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Changes in Bioactive Compounds of Cherry Laurel Fruit (*Prunus Laurocerasus L.*) Treated with Modified Atmosphere Packaging and *Aloe vera* During Shelf Life

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ABSTRACT: In the study, the changes in vitamin C, total phenolic compounds, total monomeric anthocyanin, and antioxidant activity (according to ABTS and FRAP test) of cherry laurel fruits treated with modified atmosphere packaging (MAP) and *Aloe vera* gel kept in shelf life (21±1 °C and 80±5%) for 3 days after fruit were stored in cold storage (0±0.5 °C and 90±5%), were investigated. In general, bioactive compounds decreased with progressing storage periods. In the last shelf life measurement (60+3 days), vitamin C and total phenolics of fruit with MAP and *Aloe vera*-treated were significantly higher than the control. When the anthocyanin content was examined, the anthocyanin content of the fruit kept in MAP was found to be significantly higher than the control fruits. In the last three shelf-life measurements, the antioxidant activity of MAP and *Aloe vera*-treated fruit was measured significantly higher than the control in both ABTS and FRAP tests. As a result, it has been revealed that MAP and *Aloe vera* can be used as a tool to delay the losses of quality that occurs during the shelf life of cherry laurel fruit.

Keywords – *Anthocyanin, Antioxidant, Phenolics, Prunus laurocerasus, Vitamin C*

1. Introduction

In recent years, fruit types with rich nutritional content and different taste are more preferred by consumers. One of these fruits is the cherry laurel (*Prunus laurocerasus* L.) fruit. As well as being easy to digest, cherry laurel fruits can be used as a dietary product in human nutrition with the feature of keeping people full. It is a product that is consumed in abundance as fresh fruit by the people especially in the regions where it is grown but also used as a dried product, pickles, molasses, jam, and marmalade, as a fragrance and flavoring in cake and fruit juice (İslam, 2002; Liyana-Pathirana et al., 2006; Öztürk et al., 2017; Öztürk et al., 2019a).

With the seeds that used for the kidney stones dropping, stomach ulcer and bronchitis treatment, digestive system disorders, bones strengthening, and the acid-base balance of the blood establishing; and fruits that used for the treatment of diuretics, antispasmodic and cough, eczema, and hemorrhoids make this fruit so privileged especially for human health (Karahalil and Şahin, 2011; Yıldız et al., 2014). It is reported that high consumption of fruits and vegetables is associated with the reduction of the negative effects of quarantine in epidemic diseases such as Covid-19, along with stroke, digestive system disorders, cancer, coronary heart diseases, vascular occlusion, and cardiovascular diseases (Karahalil and Şahin, 2011; Muscogiuri et al., 2020). Again, one of the main reasons for the increase of people's interest in fruits with high antioxidant activity is their use as a natural product in the struggle against acute and chronic diseases (cancer, cardiovascular diseases, diabetes, Alzheimer's disease, paralysis, some immune system disorders and aging) caused by oxidative stress (Alasalvar et al., 2005; Sun et al., 2011).

Harvested fruits are metabolically active, undergoing ripening and senescence processes that must be controlled to prolong postharvest quality. Therefore, different strategies can be implemented with the aim to increase the shelf life of fruits. In recent years, modified atmosphere packaging (MAP) and edible film coating technologies such as alginate, chitosan, and *Aloe vera* have been used to slow down metabolic events that are effective in ripening, especially respiration (Sandhya, 2010; Öztürk et al., 2019b; Lufu et al., 2020).

The gas exchange of the atmosphere within the packages provided as a result of the packaging material used of gas diffusion properties and the respiration rate of the plant tissue. As a result of the formation of a semi-permeable layer on the shell surface of the products with coating treatments, moisture loss, respiration, and oxidative reactions are reduced. Thus, the natural ripening process of the fruit is delayed (Vargas et al., 2008).

