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EFFECTS OF SOME EDIBLE COATING ON QUALITY OF READY-TO-EAT AMASYA APPLES

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ABSTRACT

This study aimed to investigate the changes of some physicochemical and sensory quality of ready-to-eat Amasya apples coated with chitosan and stevia combinations. Cube-shaped apples divided into three sample groups: i- Control (C, without coating); ii- Chitosan (CH, dipped into film mixture consisting of 0.75% chitosan, 1.5% glycerol, 2% ascorbic acid); iii- Chitosan-stevia (CHS, same CH film additionally contains 2.5% stevia extract). Some physicochemical and sensory analyzes were performed. Film coatings decreased respiration rate, increased titration acidity but no weight loss was observed in all samples. CH and CHS increased fruit hardness value at the beginning of storage, but in other days, statistically difference wasn't observed between samples (P >0.05). At the end of storage, a decrease was seen in L* values; but an increase was seen in a* and b* values of samples ($P \leq 0.05$). In addition, CHS samples wasn't approved due to herbaceous smell and taste of stevia.
Keywords: Apple, chitosan, edible film, shelf life, stevia.

BAZI YENİLEBİLİR KAPLAMA UYGULAMALARININ YEMEYE HAZIR AMASYA ELMASININ KALİTESİNE ETKİLERİ

ÖZ

Bu çalışmada, kitosan ve kitosanın stevia içeren kombinasyonları ile kaplanmış yemeye hazır Amasya elmalarında oluşan bazı fizikokimyasal ve duyusal değişimlerin incelenmesi amaçlanmıştır. Küp doğranan elma örnekleri üç gruba ayrılmıştır: i- Kontrol (C, kaplanmamış); ii- Kitosan (CH, %0.75 kitosan, %1.5 gliserol, %2 askorbik asit içeren filme daldırılmıştır); iii- Kitosan-stevia (CHS, %2.5 stevia ekstraktı içeren aynı CH filmi kullanılmıştır). Bazı fizikokimyasal ve duyusal analizler yürütülmüştür. Film kaplamalar solunum hızını azaltmış, titrasyon asitliğini arttırmış, ancak örneklerde ağırlık kaybı gözlenmemiştir. CH ve CHS, depolama başlangıcında meyve sertlik değerini arttırmış, ancak diğer günlerde örnekler arasında istatistiksel olarak anlamlı bir fark bulunmamıştır (P >0.05). Depolama sonunda, L* değerlerinde bir azalma görülmüş; ancak örneklerin a* ve b* değerlerinde bir artış görülmüştür ($P \leq 0.05$). Ayrıca, otsu kokusu ve stevia tadı nedeniyle CHS örneği kabul görmemiştir.
Anahtar kelimeler: Elma, kitosan, yenilebilir film raf ömrü, stevia.

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INTRODUCTION

As it stated in the report of the World Health Organization and specific dietary guidelines of Turkey; it is recommended to consume at least five portions of fruit and vegetables per day for control of body weight and adequate and balanced nutrition. According to this recommendation, consumption of fruits and vegetables in our country is inadequate (Anonymous, 2015). For this reason, it has been observed that edible films and coatings have been studied especially for slicing and selling fruits and vegetables in recent years. Edible coatings are packages that surround food externally and can be consumed with food. They can be obtained from proteins, lipids and polysaccharides (Chiabrando et al., 2016). One of the polysaccharide based edible film/coating is chitosan. It is obtained by deacetylation of chitin (Taştan and Baysal, 2013). This biopolymer can also be the carrier of many materials such as antioxidants, antimicrobials, etc. Thus, the efficiency of chitosan can be increased. The use of edible films with modified atmosphere packaging (MAP) is indicated in studies. By MAP application, the O_2 concentration in the package is limited and the respiration rate of the product is controlled and accordingly the shelf life of the product can be extended (Farber et al., 2003; Müftüoğlu, 2010).

Stevia rebaudiana plant originating in South America, which is known for its sweetening properties and also has antioxidant and antimicrobial properties, has recently been on the agenda (Gantait et al., 2015; Barba et al., 2015). The dry extract of *Stevia rebaudiana* leaves contains phenolic compounds, flavonoids and other antioxidant compounds. However, there are few studies on the antioxidant activity of stevia extracts (Criado et al., 2015).

