Heat shock and salicylic acid on postharvest preservation of organic strawberries

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ABSTRACT

Heat shock and salicylic acid have been studied on shelf-life extension of fruits. The benefits of these techniques have been related to their effect on inducing physiological defense responses against the oxidative stress and pathogen development. The objective of this study was to evaluate the effect of heat shock and salicylic acid on the postharvest preservation and contents of total phenolics, anthocyanins, ascorbic acid, fresh weight loss and microbiological quality of organic strawberries cv. Dover. Strawberries produced organically and stored at 5 °C were subjected to heat shock (45 °C ± 3 °C for 3 h), application of salicylic acid (soaking in 2.0 mmol L^{-1} solution), heat shock in combination with salicylic acid and control. After treatment, the fruits were packed and stored in a climatic chamber at 5 ºC ± 2 ºC. At 1, 7 and 14 days, the experimental units were removed from refrigeration and kept at room temperature of approximately 20 ºC for two days. There was no effect of treatments on fresh weight loss, incidence of pathogens or chemical variations in strawberry fruits during the storage period. In natural conditions, organically grown strawberries remained in good condition for sale up to seven days of storage in all treatments.

Key words: *Fragaria x ananassa* Duch, antioxidant compounds, microbiological quality.

RESUMO

Conservação pós-colheita de morangos orgânicos, tratados com choque térmico e ácido salicílico

O choque térmico e o uso do ácido salicílico têm sido estudados como técnicas de extensão da vida útil de frutos. A ação benéfica dessas técnicas tem sido relacionada com seus efeitos na indução de respostas fisiológicas de defesa contra estresses oxidativos e desenvolvimento de patógenos. O objetivo deste trabalho foi avaliar o efeito do choque térmico e do ácido salicílico na conservação pós-colheita e nos teores de compostos fenólicos totais, antocianinas, ácido ascórbico, perda de matéria fresca e qualidade microbiológica de frutos de morangos 'Dover', produzidos organicamente e armazenados a 5 ºC. Os morangos foram submetidos aos tratamentos de choque térmico (45 ºC ± 3 ºC, por 3 h), aplicação de ácido salicílico (imersão em solução aquosa 2,0 mmol L^{-1}), combinação de choque térmico com ácido salicílico e controle. Após tratamento, os frutos foram embalados e armazenados em câmara climatizada, a 5 ºC ± 2 ºC. Em intervalos de 1, 7 e 14 dias, as unidades experimentais foram retiradas da refrigeração e mantidas em ambiente a aproximadamente 20 ºC, por dois dias. Não houve influência dos tratamentos sobre a perda de matéria fresca e a incidência de patógenos ou variações químicas em frutos de morango, durante o período de armazenamento. Naturalmente, os morangos produzidos organicamente mantiveram-se em boas condições para a comercialização até sete dias de armazenamento, em todos os tratamentos.

Palavras-chave: *Fragaria* x *ananassa* Duch, compostos antioxidantes, qualidade microbiológica.

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INTRODUCTION

Strawberry (*Fragaria x ananassa* Duch.) is a widely consumed fruit worldwide, both fresh and processed. It has attractive color, flavor and aroma and is a source of vitamin C (ascorbic acid) and bioactive compounds such as flavonoids and other phenolic compounds (Robards *et al.*, 1999). The attractive color is derived from glycosylated anthocyanidins (anthocyanins), which are important for assessing fruit ripening. The main anthocyanin in strawberry is pelargonidin 3-glucoside and in smaller quantities are cyanidin-3-glucoside and pelargonidin 3-rutinoside (Cordenunsi *et al.*, 2005). Vitamin C (L-ascorbate or ascorbic acid) is one of the most important free radical scavengers present in plants and animals. The antioxidant activity comes from its reaction with hydrogen peroxide (H_2O_2), superoxide (O_2^-) and singlet oxygen ($^1O_2$) (Buettner & Schafer, 2004).

Strawberry has a non-climacteric respiratory pattern and a very fragile physical structure. It has high metabolic postharvest activity and high susceptibility to fungal diseases, especially *Botrytis cinerea*, *Rhizopus stolonifer* and *Penicillium* sp. (Baths-Bautista *et al.* 2003). These characteristics of strawberry fruits result in high perishability and short postharvest life. The main control measure these diseases is the use of chemical fungicides (Hernández-Muñoz *et al.*, 2006). The increasing consumer demand for healthy products has required special attention to post-harvest handling of strawberries. Research is needed on alternative methods of conservation that are supplementary to refrigerated storage and add safety to the product.

