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The Activities of SOD, POD, and CAT in 'Red Spur Delicious' Apple Fruit Are Affected by DPA but Not Calcium in Postharvest Drench Solutions

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Abstract

This study was designed to determine the effect of diphenylamine (DPA) and $CaCl_2$ on activities of antioxidant enzymes: superoxide dismutase (SOD: EC 1.1.15.1.1), catalase (CAT: EC 1.11.1.6), and peroxidase (POD: EC 1.11.1.7) during storage of apple fruits and to correlate changes in these enzymes to scald development and fruit maturity. Fruits from 'Red Spur Delicious' apples at early mature, full mature, and over mature stages were treated with 2000 ppm DPA (diphenylamine) shortly after harvest and stored at -1°C. DPA treatment did not affect flesh firmness, soluble solids, or starch rating at harvest or after 6 months in storage. DPA treatment prevented the storage related increase in POD activity, enhanced the SOD and CAT activities and reduced the scald development during storage in early and full mature fruit. The $CaCl_2$ treatments (1 and 2%) maintained higher flesh firmness and increased CAT activity, but had no significant effect on POD and SOD activities.

Introduction

Synthetic antioxidant diphenylamine (DPA) is used to control superficial scald commercially in apple fruit (*Malus xdomestica* Borkh) (32), and was found to maintain a high level of reducing power and hence slowed the senescence process (21).

Superficial scald is an important physiological storage disorder of apple fruit. The importance of oxidation processes in the etiology of scald development has led many authors to evaluate natural and synthetic molecules with antioxidant properties capable of reducing scald (3). Endogenous high levels of antioxidants at harvest have been associated with reduced susceptibility to scald (23). However, the role of naturally occurring antioxidant enzymes, such as superoxide dismutase (SOD; EC 1.1.15.1.1), catalase (CAT: EC 1.11.1.6), and peroxidase (POD; EC 1.11.1.7) in scald development has not been fully investigated. These enzymes have been described as a defensive team, whose combined effect is to minimize exposure of cells to reactive intermediates of dioxygen reduction (12, 13). In addition to the endogenous antioxidants pool, mineral elements (like calcium) were also found to be negatively correlated with scald development (4, 7).

The objectives of the present study were to determine the influence of postharvest drench materials, DPA and $CaCl_2$, on activities of the antioxidant enzymes (SOD, CAT, and POD) and the association of these enzymes activities with scald development in apple fruit.

Materials and Methods

Fruits for this study were harvested from 25-year-old-apple (*Malus xdomestica* Borkh cv. 'Red Spur Delicious') trees grafted on MM.106 rootstock grown at the Pomology Research Center, University of Illinois at Urbana-Champaign. To determine the effect of DPA on enzymes activities and scald development, fruits harvested at early mature, commercially mature, and at over mature stages were dipped in a solution of 2,000 ppm DPA immediately after harvest. Flesh firmness, soluble solids, and starch content were

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measured on treated and untreated (control) fruit before and after six months storage at –1°C.

A 5-6 g fresh peel (from 5 fruits) per treatment from each of the three replications was ground in liquid nitrogen using a mortar and pestle. The powdered tissue was suspended in 15 ml of 100 mM KPO_4 buffer (pH 7.8), containing 0.5% [v/v] triton X-100 and 1g PVPP. The mixture was centrifuged at 18,000xg at 4ºC for 30 min and the supernatant was collected and stored at -80°C for the analysis of enzymes activities. SOD, CAT and POD enzymes activities were assayed in the supernatant from fruits of each treatment according to Abassi et al. (1).

A scald rating was recorded after removal of fruit from storage. Scald severity ratings were: light (less than about 10% of the surface affected), medium (10-50% of surface affected), and severe scald (50-100% of surface affected). The following equation was used to calculate scald index:

Scald index = [(% incidence of light scald x 1) + (% incidence of medium scald x 2) + (% incidence of severe scald x 4)]/4

Another set of fruits harvested at commercial maturity was treated with 0%, 1%, 2% $CaCl_2$ and then stored at -1°C for 3 months. Fruits were evaluated for ethylene evolution, flesh firmness, soluble solids,and starch, and the enzyme activities were completed as previously noted.