The study hypothesizes that the MAP and *Aloe vera* treatments can be reduced the quality losses of cherry laurel fruit during the shelf life. It was aimed to determine the effect of MAP and *Aloe vera* treatments on the changes in the bioactive compounds of cherry laurel fruits, which are known to be an important source of antioxidants with their high vitamin and phenolic content, during their shelf life.

2. Material and Methods

2.1. Plant materials and experimental design

In the study, the promising O-44 genotype cherry laurel fruits (*Prunus laurocerasus* L.) that harvested by hand at the 15.0% soluble solids content (SCC) and 3.4 Newton (N) firmness (30 July 2015) were used. Fruits were placed in 5 kg plastic boxes and transferred to the laboratory within 1 hour using a refrigerated vehicle with 10±1.0 °C and 80±5.0% relative humidity.

Fruits the same size, homogeneously colored, uniform and without signs of disease were selected for research. Firstly, fruits were divided into two groups. The first group only distilled water, and the second group 33% *Aloe vera* gel (Forever Living Products, Turkey) were dipped for 5 seconds. Fruits were dried on blotter paper for 1 h under room conditions (21±1 °C and 80 ± 5% relative humidity). Subsequently, the fruits were divided into 2 groups again. Half of these were placed in a modified atmosphere package. The other half was not treated MAP. The trial was set up with 4 different treatments, which were control, MAP, *Aloe vera*, and *Aloe vera*+MAP treatments, respectively. The fruits were placed in the plastic boxes (39x29x21 cm, Plastas, Turkey) that approximately 750 g of fruit in each. Later, the fruits were pre-cooled with cold air for 24 h at 4±0.5 °C and 90±5% relative humidity, and then the MAP packages were closed with clips. Fruits were kept in cold storage for 60 days at 0±0.5 °C and 90±5% relative humidity. In addition to the harvest period, the fruit was taken out of the cold storage on the 15th, 30th, 45th, and 60th days and kept at 21±1 °C and 80±5% relative humidity for 3 days, and then their measurements were made. In each measurement period, 3 boxes were analyzed for each treatment and each box represented a repetition.

2.2. Methods

In each repetition of each treatment in each measurement period, 90 fruit were washed with distilled water, and then the stones were removed by hand. Then, the fruits were chopped with an electric blender and made homogeneous. For the measurement of vitamin C, fruit juice was obtained by passing the pulp through a cheesecloth. For the measurement of phenolic compounds, anthocyanin, and antioxidant activity, fruits were placed in a 50 mL falcon tube and kept at -20 °C until analysis.

For vitamin C measurement, 0.5 mL juice was taken, and 5 mL of 0.5% oxalic acid was added to it. The ascorbic acid test strip was then taken from a sealed gas-tight tube. The reflectometer (Merck RQflex plus 10) was started. The test strip was plunged into the solution for two seconds, then removed from the solution. It was then held for 8 seconds, and reading was performed at the end of the 15th second. Results were stated as mg 100 g^{-1} (Öztürk and Ozer, 2019; Öztürk et al., 2019c).

The total phenolics were measured with the aid of an automated UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) according to principles described by Saraçoğlu et al. (2017). Gallic acid was determined as the standard. The results were stated as μg gallic acid equivalents (GAE) g^{-1} fresh weight (fw).

Total monomeric anthocyanin contents were measured with pH differential method with the principles described by Giusti et al. (1999). The results were expressed as μg cyanidin 3-glucoside (cy-3-glu) g^{-1} fw.

The antioxidant activity of the cherry laurel fruit was measured with two different procedures as Ferric ions (Fe^{+3}) reducing antioxidant power assay (FRAP) (Benzie and Strain, 1996) and ABTS (2.2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity (Pellegrini et al., 1999). Results were stated as μmol Trolox equivalent (TE) g^{-1} fw.

2.3. Statistical analysis

Whether the data was normally distributed was checked by the Kolmogorov-Smirnov Test. Homogeneity control of variances was confirmed by Levene's test. After the variance analysis of the data, Tukey's multiple-comparison test was used to check whether there were significant differences ($p < 0.05$) between treatments. The statistical analyses were performed by using SAS software (SAS 9.1 version, USA).

