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THE IMPACT OF ENVIRONMENTALLY FRIENDLY POSTHARVEST TREATMENTS ON THE ANTIOXIDANT ACTIVITY OF STRAWBERRY FRUITS DURING STORAGE

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SUMMARY

Proper postharvest storage is an effective way to maintain the quality and nutritional values of fruits. The aim of this study was to determine how environmentally friendly postharvest treatments with salicylic acid solution, colloidal silver solution and ozone, affect the antioxidant activity of strawberry fruits (*Fragaria x ananassa* Duch. cv. Albion) during 7 days of storage at 4°C. The content of ascorbic acid, total phenols and antioxidant activity of strawberry fruits were determined spectrophotometrically. After 7 days of storage in strawberry fruits treated with all three treatments separately, the contents of ascorbic acid were higher than in the control fruits, supporting the usefulness of these treatments for preserving fruit quality and nutritional value during storage. The treatment with salicylic acid solution showed the most beneficial effect during storage causing a significant increase in the content of ascorbic acid, phenols and antioxidant activity at the end of the storage period.

Key-words: strawberries, postharvest treatments, phenols, ascorbic acid, antioxidant activity

INTRODUCTION

Strawberries have a very short shelf life due to their sensitivity to fungal attack and excessive texture softening caused by the natural ripening process. Proper fruit storage is an effective way to maintain the quality of fruits after harvest. High temperatures can affect not only the strawberry shelf life, but also its nutritional value, in terms of soluble sugars, ascorbic acid and antioxidant compounds (Cordenunsi et al., 2005). Due to the high ascorbic acid and phenols content, strawberries have antioxidant, anticancer, antiatherosclerotic and anti-neurodegenerative properties (Hannum, 2004; Seeram et al., 2006). To compensate the losses associated with handling and storage of strawberries and other small fruits, some postharvest techniques, such as the utilization of low temperatures or high CO₂ concentrations, as well as controlled atmosphere or a combination of these processes, are widely used to extend their shelf life (Gil et al., 1997; Allende et al., 2007). Controlled atmosphere has the undeniable benefit in reducing the postharvest decay of fruits, although a CO₂-enriched atmosphere with low O₂ concentration can affect total

ascorbic acid and anthocyanin contents adversely, with a negative consequence on fruit colour and its nutritional value (Holcroft and Kader, 1999). Temperatures around 0°C are considered to be optimal for strawberry storage because they cause only minor changes in fruit quality (Cordenunsi et al., 2005).

Salicylic acid (SA) is a phenolic phytohormone found in plants with roles in plant growth and development, such as seed germination, flowering, heat production and fruit ripening. It is also part of a signalling pathway induced by a number of biotic and abiotic stresses. A lot of data exist on the protective effect of SA in plants against ultraviolet light, high temperatures, salinity, drought and pathogens (Raskin, 1992; Yalpani et al., 1994; Dat et al., 1998). There are several studies indica-

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ting beneficial influences of SA treatment on fruit quality during storage. During kiwifruit ripening, the pattern of decrease in endogenous SA levels was related to accelerated softening, while the application of acetylsalicylic acid (a derivative of SA) slowed down the softening rate of kiwifruit by inhibiting ethylene production and maintaining higher endogenous SA levels. Salicylic acid also prevents softening of banana fruit during ripening (Zhang et al., 2003) and improves postharvest fruit quality of strawberry (Shafiee et al., 2010). Beside the described postharvest techniques, the treatment with ozone, a highly reactive molecule demonstrating excellent bactericidal, antiviral and fungicide properties is widely applied (Kim et al., 1999). Ozone has been reviewed for food processing applications (Graham et al., 1997). The use of ozone was also described previously to reduce decay and induce the highest resveratrol content in stored grapes (Cayuela et al., 2009), to inhibit the decrease of ascorbic acid and to reduce the weight loss rate in stored strawberries (Zhang et al., 2011) and to reduce fruit damage and excessive softening of tomato fruits (Rodoni et al., 2010). Ozone treatment may affect fruit quality while its application possibly destructs produced ethylene (Dickson et al., 1992). Ethylene is valuable component of the ripening initiation process in several fruits, but it can also be very harmful to many fruits, vegetables, flowers, and plants by accelerating the aging process and decreasing the product quality and shelf life. Colloidal silver is among the relatively new antimicrobial agents being tested on plants. In small concentrations it is a non-toxic material which shows high capabilities in eliminating microorganisms, e.g., fungus, bacteria and viruses (Rostami and Shahsavari, 2009). Although, chronic exposure to silver may produce toxic effects to human health like liver and kidney damage, irritation of the eyes, skin, respiratory and intestinal tract (Panayala et al., 2008). So far, the effects of colloidal silver treatments on storage of fruits have not been established, although they are expected to be positive due to the proven antimicrobial activity (Rostami and Shahsavari, 2009) and to the inhibition of ethylene action (Kumar et al., 2009).

