Carbon Dioxide, Oxygen, and Ethylene Changes in Relation to the Development of Scald in Granny Smith Apples after Cold Storage

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To control the development of scald during storage at 0 to 1 °C, Granny Smith apples (*Malus domestica*) were treated with diphenylamine (DPA) or the sucrose ester-based coating Semperfresh, either alone or formulated with the food-approved antioxidant ascorbyl palmitate. Scald control by DPA was not associated with alterations in the internal atmosphere of the apples. However, the alterations in the internal gas atmosphere brought about by the Semperfresh coatings were correlated with the partial scald control observed with ascorbyl palmitate plus Semperfresh after 4 months of storage. Under certain conditions, coating could have affected activity of the 1-aminocyclopropane-1-carboxylate (ACC) oxidase in vivo via decreased internal oxygen levels, but not via changes in the carbon dioxide levels. It is concluded that the limited control of scald by ascorbyl palmitate plus Semperfresh is partially related to the observed modification of the internal atmosphere of the apple.

Keywords: *Malus domestica; ascorbyl palmitate; diphenylamine (DPA); ethylene; carbon dioxide; oxygen; Semperfresh; superficial scald*

INTRODUCTION

Some apple varieties, such as Granny Smith, are susceptible to the storage disorder superficial scald (Fidler et al., 1973), which appears to arise from the oxidation of a sesquiterpene (α-farnesene) to conjugated triene hydroperoxides (Huelin and Coggiola, 1970; Filmer and Meigh, 1971). Amine-type antioxidants, such as diphenylamine (DPA) and ethoxyquin, effectively reduce scald (Smock, 1957), but concern over residues is leading to a search for alternative measures.

Changing the atmosphere around the stored apples has been reported to control scald, with regimes of low oxygen and high carbon dioxide (Patterson and Workman, 1962) or low oxygen (Little and Taylor, 1981) advised. In some parts of the world, these techniques are applied commercially, but where they are not cost-effective, alternatives need to be found. Consequently, we have been examining the effectiveness of treating apples with edible coatings in combination with ascorbyl palmitate, a food-compatible antioxidant. The edible coating, marketed as Semperfresh, delays ripening by altering the permeability of the coated fruit so that internal oxygen levels decrease and carbon dioxide levels increase (Smith and Stow, 1984; Banks, 1985). Thus, our approach effectively combines an antioxidant with an alteration of the gaseous conditions of fruit during storage.

Elsewhere we report in detail on the effectiveness of the treatments used (Bauchot et al., 1995). In the present paper we describe the changes in the levels of carbon dioxide, oxygen, and ethylene that are associated with the development of scald in Granny Smith apples that have undergone a variety of treatments designed to combat scald. Ethylene was included because the delay in ripening by storage in a controlled atmosphere and by the modified atmosphere created by coatings are partially due to alterations in ethylene production and action (Kader, 1986). Moreover, low ethylene (Little et al., 1985) or low oxygen and low ethylene (Lau, 1990) during storage have been shown to reduce scald development. More recently, Du and Bramlage (1994) have suggested that "ethylene has a fundamental role in changes associated with superficial scald development".

To understand further how the changes in carbon dioxide and oxygen might be controlling ethylene levels, we also examined the effect of carbon dioxide and oxygen on the enzyme in the apples that is responsible for the final step in the generation of ethylene, that is, 1-aminocyclopropane-1-carboxylate (ACC) oxidase. This enzyme uses oxygen as a substrate, and its activity is regulated by carbon dioxide levels both in vivo (Kao and Yang, 1982) and in vitro (Dong et al., 1992; Smith and John, 1993; Fernández-Maculet et al., 1993).

EXPERIMENTAL PROCEDURES

Plant Material. Experiments reported here are part of a larger multisite trial (Bauchot et al., 1995). Internal atmosphere was measured on Granny Smith apples that were harvested in Moissac, France, on September 29, 1992 (~2 weeks prior to commercial harvest), treated after harvest, and then transported to Reading, U.K., and stored at 0–1 °C under normal atmosphere. Ethylene emission was measured on early-harvested Granny Smith apples from Lérida, Spain, that were treated after harvest and immediately stored in Lérida at 1(± 1) °C under normal atmosphere. The ACC oxidase was extracted from apples purchased in Reading.