3. Results

In the study, at the 15th, 30th, and 45th days of shelf life measurements, significantly lower vitamin C has measured in *Aloe vera* treated cherry laurel fruits compared to control, while on the 60th day, the significantly higher vitamin C content of MAP and *Aloe vera* treated fruit than control. At the end of the shelf life, it was also determined that fruits treated with *Aloe vera* had higher vitamin C content than fruits treated with both MAP and *Aloe vera*+MAP (Table 1).

Total phenolic compounds were found significantly higher than control in MAP and *Aloe vera* treated fruits at the 15th, 45th, and 60th days shelf life measurement. However, while the contents of MAP and *Aloe vera* treated fruits were at similar levels on the 15th day, it was determined that the total phenol content of the MAP and *Aloe vera*+MAP treated fruits were significantly higher than compared to the *Aloe vera* treated fruits on the 45th and 60th days. At the 30th day of shelf life measurement, the total phenol content of fruit treated with the only *Aloe vera*+MAP was found to be significantly higher than the control (Table 1).

The total monomeric anthocyanin content of the MAP treated cherry laurel fruits was found to be significantly higher than the control fruits during the shelf life period. However, the anthocyanin content of the fruits treated with *Aloe vera* was found to be significantly higher than the control only in the 45th day shelf life measurement. It was also significantly higher than the control in the shelf life measurement on the 30th and 60th days. Moreover, in the measurement of shelf life on the 30th and 60th days, it was observed that the anthocyanin content of *Aloe vera*+MAP treated fruits was significantly higher than the *Aloe vera* treatment (Table 1).

Table 1. Changes in vitamin C, total phenolics and total monomeric anthocyanin of cherry laurel fruit during shelf life

Treatments	**Vitamin C (mg 100 g^{-1})**				
	0+3	15+3	30+3	45+3	60+3
Control	80.5	76.3 a	68.5 a	53.5 a	35.3 c
MAP	80.5	72.0 a	64.0 ab	54.5 a	43.5 b
Aloe vera	80.5	66.5 b	61.5 b	50.5 ab	50.0 a
***Aloe vera*+MAP**	80.5	62.5 b	56.0 c	45.5 b	44.0 b
	Phenolic compounds (µg GAE g^{-1} fw)				
Control	1304.7	1222.8 b	1211.3 b	1141.9 c	1044.0 c
MAP	1304.7	1268.6 a	1224.1 b	1208.0 a	1173.6 a
Aloe vera	1304.7	1264.7 a	1217.5 b	1181.3 b	1070.2 b
***Aloe vera*+MAP**	1304.7	1279.7 a	1245.3 a	1216.9 a	1198.0 a
	Total monomeric anthocyanin (µg cy-3-glu g^{-1} fw)				
Control	22.3	16.9 b	13.9 c	10.9 b	8.9 c
MAP	22.3	20.5 a	16.9 ab	13.4 a	11.6 ab
Aloe vera	22.3	17.2 b	15.7 bc	12.6 a	9.9 bc
***Aloe vera*+MAP**	22.3	21.5 a	18.6 a	13.9 a	12.4 a

The difference between mean values shown in the same column with the same letter is not significant according to Tukey's range test at $P < 0.05$.

Changes in the ABTS antioxidant activity of cherry laurel fruits are shown in Table 2. It was seen that all treatments had significantly different levels of antioxidant activity in the 15th day measurement, the highest value was obtained from *Aloe vera*+MAP and the lowest value was obtained from the control group fruits. Antioxidant activity was found significantly higher in MAP and *Aloe vera* treated fruits than control in the 30th and 45th days shelf life measurement. At the 45th day measurement, significantly higher antioxidant activity was obtained from the *Aloe vera*+MAP treatment compared to the *Aloe vera* and MAP. At the end of the shelf life, significantly higher antioxidant activity was measured in *Aloe vera*+MAP treated fruit compared to the control.

