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Accumulation of Antioxidants in Apple Peel as Related to Preharvest Factors and Superficial Scald Susceptibility of the Fruit

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Abstract. **Antioxidants are believed to protect against the oxidation of α-farnesene to conjugated trienes in apple (*Malus domestica*, Borkh.) peel, thus providing resistance against superficial scald development. We conducted three experiments in which apples were a) harvested weekly, during which they were exposed to increasing hours at <10C during ripening; b) induced to ripen with no hours at <10C by applying ethephon; and c) enclosed in paper bags as they ripened. Inducing ripening with ethephon increased total water-soluble reducing compounds and percentage inhibition of lipid oxidation of peel extracts, increased concentrations of α-tocopherol, carotenoids, and ascorbic acid in peel, but only slightly reduced scald. Delayed harvests increased all of these antioxidants except ascorbic acid and greatly reduced scald development. Bagging fruit before ripening decreased α-tocopherol, carotenoid, and ascorbic acid concentrations, decreased total water-soluble reducing compounds, and increased scald development. We conclude that changes in these antioxidants probably are affected more by ripening and light intensity than by low temperature before harvest. Chemical name used: (2-chloroethyl)phosphonic acid (ethephon).**

Superficial scald of apples is a disorder that develops during storage. Accumulation of the volatile sesquiterpene α-farnesene and its subsequent oxidation to conjugated trienes have been related to scald development (Huelin and Coggiola, 1968; Huelin and Murray, 1966; Meigh and Filmer, 1969). Anet (1972) reported that immature apples generally do not produce more α-farnesene than more mature apples but that they develop much more scald. Thus, their inability to prevent the oxidation of α-farnesene, possibly due to a less efficient antioxidant system, may be responsible for their higher susceptibility. In a study of 16 apple cultivars, 11 endogenous antioxidants were detected, although only three tocopherols were identified (Anet, 1974). Five of the detected antioxidants, including α-tocopherol, were found in all cultivars. There are many antioxidants that absorb at ≈200 nm (Anet, 1974), and Meir and Bramlage (1988) found a negative correlation between absorbance at 200 nm (OD 200) of a hexane extract of apple surfaces and the scald susceptibility of 'Cortland' apples. Since total antioxidant activity of the apple peel correlated with the OD 200 values, Meir and Bramlage (1988) proposed that OD 200 values estimated total antioxidant activity and may be used to predict scald susceptibility. However, additional data have not shown consistent correlation of OD 200 values and scald susceptibility (Bramlage et al., 1993).

Our study examined the hypothesis that the development of scald resistance in apples results from increased antioxidant activity in the fruit peel before harvest. These endogenous antioxidants may inhibit the oxidation of α-farnesene and thus block the sequence of events suggested by Huelin and Coggiola (1970). Factors examined for effects on scald resistance were preharvest temperature (as accumulated hours at <10C), ripening, and exposure to light. Of these, low temperature greatly reduced, ripening only slightly reduced, and low light levels during ripening substantially increased susceptibility (Barden and Bramlage, 1994). Thus, according to the hypothesis, preharvest hours at <10C should have caused endogenous antioxidants to increase substantially, ripening should have caused a slight increase to occur, and low light levels should have reduced antioxidant activity substantially. Here we report chemical analyses of fruit peel at harvest that test the hypothesis.

Materials and Methods

Field experiments. Experiments were conducted at the Horticultural Research Center, Belchertown, Mass., 1990–91. To measure effects of preharvest temperature, 'Cortland' and 'Delicious' apples were harvested at about weekly intervals during the harvest season, and cumulative preharvest hours at <10C at each harvest were obtained from a recording thermometer in the orchard. At each harvest, ≈100 fruit/tree were selected at random. There were six two-tree replications per cultivar. To assess effects of ripening, ethephon (0.25 or 0.5 g·liter^{-1}) plus surfactant was applied to 'Cortland' trees to the drip point on 20 Aug., and ≈100 fruit/tree were harvested on 1 and 6 Sept. Five two-tree replications were used. To assess effects of light levels, ≈200 fruit/tree of 'Cortland' were enclosed in brown kraft paper bags in mid-August, and ≈100 fruit/tree were harvested on 1 and 9 Oct. This experiment used five single-tree replications.