Treatments. Replicates of 10 apples each were dipped after harvest in DPA at 2500 ppm, 1% Semperfresh, or ascorbyl palmitate at 1875 ppm in 1% Semperfresh. Active ingredients of Semperfresh are sucrose esters of fatty acids (E473) formulated with sodium carboxymethylcellulose and monoacyl- and diacylglycerols. Emulsions were prepared and supplied as concentrates by Surface Systems International, East Challow, U.K. Controls were not dipped.

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Table 1. Effect of Different Postharvest Treatments on the Incidence of Scald and Internal Atmosphere of Granny Smith Apples Stored at 0−1 °C for 4 and 6 Months[a]

			internal atmosphere					
	scald score		CO_2 (%)		O_2 (%)		C_2H_4 (ppm)	
treatment	day 1	day 10	day 1	day 10	day 1	day 10	day 1	day 10
			After 4 Months of Storage					
control	1.9[a]	3.0[a]	5.2[a]	6.4[c]	15.6[b]	17.2[a]	105[c]	289[c]
DPA	1.0[b]	1.2[c]	5.8[a]	6.2[c]	17.2[ab]	16.1[b]	140[c]	272[c]
Semperfresh	1.7[a]	3.0[a]	2.4[c]	8.2[b]	19.0[a]	15.7[b]	389[b]	463[b]
Semperfresh + ascorbyl palmitate	1.1[b]	2.3[b]	3.8[b]	8.9[a]	9.3[c]	13.2[c]	595[a]	649[a]
			After 6 Months of Storage					
control	3.5[a]	3.9[a]	8.5[c]	7.0[b]	16.7[a]	15.5[b]	29[c]	143[d]
DPA	1.5[c]	1.6[b]	6.1[d]	4.3[c]	18.3[a]	16.8[a]	19[c]	211[c]
Semperfresh	2.9[ab]	4.0[a]	14.0[a]	9.2[a]	7.7[b]	11.8[c]	187[b]	389[b]
Semperfresh + ascorbyl palmitate	2.5[b]	3.6[a]	9.2[b]	8.6[a]	9.3[b]	11.8[c]	283[a]	459[a]

[a] Measurements were made on removal from the cold store (day 1) and after 10 days of storage at room temperature. Mean separation within columns for the same length of storage by Duncan's new multiple range test, 5% level. Values followed by different letters are significantly different.

Scald Scoring. After 4 and 6 months of storage, three to five replicates of each treatment were removed at room temperature and scald development was estimated on days 1 and 10. Intensity of scald was visually estimated according to the percentage of scalded surface with a scale from 0 to 4, where 0 = none, 1 = slight (<10% of the surface area affected), 2 = moderate (10−25%), 3 = severe (25−50%), and 4 = very severe (>50%). Mean scores were calculated for the replicates. Statistical analyses were carried out with appropriate SAS packages by the GLM procedure.

Emission of Ethylene. Five apples of the same batch of Spanish apples were enclosed in a 3-L jar. After 1 h, 1 mL of atmosphere was withdrawn from the jar through a septum with a hypodermic syringe. The ethylene content of the samples was determined as previously described (Mitchell et al., 1988).

Internal Atmosphere. For measurements of the internal atmosphere, 10 fruits from each treatment group were plunged in water and two 0.5-mL samples of the internal atmosphere were withdrawn from the core through the calyx end with hypodermic syringes. Carbon dioxide and oxygen were measured with a Pye Unicam gas chromatograph (GC) fitted with a Poropak R column and a thermal conductivity detector (TCD). Concentrations of the gases were determined by reference to air for oxygen and to a volumetrically prepared standard for carbon dioxide in nitrogen.

Preparation of ACC Oxidase-Enriched Fractions. The methods used were based on procedures published for other varieties of apple (Fernández-Maculet and Yang, 1992; Kuai and Dilley, 1992; Dupille et al., 1993). Apple pulp (100 g) was homogenized in a blender with 100 mL of 0.1 M Tricine-HCl (pH 8.0) containing 10% glycerol, 3 mM DTT, 1% PVP, and 0.1% Triton X-100. After filtration through a double layer of muslin, the homogenate was centrifuged for 20 min at 20 000×*g*, and the supernatant was adjusted to 30% $(NH_4)_2SO_4$. After centrifugation, the supernatant was adjusted to 90% $(NH_4)_2SO_4$ and centrifuged again.

