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Research Article/Araştırma Makalesi

Effects of hot water, calcium chloride and 1-MCP on the activity of cell wall degrading enzymes in sweet cherry (*Prunus avium*)

Sıcak su, kalsiyum klorür ve 1-MCP' nin kirazda (Prunus avium) hücre duvarını parçalayıcı enzimlerinin aktiviteleri üzerindeki etkileri

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ABSTRACT

The objective of the study was to determine the changes in the activities of the enzymes degrading the cell wall in response to hot water, calcium chloride ($CaCl_2$) and 1-methlylcyclopropene (1-MCP) treatments during post-harvest storage in the sweet cherries. For this purpose, 0.5, 1, and 5 µl L^{-1} 1-MCP, 1% and 2% $CaCl_2$ and 45, 50, and 55 ^oC hot waters were applied to the sweet cherries. As a result of the study conducted with the enzymes degrading the cell wall, it was observed that very significant increases occured during the storage in the activities of polygalacturonase, pectin methyl esterase, xyloglucanase, beta 1-4 endoglucanase and alpha and beta galactosidases. Therefore, it seems that these enzymes make a significant contribution to the softening observed during the storage after harvesting in sweet cherries. The changes observed in the activities of the enzymes degrading the cell wall have been significantly reduced by the treatments. As a result, 1-MCP, $CaCl_2$ and hot water treatmens can be used to prolong the postharvest life in sweet cherries by delaying the softening and preventing the quality losses observed after harvesting in the cherries.

Key Words: 1-MCP, $CaCl_2$, Polygalacturonase, Pectin methyl esterase, Xyloglucanase

ÖZ

Çalışmada, kirazda hasat sonrası depolama sırasında sıcak su, $CaCl_2$ ve 1-MCP uygulamalarına cevaben hücre duvarını parçalayıcı enzimlerin aktivitelerindeki değişimlerin belirlenmesi amaçlanmıştır. Bu amaç doğrultusunda kiraz meyvelerine, 0.5, 1, ve 5 µl L^{-1} konsantrasyonlarında 1-MCP, % 1 ve % 2'lik $CaCl_2$ ve 45, 50, ve 55^oC sıcak su uygulamaları yapılmıştır. Hücre duvarını parçalayıcı enzimler ile yapılan çalışma sonucunda poligalakturonazlar, pektin metil esterazlar, ksiloglukanazlar, beta 1-4 endoglukanazlar ve alfa ve beta galaktosidazların aktivitelerinde depolama sırasında çok önemli artışların gerçekleştiği görülmektedir. Dolayısıyla bu enzimlerin kirazda hasattan sonra depolama sırasında görülen yumuşamaya önemli katkılarda bulundukları görülmektedir. Hücre duvarını parçalayıcı enzimlerin aktivitelerinde görülen değişimler yapılan uygulamalarla önemli ölçüde azaltılmıştır. Sonuç olarak kirazlarda 1-MCP, $CaCl_2$ ve sıcak su uygulamaları kirazda yumuşamayı geciktirmek suretiyle hasat sonu ömrün uzatılması ve hasattan sonra görülen kalite kayıplarının önüne geçilmesi amacıyla kullanılabilir.

Anahtar Kelimeler: 1-MCP, $CaCl_2$, Poligalaktronaz, Pektin metil esteraz, Ksiloglukanaz

Introduction

While sweet cherry production in the world is 2.609.550 tons, Turkey ranks first with 724.944 tons (Faostat, 2020). At least 25-40% of the fresh fruits produced are thrown away before they reach to the consumer. Sweet cherry (*Prunus avium* L.) is a fruit species with a high market

value due to its seasonal production and the contents of anthocyanins and other health-promoting compounds. Although they are non-climacteric fruits, the sweet cherries undergo biochemical changes like climacteric fruits and attain ideal quality characteristics such as the sweetness, softness (cell wall softening), decrease in acidity, and increases in polyphenols and anthocyanins.

Sweet cherry is a very delicate type of fruit. One of the main problems encountered in the sale and transportation of sweet cherries is that it has a short postharvest shelf life. Due to the short production season and high consumer demand, it is very important to preserve the quality and extend the postharvest life of sweet cherry fruits. The cold storage of sweet cherry fruit can slightly extend the shelf life. As the storage period increases, fruit quality decreases significantly. The most important sweet cherry quality characteristics for consumer preferences are skin color, fruit firmness and size (Sloulin, 1990). The quality loss is mostly due to the increases in the fruit firmness and pectin solubility during storage (Fils-Lycaon and Buret, 1990). Therefore, the cell wall enzymes and changes in the activities of these enzymes are important. These changes have been associated with the fruit firmness (Meheriuk et al., 1997, Esti et al., 2002). The change in the firmness correlates with the change in the skin color (Bernalte et al., 1999).