Sampling fruit after harvest. In all experiments, on the day of sampling, 10 fruit/sample were 1) tested for ripeness (Barden and Bramlage, 1994), 2) extracted in 100 ml of high-performance liquid chromatography (HPLC)-grade hexane (Fisher Scientific, Pittsburgh) according to Meir and Bramlage (1988), and 3) the peel was freeze-dried and stored at –20C until analysis. The remaining fruit were stored at 0C for 20 weeks, then transferred to 20C for 1 week and examined for scald development.

Percentage inhibition of lipid oxidation. To assay percentage inhibition of oxidation as an indication of lipid-soluble antioxidant activity, 0.5 g of freeze-dried apple peel was extracted overnight in 10 ml of HPLC-grade hexane at room temperature in darkness. The

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procedure of McKersie et al. (1982) was used, as modified by Meir and Bramlage (1988), with the following changes. Dried samples in test tubes were redissolved in 0.2 ml of 100% ethanol. To each tube, 0.2 ml of 0.2 M linoleic acid was added. After adding KH_2PO_4, 1.5 ml of 0.2 mM $FeSO_4$ was added to each emulsion to initiate the reaction. Samples were incubated at 37C for 3 h. To clear the emulsion, 2 ml of 0.1 N NaOH was added. Results of the assay are reported as percentage inhibition of lipid oxidation. Absorbance at 232 nm measures conjugated dienes and is an indicator of oxidation. The standard curve with α-tocopherol showed that oxidation decreased in this system with increased antioxidant concentrations. We assume here that changes in oxidation caused by sample additions to the system were due to the presence of lipid-soluble antioxidants rather than the presence of less pro-oxidants. However, care should be taken in interpreting these results.

α-Tocopherol. α-Tocopherol content was determined by the HPLC method of Spychalla and Desborough (1990), modified as follows. Freeze-dried peel (1 g) was mixed by agitation with 25 ml of 80% ethanol in water (v/v) and the filtrate was mixed with 10 ml of petroleum ether. The mobile phase in HPLC was 97% methanol at a flow rate of 2 ml·min^{-1}. Samples were compared to external standards.

Total water-soluble reducing capacity (TWRC). TWRC was determined by monitoring iron reduction using an unpublished colorimetric method (S. Meir, personal communication). Freeze-dried samples (0.05 g) were homogenized in 10 ml of acetate buffer (pH 4.5) and centrifuged for 10 min at 1800× *g*. Aliquots (0.1 ml) of extract, 0.9 ml of buffer, and 1 ml of $FeCl_3$ (made up as 24.3 mg/100 ml H_2O + 50 µl H_2SO_4) were placed in tubes and allowed to incubate in darkness at room temperature for 24 h. After incubation, 0.8 ml of 1.3 M ammonium acetate and 0.2 ml of ferrozine reagent {75 mg ferrozine [3-(2-pyridyl)-5-6-bis (4-phenyl-sulfonic acid)1,2,4-triazine] and 75 mg Neocuproine} (Sigma, St. Louis) were added. The absorbance at 562 nm was measured 1 h after adding ferrozine. Total reducing capacity (units/g dry weight) was defined as the Fe^{+3}-reducing capacity of the tissue resulting in the absorbance of 1 OD at 562 nm.

Ascorbic acid. Ascorbic acid content was determined by a modification of the colorimetric method of Arakawa et al. (1981), as modified by Senaratna et al. (1988). Samples (0.1 g) were extracted in 10 ml of 5% trichloroacetic acid and centrifuged, and the step to convert dehydroascorbic acid to ascorbic acid was omitted.