The resulting pellet was resuspended in 10 mL of buffer A (20 mM Tricine-HCl, pH 7.5; 10% glycerol; 3 mM DTT) containing 1 M $(NH_4)_2SO_4$ and loaded onto a 25-mL Phenyl-Sepharose CL-4B column (Pharmacia, Uppsala). Fractions of 5 mL were collected from the column that was eluted at 1 mL min^{-1} with buffer A [50 mL adjusted to 1 M $(NH_4)_2SO_4$, then 50 mL at 0.5 M, 50 mL at 0.1 M, and eventually 90 mL at 0 M]. Activity of ACC oxidase was eluted with buffer A alone. The eight most active fractions were concentrated to 25 mL with Centricon 10 cartridges (Amicon, Beverly, MA) and desalted on PD-10 column (Sephadex G-25 M, Pharmacia) then, 5-mL fractions were loaded onto a 1-mL Mono-Q HR 5/5 column (Pharmacia) equilibrated with buffer A and eluted with a NaCl gradient at a flow rate of 1 mL min^{-1}. Fractions containing ACC oxidase were pooled and desalted with a column of Sephadex G-25 M.

ACC Oxidase Activity Assays. Enzyme activity was assayed by measuring the ethylene produced (Mitchell et al., 1988) after incubation of 200 μL of extract for 15 min at 30 °C in a 7-mL vial containing 800 μL of reaction mixture (0.1 M Tricine-HCl, pH 7.5; 10% glycerol; 3 mM DTT; 10 μM $FeSO_4$; 1 mM ACC; 20 mM $NaHCO_3$; and 20 mM sodium ascorbate). When the concentration of oxygen was varied, ascorbate was initially omitted from the reaction mixtures and the vials were flushed with nitrogen. One milliliter of the vial gas phase was replaced by a gaseous mixture containing varying proportions of oxygen and nitrogen. After 1 h of equilibration at room temperature, the enzyme extract and ascorbate were added. After the assay was completed, the concentrations of oxygen and carbon dioxide in the headspace were measured by GC-TCD. All determinations were replicated at least four times, and the results are expressed as means.

RESULTS AND DISCUSSION

Scald Incidence. After withdrawal from 4 months of storage, the untreated apples suffered from scald on an average of 10−25% of their surface (Table 1). After 10 days at room temperature, scald increased to affect an average of 25−50% of the apple surface. Treatment with DPA controlled scald so that it never affected >10% of the apple surface. The formulation of ascorbyl palmitate plus Semperfresh also provided a slight control over scald at the beginning of the shelf life (Table 1).

After a further 2 months of storage, there was a corresponding increase in the severity of scald both in control and coated apples. Again, however, DPA treatment provided an effective control (Table 1). Semperfresh alone did not provide any benefit, but ascorbyl palmitate plus Semperfresh showed a modest measure of control. This modest control was lost after the apples remained at room temperature for 10 days (Table 1). In general, these findings are consistent with those of Kerbel et al. (1989) who found that Semperfresh alone did not prevent scald, Kallay (1994) who found that Semperfresh−antioxidant combinations were effective with Granny Smith apples, and Manseka and Vasilakakis (1993) who found some scald control with ascorbic acid on Starking Delicious apples. The apples used in our experiments were harvested early to exacerbate scald development, and we have described elsewhere (Bauchot et al., 1995) how the Semperfresh−antioxidant combination was more effective when ripe fruit was used.

Internal Carbon Dioxide and Oxygen. There was no effect of DPA on the internal levels of carbon dioxide and oxygen after storage for either 4 or 6 months (Table 1). By contrast, coating the apples with Semperfresh