In this study, it was aimed to investigate the changes of some physicochemical and sensory quality of ready-to-eat Amasya apples coated with chitosan and stevia combinations under passive-MAP (polypropylene-PP, 30μm) storage at +1 ^{o}C for 3 days.

MATERIALS AND METHODS

Materials

'Amasya' variety apples were purchased from a local wholesale distributor (Tokat, Turkey) in October (2017) and stored at +1±2 °C until processing. For film formulations, high density chitosan (>400 m Pa.s, 1% acetic acid (AS), Sigma) and dried stevia leaves (from a local herbalist) were used. In addition, glycerol (Sigma-Aldrich, Germany) was added as plasticizer and ascorbic acid (1.65 g / cm^3, E300, Tito, China) was added as an antioxidant agent. In the MAP phase, polypropylene (PP-30 μm) packaging materials (20x30 cm^2, by Packtech, impulse sealer FS 400 for PP/PE) were brought to the appropriate sizes with a heat sealer.

Chitosan film formulation: For chitosan film production, 0.75% **(**w/v) chitosan was dissolved in 1% **(**v/v**)** acetic acid (Sigma-Aldrich, Germany). Then 1.5% (v/v) glycerol was added to the film as the plasticizer. In addition, 2% ascorbic acid was added to enrich antioxidant properties. The film formulation was homogenized at 40 °C for one hour and then subjected to degassing for 30 min (Duran, 2013) by an ultrasonic bath (Elmasonic S 100 (H), Elma, Germany). Dried *Stevia rebaudiana* leaves were added to pure water at 100 °C and left for 30 min (8.33 g stevia/100 ml). This extract was added to the chitosan film formulation as it will be a final concentration of 2.5%. This ratio showed the best antimicrobial activity in previous studies (Carbonell-Capella et al., 2015).

Application of Edible Films to Apple Slices: The apples were washed with tap water, dried, the core houses removed and cut shelly into cube-shape (1.8x1.8x1.8 cm^3, with laboratory knife). The sliced apples were dipped in the edible films for 30 min by dipping method. The coated apples with film was filtered for 20 min and then dried for 60 min in drying oven (Memmert 100-800, Schwabach, Germany, at room temperature). Coated and uncoated samples were stored in PP packaging materials under passive-MAP conditions at +1±2 °C (at the beginning gas composition: 21% O_2, 0.03% CO_2 and other gases) for 3 days (Capri, CSS 501, 1.38 m^3 ±2, Turkey, 80-90% relative humidity).

Respiration rate: All samples were stored in jars at +1±2 °C and gas measurements were made by using Gaspace2 (England) gas analyzer. For each measurement 1 kg sample was used, and at the end of 24 h, the O_2% and CO_2% concentrations were determined and this concentrations were used for calculation of respiration rates. Detailed information is provided by Demirdöven (2003).

Titration acidity: 15 g apple sample pulped with Ultraturax and placed in a 150 ml erlenmeyer and topped with hot water and kept for 2 hours, filtered. The titration acidity of prepared sample was calculated as "%" malic acid (g/100 fruit) considering the dilution factor (Stevens et al., 1979).

Weight loss: It was expressed as "%" by weighing at the beginning and at the end of shelf life of the apples (Tokatlı, 2016).

Texture value: The maximum force required to drill 10 mm of the apples from the vertical dimension was determined by measuring in Newtons. In the measurement, Zwick Z 0.5 Tester (USA) with 10 mm diameter stainless steel head was used (Anonymous, 2002).