Results and Discussion

DPA treatment did not affect flesh firmness, soluble solids, or starch rating after 6 months of storage. However, this treatment reduced scald rating significantly in early mature fruits (Table 1). Previous studies have shown inconsistent results regarding DPA effect on fruit firmness. Few studies (20, 21) have reported higher firmness retention in DPA treated apple fruits. A later study (22)

Table 1: Effect of storage and DPA treatments on firmness, soluble solids, starch, and scald of 'Red Spur Delicious' apples.

Maturity	Storage (Months)	DPA 2000ppm	Firmness (N)	S.S. (%)	Starch[a] index	Scald index[b]
Early	0	Control	85.4 a	9.3 e	1.4 d	--
mature	0	DPA	82.9 a	9.4 e	1.3 d	--
	6	Control	66.6 cd	12.8 a	9.0 a	42.5 a
	6	DPA	68.1 c	12.5 a	9.0 a	15.8 b
Full	0	Control	77.4 b	9.9 d	3.7 c	--
mature	0	DPA	77.4 b	10.1 cd	3.8 c	--
	6	Control	65.1 cd	12.7 a	9.0 a	4.2 bc
	6	DPA	65.7 cd	12.8 a	9.0 a	1.7 cd
Over	0	Control	76.2 b	10.5 bc	5.1 b	--
mature	0	DPA	75.4 b	10.9 b	5.2 b	--
	6	Control	64.3 d	13.1 a	9.0 a	1.0 cd
	6	DPA	65.2 cd	13.1 a	9.0 a	0.0 d

Different letters within column denote significant difference at ρ = 0.05; means are separated according to Fisher's LSD.
[a]1 = 100% starch and 9 = 0% starch.
[b]Scald Index = {(%light scald x 1) + (%medium scald x 2) + (%severe scald x 4)}/4

Table 2: Effect of storage and DPA treatments on enzymes activities of 'Red Spur Delicious' apples.

Maturity	Storage (months)	DPA 2000ppm	POD ΔO.D. $min^{-1}mg^{-1}$P	SOD $u.g^{-1}$fr.wt	Catalase $u.g^{-1}$fr.wt	Scald index[a]
Early	0	Control	0.58 d	34.16 d	13.78 e	--
mature	0	DPA	0.62 d	35.25 d	14.86 e	--
	6	Control	1.68 a	69.95 c	20.32 d	42.5 a
	6	DPA	0.95 c	89.43 b	28.70 c	15.8 b
Full	0	Control	0.49 d	23.11 e	12.61 e	--
mature	0	DPA	0.50 d	24.08 e	15.42 e	--
	6	Control	1.18 b	87.29 b	32.25 c	4.2 bc
	6	DPA	0.51 d	105.29 a	47.95 a	1.7 cd
Over	0	Control	0.69 d	19.00 f	15.93 e	--
mature	0	DPA	0.52 d	21.65 ef	16.47 de	--
	6	Control	1.11 bc	90.57 b	41.64 b	1.0 cd
	6	DPA	0.51 d	103.50 a	42.20 b	0.0 d

Different letters within column denote significant difference at $\rho = 0.05$; means are separated according to Fisher's LSD.
[a]Scald Index = {(%light scald x 1) + (%medium scald x 2) + (%severe scald x 4)}/4

did not find any effect of DPA treatment on flesh firmness. Our data support studies that show no significant effect of DPA on flesh firmness.

Results in Table 2 indicate that there was an increase in POD activity with storage and DPA inhibited this increase. SOD and CAT activities were enhanced in DPA treated fruit during storage. Full mature fruit had lower POD activity while higher SOD and CAT activities as compared to early-mature fruit after 6 months storage. Increase in POD activity during storage confirms previous findings that POD activity peaks during fruit ripening and senescence (16, 28). These changes in POD activity might be linked to changes in its substrate (H_2O_2) concentration. H_2O_2 was reported to increase with the climacteric rise in ethylene production (6, 18). Previous studies have shown that in addition to its role in H_2O_2 removal, POD has been implicated in regulation of cell elongation (15), phenol oxidation (31), ethylene production (14), auxin breakdown (11), and chlorophyll degradation (17). POD is also involved in polymerization of phenolics into brown pigments (19). Since scald results from polymerization of phenolic compounds, it is possible that DPA inhibition of scald may have resulted, at least in part, from inhibition of POD activity.