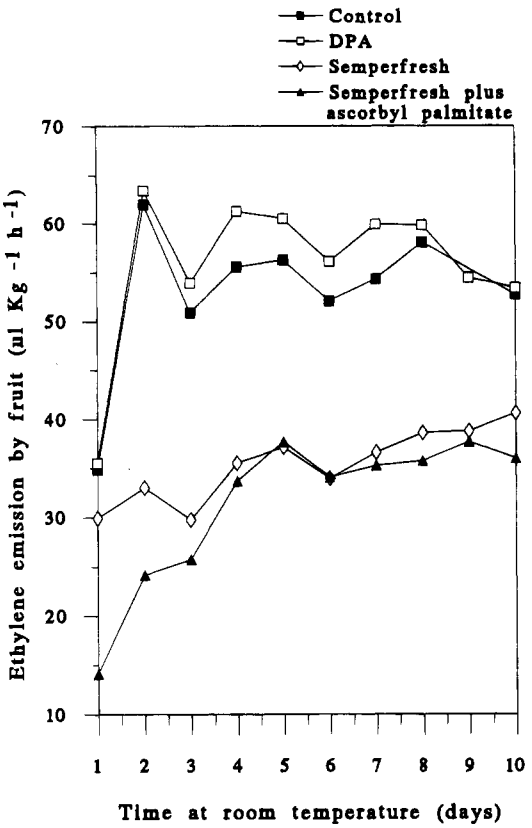

Figure 1. Ethylene emission by apples maintained at room temperature after 4 months of storage at 1 °C.

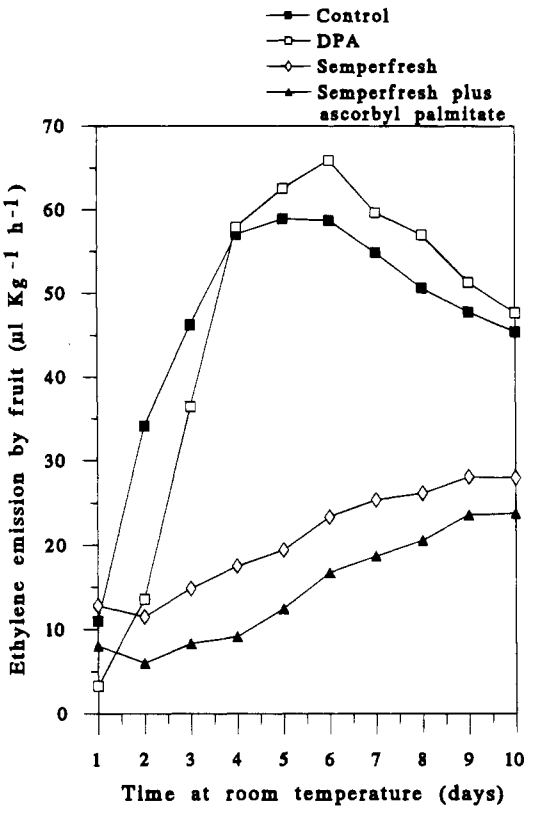

Figure 2. Ethylene emission by apples maintained at room temperature after 6 months of storage at 1 °C.

affected carbon dioxide and oxygen levels dramatically. Specifically, coating caused carbon dioxide levels to rise during the 10-day shelf life after 4 months of storage; but, this effect was not observed after 6 months of storage (Table 1). Moreover, the internal carbon dioxide levels were lower on withdrawal from 4 months of cold storage in coated apples compared with all the other treatments (Table 1).

Changes in the internal levels of oxygen were apparent with the ascorbyl palmitate plus Semperfresh after withdrawal from either 4 or 6 months of cold storage (Table 1). The relative stability of the oxygen levels during the shelf life of the coated apples after storage, in contrast to the accumulation of carbon dioxide that resulted from coating, suggests that the Semperfresh was more permeable to oxygen than to carbon dioxide with Granny Smith (see Banks, 1985).

Ethylene Accumulation and Emission. Compared with the untreated control, DPA treatment had only a slight effect on both the internal ethylene levels (Table 1) and on the rates of ethylene emission (Figures 1 and 2), despite the greater tissue damage suffered by the scalded control (Table 1). By contrast, coating with Semperfresh, either alone or with ascorbyl palmitate, raised the internal ethylene levels (Table 1) and depressed ethylene emission (Figures 1 and 2). The presence of ascorbyl palmitate plus Semperfresh increased the internal ethylene levels after 4 and 6 months of storage (Table 1) compared with Semperfresh alone, but otherwise ascorbyl palmitate plus Semperfresh had no effect the rate of ethylene emission (Figure 1). The surge in ethylene emission by the uncoated apples during the 10 days of shelf life (Figures 1 and 2) was matched by a dramatic increase in the internal ethylene levels in these uncoated apples during this period (Table 1). By contrast, the coated apples, having accumulated ethylene during cold storage (Table 1), had overall lower emission rates during the subsequent shelf life (Figures 1 and 2). These results are consistent with those of previous authors who had noted that sucrose ester-based coatings, such as Semperfresh, formed an effective barrier to ethylene diffusion from treated fruit (Kerbel and Kader, 1988; Kerbel et al., 1989).