Glutathione. Glutathione content was determined using the HPLC procedure of Buwalda et al. (1988), as modified by Hariyadi and Parkin (1991), with the following changes. Freeze-dried apple peel (0.5 g) was extracted in 10 ml of 80 mM sulfosalicylic acid with 1 mM EDTA. The sulfosalicylic acid and EDTA were dissolved separately and then mixed to avoid insolubility. Samples were centrifuged at 27,000× *g* for 15 min and filtered through a 0.45 µm nylon filter. 5,5´-Dithio-bis (2-nitrobenzoic acid) was dissolved at pH 8.0 and then brought to pH 7.0. Samples were derivatized for 5 min and then neutralized. The HPLC column was a C18 reverse-phase 250 × 4.0-mm column (Phenomenex, Torrance, Calif.) at 22C. The mobile phase was 2% acetonitrile in 30 mM sodium phosphate buffer, pH 7.0, with a flow rate of 1 ml·min^{-1}. Absorbance was determined at 280 nm and compared to external standards. A 7-min cleaning phase with 33% acetonitrile was run after each sample.

Carotenoids. Carotenoids were determined by extracting 0.5 g of freeze-dried peel tissue in 10 ml of hexane. The samples in hexane were placed in vials, purged with N, left in darkness at room temperature for 2 h, and centrifuged at 39,000× *g* to remove all cloudiness. Absorbance was measured at 440 nm, and an extinction coefficient of 2500 was used to calculate the concentrations.

Statistical analyses. Blocking was done where possible and, in experiments involving treatments, randomly selected trees (replications) were treated. Statistical analyses included analyses of variance, correlations, and analyses of covariance, where appropriate. The analyses of covariance were run using replications as the noncontinuous independent variable and the other variable as the continuous covariate. Because the replication effect was not of interest, results were interpreted using the variance associated with the linear or quadratic covariates, presented as a percentage of the total variance with replication removed (i.e., percentage of the nonreplicate sums of squares). SAS Statistical Software (SAS Institute, Cary, N.C.) was used to analyze the data.

Results

Estimates of antioxidant activity in apple peel at harvest. As harvest progressed, the OD 200 values, percentage inhibition of lipid oxidation (as indications of lipid-soluble antioxidant activity), and TWRC increased in fruit peel of both cultivars (Table 1). Preharvest hours at <10C increased linearly with delayed harvest, and these hours were related strongly to all three antioxidant estimates. The OD 200 values were correlated strongly with percentage inhibition of lipid oxidation in both cultivars (r = 0.90 and 0.85 for 'Cortland' and 'Delicious', respectively).

Scald susceptibility decreased markedly with later harvest (Table 1). Covariance of OD 200 values and scald was highly significant in both cultivars. In preliminary studies, the covariance of OD 200 and scald also was significant in 1988 and 1989 ($P \leq 0.01$) for 'Cortland' and for 'Delicious' in 1988 ($P \leq 0.05$) (data not shown). Scald vs. percentage inhibition of oxidation was significant for 'Cortland' and 'Delicious' apples in 1990. In 1989, however, these correlations were not significant (data not shown). TWRC values were correlated significantly with scald development, although they accounted for a smaller portion of the nonreplicate variance in scald than percentage inhibition of lipid oxidation (Table 1).

Ethephon greatly increased fruit ripeness, as shown by starch hydrolysis, internal ethylene concentrations, and fruit firmness (Barden and Bramlage, 1994). The at-harvest OD 200 values were much higher after ethephon treatment, as were the percentage inhibition of lipid oxidation values (Table 2). TWRC values also were increased by treatment. At least 90% of the fruit developed scald, although ethephon produced a significant reduction. Covariance analysis showed that percentage inhibition of lipid oxidation was significant, but accounted for only 32% of the nonreplicate variance in scald development, a result indicating a relatively weak relationship (data not shown). In this experiment, TWRC values were not correlated with scald development. From these results, ethephon-induced ripening seemed to increase antioxidant activity in apple peel, with a greater effect on lipid-soluble than water-soluble compounds. However, little resistance to scald was associated with these changes.

Mid-August bagging had no effect on fruit ripeness at harvest (Barden and Bramlage, 1994), but it resulted in substantially greater scald susceptibility of the fruit (Table 3). Bagging had no significant effect on OD 200 values or percentage inhibition of lipid oxidation, but it decreased TWRC. Thus, bagging seemed to a) reduce the accumulation of water-soluble antioxidants in the fruit, b) not influence lipid-soluble antioxidant levels, and c) increase scald susceptibility greatly.