Color: Color of the apples were measured on the fruit skin free surface with Minolta color meter (Chroma meter, CR-300, Japan). First it was calibrated on a standard plate of white and black; L* (brightness), a* (red, green), b* (yellow, blue), then values were determined at three different points on the apple using Hunter color measurement parameters (Anonymous, 1991). ΔE, ΔC and Hue angle values were calculated using L*, a* and b* values, as follows (calculations were made with reference of the L*, a* and b* values of 0th day of the control sample):

$$\Delta E = [(L^*-L_{ref}^*)^2 + (a^*-a_{ref}^*)^2 + (b^*-b_{ref}^*)^2]^{1/2} \quad (1)$$

$$\Delta C = [(a^*-a_{ref}^*)^2 + (b^*-b_{ref}^*)^2]^{1/2} \quad (2)$$

$$Hue\ angle = tan^{-1}(b^*/a^*) \quad (3)$$

Sensory analysis: The samples were evaluated in terms of sweetness, sourness, apple flavor, brittleness, hardness and general acceptability. Sensory analysis was performed by 9 panelists using a graph scale of 0-5 points for each feature. The sections marked by the panelist are based on their average values in the scale (Altuğ and Elmacı, 2005).

Statistical analysis: Statistical evaluations were carried out using the SPSS 16 packet program. Differences in practice were assessed according to the "One-Way Anova for Repeated Measures" analysis for the related samples. The mean values were compared with the "Tukey" multiple comparison test at 95% confidence level (Tokatlı, 2016).

RESULTS AND DISCUSSION

Respiration rate: O_2 consumption was highest in the CH coated samples ($P < 0.05$) among the C and CHS coatings and CO_2 production was highest in C samples ($P \leq 0.05$). According to the interaction between respiration rates and shelf life of fruits and vegetables, the storage life is shortened when the respiration rate of a product increases (Cemeroğlu et al., 2001). However, edible coatings reduce the respiration rate by providing a semi-permeable structure against the motion of oxygen, carbondioxyde, moisture and solid materials (Baldwin et al., 1995; Perez-Gago et al., 2010; Öz and Süfer, 2012).

As seen in Figure 1, the film coating slowed down the respiration rate. In a related study, the respiration rate of sponge pumpkin samples treated with 0.5% and 1% chitosan stored at 25 °C for 10 days were detected as 65.43 mg CO_2 kg^{-1} s^{-1} in the control samples, 48.66 mg CO_2 kg^{-1} s^{-1} in coated with 0.5% chitosan and 41.37 mg CO_2 kg^{-1} s^{-1} in 1% chitosan-coated samples (Han et al., 2014; Tokatlı, 2016). Petriccione et al., (2015) found that the respiration rate of cherries coated with chitosan (0.5% chitosan) at 24 °C is lower than the control sample. In this study the minimum O_2 consumption value of C, CH and CHS samples was seen in the sample of CHS with 19.75 ml/kg.s (24 h).

