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Combined Salicyclic Acid and Ultrasound Treatments for Reducing the Chilling Injury on Peach Fruit

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ABSTRACT: The effects of salicylic acid (SA; 1 mmol L^{-1}) and ultrasound treatment (40 kHz, 10 min) either separately or combined on the chilling injury (CI) in cold-stored peach fruit (*Prunus persica* Batsch cv. Baifeng) were investigated. The results showed that SA treatment alone alleviated CI during storage. Ultrasound alone had no influence, but when it was combined with SA, it resulted in greater inhibition of CI than SA alone. The activities of antioxidant enzymes, such as catalase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase, were induced by a combination of SA with ultrasound. In addition, the combined treatment also increased the endogenous SA concentrations in peaches. These results suggested that the induced tolerance against CI by the combination of ultrasound and SA treatment in cold-stored peach fruit was related to the induction of antioxidant enzymes and the increase in the SA concentration.

KEYWORDS: *Peach, salicylic acid, ultrasound, chilling injury, antioxidant enzymes*

INTRODUCTION

One of the most significant reasons for postharvest loss is due to peach fruit susceptibility to chilling injury (CI) when stored at low but nonfreezing temperatures.[1] Chilling-induced symptoms in peaches include flesh browning, flesh mealiness, failure to ripen normally, increased susceptibility to decay, and accelerated senescence.[2]

Salicylic acid (SA) is a natural phenolic compound involved in the regulation of many processes during plant growth and development, fruit ripening, and responses to environmental stress.[3,4] In recent years, a few studies have reported that SA or methyl salicylate (MeSA) treatment increased resistance to CI and maintained postharvest quality in horticultural crops, such as tomato, sweet pepper, peach, pomegranate, and plum fruit.[5−10] The suppression of CI by SA was associated with reducing leakage, malondialdehyde content, and enhanced polyamine accumulation.[10] The increase in transcript levels of heat-shock proteins and activities of antioxidant enzymes has been observed in peach fruit after SA treatment, which was associated with the reduction of CI.[7,8] The results suggest that SA has potential application as a postharvest treatment for alleviating CI and maintaining a high-quality product.

Also, ultrasonic technology has emerged as a promising alternative in food processing and preservation. Studies on bioactive compound extraction and nondestructive testing of horticultural crops have shown that ultrasound may be a useful processing tool.[11,12] Our previous studies showed that ultrasound treatment was effective in reducing fruit decay and maintaining quality in strawberry fruit.[13,14] Furthermore, a combination of ultrasound and SA treatment may be a useful technique to reduce blue mold in peach fruit.[15] However, to our knowledge, there are no reports on the effect of a combination of SA and ultrasound on the control of CI in postharvest fruit.

It is widely reported that symptoms of CI are a consequence of oxidative stress in fruit tissues from excess reactive oxygen species (ROS).[16] Regulation of ROS can be controlled by antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX). Preconditioning treatments of fruit with SA may induce chilling tolerance by modulating antioxidant systems that would prevent the accumulation of ROS.[7,8]

Thus, the objectives of this study were to evaluate the effects of SA and ultrasound used separately or in combination on controlling postharvest CI of peach fruit and to investigate their influence on the activity of antioxidant enzymes, including SOD, CAT, APX, glutathione peroxidase (GPX), glutathione-*S*-transferase (GST), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). In addition, the effects of the combined treatment on endogenous SA were also assessed.

MATERIALS AND METHODS

Plant Material and Treatment. Peach fruit (*Prunus persica* Batsch cv. Baifeng) were hand-harvested at 12.1 N firmness and 11.08% total soluble solids (TSS) from a commercial orchard in Nanjing, China, selected for uniform size, color, and absence of defects, and then randomly divided into four groups of 90 fruit each.

Ultrasound treatment was applied in a water bath (20 °C) with dimensions of 500 × 300 × 150 mm in the ultrasonic chamber (SB-