Heat shock treatments have been used to control fungal diseases in postharvest fruit and vegetables (Patras *et al.*, 2009). This can be a promising alternative to replace or to reduce chemical treatments in strawberries. A moderate heat stress on the fruit mobilizes antioxidant defense responses and induces changes in the metabolism. The production of antioxidant enzymes involved in inactivating oxygen radicals keeps the levels of harmful free radicals under intracellular control (Vincent *et al.*, 2006). The non-lethal heat shock temperature, around 45 °C for three hours, may reduce fruit decay by pathogens and increase the shelf life (Vincent *et al.*, 2002).

Salicylic acid has been studied in the post-harvest conservation of strawberries applied alone (Srivastava & Dwivedi, 2000; Vincent *et al.*, 2002; Vincent *et al.*, 2006), or combined with thermal shock by immersing in warm water (Shafiee *et al.*, 2010). Its exogenous application in strawberry fruits can increase resistance against pathogens (Babalar *et al.*, 2007; Zhang *et al.*, 2010). This phenolic compound is present in many plants and is an important component in the signal transduction pathway, inducing defense responses (Zhang *et al.*, 2010).

The efficiency of heat shock and the application of salicylic acid in increasing the shelf life of strawberries have been proven. However, the application of salicylic acid combined with heat shock by heated air has not yet been tested in strawberry postharvest preservation. The objective of this study was to evaluate the effect of heat shock and salicylic acid, alone or in combination, in the postharvest preservation and microbiological quality of organic strawberries.

MATERIALS AND METHODS

Strawberry c.v. Dover was produced in the organic system in the municipality of Marechal Candido Rondon, PR, Brazil, between the coordinates 24º 26' S and 53º 57' W, at 420 m altitude. Fruits were harvested in July 2011, selected for uniform size, without external blemishes, healthy, with 75% of the surface of predominant red color and packed in polystyrene trays. Groups of ten fruits constituted the experimental units. The treatments applied consisted of: 1) heat shock (45 °C ± 3 °C for 3 h in an oven); 2) salicylic acid (immersion in aqueous solution to 2.0 mmol L^{-1} for 5 minutes, followed by drying on a bench for 1 h); 3) Salicylic acid (immersion in aqueous solution to 2.0 mmol L^{-1}, 5 minutes), followed by heat shock (45 °C ± 3 °C for 3 h in an oven); and 4) control. After the treatments, the trays were covered with PVC film and stored in a climatic chamber at 5 ºC ± 2 ºC. and 90% ± 5% RH. The control group of untreated strawberries and also packed was stored under the same conditions. After 1, 7 and 14 days of storage, the experimental units were removed from refrigeration and moved to room at 20 °C and 60-65% RH. After 48 h in this condition, the samples were frozen in liquid nitrogen and stored at -24 ºC until analysis. All analyzes were performed in triplicate.

The concentration of ascorbic acid (vitamin C) was determined by titration with 2,6-dichlorophenol-indophenol sodium salt (Benassi & Ali 1988). The total phenolic compounds were determined by the colorimetric method, using the Folin-Ciocalteu reagent (Singleton *et al.*, 1999) and read in a spectrophotometer at 760 nm. The anthocyanin content was determined in a spectrophotometer at 537 nm (Sims & Gamon, 2002). The variation in weight (%) was determined in a semi-analytical balance by subtracting the weight of each storage interval from the initial weight.

Pathogen incidence (fungi) was determined visually and expressed as the percentage (%) of attacked fruits (Hernández-Muñoz *et al.*, 2006). Fungal identification was based on microscopic examination (Franco & Landgraf, 1996).

The experiment was arranged in a completely randomized 3x4 factorial design, with three storage periods combined with the three treatments and the control, with four replications. Analysis of variance and comparison of means (Tukey, $p < 0.05$) were performed with the statistical software SAEG 9.1 (System for Genetic Analysis and Statistics, 2007).

RESULTS AND DISCUSSION

The high respiratory intensity and high susceptibility to damages induced by pathogen infection are common features in strawberries and lead to the relatively short shelf life that normally occurs with this fruit (Pelayo *et al.* 2003). This relevant behavior was also observed in this experiment.

Up to seven days of storage, there were significant decreases in total phenolic compounds in the fruits caused by all the treatments. Between 7 and 14 days of storage, only the treatment with heat shock resulted in decreasing of total phenolic compounds, whereas in the control, there was increase in these phenols (Table 1). Opposite results were observed in the cv. Selva treated with heat shock, which showed a significant increase of total phenolics in the control until seven days of storage (Vicente *et al.*, 2003).