Table 2. Correlation Coefficients between the Concentrations of Internal Gases (O_2, CO_2, and C_2H_4) in the Apples and Scald Development[a]

	storage duration					
	4 months			6 months		
parameter	O_2	CO_2	scald	O_2	CO_2	scald
C_2H_4	−0.92[b]	0.98[b]	0.83[b]	−0.95[b]	NS[c]	NS
O_2	—	−0.97[b]	−0.97[b]	—	NS	NS
CO_2	—	—	0.92	—	—	NS

[a] All measured after 4 and 6 months of cold storage and a 10 day shelf life. Data from all treatments except DPA. [b] Significant at 0.01 level. [c] NS, Nonsignificant.

Relationship between Internal Gases and Scald Incidence on Coated Apples. It is clear that the effectiveness of DPA is not related to changes in the internal atmosphere (Table 1). However, when ascorbyl palmitate plus Semperfesh delayed scald development during the early stage of shelf life after 4 months of storage, this treatment also induced a higher accumulation of ethylene and carbon dioxide and a lower accumulation of oxygen in the internal atmosphere of the apples compared with Semperfresh alone (Table 1). The correlations shown in Table 2 confirm that internal gas levels at day 10 are strongly correlated with scald development after 4 months of storage when the data obtained from DPA treatment are excluded. Thus, our results suggest that internal atmosphere may be related to scald development.

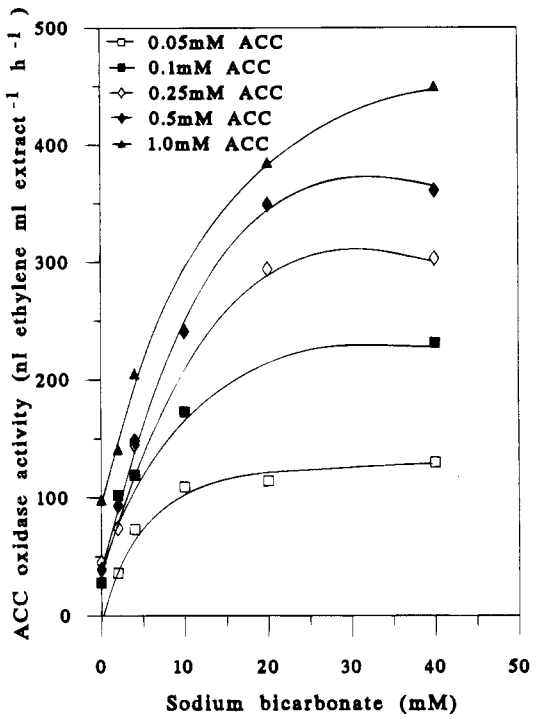

Figure 3. Effect of the concentration of sodium bicarbonate added to the reaction mixture on the activity of a partially purified ACC oxidase in the presence of different concentrations of ACC.

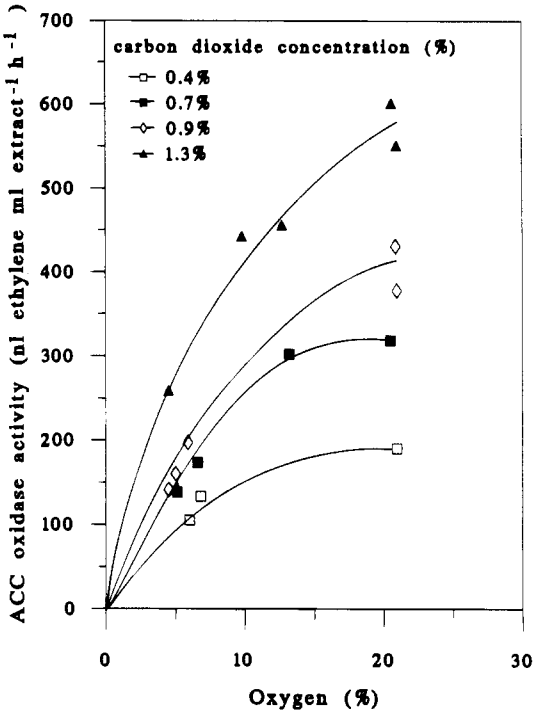

Figure 4. Effect of the oxygen concentration on the activity of a partially purified ACC oxidase in the presence of different concentrations of carbon dioxide. The carbon dioxide levels were varied by adding graded amounts of sodium bicarbonate to the reaction mixture. The actual concentrations of oxygen and carbon dioxide in the head space of the reaction vials were measured by GC-TCD at the end of the enzyme assay.