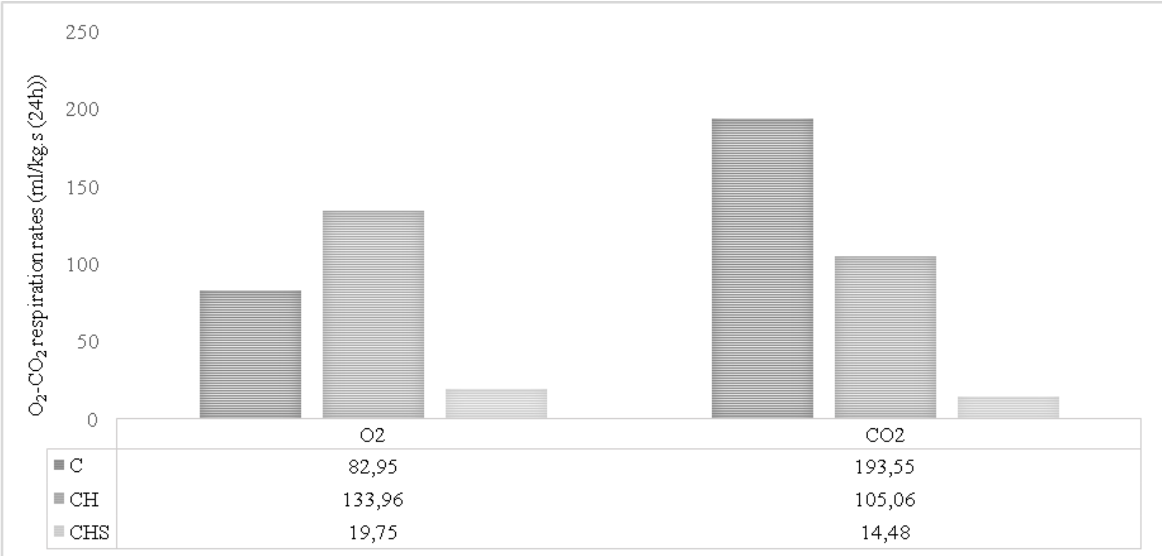
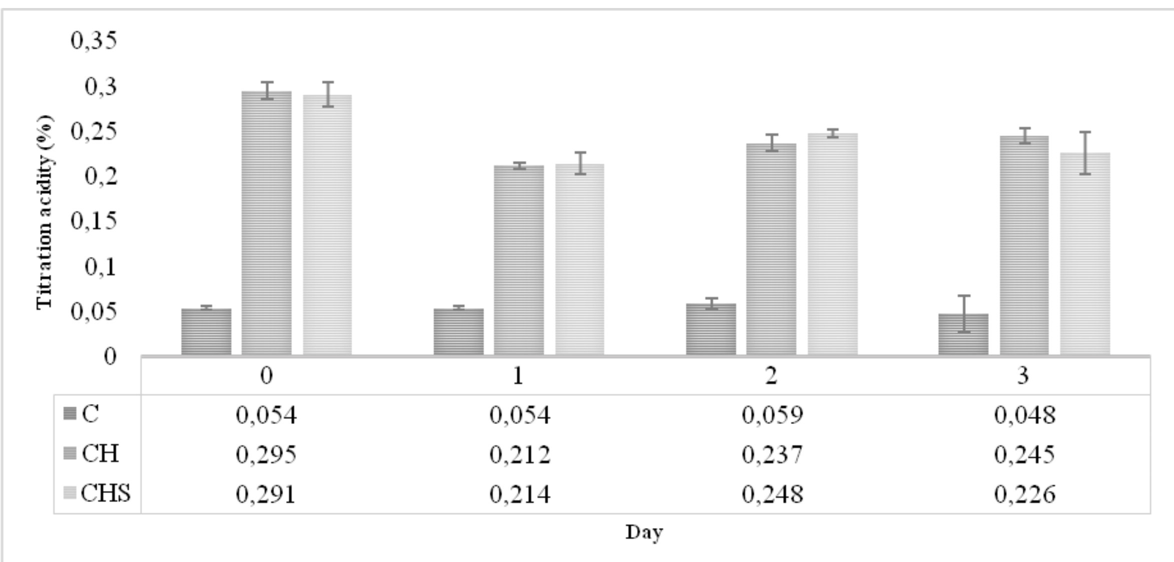


Figure 1. O_2-CO_2 respiration rates (ml/kg.s(24h)) of C (Control), CH (Chitosan), CHS (Chitosan-Stevia) samples in MAP

Titration acidity: The titration acidity values of all samples were decreased at the end of storage. This was not significant in the control sample (*P* >0.05) but it was significant in the CH and CHS samples (*P* ≤0.05) (Figure 2). Saraçoğlu (2007) also stated that during the storage of apples, titratable acidity is decreased in contrast to pH value. Şumnu and Bayındırlı (1995) stated that the malic acid value of Amasya apple in postharvest was determined as 3.24 g / kg, and showed a significant decrease after one week of harvest. This is expressed by the consumption of organic acids as a substrate during respiration.



* n=4, (┬ ┴ standard deviation)

Figure 2. Titration acidity (%) values of C (Control), CH (Chitosan) and CHS (Chitosan-Stevia) samples