1-MCP, calcium chloride and hot water treatments are frequently used in order to extend the shelf life of fruit after harvest. When 1-MCP is applied to the plant, it binds to ethylene receptors, preventing ethylene from binding to this region, and therefore slowing down the rate of ethylene-related biochemical reactions (Kasım and Kasım, 2007). Post-harvest calcium application not only prevents cell wall deterioration but also prevents softening of fruits and vegetables by maintaining proper cell membrane function and turgor pressure (Pareek, 2017). Hot water application in fruits can be preferred to eliminate fungal factors and prevent chilling damage. It is stated that this physiological disorder, which occurs especially in early harvested apples, can be reduced by temperature applications (Calhan, 2014).

This rapid loss of the quality in sweet cherry fruit creates a problem in marketing and the producer cannot deliver the product to distant markets, which negatively affects both the producers and the country's economy, and reduces the flow of money arising from the marketing of sweet cherry in our country.

In this study, it was aimed to determine the changes in the activities of cell wall-degrading enzymes in response to the hot water, calcium chloride and 1-MCP treatments during the storage in sweet cherry.

Material and Methods

The sweet cherry used in the study (‘0900 Ziraat’ cultivar) was obtained from a local cherry producer in Isparta, Türkiye. According to the results of soil analysis, it was stated that the orchard was suitable for cherry cultivation (Senol et al., 2020).The sweet cherry fruit were hand-harvested at the commercial maturity stage based on the criterias including the fruit skin color, size, number of days since flowering and fruit flesh firmness.

Immediately after the harvest, the fruit were taken to a 4^{o}C and 90±5% relative humidity (RH) conditions and transported to the laboratory after the necessary treatments were made from there, they were taken to the storage. The sweet cherry fruit brought to the laboratory were controlled and the damaged, stained and diseased ones were discarded. Then, the remaining fruit were separated into different groups for the treatments. After that the fruit were treated with 1-MCP, $CaCl_2$, hot water, 1-MCP + $CaCl_2$, 1-MCP + hot water, 1-MCP + hot water + $CaCl_2$, $CaCl_2$ + hot water. The treatments were carried out as given below.

1-MCP treatment

1-MCP was applied at the concentrations of 0.5, 1, and 5 µl L^{-1} at 4^{o}C and 90±5% RH in the 2-

liter glass jars for 24 h (Ergun et al., 2007; Sharma et al., 2010).

CaCl2 treatment

For $CaCl_2$ treatment, 1% and 2% solutions were prepared and sweet cherry fruit were dipped into these solutions and kept for two min (Aguayo et al., 2006).

Hot water treatment

Different temperature and time combinations are used in the hot water treatment. For this purpose, the fruit were treated with 45, 50, and 55°C temperatures for 45, 60 and 75 s (Karabulut et al., 2004).

Fruit that were not subjected to any treatment were selected as control treatment. After the treatment procedures were completed, the sweet cherry fruit were kept in the storage at 4°C and 55-60% RH until they rotted. Each treatment was performed with 5 replications and 20 fruit were used for each replication. The samples were taken from each treatment and the control at four-day intervals (0, 4, 8, 12, etc.), frozen with liquid nitrogen, and stored for analysis at -80°C.

Polygalacturonase, pectin methyl esterase, alpha and beta galactosidase, beta 1,4 endoglucanase and xyloglucanase activities of the cell wall degrading enzymes were determined using the methods as described by Karakurt and Huber (2002 and 2003).

Statistical analysis

The data were subjected to the analyses of variance (ANOVA) at $p \leq 0.05$ using MINITAB statistical software (MINITAB Inc., Coventry, UK). Means were separated by Tukey's multiple range test at $p \leq 0.05$.

Results and Discussion

Polygalacturonase enzyme activity showed significant changes both in the control group and in response to 1-MCP, $CaCl_2$ and hot water treatments (Table 1). In the control group, the enzyme activity increased significantly from the beginning of the storage and reached to the maximum level on the tenth day of storage. Then, the activity decreased towards the end of the storage.