Exposure of fruits to heat shock induces synthesis of a unique set of proteins called "heat shock proteins" (Saltveit, 2000). The synthesis of these proteins is accompanied by a general inhibition of protein synthesis in the phenylpropanoid pathway with a reduction in the levels of phenolic compounds. This may explain the decreases in the total phenolic content occurred in all treatments up to seven days of storage (Table 1).

The increase in the content of phenolic compounds in strawberries treated with salicylic acid and the combination heat shock+salicylic acid after 14 days of storage may have occurred because of stress damages related to the incidence of pathogens in the fruits (Table 1). Stresses caused by physical damage, such as pathogen infection, lead to physiological disorders that, through gene expression signals, lead to the induction of specific proteins of the phenylpropanoid metabolism. This increased activity causes the accumulation of phenolic compounds (Lopez-Galvez *et al.*, 1996; Peiser *et al.*, 1998), which seems to be related to defense mechanisms of the fruit via oxidative metabolism enzymes such as peroxidases (PO) and polyphenoloxidases (PPO). These enzymes lead to oxidative degradation of phenolic compounds, close to the site of cell decompartmentalization caused by pathogens (Campos *et al.* 2003). The damage caused by pathogens to cells also contribute to the increased levels of pre-existing phenolic compounds released from vacuoles, oxidized to quinones (Thipyapong *et al.*, 2004), which exhibit antimicrobial activity (Jung *et al.*, 2004) .

There was no correlation between the levels of phenolic compounds and the weight loss and pathogen incidence (Table 2). However, there is evidence of positive associations between levels of total phenolics and pathogen indices between 7 and 14 days of storage (except for the treatment with heat shock), suggesting that high rates of decay by pathogens, at the end of storage, have caused increases in the content of phenolic compounds, in agreement with observations that indicate higher content of phenolic compounds in strawberries associated with higher levels of physical damage (Vincent *et al.*, 2003). Physiological stresses caused by injuries or infections can induce the activity of oxidative enzymes (PPO and POD), decreasing the contents of phenolic compounds, and can also increase the activity of phenylalanine ammonia-lyase (PAL) (Thomas-Barberán & Espín, 2001), which is considered a key enzyme in the biosynthesis of phenolic compounds from the phenylpropanoid pathway (Andersen & Markham, 2005). The results show evidence that after 14 days of storage, the heat shock alone influenced phenol oxidation processes, whereas the salicylic acid influenced the increase in activity of the biosynthetic phenylpropanoid pathway.

The anthocyanin content in strawberries increased in all treatments up to seven days of storage (Table 3). Increases of total anthocyanins in strawberries were also reported for cultivars Dover, Campineiro (Cordenunsi *et al.*, 2005), Earliglow and Allstar (Jin *et al.*, 2011), during the same period of refrigerated storage. Between seven and 14 days, only the treatment with heat shock alone showed reduced anthocyanin contents. Civello *et al.* (1997)

Table 1. Total phenolic compounds in strawberry cv. Dover stored after treatment with heat shock (HS) and salicylic acid (SA)

Storage (days[1])	Total phenolic compounds ($mg\ g^{-1}$)			
	Control	HS	SA	HS+SA
1+2	3.79 ^{aA}	3.78 ^{aA}	3.43 ^{bB}	3.05 ^{bC}
7+2	3.08 ^{cB}	3.20 ^{bA}	3.08 ^{cB}	2.84 ^{cC}
14+2	3.58 ^{bB}	2.22 ^{cD}	3.76 ^{aA}	3.14 ^{aC}

Means followed by the same small letter in the column and capital letter in the row are not significantly different by the Tukey's test ($p \geq 0.05$).

[1]Days at 5 °C + 2 days at 20 ° C.

also found reduction in the anthocyanin content in thermally treated fruits of cv. Selva, after seven days of storage. However, Vincent *et al.* (2002) found increases of anthocyanins in cv. Dover, both thermally treated and untreated, up to 14 days of storage, suggesting a possible influence of the cultivar in the response of fruits under high stress by long-term storage.

After the application of heat shock and/or salicylic acid, the anthocyanin content in fruits was higher than that of the control at 7 and 14 days of storage (Table 3), suggesting that the treatments were effective in inducing accumulation of anthocyanins, except for the treatment heat shock alone at 14 days, which showed results similar to the control. However, Vincent *et al.* (2002) found lower total anthocyanin content at seven and 14 days of storage, which were similar to the control in heat-treated cv. Selva. Similarly, Civello *et al.* (1997) found that heat-treated Selva strawberries had lower contents of anthocyanins than the control, during three days of storage and related this result to the inhibition of the biosynthetic pathway by the decrease in the PAL activity.