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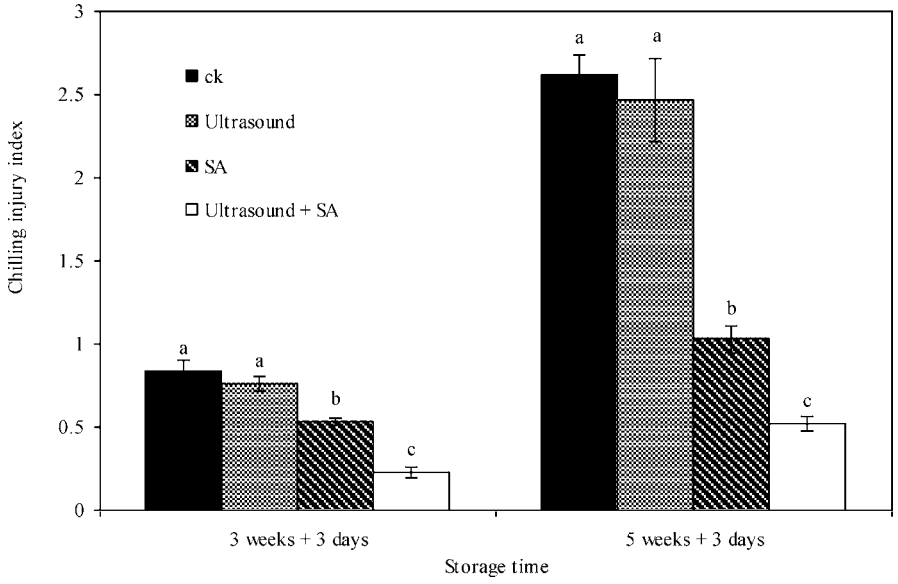

Figure 1. Effects of SA and ultrasound treatment on the CI index of peach fruit after 3 or 5 weeks of storage at 1 °C plus 3 days of shelf life at 20 °C. Vertical bars represent the standard errors of the means. Values with different letters within the same figure are significantly different at $p = 0.05$.

500DTY, Ningbo Xinzhi Science and Technology Co.) and treated with 40 kHz frequency operating at a power of 350 W for 10 min. A total of 1 mmol L^{-1} SA was chosen as the optimal concentration for our experiment based on previous results.[8] Treatments were applied as follows: (1) Fruit immersed into sterile-distilled water at 20 °C for 10 min were designated as the control. (2) Fruit immersed into solution at 1 mmol L^{-1} SA at 20 °C for 10 min were designated as the SA treatment. (3) Fruit were ultrasound-treated in an ultrasonic chamber (40 kHz and 350 W) at 20 °C for 10 min. (4) Fruit treated with 1 mmol L^{-1} SA in the ultrasonic chamber (40 kHz and 350 W) at 20 °C for 10 min were designated as the SA + ultrasound treatment.

After treatment, all fruit were then air-dried for approximately 30 min and stored at 1 °C and 80−90% relative humidity. Samples were collected from five fruit after 0, 3, or 5 weeks of storage at 1 °C for measurements for levels of endogenous SA and jasmonic acid (JA) and activities of SOD, CAT, APX, GPX, GST, MDHAR, DHAR, and GR. Another sample of 10 fruit was removed after 3 or 5 weeks of storage at 1 °C and held at 20 °C for 3 days to simulate shelf conditions for CI evaluation. Each treatment was replicated 3 times, and the experiment was conducted twice with similar results; therefore, only the result in the first experiment was presented. At each time point, pulp tissues were taken from each fruit, frozen in liquid nitrogen, and stored at −80 °C until analyzed.

CI Evaluation. Flesh browning is usually associated with advanced CI symptoms and follows or coexists with symptoms of peach fruit during cold storage. Internal browning is used to evaluate the development of CI in peach fruit. The extent of flesh browning was divided into four classes: 0, no browning; 1, browning covering <25% of the cut surface; 2, browning covering ≥25% but <50% of the cut surface; and 3, browning covering ≥50%. The CI index was calculated using the following formula:

$$\text{CI index} = \sum [(\text{browning level})(\text{number of fruit at the browning level})]/(\text{total number of fruit in the treatment})$$

Enzyme Activity Determination. All enzyme extract procedures were conducted at 4 °C. For SOD, 2 g of flesh tissue was ground with 5 mL of 50 mmol L^{-1} sodium phosphate buffer (pH 7.8). Flesh tissue (2 g) was ground with 5 mL of 50 mmol L^{-1} sodium phosphate buffer (pH 7.0) for CAT. For APX, MDHAR, and DHAR, flesh tissue (2 g) was ground with 5 mL of 50 mmol L^{-1} sodium phosphate buffer (pH 7.0) containing 0.1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA), 1 mmol L^{-1} acetylsalicylic acid (AsA), and 1% (w/v) polyvinylpyrrolidone. A total of 2 g of fruit tissue was homogenized in 5 mL of 0.1 mol L^{-1} Tris-HCl buffer (pH 7.8) containing 2 mmol L^{-1} EDTA and 2 mmol L^{-1} dithiothreitol for GR, GST, and GPX. The extracts were then homogenized and centrifuged at 20000*g* for 20 min at 4 °C. The supernatants were used for the enzyme assays. All of the above antioxidant enzymes were measured as in our previous study.[17] The protein content in the enzyme extracts was estimated according to the method by Bradford,[18] using bovine serum albumin as a standard. The specific activity of the enzymes was expressed as units per milligram of protein.