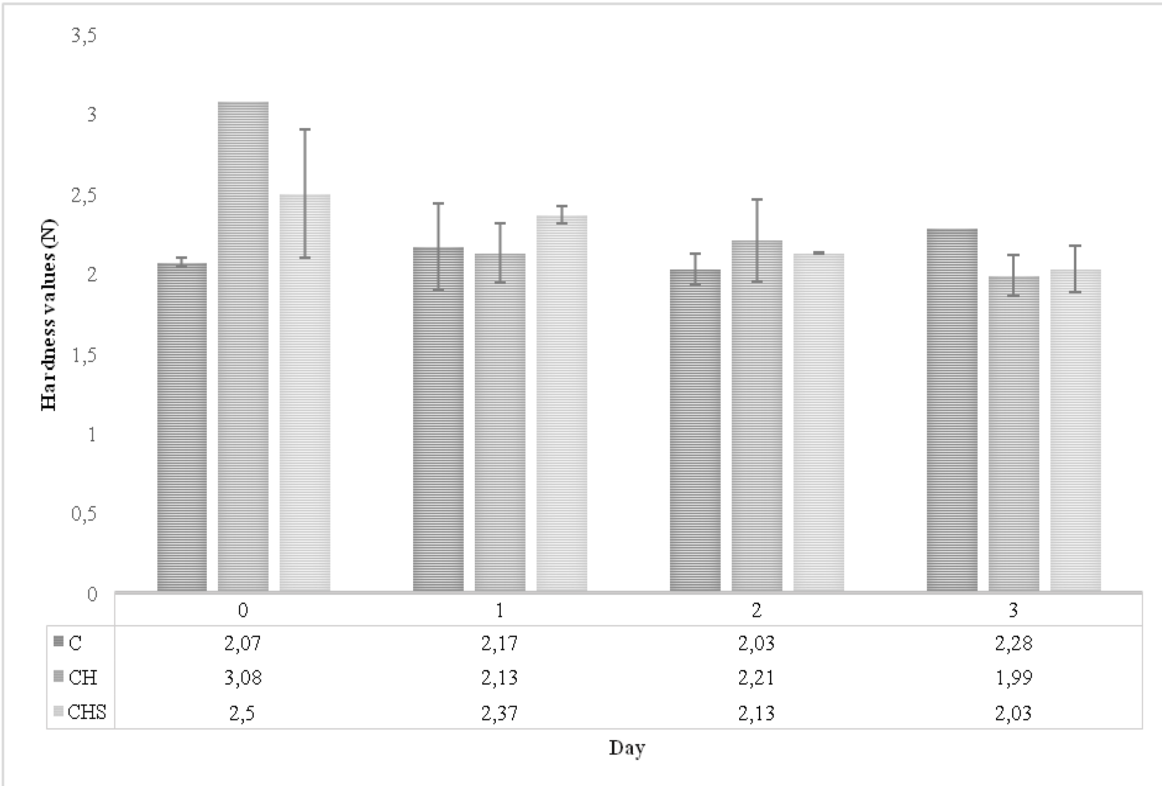
Titration acidity values from day 0 to day 1 showed a higher decrease compared to other days. However, the titration acidity of the CH and CHS samples showed a decrease at the end of storage. This is thought to be due to the degradation of ascorbic acid that available in edible film formulations. For this reason, the titratable acidity of the film-coated samples decreased more than the C samples. It is supported by the fact that despite the respiration of the C sample is rapid, the decrease in titratable acidity at the end of storage is not significant. Gardesh et al. (2016) reported that the titration acidity of nanochitosan-coated samples was slightly slower than control fruits during storage period, and this was due to the slow respiration of the coated samples. They also indicated that the titration acidity values of the coated samples were significantly higher than the values of the control samples.

The difference between the titration acidity values of the CH and CHS samples and the control samples was found to be statistically significant ($P \leq 0.05$). It is thought that dissolution of CH in 1% acetic acid and the addition of ascorbic acid into the film formulations composed this difference. However, there is no statistical difference between CH and CHS samples ($P > 0.05$). El Ghaouth et al., (1991) was determined that the titration acidity of strawberries coated with 1% and 1.5% chitosan and stored at 4 °C was higher during the storage period.

Weight loss: The weight of fruits and vegetables was reduced due to the on-going respiration after harvest and the water loss. These reductions in the weight of the products cause huge damages to the economy during the shelf life. For this reason, the shelf life determined for fruits and vegetables is important. Since the storage time is as short as 3 days, the weights of the apple slices coated with CH and CHS film were not changed. In other words, it was determined under the critical limit of 4-6% (Kays, 1991).