When the FRAP antioxidant activity test data were examined, as in the ABTS test the antioxidant activity was found to be significantly higher in MAP and *Aloe vera* applied fruits in all measurement periods except for the shelf life measurement on the 15th day. In the 15th day shelf life measurement, the antioxidant activity of only *Aloe vera*+MAP treated fruits was significantly higher than the control. At the same time, it was determined that the antioxidant activity of Aloe vera+MAP treated fruits were significantly higher than the MAP and Aloe vera treated fruits in the 30th and 45th day shelf life measurements, while it was found to be at a similar level on the 60th day (Table 2).

Table 2. Changes in antioxidant activity of cherry laurel fruit during shelf life

Treatments	**ABTS (µmol TE g^{-1})**				
	0+3	15+3	30+3	45+3	60+3
Control	6.12	3.85 d	3.77 c	3.23 c	2.75 b
MAP	6.12	4.74 b	4.24 ab	3.90 b	2.86 ab
Aloe vera	6.12	4.08 c	4.04 b	3.73 b	2.81 b
***Aloe vera*+MAP**	6.12	5.05 a	4.32 a	4.27 a	3.03 a
	FRAP (µmol TE g^{-1})				
Control	4.13	3.47 b	2.83 c	2.57 c	2.39 b
MAP	4.13	3.68 b	3.28 b	3.24 b	2.63 a
Aloe vera	4.13	3.66 b	3.21 b	3.14 b	2.56 a
***Aloe vera*+MAP**	4.13	4.48 a	3.60 a	3.56 a	2.66 a

ABTS: 2.2-azino-bis- 3-ethylbenzothiazoline-6-sulfonic acid. FRAP: Ferric ions (Fe^{+3}) reducing antioxidant power. The difference between mean values shown in the same column with the same letter is not significant according to Tukey's range test at $P < 0.05$.

4. Discussion

In the world, in parallel with the rapid population growth, the need for food is increasing day by day. While a certain part of the population meets their food needs sufficiently, the rest cannot reach the food they need. On the other hand, the section that can reach food has the desire to consume fruits that are suitable for different tastes. Muscogiuri et al. (2020) reported that the consumption of cherries may play an important role in reducing the negative effects of the quarantine process in epidemic diseases (such as Covid-19). Cherry laurel fruits attracting the attention of consumers in recent years in Turkey due to its rich vitamins and phenolic compounds. Cherries are fruit species with a short storage life. It rapidly loses its quality after harvest. In this respect, quality losses can be delayed with postharvest technologies (Wani et al., 2014). In our study, it is seen that there are losses in the contents during storage. However, on the 60th day measurement, it was determined that the loss of vitamin C, total phenolics, total monomeric anthocyanin, and antioxidant activity was delayed by MAP and *Aloe vera* treatments. In the study, MAP and *Aloe vera* treatments were effective in delaying the losses. The possible reason for the lower loss of vitamin C in MAP and *Aloe vera* coating treatment is the prevention of self-oxidation that occurs when ascorbic acid is combined with oxygen in the air. The MAP and *Aloe vera* coating treatment act as a protective layer outside the fruit by controlling the oxygen and carbon dioxide permeability (Diaz-Mula et al., 2011; Guillen et al., 2013).

Treatments such as MAP and *Aloe vera* are reported to delay the accumulation of phenolic compounds and anthocyanins by inhibiting ethylene synthesis (Carrillo-Lopez et al., 2000; Artes et al., 2006). In this study, on the contrary, total phenolics and anthocyanin content of *Aloe vera*+MAP treatment were found to be higher than the control during the shelf life. As a result, the antioxidant activity of fruits that were treated with *Aloe vera*+MAP in general during the shelf life was higher than the control treatment. Similarly, Vieira et al. (2016) in blueberry fruits treated with *Aloe vera*; Sophia et al. (2015) in mango fruits preserved in MAP, found higher phenolic compounds and antioxidant activity compared to control fruits.

5. Conclusion

As a result, it can be stated that vitamin C, phenolic compounds, and anthocyanin content, which contributes significantly to antioxidant activity during the shelf life, can be preserved for a longer time with MAP and *Aloe vera* coating treatments.

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