Endogenous SA Assay. The quantitative determination of SA was carried out via an enzyme-linked immunosorbent assay (ELISA). Flesh tissue (2.0 g) was extracted with 5 mL of 80% (v/v) methanol. The extract was centrifuged at 12000*g* for 15 min. After that, the supernatant was then reduced to the aqueous phase by rotary evaporation and then re-extracted with 30% (v/v) methanol and 0.2% (v/v) acetic acid. After centrifugation at 12000*g* for another 15 min, the supernatant was passed through a Sep-Pak C_{18} Cartridge (Waters, Milford, MA) and the eluate was used for SA analysis. The SA content was measured using the Plant SA ELISA Kit (Research and Diagnostics Systems, Inc., Minneapolis, MN) as described in the protocol of the manufacturer. The concentration of SA in the samples was then calculated according to the standard curve.

Data Analysis. Experiments were performed using a completely randomized design. All statistical analyses were performed with SPSS (SPSS, Inc., Chicago, IL). The data were analyzed by two-way analysis of variance (ANOVA) with the treatment and storage time as factors. The means were separated by Tukey's test, and differences at $p \leq 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

SA is a small signaling molecule in plants that mediates the defense response to chilling stress.[19] Our results are in agreement with previous finding on the effect of SA in reducing CI in peaches[7,8] (Figure 1). The control peach fruit developed chilling symptom severely, and the index was 1.2 and 1.9 after 3 and 5 weeks at 1 °C plus 3 days at 20 °C, respectively. SA treatment effectively reduced CI incidence, and the index is only 1.2 at the end of storage. Ultrasound treatment had no influence on CI incidence in peaches during storage; however, the combined application of the two

treatments was much more effective than the application of SA alone and reduced the CI index to 0.3 and 0.6 after 3 and 5 weeks at 1 °C plus 3 days at 20 °C, respectively (Figure 1), which suggested that the two treatments in combination activate more defensive capacity against chilling in peach fruit. Therefore, ultrasound combined with SA could be considered by processors prior to its adoption as a preservation technique.

SA, as a signal molecule, could directly or indirectly change cold tolerance during chilling stress, which had an ability to alleviate chilling-induced injury.[19,20] An increase in the endogenous levels of SA in postharvest loquat fruit after treatment with AsA has been reported to coincide with the activation of the chilling tolerance.[20] Similarly, we found that an enhanced chilling tolerance in peach fruit treated with SA plus ultrasound was associated with increased SA concentrations during cold storage, while the combined treatment induced a higher chilling tolerance than that in SA-treated fruit alone (Figure 2). Hence, these results suggested that an increased SA

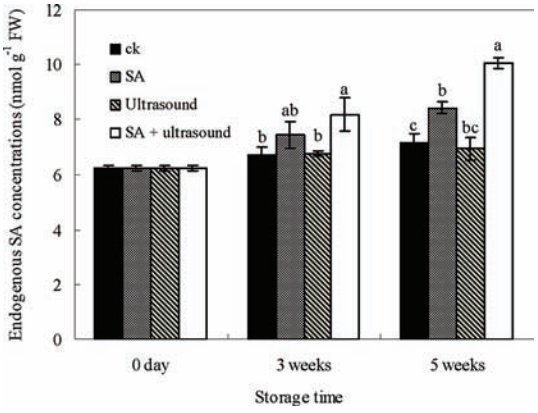

Figure 2. Effects of SA and ultrasound treatment on the endogenous SA content in peach fruit after 3 or 5 weeks of storage at 1 °C plus 3 days of shelf life at 20 °C. Vertical bars represent the standard errors of the means. Values with different letters within the same figure are significantly different at $p = 0.05$.

content may be involved in the induction of the chilling tolerance. It is known that ultrasound generates macroturbulence, high-velocity interparticle collisions, and perturbation in microporous particles of the biomass.[21] SA could infiltrate into peaches more easily when ultrasound was used, which might be used to explain the higher SA content in peach fruit with treatment of SA and ultrasound in combination. The additional synergistic effect of ultrasound treatment on CI in peach fruit, not previously reported, is probably caused by an enhanced SA content.