Table 1. The changes in the polygalacturonase activity (µmol galacturonic acid mg protein^{-1} min $^{-1}$) in response to different 1-MCP, $CaCl_2$ and hot water treatments during the storage

Treatment	**Storage period (day)**						
	0	5	10	15	20	25	30
Control	3.12c	3.94c	6.74a	5.15b	4.12bc	3.7c	1.4d
1-MCP (0.5 µl L^{-1})	3.12bc	3.20bc	5.10a	3.62bc	3.02bc	2.65c	1.2d
1-MCP (1 µl L^{-1})	3.12ab	3.20ab	3.8a	3.5ab	2.4bc	1.6cd	1.1d
1-MCP (5 µl L^{-1})	3.12a	3.16a	3.3a	3.6a	3.0a	1.2b	0.8b
$CaCl_2$ 1%	3.12cd	3.25cd	4.75ab	5.03a	3.9bc	2.2de	1.3e
$CaCl_2$ 2%	3.12b	3.8b	5.2a	5.1a	3.6b	1.9c	1.1c
Hot water 45 °C 45 s	3.12c	3.8c	6.8a	6.1ab	5.2b	3.3c	1.9d
Hot water 45 °C 60 s	3.12c	3.7c	6.9a	6.3ab	5.7b	2.9c	1.3d
Hot water 45 °C 75 s	3.12c	3.5c	6.7a	6.0ab	5.2b	2.8c	1.4d
Hot water 50 °C 45 s	3.12b	3.3b	6.4a	5.5a	4.2b	3.1b	1.2c
Hot water 50 °C 60 s	3.12c	3.4bc	6.5a	5.5a	4.3b	3.0c	1.3d
Hot water 50 °C 75 s	3.12bc	3.2bc	6.1a	5.2a	3.8b	2.6c	1.1d
Hot water 55 °C 45 s	3.12c	3.3c	5.8a	4.6b	3.2c	1.9d	1.1d
Hot water 55 °C 60 s	3.12b	3.1b	5.2a	3.3b	2.6bc	1.7cd	1.0d
Hot water 55 °C 75 s	3.12b	3.14b	4.9a	3.1b	2.2bc	1.4cd	0.8d

The difference between the means of different letters in the same line is significant ($p<0.05$)

The increase in the polygalacturonase activity was significantly affected by the treatments. The greatest decrease in the enzyme activity occurred in 5 µL L^{-1} 1-MCP treatment (3.6), followed by 55 °C 75 s hot water (4.9) and 1% $CaCl_2$ (5.03) treatments, respectively. In the 5 µL L^{-1} 1-MCP treatment, there was no significant change in the enzyme activity until the 20th day of storage,

while a significant decrease was observed in the activity towards the end of the storage. $CaCl_2$ treatments also caused significant decreases in the polygalacturonase enzyme activity. The highest decrease was observed in the 2% $CaCl_2$ treatment. With this treatment, the enzyme activity increased significantly on the 15th day of storage, but then decreased significantly towards the end of the storage. Similarly, hot water treatments caused significant decreases in the polygalacturonase enzyme activity compared to the control. Among the hot water treatments, the most effective one was the 55 ˚C 75 s treatment, and in this treatment, a significant increase in the enzyme activity was obtained on the 10th day of storage. However, significant decreases in the activity were obtained towards the end of the storage.

Alpha galactosidase enzyme activity showed significant changes both in the control group and in response to the 1-MCP, $CaCl_2$ and hot water treatments (Table 2). In the control group, the enzyme activity increased significantly from the beginning of the storage and reached to its maximum level on the 15th day of storage. Then the activity decreased towards the end of the storage.

Table 2. The changes in the alpha galactosidase activity (mol pNO_2 phenol kg protein^{-1} min^{-1}) in response to different 1-MCP, $CaCl_2$ and hot water treatments during the storage.