Effect of Carbon Dioxide and Oxygen on ACC Oxidase Activity. After 4 months of storage, ethylene levels are strongly correlated with oxygen and carbon dioxide levels (Table 2). Thus, it was appropriate to confirm whether ethylene synthesis could be affected directly by the internal levels of oxygen and carbon dioxide that had been measured.

When the ACC oxidase is assayed in vitro it is more convenient to supply defined levels of carbon dioxide in the form of graded concentrations of bicarbonate (Smith and John, 1993). The ACC oxidase from Granny Smith apples responded to increasing bicarbonate concentrations up to 10 to 20 mM added bicarbonate, depending on the ACC concentration (Figure 3). The ACC concentration at the site of the ACC oxidase in situ is unknown. The saturating value of 20 mM bicarbonate corresponded to a value of ~1.3% carbon dioxide measured in the headspace above the assay solution (Butler, 1982). Carbon dioxide levels in the apples studied here exceeded this value, varying from 2.5% to almost 14% (Table 1). Therefore, different carbon dioxide levels resulting from the different postharvest treatments applied probably did not act to regulate ACC oxidase activity in vivo, although the carbon dioxide may have inhibited ethylene action at these levels (Abeles et al., 1992).

The ACC oxidase activity from Granny Smith apples was half-saturated at ~7% oxygen when supplied with saturating carbon dioxide levels, but this value was lower when carbon dioxide was limiting (Figure 4). Thus, the range of internal oxygen concentrations measured (Table 1) after the different postharvest treatments (8–19%) could have affected in vivo ACC oxidase activity and thus ethylene synthesis. After 4 months of storage, the apples coated with ascorbyl palmitate plus Semperfresh, and, after 6 months of storage, all the coated apples, showed both lower internal oxygen levels (Table 1) and lower rates of ethylene emission (Figures 1 and 2) compared with the uncoated apples. However, we also note that after 4 months of storage, apples coated with Semperfresh alone had exceptionally high oxygen levels (Table 1) and low rates of ethylene emission (Figure 1). These were the conditions that gave the lowest carbon dioxide levels (Table 1), and if the carbon dioxide levels were even lower at the site of the ACC oxidase, they may have restricted its activity.

ABBREVIATIONS USED

ACC, 1-aminocyclopropane-1-carboxylic acid; DPA, diphenylamine; GC, gas chromatography; TCD, thermoconductivity.

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LITERATURE CITED

Abeles, F. B.; Morgan, G. W.; Saltveit, M. E. Regulation of Ethylene Production by internal, environmental, and stress factors. in *Ethylene in Plant Biology*; Academic: New York, 1992; pp 56–119.

Banks, N. H. Internal atmosphere modification in pro-long coated apples. *Acta Hortic.* **1985,** *157*, 105–112.

Bauchot, A. D.; John, P.; Soria, Y.; Recasens, I. Sucrose ester-based coatings formulated with food compatible antioxidants in the prevention of superficial scald in stored apples. *J. Am. Soc. Hortic. Sci.* **1995**, *120*, 491–496.

Butler, J. N. The basic equations. In *Carbon Dioxide Equilibria and their Applications*; Addison-Wesley: Reading, MA, 1982; pp 15–43.

Dong, J. G.; Fernández-Maculet, J. C.; Yang, S. F. Purification and characterization of 1-aminocyclopropane-1-carboxylate oxidase from apple fruit. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9789–9793.

Du, Z.; Bramlage, W. J. Roles of ethylene in the development of superficial scald in cortland Apples. *J. Am. Soc. Hortic. Sci.* **1994**, *119*, 516–523.

Dupille, E.; Rombaldi, C.; Lelièvre, J-M.; Cleyet-Marel, J-C.; Pech, J-C.; Latché, A. Purification, properties and partial amino-acid sequence of 1-aminocyclopropane-1-carboxylate oxidase from apple fruits. *Planta* **1993**, *90*, 65–70.

Fernández-Maculet, J. C.; Yang, S. F. Extraction and partial characterization of the ethylene-forming enzyme from apple fruit. *Plant Physiol.* **1992**, *99*, 751–754.