The aim of this study was to determine how environmentally friendly postharvest treatments (salicylic acid solution, colloidal silver solution and ozone) affect the antioxidant activity of strawberry fruits (*Fragaria x ananassa* cv. Albion) during seven days of storage at 4°C, by determining the concentration of ascorbic acid, total phenols and total antioxidant activity.

MATERIAL AND METHODS

Strawberries were grown in pots filled with mix of peat at semicontrol environment in greenhouse and harvested 28 May. A total of 400 strawberry fruits (*Fragaria x ananassa* cv. Albion) were used in this investigation and were divided in four groups (each treatment containing 100 strawberries). Additionally, we used 25 fruits more for entry measurements. The first group represen-

ted the control (C). The second group was treated by immersion in 1 mM salicylic acid solution (SA), third group was treated by immersion in a colloidal silver solution (30 ppm) (AG), while the last group was treated with ozone (OZ) for 15 minutes. Ozone was produced by ozone generator (max. output 150 mg/h) and ozone concentration was measured using a portable ozone detector. The partial pressure in closed container was 141 Pa with concentration 0.05787 mole/m³ (mole fraction 0.139%). After all treatments, strawberry fruits were stored at 4°C in cold room at 90% humidity until the biochemical analyses. The analyses were carried out at the initial time point 0 as well as on the 1st, 3rd, 5th and 7th day of storage. All measurements were made in 5 replicates. Strawberry fruits were mashed using the stick blender, and aqueous extracts were made by adding 10 mL of distilled water on 0.5 g of strawberry juice. The concentration of ascorbic acid was determined spectrophotometrically, from the aqueous extract, using the method of Benderitter et al. (1998). Results were expressed as mg of ascorbic acid/100 g FW. Strawberry extracts for determination of total phenolic content were obtained by adding 2.5 mL of 80% ethanol to 1 g of mashed strawberry juice and the values were determined spectrophotometrically using the method of Folin-Ciocalteu (Singleton and Rossi, 1965). The results were expressed as g of gallic acid/100 g FW. The total antioxidant activity of ethanol extracts was determined using the free radical-scavenging method described by Brand-Williams et al. (1995). The total antioxidant activity of the extracts was subsequently calculated using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as standard, and expressed as μ mol Trolox equivalents/g FW. Statistical analysis was done by using the Statistica software package version 8.0. Differences between means were first analyzed by two-way ANOVA test followed by LSD (least significant difference) test ($P < 0.05$).

RESULTS AND DISCUSSION

The concentration of ascorbic acid in fruits depends on many variables such as weather conditions before harvest, conditions during the storage, but also the genetic factors specific for different cultivars (Lee and Kader, 2000). According to the research of Da Silva Pinto et al. (2008), the average content of ascorbic acid in strawberries is about 82 mg/100 g FW, while Lester et al. (2012) showed that the content of ascorbic acid in cv. Albion was about 100 mg/100 g FW. In our study, the average content of AA in the strawberry fruits (*Fragaria x ananassa* cv. Albion) was 87.09 mg/100 g FW. Ascorbic acid is very susceptible to chemical and enzymatic oxidation during storage of fruits and vegetables (Lee and Kader, 2000). In our study, the total AA concentration in control fruits started to decline on the 5th day of storage, with the values decreased by 43% on the 7th day of storage when compared to the values obtained in the control fruits at the 1st of storage

(Fig. 1). Hansawasdi et al. (2006) also showed that low temperatures during storage cause the reduction in the concentration of ascorbic acid. Therefore, temperature management after harvest seems to be the most important factor to maintain the ascorbic acid content in fruits.

In our study, based on the whole experiment level (Tab. 1) there was highly significant influence of independent variables (treatment, days of storage and their interaction) on observed parameters (AA, PHE and TAA).

Table 1. Two-way ANOVA for AA, PHE and TAA in strawberry fruits ($p < 0.05$)

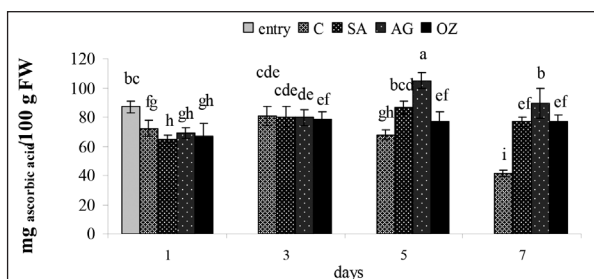
Tablica 1. Dvofaktorijalna analiza varijance za askorbinsku kiselinu, fenole i ukupne antioksidacijske aktivnosti u plodovima jagoda ($p < 0,05$)

	AA		PHE		TAA	
	F test	p	F test	p	F test	p
Days of storage	29.86	<.0001	11.24	<.0001	14.88	<.0001
Treatments	53.44	<.0001	16.64	<.0001	168.71	<.0001
Treatments * Days of storage	19.79	<.0001	5.21	<.0001	24.58	<.0001

The average concentration of phenolic compounds (PHE) in strawberry fruits was 0.34 g gallic acid/100 g FW. Lester et al. (2012) showed similar results for cv. Albion (0.46 and 0.20 g gallic acid/100 g FW for two different years of harvesting). The total concentration of phenols varies depending on the fruit variety (Waterhouse and Walzem, 1998), maturity (Wang and Lin, 2000), and environmental factors such as light and temperature (Kalt et al., 1999).