Hardness value: In CH coated samples, a decrease in the fruit hardness value was detected at the end of storage ($P \leq 0.05$) (Figure 3). In addition, at the end of storage, the values of fruit hardness of the CHS and C samples did not show any statistically significant differences ($P > 0.05$). The respiration increased the softening. Even though the CH sample has lower respiration rate than C sample, it was determined that of the CH sample showed more softening. Ponting et al. (1971) reported that the softening observed in fresh apples may result from pectic acid passing through the acid hydrolysis process. In addition, the respiration rate also reduces the hardness (Kartal, 2010). However, the oxygen in the ambiance increases the activities of pectolytic enzymes and causes softening in hardness (Cemeroğlu et al., 2001).

CH and CHS coating increased fruit hardness in comparison with control samples after coating ($P \leq 0.05$). Xiao et al., (2011) have stated that the hardnesses formed may be affected by other factors, and that, when evaluating the results, it is not true to say that the application of chitosan coating protects the fresh-cut fruit hardness. Thus; the obtained data showed that the fruit hardness value of 3.08 on the chitosan decreased to 2.13 on the next day and gave almost the same value as the control sample. It is thought that this increase in the beginning of storage may be due to the resistance developed after the drying of the chitosan. In a study on papaya, it was detected that the hardness loss of chitosan coating was decreased and the hardness value increased due to the increase of chitosan concentration (Ali et al., 2011). Qi et al., (2011) reported that softening of chitosan coating was retarded and hardness loss was minimized. As seen in this study, chitosan increased the hardness value of fruit at the beginning of storage but on other days there was no statistically difference among the control sample ($P > 0.05$).



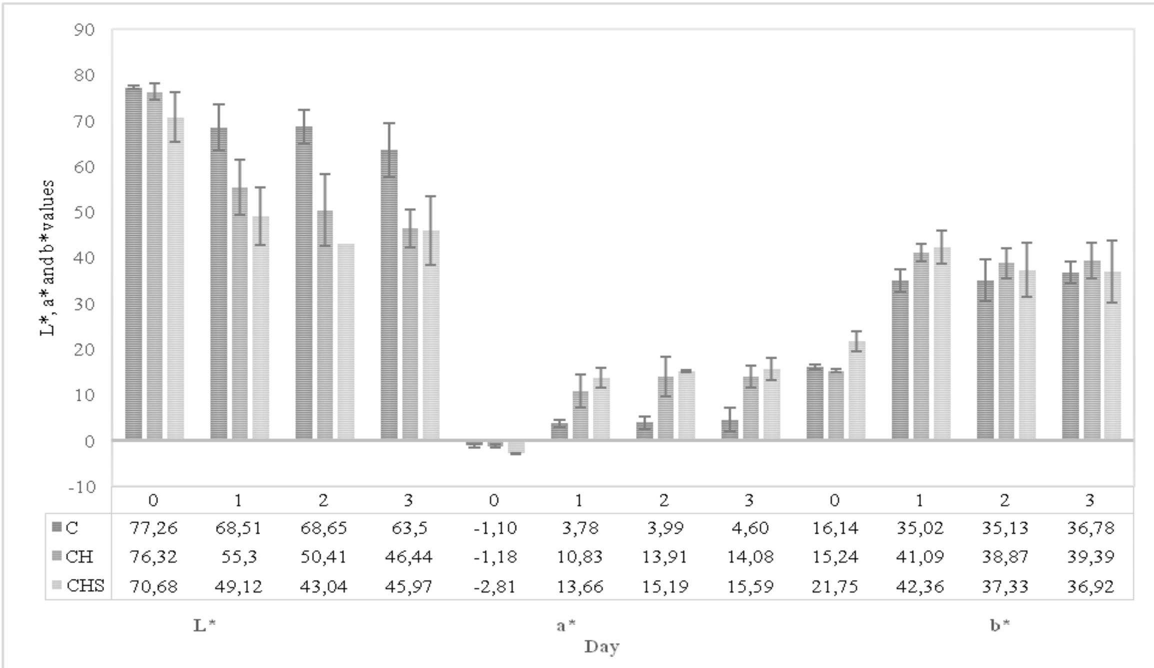
* n=4, (┬ ┴ standard deviation)