Measurements of specific antioxidants in apple peel at harvest.

Table 1. At-harvest estimates of antioxidant activity in peel from 'Cortland' and 'Delicious' apples and scald development after storage for 20 weeks at 0C in 1990. Relationships are shown as a percentage of nonreplicate sums of squares (SS) with the linear (L) or quadratic (Q) portions of the model or both.

Statistic	Harvest date	Hours <10C	OD 200 (OD × 1000/cm^2)	Oxidation[z] (% inhibition)	TWRC[y] (units/g dry wt)	Scald (%)	Scald score[x]
			Cortland				
	17 Sept.	21	5.5	40	11.2	98	2.7
	24 Sept.	79	7.7	60	11.3	78	1.9
	3 Oct.	127	17.9	76	12.0	46	1.6
	11 Oct.	150	23.5	87	13.8	49	1.6
Covariance with hours			$L^{***}Q^{***}$	L^{***}	$L^{***}Q^{*}$	L^{***}	L^{***}
Percentage nonreplicate SS			97%	93%	69%	82%	66%
Covariance with scald			L^{***}	L^{**}	L^{**}		
Percentage nonreplicate SS			78%	71%	45%		
			Delicious				
	21 Sept.	62	2.4	22	13.9	94	2.7
	26 Sept.	104	5.5	34	14.1	88	2.8
	3 Oct.	127	10.9	44	14.9	68	2.1
	11 Oct.	150	18.1	55	15.8	51	1.7
Covariance with hours			L^{***}	L^{***}	L^{**}	$L^{***}Q^{*}$	$L^{***}Q^{**}$
Percentage nonreplicate SS			98%	86%	60%	79%	76%
Covariance with scald			L^{***}	L^{**}	L^{**}		
Percentage nonreplicate SS			76%	68%	60%		

[z]Percentage inhibition of lipid oxidation as estimated by conjugated dienes.
[y]TWRC = total water-soluble reducing capacity.
[x]Score reflects percentage of surface affected: 1 is ≥ 1% ≤ 10%; 2 is ≥ 11% ≤ 33%; 3 is ≥ 34% ≤ 66%; 4 is ≥ 67% ≤ 100%.
*,**,***Significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2. Effects of ethephon on estimates of antioxidant activity in peel of 'Cortland' apples at harvest and scald development after storage at 0C.

Harvest date	Ethephon (ppm)	OD 200 (OD × 1000/cm^2)	Oxidation[z] (% inhibition)	TWRC[y] (units/g dry wt)	Scald (%)	Scald score[x]
1 Sept.	0	4.2	61	9.6	97	2.4
	250	28.8	89	11.2	90	2.8
	500	32.1	88	11.6	92	2.8
6 Sept.	0	6.0	62	11.3	99	2.4
	250	29.5	90	13.9	91	3.2
	500	33.9	91	14.2	96	3.2
Significance						
Ethephon		***	***	*	**	***

[z]Percentage inhibition of lipid oxidation as estimated by conjugated dienes.
[y]TWRC = total water-soluble reducing capacity.
[x]Score reflects percentage of surface affected: 1 is ≥ 1% ≤ 10%; 2 is ≥ 11% ≤ 33%; 3 is ≥ 34% ≤ 66%; 4 is ≥ 67% ≤ 100%.
*,**,***Significant at $P \leq 0.05$, 0.01, or 0.001, respectively, by analysis of variance.

Table 3. Effects of mid-August bagging of 'Cortland' apples on at-harvest estimates of antioxidant activity in peel and on scald development after storage at 0C, 1990.