Treatment	**Storage period (day)**						
	0	5	10	15	20	25	30
Control	2.2e	2.9de	4.9ab	5.2a	4.1bc	3.3cd	2.1e
1-MCP (0.5 µl L^{-1})	2.2c	2.8bc	5.1a	5.1a	3.7b	2.9bc	1.9c
1-MCP (1 µl L^{-1})	2.2bc	2.6bc	4.1a	4.1a	3.2ab	2.1c	1.8c
1-MCP (5 µl L^{-1})	2.2ab	2.4ab	2.9a	2.9a	2.2ab	1.7bc	1.1c
$CaCl_2$ 1%	2.2b	2.8b	4.7a	5.3a	4.4a	3.2b	2.3b
$CaCl_2$ 2%	2.2c	2.7bc	4.6a	4.9a	3.4b	2.7bc	1.9c
Hot water 45 ºC 45 s	2.2bc	2.8bc	5.1a	5.7a	4.8a	3.2b	2.1c
Hot water 45 ºC 60 s	2.2b	2.6b	5.0a	5.3a	4.6a	2.5b	1.7b
Hot water 45 ºC 75 s	2.2c	2.5c	4.6ab	5.0a	3.9b	2.1c	1.9c
Hot water 50 ºC 45 s	2.2b	2.4b	4.1a	4.5a	3.7a	1.9b	1.6b
Hot water 50 ºC 60 s	2.2bc	2.2bc	4.0a	4.0a	3.2ab	2.7bc	1.9c
Hot water 50 ºC 75 s	2.2ab	2.3ab	2.9a	3.1a	2.6ab	2.2ab	1.6b
Hot water 55 ºC 45 s	2.2b	2.4ab	2.8a	3.4a	3.1a	2.1b	1.5b
Hot water 55 ºC 60 s	2.2ab	2.2ab	2.6ab	2.8a	2.4ab	1.9ab	1.7b
Hot water 55 ºC 75 s	2.2ab	2.1ab	2.4ab	3.1a	2.1ab	2.1ab	1.6b

The difference between the means of different letters in the same line is significant ($p<0.05$).

The increase in the alpha-galactosidase activity was significantly affected by the treatments. The highest decrease in the enzyme activity occurred in 5 µLL^{-1} 1-MCP treatment (2.9), followed by 55 ˚C 60 s hot water (2.9) and 2% $CaCl_2$ (4.9) treatments, respectively. In the 5 µL L^{-1} 1-MCP treatment, there was no significant change in the enzyme activity until the 20th day of storage, while a statistically significant decrease was observed in the activity towards the end of the storage. $CaCl_2$ treatments also caused significant decreases in the alpha galactosidase enzyme activity. The highest decrease was observed in the 2% $CaCl_2$ treatment. With this treatment, the enzyme activity increased significantly on the 10th day of storage, but then decreased significantly towards the end of the storage. Similarly, hot water treatments caused significant decreases in the alpha galactosidase enzyme activity as compared to the control. Among the hot water treatments, the most effective one was the 55 ˚C for 60 s treatment, and in this treatment, the enzyme activity did not change significantly until the 25th day of storage. However, a significant decrease in the activity was obtained towards the end of the storage.

Beta galactosidase enzyme activity showed significant changes both in the control group and in response to 1-MCP, $CaCl_2$ and hot water treatments (Table 3). In the control group, the

enzyme activity increased significantly from the beginning of the storage and reached to its maximum level on the tenth day of the storage. Then, the activity decreased towards the end of the storage.

Table 3. The changes in beta galactosidase activity (mol pNO_2 phenol kg protein^{-1} min^{-1}) in response to different 1-MCP, $CaCl_2$ and hot water treatments during the storage

Treatment	Storage period (day)						
	0	5	10	15	20	25	30
Control	3.4cd	4.9bc	7.2a	6.3ab	4.1c	3.2d	2.8d
1-MCP(0.5µl L^{-1})	3.4bc	5.6a	6.7a	5.8a	3.7b	2.6bc	1.9c
1-MCP(1 µl L^{-1})	3.4b	3.5b	5.6a	5.1a	3.2b	2.1bc	1.6c
1-MCP (5 µl L^{-1})	3.4a	3.4a	3.2a	3.1ab	2.5ab	1.6bc	0.9c
$CaCl_2$ 1%	3.4cd	3.8c	6.2a	6.6a	5.8ab	4.3bc	2.2d
$CaCl_2$ 2%	3.4de	3.9cd	6.6ab	6.2a	5.1bc	3.1de	2.1e
Hot water 45 °C 45 s	3.4e	5.1cd	8.3a	6.8ab	5.7bc	4.1de	2.9e
Hot water 45 °C 60 s	3.4d	5.2c	8.1a	7.3ab	6.4bc	5.2c	3.1d
Hot water 45 °C 75 s	3.4cd	4.9bc	7.4a	6.3ab	5.2b	3.7cd	2.5d
Hot water 50 °C 45 s	3.4cd	4.2c	6.8a	5.9ab	4.4bc	3.1cd	2.1d
Hot water 50 °C 60 s	3.4cd	4.1bc	6.9a	5.4ab	3.7c	3.0cd	2.1d
Hot water 50 °C 75 s	3.4ab	3.9a	3.5ab	3.4ab	3.2ab	2.9ab	2.2b
Hot water 55 °C 45 s	3.4ab	3.8a	4.9a	4.8a	3.7a	3.2b	2.1b
Hot water 55 °C 60 s	3.4a	3.7a	4.5a	4.1a	3.2ab	2.5b	1.8b
Hot water 55 °C 75 s	3.4ab	3.4ab	4.6a	3.9a	2.8b	2.8b	2.2b