Figure 3. Hardness values of C (Control), CH (Chitosan) and CHS (Chitosan-Stevia) samples

Color values: Browning of fresh cut apples are mainly indicated by a decrease in the brightness of the fruit hardness (L* value) and an increase in the a* (redness) value which means a decrease in the hue angle value (Soliva-Fortuny et al., 2001). In this direction, according to the results, a decrease in L* value of fruit and an increase in fruit a* and b* value of C, CH and CHS samples were seen at the end of storage period ($P \leq 0.05$) (Figure 4). In addition, the most significant decrease in fruit L* values and the most significant increase in fruit a* and b* values in all samples were seen between 0th and 1st day. However, it is clear that L*, a* and b* values of the CHS coated samples were different from the other samples. It is thought that this difference due to the fact that the film formulation has different L*, a* and b* values.

At the beginning of storage, the lowest fruit a* and L* values and the highest fruit b* values were observed in the CHS coated samples, and the difference between C sample was statistically significant ($P \leq 0.05$). At the end of the storage, the L* value of C sample is higher than other samples.

On the other hand, it was stated that in rose apples the chitosan coating decreased the L* value and caused the fruit to become a little darker. However, because of chitosan application can reduce the respiration rate and ethylene production, a decrease in color change may occured (Ali et al., 2011). Qi et al. (2011) reported that a decrease in L* and an increase in the value of a* occurred due to the enzymatic browning during storage of the apple slices. Compared to the control samples, the apples coated with chitosan were discovered to exhibit almost stable L* and a* values at 20 °C for 24 hours, and they indicated that chitosan inhibited browning in apple pieces, and that ascorbic acid and citric acid effectively increased anti-browning activity.



* n=4, (┬┴ standard deviation)

Figure 4. L*, a* and b* values of C (Control), CH (Chitosan) and CHS (Chitosan-Stevia) samples

Table 1. ΔE, ΔC and Hue° values of C (Control), CH (Chitosan) and CHS (Chitosan- Stevia) samples

	Storage time (day)					
Samples	0			1		
	ΔE	ΔC	Hue°	ΔE	ΔC	Hue°
C	0.00±0 aA	0.00±0 aA	82.96±1 aA	21.64±3 bA	19.7±1 bA	83.82±1 aA
CH	1.39±0 aB	1.21±0 aB	85.56±2 aA	35.52±4 bB	28.10±1 bB	75.23±4 bB
CHS	8.48±0 aC	5.67±0 aC	82.62±0 aA	41.08±2 bB	30.14±3 bB	69.93±10 bB
	2			3		
C	21.64±6 bA	19.91±5 bA	83.30±1 aA	25.66±5 bA	21.67±2 bA	83.30±3 aA
CH	38.82±4 bB	27.92±1 bB	70.92±6 bB	41.63±1 bB	28.27±1 bB	70.32±3 bB
CHS	42.45±2 bB	25.43±4 bB	56.79±0 bC	41.36±2 bB	26.71±5 bB	67.10±6 bB

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

Findings of changes in fruit ΔE, ΔC, hue angle values were seen in Table 1. When the fruit ΔE and ΔC values of C, CH and CHS samples were examined, an increase was observed in all samples (*P* ≤0.05). At the storage end ΔE and ΔC values of all samples, it was seen that the film-coated samples show higher values than the C sample (*P* ≤0.05). In addition, during the storage period the largest color change was seen between the 0th and 1st days. During the storage period of the CH and CHS coated samples, the fruit hue angle values decreased per day (*P* ≤0.05). However, in the C sample, the difference is not statistically significant (*P* >0.05). At the beginning of storage there was no statistically significant difference between the hue angle values of the film-coated and control samples (*P* >0.05), but there was a statistically significant difference between the storage end values (*P* ≤0.05). The storage end hue angle values of film coated samples were lower than control sample. There is no statistical difference between storage end values of CH and CHS coated samples (*P* >0.05). Sánchez et al. (2015) reported that the decrease in hue angle showed a similar tendency in all treatments, and after 10 days, they observed only a slight difference between control and chitosan coated pear slices. In addition, similar effects of chitosan were observed in color preservation of fresh cut fruits such as strawberry, papaya, peach, pear, kiwi and mango (Du et al., 1997; Chien et al., 2007; Campaniello et al., 2008; Gonzalez-Aguilar et al., 2008).