Plants which over-express SOD activity by genetic manipulation, were found to have enhanced oxidative stress protection (2, 8, and 26). Our finding of relatively higher SOD activity in mature apple fruits, which are less susceptible to scald (9), shows that mature fruits might have had higher ability to combat free radical damage by expressing higher SOD activity. Our study indicates that application of DPA enhanced the activity of the antioxidant enzyme SOD during storage and significantly reduced scald incidence. This suggests that DPA, in addition to its many other effects in controlling scald, might have also been involved in inducing the antioxidant enzyme SOD and hence increasing the endogenous antioxidant pool to prevent fruit damage by free radicals. These results disagree with the finding where DPA treatment had no significant effect on SOD activity as compared to non-treated fruit (10).

Mature fruits have higher ability to produce antioxidants, including catalase, in response to free radicals to avoid possible membrane damage than do immature fruits (24, 29). Increase in catalase activity in mature, over-mature, and DPA treated fruits in this study (Table 2) is likely in response to an increase in H_2O_2 build-up.

Effect of $CaCl_2$ After three months in cold storage apple fruits treated with 2% $CaCl_2$ maintained significantly higher firmness compared to control fruits. Soluble solids, starch, and ethylene synthesis were not affected (Table 3). Ca plays a special role in maintaining the cell wall structure in fruits and other storage organs by interacting with the pectic acid in the cell walls to form calcium pectate. Thus, fruits treated with Ca are generally firmer than control (27, 30).

Only CAT activity at 2% $CaCl_2$ treatment was significantly increased while SOD and POD activities remained unaffected as compared to untreated fruit (Table 4). However, the increase in CAT activity was not paralleled by an increase in SOD activity. The build-up of H_2O_2, which might has stimulated catalase activity in this case, may have been produced from other sources i.e. photorespiration and lipid peroxidation (13, 32).

In conclusion, this study has identified a possible relationship between enzyme activities and DPA treatment during ripening, and scald development. In contrast to DPA, CaCl2 treatment had no effect on antioxidant enzyme activities except catalase. DPA treatment and maturity were found to be involved in the inhibition of POD activities and enhancement of SOD and CAT activities during storage. This study also provides evidence that DPA treatment controls scald not only by inhibiting α-farnesene oxidation but also by affecting activities of enzymes associated with oxidative reactions. Association of increased SOD and CAT activities in mature and DPA treated fruits with reduced scald incidence, further support evidence that free radicals play an important role in the development of this disorder. However, biological levels of antioxidant enzymes are not sufficient to protect living tissue from harmful effects of free radicals (25). Therefore, genetical or physiologic manipulation of SOD and CAT along with other antioxidant enzymes may result in reducing scald damage and prolonging the shelf life of harvested produce.

Table 3: Changes in firmness, soluble solids, starch, and ethylene in $CaCl_2$ treated 'Red Spur Delicious' apples stored for three months

Storage (mo)	$CaCl_2$	Firmness (N)	Soluble solids (%)	Starch[a]	Ethylene $\mu l\ kg^{-1}\ hr^{-1}$
0	----	76.7 a	11.0 b	5.5 b	0.8 b
3	0%$CaCl_2$	69.9 b	12.7 a	7.5 a	8.5 a
3	1% $CaCl_2$	73.1 ab	12.7 a	7.3 a	7.8 a
3	2% $CaCl_2$	73.8 a	12.8 a	7.3 a	8.2 a

Different letters within column denote significant difference at $\rho = 0.05$; means are separated according to Fisher's LSD.

[a]1 = 100%, 9 = 0% starch.

Table 4: Enzyme activity in $CaCl_2$ treated 'Red Spur Delicious' apples stored for three months

Storage	$CaCl_2$	POD	SOD	Catalase
		ΔO.D. min^{-1} mg^{-1} P	$u.g^{-1}fr.wt$	
0	-----	0.74 a	20.13 c	15.61 b
3	0% $CaCl_2$	0.92 a	87.64 b	18.04 b
	1% $CaCl_2$	0.75 a	88.40 b	17.19 b
	2% $CaCl_2$	0.70 a	88.91 b	23.17 a

Different letters within column denote significant difference at ρ = 0.05; means are separated according to Fisher's LSD.

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