The difference between the means of different letters in the same line is significant ($p<0.05$).

The increase in the beta galactosidase activity was significantly affected by the treatments. The greatest decrease in the enzyme activity was occurred in 5 µL L^{-1} 1-MCP treatment (0.9), followed by 55 °C 60 s hot water (1.8) and 2% $CaCl_2$ (2.2) treatments, respectively. In the 5 µL L^{-1} 1-MCP treatment, there was no significant change in the enzyme activity until the 20th day of storage, but a significant decrease was observed in the activity towards the end of the storage. $CaCl_2$ also caused significant decreases in the beta galactosidase enzyme activity as compared to the control. The greatest decrease was observed in the 2% $CaCl_2$ treatment. With this treatment, the enzyme activity increased significantly on the 10th day of storage, but then decreased significantly towards the end of the storage. Similarly, the hot water treatments caused significant decreases in the beta galactosidase enzyme activity as compared to the control. Among the hot water treatments, the most effective one was the 55 °C 60 sec treatment, and in this treatment, the enzyme activity did not show a significant change until the 20th day of storage. However, significant decreases in the activity were obtained towards the end of the storage.

Pectin methyl esterase enzyme activity showed significant changes both in the control group and in response to the 1-MCP, $CaCl_2$ and hot water treatments (Table 4). In the control group, the enzyme activity increased significantly from the beginning of the storage and reached to its maximum level on the tenth day of storage. The activity then decreased towards the end of the storage.

The increase in the pectin methyl esterase activity was significantly affected by the treatments. The greatest decrease in the enzyme activity occurred in 5 µL L^{-1} 1-MCP treatment (0.8), followed by 55 °C 75 s hot water (0.6) and 2% $CaCl_2$ (1.1) treatments, respectively. In the 5 µL L^{-1} 1-MCP treatment, there was no significant change in the enzyme activity until the 25th day of storage, while there was a significant decrease in the activity towards the end of the storage. $CaCl_2$ treatments also caused significant decreases in the pectin methyl esterase enzyme activity. The highest decrease was observed in the 2% $CaCl_2$ treatment. With this treatment, the enzyme activity increased significantly on the 10th day of storage, but then decreased significantly towards the end of the storage. Similarly, the hot water

treatments caused significant decreases in the pectin methyl esterase enzyme activity as compared to the control. Among the hot water treatments, the most effective treatment was 55 ˚C 75 sec. In this treatment, the enzyme activity did not show a significant change until the 20th day of storage. However, significant decreases in the activity were obtained towards the end of the storage.

Table 4. The changes in pectin methyl esterase activity (mol H^+ equivalent kg protein^{-1} min^{-1}) in response to different 1-MCP, $CaCl_2$ and hot water treatments during the storage