There were no differences in the anthocyanin content among the treatments with heat shock and salicylic acid applied alone or combined at 7 days of storage (Table 3). However, at 14 days, heat shock-treated strawberries had lower content than the other treatments. Exogenous application of salicylic acid can activate the metabolic pathway for the synthesis of phenylpropanoids (flavonoid compounds) involved in mechanisms of resistance to plant pathogens (Campos *et al.*, 2003). The defense responses induced by salicylic acid are probably involved in the expression of a number of defense genes, especially those encoding pathogenesis related proteins such as chitinase, â-1,3-glucanase and peroxidase (Qin *et al.*, 2003). However, there was no significant correlation between the variables anthocyanins and pathogen incidence (Table 2).

Strawberries treated with heat shock and heat shock+salicylic acid showed increased ascorbic acid content, up to 7 days of storage, with higher contents than the control (Table 4). The use of salicylic acid alone resulted in reduced ascorbic acid. However, there was an increase in the content at 14 days of storage, which was higher than the other treatments. The application of heat shock and/or salicylic acid resulted in higher ascorbic acid contents than the control at 14 days of storage. The maintenance of levels of ascorbic acid involves a recycling mechanism rigidly controlled within the plant cell. Because of its antioxidant function, this recycling route in the fruit is especially important during the response to oxidative stress, when the reduced ascorbic acid is oxidized to the unstable form of dehydroascorbate, which is easily degraded. The reduced form of ascorbic acid may be exhausted if the oxidized forms are not recovered by the reductase enzymes (monodehydroascorbate and dehydroascorbateredutase) genetically expressed in response to oxidative stress (Smirnoff & Wheeler, 2000). This recycling route and synthesis processes can explain the maintenance and increase in the content of ascorbic acid, especially in response to heat shock and salicylic acid treatments.

Vincent *et al.* (2006) reported no differences in ascorbic acid content between heat shock-treated Selva strawberries and the control during storage at 0 °C + 2 days at 20 °C for 7 and 14 days. Cordenunsi *et al.* (2005) found that the content of ascorbic acid was maintained around 60 mg 100 g^{-1} during storage of strawberries Dover at 6 ºC for six days. This is related, most likely, to the

Table 2. Pearson coefficients of correlation for combinations between chemical and physical variables of strawberry cv. Dover stored after treatment with heat shock and salicylic acid

Variables	Anthocyanins	Ascorbic acid	Loss of weight	Incidence of pathogens
Total phenolic	-0.18 ^{ns}	-0.03 ^{ns}	-0.20 ^{ns}	-0.16 ^{ns}
Anthocyanins		0.57 **	0.29 *	0.18 ^{ns}
Ascorbic acid			0.45 **	0.33 *
Loss of weight				0.86 **

* and ** Significant at $p < 0.05$ and $p < 0.01$, respectively. ^{ns} Non-significant at $p \geq 0.05$.

Table 3. Anthocyanins in strawberry cv. Dover stored after treatment with heat shock (HS) and salicylic acid (SA)

Storage (days[1])	Anthocyanins (mg 100 g^{-1})			
	Control	HS	SA	HS+SA
1+2	75.06 ^{bC}	86.27 ^{bB}	77.52 ^{bC}	90.49 ^{bA}
7+2	83.28 ^{aB}	97.48 ^{aA}	94.98 ^{aA}	96.24 ^{aA}
14+2	83.05 ^{aC}	83.56 ^{bC}	97.56 ^{aA}	94.77 ^{aA}

Means followed by the same small letter in the column and capital letter in the row are not significantly different by the Tukey's test ($p \geq 0.05$).

[1]Days at 5 °C + 2 days at 20 º C.

Table 4. Ascorbic acid in strawberry cv. Dover stored after treatment with heat shock (HS) and salicylic acid (SA)

Storage (days[1])	Ascorbic acid (mg 100 g^{-1})			
	Control	HS	SA	HS+SA
1+2	48.67 ^{bB}	50.69 ^{cB}	54.30 ^{bA}	48.16 ^{bB}
7+2	53.28 ^{aB}	60.45 ^{aA}	50.72 ^{cB}	60.25 ^{aA}
14+2	50.20 ^{abD}	54.30 ^{bC}	68.14 ^{aA}	58.40 ^{aB}

Means followed by the same small letter in the column and capital letter in the row are not significantly different by the Tukey's test ($p \geq 0.05$).