CI can induce the accumulation of ROS, such as singlet oxygen, superoxide radical, hydrogen peroxide (H_2O_2), and hydroxyl radical, which cause oxidative damage to plants.[16] Plants can protect themselves against oxidative damage by the antioxidant system, including antioxidative enzymes and nonenzymatic compounds.[22] SOD, CAT, GPX, and GST are a part of protective enzymes that respond to oxidative stress. The application of SA treatment alone led to an increase in SOD and GST contents during storage compared to control peach fruit (Table 1), which might be related to the reduction in CI of peach fruit when treated by SA alone. On the other hand, our present study also showed that the combined treatment induced higher CAT activity than the application of SA treatment alone (Table 1), which could be used to explain

Table 1. Effect of Ultrasound and SA Treatment on Activities (Units per Milligram of Protein) of SOD, CAT, GPX, and GST of Peach Fruit after Storage at 1 °C for 3 or 5 Weeks[a]

storage time	treatment	SOD	CAT	GPX	GST
0 week		96.78	30.60	1.35	10.35
	control	84.52 b	32.94 bc	1.04 a	7.84 b
	SA	95.70 a	39.88 b	1.45 a	10.77 a
3 week	ultrasound	74.34 c	31.03 c	1.10 a	10.83 a
	SA + ultrasound	78.06 bc	48.96 a	1.36 a	11.60 a
	control	77.85 c	34.26 b	1.33 a	9.30 b
	SA	91.27 a	30.22 b	1.21 a	13.10 a
5 week	ultrasound	81.79 bc	28.15 b	0.96 a	8.89 b
	SA + ultrasound	83.50 b	44.31 a	0.99 a	13.30 a
significance[b]					
treatment (T)	df 3	sig	sig	ns	sig
during (D)	df 2	sig	sig	ns	sig
$T \times D$	df 6	sig	ns	ns	sig

[a]Data were expressed as the mean of triplicate assays. Values within the same column for a given storage period followed by a different letter were significantly different at $p = 0.05$. [b]ns, not significant; sig, significant at $p \leq 0.05$.

partly why the combined treatment was more effective in reducing CI, as compared to SA treatment alone (Figure 1). APX, MDHAR, DHAR, and GR are key enzymes involved in the ascorbate−glutathione cycle.[23] A higher activity of these enzymes may be associated with the chilling tolerance. For example, activities of these four enzymes were found to be higher in chilling-tolerant loquat fruit[24] or in other postharvest crops.[25,26] Our present study showed that SA combined with ultrasound induced higher activities of APX, MDHAR, DHAR, and GR than the control or SA treatment alone (Table 2). Preconditioning treatments of fruit with SA may induce the

Table 2. Effect of Ultrasound and SA Treatment on Activities (Units per Milligram of Protein) of APX, MDHAR, DHAR, and GR of Peach Fruit after Storage at 1 °C for 3 or 5 Weeks[a]

storage time	treatment	APX	MDHAR	DHAR	GR
0 week		20.08	1.27	0.92	0.50
	control	10.36 b	0.78 a	0.70 a	0.36 a
	SA	13.86 ab	0.61 a	0.82 a	0.41 a
3 week	ultrasound	11.79 b	0.78 a	0.52 a	0.41 a
	SA + ultrasound	19.23 a	0.61 a	0.51 a	0.41 a
	control	12.45 b	0.92 b	0.74 b	0.30 c
	SA	10.71 b	0.77 b	0.68 b	0.49 b
5 week	ultrasound	14.10 ab	0.80 b	0.81 ab	0.29 c
	SA + ultrasound	18.36 a	2.01 a	1.45 a	0.70 a
significance[b]					
treatment (T)	df 3	sig	sig	ns	ns
during (D)	df 2	sig	sig	sig	ns
$T \times D$	df 6	sig	sig	ns	ns

[a]Data were expressed as the mean of triplicate assays. Values within the same column for a given storage period followed by a different letter were significantly different at $p = 0.05$. [b]ns, not significant; sig, significant at $p \leq 0.05$.

chilling tolerance by modulating antioxidant systems that would prevent the accumulation of ROS.[7,8] Therefore, in the present study, the additionally enhanced antioxidant enzyme activities (in a synergistic way) by ultrasound treatment are due to the enhancement of the SA content in peach partly. This finding is possibly another evidence of alleviating CI in peach fruit treated by SA combined with ultrasound treatment.

In conclusion, the results of our study suggested that the combination of ultrasound and SA was more effective in alleviating CI in peach fruit than the individual application of SA. The effect of SA combined with ultrasound treatment on enhancing the chilling tolerance was related to its effect on inducing the antioxidant system, such as CAT, APX, MDHAR, DHAR, and GR, and the endogenous SA content. Thus, it is considered that ultrasound combined with SA could be a reliable method to control CI of postharvest peach fruit during refrigerated storage.

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