Treatment	Storage period (day)						
	0	5	10	15	20	25	30
Control	1.7d	2.2cd	3.9a	3.6ab	2.9bc	2.1cd	1.8d
1-MCP(0.5 µl L^{-1})	1.7c	2.1c	3.8a	3.1ab	2.2bc	1.6c	1.6c
1-MCP (1 µl L^{-1})	1.7bc	2.2ab	3.1a	2.7a	2.0bc	1.7bc	1.2c
1-MCP (5 µl L^{-1})	1.7ab	2.0a	2.1a	1.9a	1.9a	1.2ab	0.8b
$CaCl_2$ 1%	1.7d	2.3cd	3.6ab	3.9a	2.8bc	2.1cd	1.8d
$CaCl_2$ 2%	1.7bc	2.3b	3.7a	3.7a	2.4b	1.9bc	1.1c
Hot water 45 ºC 45 s	1.7d	2.2cd	3.8a	4.1a	3.4ab	2.9bc	2.1cd
Hot water 45 ºC 60 s	1.7c	2.1c	3.6a	3.9a	3.1ab	2.4bc	1.8c
Hot water 45 ºC 75 s	1.7c	2.0bc	3.5a	3.5a	2.9ab	2.4bc	1.9c
Hot water 50 ºC 45 s	1.7b	2.0b	3.6a	3.4a	3.4a	2.2b	1.9b
Hot water 50 ºC 60 s	1.7c	1.8c	3.4a	3.1ab	2.8ab	2.2bc	1.6c
Hot water 50 ºC 75 s	1.7bc	1.5c	3.1a	2.5ab	2.1b	1.8bc	0.9c
Hot water 55 ºC 45 s	1.7bc	1.5bc	3.1a	2.6ab	2.2abc	1.9b	1.5c
Hot water 55 ºC 60 s	1.7bc	1.6bc	2.8a	2.3ab	2.0ab	1.7bc	0.8c
Hot water 55 ºC 75 s	1.7abc	1.3bc	2.4a	2.1ab	2.1ab	1.2bc	0.6c

The difference between the means of different letters in the same line is significant ($p<0.05$).

The beta 1,4 endoglucanase enzyme activity showed significant changes both in the control group and in response to 1-MCP, $CaCl_2$ and hot water treatments (Table 5). In the control group, the enzyme activity increased significantly from the beginning of the storage and reached its maximum level on the tenth day of storage. The activity then decreased towards the end of the storage.

Table 5. The changes in the beta 1,4 endoglucanase activity (Unit mg protein^{-1}) in response to different 1-MCP, $CaCl_2$ and hot water treatments during the storage

Treatment	Storage period (day)						
	0	5	10	15	20	25	30
Control	2.9b	3.6b	5.9a	5.5a	4.1b	3.6b	1.2c
1-MCP (0.5 µl L^{-1})	2.9bc	3.4b	5.5a	4.9a	3.6b	3.6b	1.8c
1-MCP (1 µl L^{-1})	2.9ab	3.4ab	4.1a	3.8a	3.2a	2.4b	1.1c
1-MCP (5 µl L^{-1})	2.9a	2.8a	3.2a	3.1a	2.4a	2.1a	0.8b
$CaCl_2$ 1%	2.9bc	2.9bc	3.9b	5.2a	3.7b	3.1bc	2.2c
$CaCl_2$ 2%	2.9cd	3.1cd	5.4a	5.1ab	3.9bc	3.0cd	1.9d
Hot water 45 ºC 45 s	2.9d	3.7cd	6.1a	5.8ab	4.6bc	3.8cd	2.6d
Hot water 45 ºC 60 s	2.9cd	3.1cd	6.4a	6.2a	4.8b	4.1bc	2.3d
Hot water 45 ºC 75 s	2.9bc	3.4b	6.0a	5.2a	3.7b	3.7b	1.9c
Hot water 50 ºC 45 s	2.9c	3.1c	5.2a	4.8ab	3.8bc	2.7c	1.4d
Hot water 50 ºC 60 s	2.9c	3.1bc	4.9a	4.2ab	3.1bc	2.2cd	1.0d
Hot water 50 ºC 75 s	2.9a	2.9a	3.4a	3.1a	2.8a	2.8a	2.2a
Hot water 55 ºC 45 s	2.9b	2.8b	4.9a	4.5a	4.1a	2.3b	2.0b
Hot water 55 ºC 60 s	2.9ab	3.0ab	3.4a	3.4a	2.7ab	2.2ab	1.9b
Hot water 55 ºC 75 s	2.9ab	2.9ab	3.2a	3.2a	3.1a	2.5ab	1.7b

The difference between the means of different letters in the same line is significant ($p<0.05$).