Fernández-Maculet, J. C.; Dong, J. G.; Yang, S. F. Activation of 1-aminocyclopropane-1-carboxylate oxidase by carbon dioxide. *Biochem. Biophys. Res. Commun.* **1993**, *193*, 1168–1173.

Fidler, J. C.; Wilkinson, B. G.; Edney, K. L.; Sharples, R. O. Injuries to the skin of the fruit. In *The Biology of Apple and Pear Storage*; Fidler, J. C., Ed.; Commonwealth Agricultural Bureau: East Malling, U.K., 1973; Part 2, pp 67–75.

Filmer, A. A. E.; Meigh, D. F. Natural skin coating of the apple and its influence on scald during storage: oxidation products of α-farnesene. *J. Sci. Food Agric.* **1971**, *22*, 188–190.

Huelin, F. E.; Coggiola, I. M. Superficial Scald, a functional disorder of stored apples: VI. effect of applied α-farnesene, temperature and diphenylamine on the concentration and oxidation of α-farnesene in the fruit. *J. Sci. Food Agric.* **1970**, *21*, 584–589.

Kader, A. A. Biochemical and physiological basis for effects of controlled and modified atmosphere on fruits and vegetables. *Food Technol.* **1986**, *40*, 99–104.

Kao, C. H.; Yang, S. F. Light Inhibition of the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene in leaves is mediated through carbon dioxide. *Planta* **1982**, *155*, 261–266.

Kallay, T. New measures against superficial scald in apples *Acta Hortic.* **1994**, *368*, 220–224.

Kerbel, E.; Kader, A. Comparison between "Semperfresh" and "Nutri-Save" coatings on Granny Smith apples. In *Perishable Handling, Postharvest Technology of Fresh Horticultural Crops*; Cooperative Extension, University of California: Davis, 1988; No. 63, pp 4–5.

Kerbel, E. L.; Mitchell, F. G.; Kader, A. A.; Mayer, G. Effects of semperfresh coating on postharvest life, internal atmosphere modification and quality maintenance of Granny Smith apples. In *Proceedings of the Fifth International Controlled Atmosphere Research Conference*, June 14–16, 1989, Wenatchee, WA; Vol. 1.

Kuai, J.; Dilley, D. R. Extraction, partial purification and characterization of 1-aminocyclopropane-1-carboxylate oxidase from apple fruit. *Postharv. Biol. Technol.* **1992**, *1*, 203–211.

Lau, O. L. Efficacy of diphenylamine, ultra-low O_2, and ethylene scrubbing on scald control in Delicious apples *J. Am. Soc. Hortic. Sci.* **1990**, *115*, 959–961.

Little, C. R.; Taylor, H. J. Orchard locality and storage factors affecting the commercial quality of Australian Granny Smith apples. *J. Hortic. Sci.* **1981**, *56*, 323–329.

Little, C. R.; Taylor, H. J.; McFarlane, F. Postharvest and storage factors affecting superficial scald and core flush of Granny Smith apples. *HortScience* **1985**, *20*, 1080–1082.

Manseka, V. S.; Vasilakakis, M. Effect of storage maturity, postharvest treatments and storage conditions on superficial scald and quality of apples. *Acta Hortic.* **1993**, *326*, 213–224.

Mitchell, T.; Porter, A. J. R.; John, P. Authentic activity of the ethylene-forming enzyme observed in membranes obtained from kiwifruit (*Actinidia deliciosa*). *New Phytol.* **1988**, *106*, 313–319.

Patterson, M. E.; Workman, M. The influence of oxygen and carbon dioxide on the development of apple scald. *J. Am. Soc. Hortic. Sci.* **1962**, *80*, 130–136.

Smith, J. J.; John, P. Activation of 1-aminocyclopropane-1-carboxylate oxidase by bicarbonate/carbon dioxide. *Phytochemistry* **1993**, *32*, 1381–1386.

Smith, S. M.; Stow, J. R. The Potential of a sucrose ester coating material for improving the storage and shelf-life qualities of Cox's Orange Pippin apples. *Ann. Appl. Biol.* **1994**, *104*, 383–391.

Smock, R. M. A comparison of treatment for control of the apple scald disease. *Proc. Am. Soc. Hortic. Sci.* **1957**, *69*, 91–100.

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