[1]Days at 5 °C + 2 days at 20 º C.

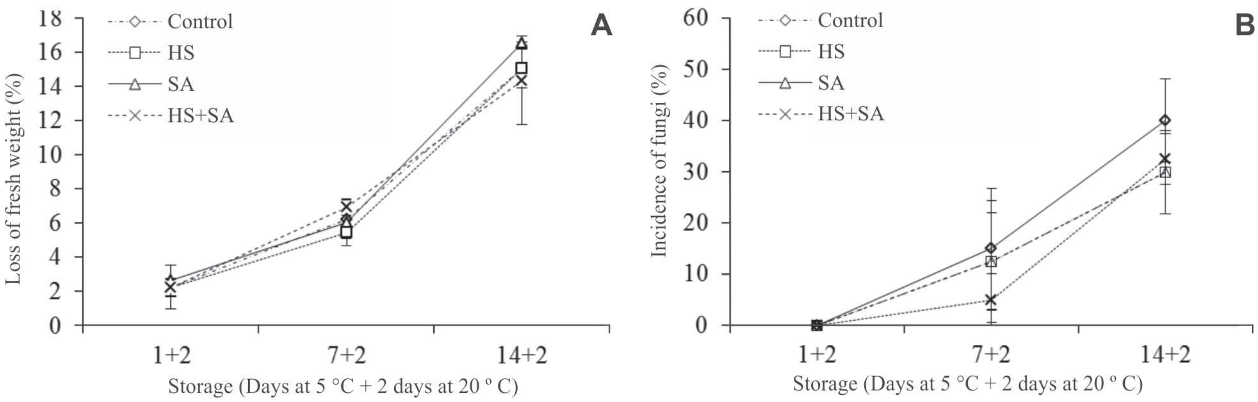

Figure 1. Loss of fresh weight (a) and incidence of pathogens (b) in strawberry cv. Dover stored after treatment with heat shock (HS) and salicylic acid (SA). Vertical bars mean standard deviation.

positive influence of the low storage temperature of strawberries (Lee & Kader, 2000; Pelayo *et al.*, 2003). The same was observed in Camarosa strawberries after treatment with heat shock and salicylic acid and stored for seven days at 2 °C (Shafiee *et al.*, 2010).

The effect of the storage time on the loss of fruit weight was around 6% at 7 days and 14-16% at 14 days, which is not influenced by the treatments (Figure 1a), disagreeing with the findings by Vincent *et al.* (2003), who found reduction in weight loss in strawberries treated with heat shock. Shafiee *et al.* (2010) also observed decrease in weight loss in Camarosa strawberries treated with salicylic acid, with or without heat shock, after seven days of storage at 2 °C. Losses higher than 10% of weight lead to wrinkled and opaque skin, compromise the fruit appearance and can lead to rejection by consumers (Flores-Cantillano, 2003; Hernández-Muñoz *et al.*, 2006). The increased incidence of fungi (Figure 1b) and its correlation with weight loss (Table 2) may have accelerated metabolic processes related to senescence, with consequent increase of water content and higher rate of weight loss by evaporation (Cordenunsi *et al.*, 2002; Chitarra & Chitarra, 2005).

There was an increased incidence of *Rhyzopus nigricans* and *Penicillium* sp during the storage period, with the highest rates occurring at 14 days (Figure 1b), which was not influenced by the treatments. This result disagrees with the reports by Shafiee *et al.* (2010), who found reduction in both the incidence and lesion diameter of *Rhizopus* in Camarosa strawberries treated with salicylic acid and stored at 2 °C. Babalar *et al.* (2007) also found a reduction of fungal decay in Selva strawberries treated with salicylic acid and stored at 2 °C and related this result to the activation of the defense system of the body, caused by the application of salicylic acid.

The major changes in strawberries during refrigerated storage were physical and microbiological, which were influenced by the senescent metabolism of the organ. Erkan *et al.* (2008) found no visible infection in strawberry fruits during the first five days of storage at 10 ºC, while at 20 days, it reached approximately 90%. Vieites *et al.* (2006) observed incidence, mainly of *Botrytis cinerea*, in strawberry cv. Oso Grande, from day 8 of storage at 0 °C. The increased infestation of rot diseases in strawberry is associated with temperature and storage time (Reddy *et al.*, 2000).

CONCLUSIONS

The heat shock and/or salicylic acid applied post-harvest did not influence the loss of fresh weight, incidence of pathogens or chemical variations in stored strawberry.

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