Harvest date	Treatment	Hours <10C	OD 200 (OD × 1000/cm^2)	Oxidation[z] (% inhibition)	TWRC[y] (units/g dry wt)	Scald (%)	Scald score[x]
1 Oct.	Control	107	20.5	37	10	31	1.4
	Bagged	107	19.3	40	8	62	1.4
9 Oct.	Control	150	26.3	67	10	13	1.4
	Bagged	150	26.3	49	7	42	1.2
Significance							
Bagging		NS	NS	***	*	NS	
Hours		*	**	NS	NS	NS	
Bagging × hours		NS	*	NS	NS	NS	

[z]Percentage inhibition of lipid oxidation as estimated by conjugated dienes.
[y]TWRC = total water-soluble antioxidant activity.
[x]Score reflects percent of surface affected: 1 is ≥ 1% ≤ 10%; 2% is ≥ 11% ≤ 33%; 3 is ≥ 34% ≤ 66%; 4 is ≥ 67% ≤ 100%
^{NS,*,**,***}Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, respectively, by analysis of variance.

The data in Tables 1 to 3 present only estimates of total lipid-soluble and water-soluble antioxidants in the fruit peel. The most abundant lipid-soluble antioxidants are α-tocopherol and carotenoids, and the most abundant water-soluble antioxidants are ascorbic acid and glutathione (Larson, 1988; Winston, 1990). In 'Cortland', the α-tocopherol concentration was highest in peel of fruit from the last harvest and in 'Delicious' from the last two harvests, but the concentration was not significantly related to scald development (Table 4). Carotenoids increased with hours at <10C in 'Cortland' but not in 'Delicious'. Neither ascorbic acid nor glutathione changed significantly during harvest. Scald also was not correlated significantly with ascorbic acid or glutathione concentrations in apple peel at harvest; it was correlated with carotenoid concentrations in 'Delicious', but not in 'Cortland'.

Table 4. Antioxidant concentrations in peel of 'Cortland' and 'Delicious' apples at harvest, 1990. Relationships with scald are shown as the percentage of nonreplicate sums of squares (SS) with linear (L) or quadratic (Q) portions of the model or both.

Statistic	Harvest date	Hours <10C	α-Tocopherol ($\mu g \cdot g^{-1}$ dry wt)	Carotenoids ($\mu g \cdot g^{-1}$ dry wt)	Ascorbic acid ($\mu g \cdot g^{-1}$ dry wt)	Glutathione ($\mu g \cdot g^{-1}$ dry wt)	Scald (%)	Scald score[z]
				Cortland				
	17 Sept.	21	103	22	380	91	98	2.7
	24 Sept.	79	90	21	376	106	78	1.9
	3 Oct.	127	100	23	365	139	46	1.6
	11 Oct.	150	145	33	411	81	49	1.6
Covariance with hours			L^{***}	$L^{**}Q^{**}$	NS	NS	L^{***}	
Percentage nonreplicate SS		76%	65%	17%	19%	82%		
Covariance with scald			NS	NS	NS	NS		
Percentage nonreplicate SS			16%	24%	5%	12%		
				Delicious				
	21 Sept.	62	72	18	624	39	94	2.7
	26 Sept.	104	69	15	600	39	88	2.8
	3 Oct.	127	78	17	592	55	68	2.1
	11 Oct.	150	78	20	603	35	51	1.7
Covariance with hours			L^{*}	NS	NS	NS	$L^{***}Q^{*}$	
Percentage nonreplicate SS			35%	25%	20%	15%	79%	
Covariance with scald			NS	$L^{*}Q^{**}$	NS	NS		
Percentage nonreplicate SS			36%	62%	5%	0%		

[z]Score reflects percentage of surface affected: 1 is ≥ 1% ≤ 10%; 2 is ≥ 11% ≤ 33%; 3 is ≥ 34% ≤ 66%; 4 is ≥ 67% ≤ 100%.
^{NS,*,**,***}Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 5. Effects of ethephon treatments on concentrations of specific antioxidants in 'Cortland' apple peel at harvest, 1990.

Harvest date	Ethephon (ppm)	α-Tocopherol ($\mu g \cdot g^{-1}$ dry wt)	Carotenoids ($\mu g \cdot g^{-1}$ dry wt)	Ascorbic acid ($\mu g \cdot g^{-1}$ dry wt)	Glutathione ($\mu g \cdot g^{-1}$ dry wt)
1 Sept.	0	79	25	423	87
	250	120	31	493	98
	500	116	27	511	83
6 Sept.	0	72	21	383	87
	250	149	33	479	88
	500	154	36	481	93
Significance					
Ethephon		***	NS[z]	***	NS

[z]Significance level for both dates combined at $P = 0.06$.
^{NS,***}Nonsignificant or significant at $P \leq 0.001$, respectively, by analysis of variance.