Sensory analysis results: Sensory evaluation results of film-coated and uncoated samples at the beginning of storage are given in Table 2. Sensory analysis was not performed on the other days of storage due to deterioration of sensory quality.

Table 2. Sensory analysis values of C (Control), CH (Chitosan), and CHS (Chitosan-Stevia) samples starting from storage (0-5p)

Samples	Sweetness	Sourness	Aroma	Brittleness	Hardness	Acceptability
C	$4.8±0.4^B$	$0.4±0.7^A$	$4.7±0.5^C$	$4.1±0.3^B$	$3.3±0.8^A$	$4.8±0.4^B$
CH	$3.4±0.5^A$	$2.3±1^B$	$3.8±1^B$	$4±0.7^A$	$3.4±0.7^A$	$3.8±1^B$
CHS	$3.0±0.7^A$	$2.6±0.9^B$	$2.2±0.5^A$	$3.6±0.7^A$	$3.1±0.7^A$	$1.9±0.8^A$

* n = 9, (± standard deviation), A, B, C ≤0.05 represent the differences in the same column respectively.

In general, CH and CHS coated apples tasted by the panelists are found to be sour due to acetic acid and ascorbic acid that present in the film. So, the degree of sweetness is thought to be lower than the control sample. The sourish taste is more significant. For this reason, although it is not look like the control samples taste, it is accepted and liked by the sour apple lovers. However, panelists stated that samples are similar with the control samples in terms of hardness and brittleness. CHS coated apples have not much acceptable by the panelists. This is because of herbaceous smell and harsh taste of stevia. Stevia suppressed the flavor of the apple and affected its flavor. However, in terms of other characteristics, they stated that they are similar to chitosan coated sample.

South American origin stevia plant extracts have been used for years in many countries including Japan, China, Korea and Brazil as a calorie-free, natural sweetener (especially sweetening traditional drinks) (İnanç and Çınar, 2009). However, evaluations of the sweetness ratio were differing by the idea that stevia-containing coatings were sweeter. Because of the harsh and sour taste of stevia negative evaluation were made by the panelists in terms of sweetness. However, the control sample was selected as the sweetest sample with 4.8p. The smell of stevia and harsh taste also affected the aroma of apples.

CONCLUSION

Film coating slowed down the respiration rate and stevia was effective on respiration rate. It is effective in prolonging the shelf life of a product.

So, this effect of film coating should be considered. However, the acetic acid and ascorbic acid in the film coating increased the titration acidity. This acidity has also been shown in sensory analysis and perceived as sour by panelists. This may seem negative for Amasya apple, but acceptable for sour apple varieties. There is no weight loss was observed in all samples is one of the important result. Because weight loss is associated with respiration and moisture loss and it will reduce economic losses to minimum. This is an indication that the storage period has been correctly selected. However, fruit hardness is important for consumer to prefer the fruits, especially as apple. CH and CHS increased fruit hardness value at the beginning of storage but in other days, no statistically difference was observed between coated and control samples ($P > 0.05$). But chitosan did not increase the fruit hardness in other days and it protected the hardness. Fruit color changed with enzymatic browning in a very short time in the control samples. However, enzymatic browning occurred in film-coated products after 6-9 hours. This period can be extended by complementary studies. At the end of storage period, a decrease was observed in the fruit L* value of C, CH and CHS samples; and an increase in a* and b* values ($P \leq 0.05$). The fruit L*, a* and b* values of the CHS coated samples were different from the other samples ($P \leq 0.05$). This is due to specific color of stevia. In addition, this property has negative effect on sensorial quality. Other negative effect is herbaceous taste and smell of stevia. For this reason, these negative features of stevia can be eliminated with further studies. And in order to extend the shelf life of the film coating, auxiliary agents can be added or the active-MAP can be applied on the coated apples.

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