Figure 1. The concentrations of ascorbic acid in strawberry fruits during storage at 4°C (C - control; SA - treatment with salicylic acid; AG - treatment with colloidal silver; OZ - treatment with ozone). Average values marked with the same letter do not differ according to LSD test ($p < 0.05$)

Slika 1. Koncentracije askorbinske kiseline u plodovima jagoda tijekom perioda skladištenja na 4°C (C - kontrola; SA - tretman salicilnom kiselinom; AG - tretman koloidnim srebrom; OZ - tretman ozonom). Prosječne vrijednosti označene istim slovom ne razlikuju se prema LSD testu ($p < 0,05$)

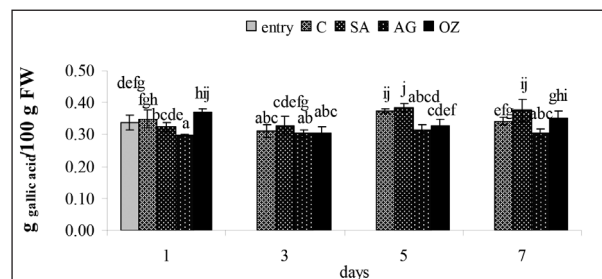


In control fruits, the concentration of PHE and the total antioxidant activity (TAA) did not change significantly during the storage (Fig. 2 and 3). According to Cordenunsi et al. (2005) and Gil et al. (2006) the content of phenols in different fruits did not change significantly during storage. As reported by Wang et al. (1996), antioxidant activity of fruits (strawberries, kiwi, bananas, grapes, apples) is directly correlated with the concentration of phenolic compounds. On the contrary, Hansawasdi et al. (2006) showed negative correlation between both phenolics and anthocyanin content to antioxidant activity during low temperature storage of strawberry fruits.

Figure 2. The concentrations of phenolic compounds in strawberry fruits during storage at 4°C (C - control; SA - treatment with salicylic acid; AG - treatment with colloidal silver; OZ - treatment with ozone).

Average values marked with the same letter do not differ according to LSD test ($p < 0.05$)

Slika 2. Koncentracije fenola u plodovima jagoda tijekom perioda skladištenja na 4°C (C - kontrola; SA - tretman salicilnom kiselinom; AG - tretman koloidnim srebrom; OZ - tretman ozonom). Prosječne vrijednosti označene istim slovom ne razlikuju se prema LSD testu ($p < 0,05$)

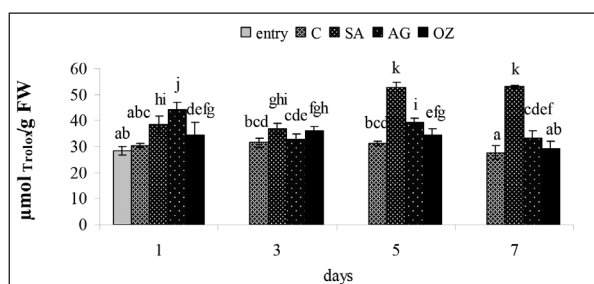


To prevent losses of strawberry fruit quality during storage, strawberries were treated in the present study with three different treatments: salicylic acid solution (SA), colloidal silver solution (AG) and ozone (OZ). In strawberry fruits treated with SA, there was a significant increase in the concentration of AA during storage. On the 7th day of storage, AA concentration was 46% higher than the control value (Fig. 1). Also, there was an increase in the concentration of PHE, until the 5th day of storage, when the concentration was the highest (Fig. 2). The TAA was increasing through the whole period of storage, with the 46% higher values on the 7th day of storage when compared to the control (Fig. 3). SA is a major phenylpropanoid compound influencing plant resistance to pathogens and other stress factors (Sharma et al., 1996). There is also evidence that SA can alter the TAA in plants (Rao et al., 1997). SA activates ascorbate peroxidase, increasing antioxidant ability and AA amount in fruits (Wang et al., 2006). The increased TAA and plant resistance to stress, induced by SA, prevent ascorbic acid destruction (Wisniewska and Chelcowski, 1999). According to Shafiee et al. (2010), SA treatment

improves the fruit firmness, prevents decay, weight loss and ascorbic acid reduction. This increase in the concentrations of AA, PHE and TAA confirms the positive effect of SA treatment on antioxidant activity and ascorbic acid amount in fruits.