Table 6. Effects of mid-August bagging of 'Cortland' apples on at-harvest antioxidant concentrations in peel, 1990.

Harvest date	Treatment	α-Tocopherol ($\mu g \cdot g^{-1}$ dry wt)	Carotenoids ($\mu g \cdot g^{-1}$ dry wt)	Ascorbic acid ($\mu g \cdot g^{-1}$ dry wt)	Glutathione ($\mu g \cdot g^{-1}$ dry wt)
1 Oct.	Control	106	23	399	102
	Bagged	91	18	324	117
9 Oct.	Control	122	30	384	103
	Bagged	93	17	297	84
Significance					
Bagging		*	*	***	NS
Hours		**	NS	NS	NS
Bagging × hours		*	NS	NS	NS

^{NS,*,**,***}Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, respectively, by analysis of variance.

Ethephon-induced ripening increased α-tocopherol and ascorbic acid but not glutathione concentrations in fruit peel (Table 5). Carotenoids also seemed to increase with ethephon treatment, but only for the 6 Sept. harvest (P = 0.06). Relationships between ethephon concentration and α-tocopherol and ascorbic acid were linear (data not shown). These data, along with the antioxidant estimates (Table 2), indicated that lipid- and water-soluble antioxidants were increased by fruit ripening.

Bagging decreased α-tocopherol, carotenoid, and ascorbic acid, but not glutathione, concentrations in apple peel (Table 6). The lack of significance for the latter undoubtedly is related to the opposite trends for the two harvest dates. These results showed that low light levels decreased lipid-soluble and water-soluble antioxidants, as also was indicated by the antioxidant estimates (Table 3).

Discussion

Estimates of antioxidant activity. OD 200 values increased greatly as harvest was delayed (Table 1) and as ripening was induced by ethephon (Table 2). Although increasing OD 200 values were correlated with increasing hours at <10C (Table 1), the greatest increases occurred during the late harvests, as fruit were ripening. Furthermore, in the absence of any hours at <10C, ethephon induced substantially higher OD 200 values in fruit peel than those for the sequential-harvests experiment (34 vs. 24 for final harvests of 'Cortland'). Thus, OD 200 values seem to have been influenced more strongly by ripening (or ethylene) than by low temperature. Since bagging had no effect on OD 200 values (Table 3), light levels evidently had little or no influence on their increase.

Meir and Bramlage (1988) proposed that OD 200 values are estimates of lipid-soluble antioxidant activity. In these experiments, the OD 200 values always were correlated highly with percentage inhibition of lipid oxidation (r = 0.90, 0.81, 0.95, and 0.67; $P \leq 0.001$). However, both of these estimates represent gross changes and may reflect differences in components other than antioxidants, such as changes in lipid composition of surface coatings. Neither OD 200 nor percentage inhibition values were correlated consistently with either α-tocopherol or carotenoid concentrations in these experiments (data not shown).

TWRC, another estimate of gross changes, also increased during the season as hours at <10C increased and ripening progressed (Table 1). The increase between the first and last harvest was almost 20% in 'Cortland' and somewhat <20% in 'Delicious', and the TWRC values were correlated with scald development in both cultivars. TWRC also increased ≈20% with ethephon treatment in the absence of low temperature, but was not correlated with scald development [r = (–0.18), nonsignificant] (Table 2). Bagging decreased TWRC ≈20%, and these reductions were correlated with greater scald development [r = (–0.54), $P \leq 0.05$]. Therefore, it seems that TWRC values were influenced more by ripening and light than by low temperature, and they were not correlated consistently with scald susceptibility.