The increase in the beta 1,4 endoglucanase activity was significantly affected by the treatments. The greatest decrease in the enzyme activity occurred in the 5 µL L^{-1} 1-MCP treatment (3.2), followed by 55 ˚C 75 s hot water (3.2) and 1% $CaCl_2$ (5.2) treatments, respectively. In the 5 µL L^{-1} 1-MCP treatment, there was no significant change in the enzyme activity until the 25th day of storage, while a significant decrease was observed in the activity towards the end of the storage. $CaCl_2$ treatments also caused significant decreases in the beta 1,4 endoglucanase enzyme activity. The highest decrease was observed in the 5 µL L^{-1} 1-MCP treatment. With this treatment, the enzyme activity increased significantly on the 10th day of storage, but then decreased significantly towards the end of the storage. Similarly, the hot water treatments caused significant decreases in the beta 1,4 endoglucanase enzyme activity as compared to the control. Among the hot water treatments, the most effective treatment was 55 ˚C 75 sec. In this treatment, the enzyme activity did not show a significant change until the 25th day of storage. However, a significant decrease in the activity was obtained towards the end of the storage.

Xyloglucanase enzyme activity showed significant changes both in the control group and in response to 1-MCP, $CaCl_2$ and hot water treatments (Table 6). In the control group, the enzyme activity increased significantly from the beginning of the storage and reached to its maximum level on the tenth day of storage. The activity then decreased towards the end of the storage.

Table 6. The changes in the xyloglucanase activity (Unit mg $protein^{-1}$) in response to different 1-MCP, $CaCl_2$ and hot water treatments during the storage

Treatment	**Storage period (day)**						
	0	5	10	15	20	25	30
Control	1.6cd	2.4bc	3.6a	3.2ab	2.8ab	1.4d	1.12d
1-MCP (0.5 µl L^{-1})	1.6cd	2.3bc	3.2a	2.8ab	2.1bc	1.1d	0.9d
1-MCP (1 µl L^{-1})	1.6bcd	2.2b	3.1a	2.4ab	1.7bcd	1.2cd	0.8d
1-MCP (5 µl L^{-1})	1.6abc	2.0ab	2.3a	1.9abc	1.4bc	1.1cd	0.5d
$CaCl_2$ 1%	1.6c	2.2bc	3.8a	2.6b	2.1bc	2.1bc	1.4c
$CaCl_2$ 2%	1.6bc	2.1b	3.4a	2.3b	1.9b	1.6bc	0.9c
Hot water 45 ºC 45 s	1.6d	1.9d	3.7ab	4.1a	2.9bc	2.3cd	1.6d
Hot water 45 ºC 60 s	1.6cd	1.8cd	3.2ab	3.4a	2.4bc	1.9cd	1.2d
Hot water 45 ºC 75 s	1.6d	1.6d	3.4ab	3.8a	2.6bc	2.1cd	1.4d
Hot water 50 ºC 45 s	1.6c	1.7c	3.6a	3.4a	2.9ab	2.1bc	1.5c
Hot water 50 ºC 60 s	1.6bc	1.6bc	3.1a	3.1a	2.4ab	1.8b	0.9c
Hot water 50 ºC 75 s	1.6ab	1.4ab	2.1a	1.9a	1.7a	1.7a	0.8b
Hot water 55 ºC 45 s	1.6b	1.9b	1.9b	3.1a	2.4ab	2.0b	1.6b
Hot water 55 ºC 60 s	1.6bc	1.7bc	3.0a	2.4ab	1.6bc	1.2cd	0.7d
Hot water 55 ºC 75 s	1.6ab	1.6ab	1.9a	1.9a	1.7a	1.1ab	0.8b

The difference between the means of different letters in the same line is significant ($p<0.05$).

The increase in the xyloglucanase activity was significantly affected by the treatments. The highest decrease in the enzyme activity occurred in the 5 µL L^{-1} 1-MCP treatment (0.5), followed by the 55˚C 75 s hot water (0.7) and 2% $CaCl_2$ (0.9) treatments, respectively. In the 5 µL L^{-1} 1-MCP treatment, the enzyme activity did not show a significant change until the 15th day of storage, while there was a significant decrease in the activity towards the end of the storage. $CaCl_2$ treatments also caused significant decreases in the xyloglucanase enzyme activity as compared to the control. The highest decrease was observed in the 2% $CaCl_2$ treatment. With this treatment, the enzyme activity increased significantly on the 10th day of storage, but then decreased significantly towards the end of the storage. Similarly, the hot water treatments caused significant decreases in the xyloglucanase enzyme activity as compared to the control. Among the hot water treatments, the

most effective one was 55 ˚C 75 s treatment, and in this treatment, the enzyme activity did not show a significant change until the 25th day of storage. However, a significant decrease in the activity was obtained towards the end of the storage.