Figure 3. The total antioxidant activity in strawberry fruits during storage at 4°C (C - control; SA - treatment with salicylic acid; AG - treatment with colloidal silver; OZ - treatment with ozone). Average values marked with the same letter do not differ according to LSD test ($p < 0.05$)

Slika 3. Ukupne antioksidacijske aktivnosti u plodovima jagoda tijekom perioda skladištenja na 4°C (C - kontrola; SA - tretman salicilnom kiselinom; AG - tretman koloidnim srebrom; OZ - tretman ozonom). Prosječne vrijednosti označene istim slovom ne razlikuju se prema LSD testu ($p < 0,05$)



The concentrations of AA in fruits treated with AG were 35% and 54% higher compared to values obtained in the control fruits on the 5th and the 7th day of storage, respectively (Fig. 1) This increase in the concentrations of AA confirms the positive effect of AG treatment on strawberry fruits during storage. The concentrations of PHE in strawberry fruits treated with AG were lower compared to the control values, except on the 3rd day of storage. Different effect after AG treatment was observed in antioxidant activity assay, where the values were significantly higher compared with the ones found in the control fruits at the end of the storage period (Fig. 2 and 3). These results are in agreement with Hansawasdi et al. (2006) who showed negative correlation between both phenolics and anthocyanin content to antioxidant activity during low temperature storage of strawberry fruits. Since the positive impact of colloidal silver solution on postharvest fruit storage has not yet been described, these results are the first to confirm the beneficial effect of colloidal silver treatment on the antioxidant activity of strawberry fruits during the storage.

The concentrations of AA in fruits treated with ozone (OZ) were greater than in the control fruits, with the 46% higher values on the 7th day of storage, as compared to the control concentrations at the same time point (Fig. 1). These results show that OZ can prevent ascorbic acid reduction. Previous stud-

ies have demonstrated the positive effect of ozone in maintaining a constant content of ascorbic acid (Luwe et al., 1993; Pérez et al., 1999; Cruz-Rus et al., 2011). The use of ozone is also recommended to reduce decay and prolong storage of fruits (Sarig et al., 1996). Contrary to AA content, the concentrations of PHE and TAA in strawberry fruits treated with ozone were not significantly different compared to control values (Fig. 2 and 3), showing that the treatment of strawberry fruits with ozone during the storage has a positive effect on the concentration of ascorbic acid while it had no effects on phenolic compounds and antioxidant activity.

CONCLUSION

During the storage at 4°C a decrease in the ascorbic acid concentration was observed in the control strawberry fruits. At the end of storage period of 7 days, the total ascorbic acid concentration has been higher than in the control fruits in all treatments, indicating their positive effect on fruit quality and potential application as environmentally acceptable treatment for maintaining the quality of strawberry fruit. No differences were found in the concentration of phenols in all three treatments, except for the last day of storage when the salicylic acid treatment caused an increase in the concentration of phenols as well as in the antioxidant activity. Based on these data, it is possible to conclude that treatment with 1 mM salicylic acid solution is the most effective method for maintaining the strawberry fruit quality during storage at 4°C.

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UTJECAJ EKOLOŠKI PRIHVATLJIVIH TRETMANA NA ANTIOKSIDACIJSKU AKTIVNOST U PLODOVIMA JAGODA TIJEKOM SKLADIŠTENJA

SAŽETAK

Pravilno skladištenje voća učinkovit je način za održavanje kvalitete i nutritivne vrijednosti plodova nakon berbe. Cilj ovog istraživanja bio je utvrditi kako ekološki prihvatljivi tretmani (otopina salicilne kiseline, otopina koloidnoga srebra i ozon) utječu na ukupnu antioksidacijsku aktivnost u plodovima jagoda (*Fragaria x ananassa* Duch.) sorte Albion tijekom 7 dana skladištenja pri 4°C. Koncentracije askorbinske kiseline, ukupnih fenola i ukupna antioksidacijska aktivnost u plodovima jagoda određeni su spektrofotometrijski. Svi primijenjeni tretmani prouzročili su povećanje koncentracije askorbinske kiseline u plodovima jagoda, što potvrđuje povoljno djelovanje navedenih tretmana na očuvanje kvalitete i nutritivne vrijednosti plodova. Tretman otopinom salicilne kiseline bio je najučinkovitiji, jer je prouzročio značajno povećanje koncentracije fenola, askorbinske kiseline i ukupne antioksidacijske aktivnosti na kraju perioda skladištenja.

Ključne riječi: jagoda, uvjeti skladištenja, fenoli, askorbinska kiselina, ukupna antioksidacijska aktivnost

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