Measurements of specific antioxidants. Concentrations of α-tocopherol measured in apple peel were similar to those reported by Gallerani et al. (1990). They increased with sequential harvests of both cultivars and were correlated significantly with hours at <10C, although they accounted for a relatively small portion of the nonreplicate variance (Table 4). Starch indexes also were correlated significantly with α-tocopherol (data not shown), and the greatest increase in α-tocopherol came between harvests 3 and 4, when fruit were ripening rapidly. Ethephon increased α-tocopherol concentration (Table 5), and the apples treated with ethephon and harvested on 6 Sept. had slightly more α-tocopherol than did those harvested on 11 Oct. in the sequential-harvest experiment (Table 4). These data suggest that ripening was a primary contributor to increased α-tocopherol concentrations in apple peel. Results of the bagging experiment (Table 6) indicated that relatively high light levels were required for high α-tocopherol concentrations to accumulate in peel, since they were decreased by bagging. Only in the bagging experiment was scald correlated with α-tocopherol concentration. Thus, it seemed that, in these experiments, α-tocopherol alone did not play as large a role in the development of scald resistance as has been proposed previously (Anet, 1974; Gallerani et al., 1990; Meir and Bramlage, 1988).

Carotenoids, the other major contributor to lipid-soluble antioxidant activity (Winston, 1990), underwent changes similar to those of α-tocopherol. They increased with hours at <10C (Table 4) and with ethephon treatment (P = 0.06) (Table 5), the magnitude of increase being similar in both cases. Bagging decreased carotenoids by 20% and 40% at the first and second harvest, respectively (Table 6). In the sequential-harvest and ethephon experiments, carotenoids were not correlated significantly with scald [r = (–0.34), –0.26, and –0.21, for 'Cortland', 'Delicious', and ethephon experiments, respectively], but in the bagging experiment a negative correlation existed [r = (–0.65), $P \leq 0.01$].

Ascorbic acid concentrations did not change significantly during sequential harvests of either 'Cortland' or 'Delicious' (Table 4). Ethephon treatments, however, increased concentrations by ≈20% (Table 5), while bagging fruit decreased concentrations by ≈20% (Table 6). These data indicate that ripening can increase ascorbic acid concentration in apple peel and that low light levels can reduce it. Scald was not correlated significantly with ascorbic acid concentrations in peel of either 'Cortland' or 'Delicious' in the sequential-harvest experiments (Table 4). In the ethephon and bagging experiments, however, significant correlations existed [r = (–0.44) and –0.54, $P \leq 0.05$, respectively]. Albrigo (1968) found that the red sides of apples contained higher ascorbic acid concentrations than the green sides, and that scald occurred more frequently on the green than on the red sides. Therefore, a strong correlative case can be made for a protective role of ascorbic acid against scald development, but low temperature does not seem to enhance its accumulation significantly in apple peel.

Glutathione concentration was not influenced consistently by low temperatures, was not affected by ethephon treatment or bagging of fruit, and was not related consistently to scald or to any of the other constituents that were measured. Therefore, it seems that glutathione concentration may not play a direct role in development of scald resistance.

The putative estimates of lipid-soluble antioxidant activity (OD 200 and percentage inhibition of lipid oxidation) indicated much greater changes in response to delayed ripening and ethephon treatment than were attributable to α-tocopherol or carotenoids, the most abundant of these compounds. Conversely, these estimates did not reflect the reductions of α-tocopherol and carotenoids that occurred in the bagged fruit. These estimates apparently are influenced extensively by other compounds, especially those that increased during ripening. In contrast, changes in the TWRC values seemed to reflect relatively closely the changes that occurred in ascorbic acid concentrations.

Conclusion

These data indicate that ripening substantially increased and bagging substantially decreased concentrations of α-tocopherol, carotenoids, and ascorbic acid in apple peel. Increasing hours at

<10C apparently also increased antioxidant concentrations, as indicated by estimates of total activity, but temperature affected antioxidants less than ripening and light. Changes in α-tocopherol, carotenoids, and ascorbic acid were at least as great during ripening at 10C (Table 5) as during ripening at <10C (Table 4). However, during these experiments, scald susceptibility was affected more by increasing hours at <10C than by ethephon-induced ripening or bagging (Barden and Bramlage, 1993), so the effects of low temperature in reducing scald susceptibility may involve significantly more than an increased concentration of the principal antioxidants in apple peel.

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