing the mesophilic and psychrophilic counts when compared to uncoated, alginate-coated, and pectin-coated apples. According to the data obtained from the storage of the C, SA, and SAS samples, the total psychrophilic aerobic bacteria count was <2 log cfu/g (Table 2). Considering the initial storage values, the yeast-mold counts in the SA samples were higher than in the C and SAS samples ($p \leq 0.05$) (Table 2). However, the SAS samples showed lower loads than the SA samples ($p \leq 0.05$). The SA data were expected to be much lower at the end of storage. As is known, molds are aerobic microorganisms, and the film coating acts as an oxygen barrier. However, it was determined that there were no differences between the C and SAS samples or the SAS and SA samples at the final stage of the storage ($p > 0.05$).

During the storage period, mold growth was observed in the C and SAS samples and the difference between them was statistically significant ($p \leq 0.05$). In this case, the SAS samples were different than the expected, which suggested that the SAS film increased the growth of the mold. This may have been associated with the hydrophobic property of SA. As the result of all these evaluations, it would not be correct to make clear statements about the behavior of SA. Indeed, the effect of SA on microorganisms is a matter of debate. Ragaert et al. [35] stated that alginate coatings do not have an inhibitory effect that is greater than the shelf-life limit of the fresh-cut fruits

and vegetables. In contrast, Rojas-Graü et al. [23] showed that alginate coated fresh-cut apples showed lower values than control samples.

Table 2. Microbiological analysis (log cfu/g) of the coated (SA and SAS) and uncoated (C) fresh-cut apples

	Total mesophilic aerobic bacteria				Total psychrophilic aerobic bacteria				Total yeast and mold			
Samples	Storage time (day)				Storage time (day)				Storage time (day)			
	0	1	2	3	0	1	2	3	0	1	2	3
C	$<2^{aA}$	$<2^{aA}$	2.54 ± 0.5^{bA}	3.31 ± 0.01^{cA}	$<2^{aA}$	$<2^{aA}$	$<2^{aA}$	$<2^{aA}$	2.58 ± 0.12^{aA}	3.21 ± 0.05^{bcA}	3.12 ± 0.01^{bA}	3.44 ± 0.23^{cA}
SA	$<2^{aA}$	2.92 ± 0.10^{bB}	3.14 ± 0.23^{bA}	3.27 ± 0.25^{bA}	$<2^{aA}$	$<2^{aA}$	$<2^{aA}$	$<2^{aA}$	3.72 ± 0.00^{aC}	3.73 ± 0.6^{aC}	3.70 ± 0.19^{aB}	3.86 ± 0.15^{aB}
SAS	$<2^{aA}$	2.71 ± 0.22^{bB}	3.08 ± 0.20^{bcA}	3.54 ± 0.28^{cA}	$<2^{aA}$	$<2^{aA}$	$<2^{aA}$	$<2^{aA}$	3.06 ± 0.12^{aB}	3.45 ± 0.6^{abB}	3.75 ± 0.27^{bB}	3.72 ± 0.23^{bAB}

n = 4, (± standard deviation), $^{a, b} \leq 0.05$ represent the differences in the same column, $^{A, B, C} \leq 0.05$, respectively, on the same line. <2 was below the detectable amount.

4. Conclusion

When compared to the C samples, the PPO activity and pH values of the coated samples were low, and the TAC and TPC values were high. The stevia enriched coatings showed low PPO activity, and high TPC and TAC when compared to the SA samples at storage end. Additionally, the difference between the TMAB counts of the C, SA, and SAS samples was not statistically significant. Nevertheless, the load of mesophilic aerobic bacteria increased in the whole samples during storage. However, the total psychrophilic aerobic bacteria count in the C, SA, and SAS samples was under the detectable limit (<2). When the TYM results of the C, SA, and SAS samples were evaluated, it was found that the SA and SAS samples showed higher values than the C samples. However, there was no difference between the SA and SAS samples. In addition, the values in the C and SAS samples increased during storage. In conclusion, it has been specified that these film coatings have a wide potential for usage in business corporations, such as hotel kitchens, catering companies, etc., to keep products fresh during the day. Additionally, better outcomes can be acquired by adding active supplementary to the films ($CaCl_2$, etc.) to improve the quality features or use active modified atmosphere packaging.

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6. Author contribution statement

In the scope of this study, author 1, contributed to the conceptualization, doing the experiment, supervision, literature review, writing original draft, writing-review & editing. Author 2, contributed author contributed to the conduct of the experiment and the interpretation of the results.

7. Ethics committee approval and conflict of interest statement

"Ethics committee approval is not required for the prepared article."

"There is no conflict of interest with any person/institution in the prepared article."

8. Kaynaklar

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