Post-harvest fruit losses are one of the biggest problems encountered in storage. Therefore, many studies have been carried out to prevent fruit losses. Karakurt and Huber (2003) investigated the enzyme activities of various membrane and cell wall hydrolases, ethylene biosynthetic enzymes and cell wall polyuronides of papaya fruit during storage. As a result of their study, they reported that injured fruits spoil earlier than fresh fruits. Sharma et al. (2010) examined the effects of hexanal and 1-MCP applications on the shelf life and fruit quality in cherries. As a result of the study, they reported that there was an increase in the antioxidant enzyme activities. Moreover, in their study performed in order to extend the shelf life of fruit after harvest, Ge et al. (2019) reported that the applications also suppressed the activities of polygalacturonic acid, pectin methyltranseliminase, pectin methylgalacturonase, polygalacturonase, cellulase and β-galactosidase. Wang et al. (2014) investigated the effects of postharvest $CaCl_2$ treatment on the calcium content, biochemical changes and quality characteristics of cherry fruit tissues. As a result of the study, they determined that there was an increase in the content of Ca in the fruit tissues, but decreases in the respiration and lipid peroxidation. Li et al. (2014) reported in their study on jujube that 1-MCP and $CaCl_2$ applications caused significant decreases in the polygalacturonase and polyphenol oxidase enzyme activities. Puthmee et al. (2008) investigated the effects of 1-MCP, $CaCl_2$ and heat treatments on the quality and cell wall enzymes of papaya fruit. In the fruits examined during storage, they found that the treated fruits were firmer as compared to the control group, and there were also decreases in the cell wall enzyme activities. Similarly, Zhang et al. (2019) reported that 1-MCP and $CaCl_2$ treatments provide significant reductions in the gene expression and enzyme activities of polygalacturonase, pectin methylesterase and pectate lyase. Ca, which is one of the most important components of plant cell wall structure, inhibits the activity of cell wall enzymes and prevents fruit ripening. Another important component that we used in our study was 1-MCP. 1-MCP delays maturation by inhibiting ethylene production in the cell. On the other hand, hot water application, contributes to the delay of fruit ripening by disrupting the structure of the enzymes in the cell wall structure. As a matter of fact, when previous studies were examined, it was observed that similar results were obtained with our study (Qiuping and Wenshui 2007; Wei et al., 2010; Amnuaysin et al., 2012; Cruz et al., 2015; Chang et al., 2017; Ge et al., 2019).

There has been a rapid increase in the sweet cherry consumption in recent years. One of the solutions in order to meet the increasing demand is to prevent post-harvest losses in the sweet cherries. However, the post-harvest life of sweet cherries is very short. This creates difficulties in the marketing and because of the rapid softening, the manufacturer cannot send the product to distant markets. Considering these problems, in this study, it was determined that the levels of cell wall-degrading enzymes, which are the main causes of softening, changed during storage after harvest and how the enzyme activity levels changed with 1-MCP, $CaCl_2$ and hot water treatments as compared to the control. As expected, the activities of all enzymes examined in the cherry fruits that were not subjected to any treatment during storage increased significantly. Especially in the first 15 days of storage, significant increases were recorded in the activity levels of all enzymes. Applications have caused significant changes in the enzyme activity levels. Among the treatments, the most effective ones on the enzyme activities were 5 µL L^{-1} 1-MCP, 2% $CaCl_2$ and 55 ˚C 60 sec hot water treatments. Considering all the results, it is thought that 1-MCP, $CaCl_2$ and hot water treatments will be

beneficial in order to extend the shelf life of cherries by reducing the cell wall degrading enzyme activities and thus delaying the softening of the cherries.

Conclusion

As a result of the study with the cell wall-degrading enzymes, it was observed that the activities of polygalacturonase, pectin methyl esterase, xyloglucanase, beta 1-4 endoglucanase and alpha and beta galactosidase significantly increased during storage. Therefore, it seems that these enzymes contribute significantly to the softening observed during storage after harvest in the cherries. The changes in the activities of the cherry cell wall-degrading enzymes have been significantly reduced by the treatments. The increases in the enzyme activities were either completely prevented or significantly reduced with the treatments. The highest effect was observed in the 1-MCP treatment. However, the effects of both $CaCl_2$ and hot water treatments on the enzyme activities are also very important. As a result, 1-MCP, $CaCl_2$ and hot water treatments can be used to delay the softening of sweet cherries, extend the postharvest life and prevent quality losses after harvest.

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