Effect of Emulsifying Agents on Inhibition of Enzymatic Browning in Apple Juice by Ascorbyl Palmitate, Laurate and Decanoate

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ABSTRACT

The effects of emulsifying agents (EA) on the stability of aqueous dispersions of ascorbyl palmitate (AP), laurate (AL), and decanoate (AD), and on the effectiveness of these compounds as browning inhibitors in apple juice were investigated. The stability of 1.14 mM AP, AL or AD, dispersed in simulated juice, was greatly improved by the addition of hydrophilic EA's such as Tween 60. More lipophilic EA's also were effective in stabilizing AL and AD dispersions. However, the addition of EA's to these browning inhibitors failed to improve their effectiveness in juice and, in the case of Tweens, had a detrimental effect. These results are attributed to the solubilization or activation of bound polyphenol oxidase.

INTRODUCTION

PREVIOUSLY, we reported that several 6-fatty acid esters of L-ascorbic acid (AA) showed promise as inhibitors of enzymatic browning in raw apple juice (Sapers et al., 1989). Cort (1982) observed that these esters did not function as oxygen scavengers in an aqueous system unless they were solubilized, i.e., by adjusting the pH to 9. Our objective was to determine whether the effectiveness of these compounds as browning inhibitors in apple juice could be improved by the addition of emulsifying agents to increase the stability of their dispersions.

MATERIALS & METHODS

THE STABILITY of aqueous dispersions of the fatty acid esters of AA was evaluated in a simulated apple juice (SAJ) containing 8% fructose, 4% sucrose, and 0.6% malic acid and adjusted to pH 3.3 with 10% NaOH. Ethanolic solutions containing 1.5% ascorbyl palmitate (AP), 1.3% ascorbyl laurate (AL) or 1.2% ascorbyl decanoate (AD) (Hoffman-La Roche, Inc., Nutley, NJ; AP was NF-FCC grade while AL and AD were experimental products) were prepared either: (1) with no emulsifying agent; (2) with an equal concentration (weight percent) of either Tween 20, Tween 40, Tween 60, Tween 80 (Sigma Chemical Co., St. Louis), Santone 8–1–0, EC-25, Durlac-100 or Dur-Em 114 (the latter 4 obtained from Durkee Industrial Foods, Cleveland, OH); (3) with half, or (4) with double the concentration of each emulsifying agent. Aliquots of these solutions were added to SAJ with rapid stirring at room temperature (20°C) to give dispersions equivalent in AA concentration to 0.57 or 1.14 mM. The solutions were observed over 6 hr at 20°C and following overnight storage at 5°C for the development of cloudiness or precipitation.

The effects of emulsifying agents on the performance of the fatty acid esters of AA as browning inhibitors was evaluated in raw juice prepared from Granny Smith apples (Sapers and Douglas, 1987; Sapers et al., 1989). Briefly, ethanolic solutions of the fatty acid esters, alone or with different proportions of various emulsifying agents, or equimolar AA solutions were added at zero time to duplicate aliquots of freshly prepared juice to give concentrations equivalent to 0.57 or 1.14 mM AA. The extent of browning during storage at room temperature (20°C) was determined by measurement of the percent reflectance at 440 nm with a spectrocolorimeter (The Color Machine, Pacific Scientific, Silver Spring, MD). Treatment effectiveness, expressed as a percent inhibition value, was determined by comparing samples containing the esters or AA with an untreated control. Percent inhibition data for each treatment were subjected to analysis of variance to determine treatment effects. The Bonferroni LSD test (Miller, 1981) was used to separate means.

RESULTS & DISCUSSION

Effects of emulsifying agents on dispersion stability

The presence of emulsifying agents greatly increased the stability of dispersions of AP, AL and AD in SAJ (Table 1). With AP, Tween 60 (polyoxyethylenesorbitan monostearate) was most effective, followed by Santone 8–1–0 (a polyglycerol ester), Tween 80 (polyoxyethylenesorbitan monooleate), and EC-25 (a propylene glycol ester). The more lipophilic emulsifying agents (Durlac 100, a lactylated glycerol ester, and Dur-Em 114, a mono- and diglyceride) tended to precipitate under the conditions of these experiments. With AL, both Tweens, Santone 8–1–0 and EC-25 were similar in effectiveness, while with AD, the Tweens and EC-25 were more effective than Santone 8–1–0. Dispersions in which the fatty acid ester concentrations were 0.57 mM generally were more stable than dispersions containing 1.14 mM AP, AL or AD. Dispersion stability could be improved by increasing the ratio of emulsifying agent to fatty acid ester within the limits of solubility of the emulsifying agent. In the absence of an emulsifying agent, AL dispersions tended to be more stable than those of AP or AD.

With AP, emulsifying agents having a higher hydrophilic-lipophilic balance (HLB) (Anon, 1986) produced more stable dispersions. In contrast to these results, we previously observed an inverse relationship between the stability of AP dis-

Table 1 – Stabilization of aqueous dispersions of 6-fatty acid esters of ascorbic acid with emulsifying agents[a]

Emulsifying agent	Ratio[b]	Time until cloudy or precipitation (min)		
		Ascorbyl palmitate	Ascorbyl laurate	Ascorbyl decanoate
None	–	5	10-20	5
Tween 60	1:2	>360	45	45
	1:1	>360c	>360c	240
	2:1	>360	>360	>360
Tween 80	1:2	30	45	5
	1:1	30	>360c	240
	2:1	20	>360	>360
Santone 8-1-0	1:2	30	45	30
	1:1	180	>360c	30
	2:1	60	45	>360
EC-25	1:2	5	120	90
	1:1	5	>360c	>360c
	2:1	5	5	5

[a] Esters in simulated apple juice at concentration of 1.14 mM.
[b] Ratio of emulsifying agent to ester.
[c] Cloudy or precipitation after overnight storage at 5°C.

Table 2—Effect of emulsifying agents on inhibition of enzymatic browning in Granny Smith juice by 6-fatty acid esters of ascorbic acid

	Percent inhibition[b]								
	Ascorbyl palmitate			Ascorbyl laurate			Ascorbyl decanoate		
Treatment[a]	2 hr	4 hr	6 hr	2 hr	4 hr	6 hr	2 hr	4 hr	6 hr
Ester only	88[c]	82[c]	72[c]	99[cd]	98[cd]	98[cd]	100[c]	100[c]	99[c]
Ester + EC-25	82[c]	75[cd]	54[d]	80[e]	82[de]	82[d]	98[c]	100[c]	100[c]
Ester + Santone 8-1-0	81[c]	63[de]	38[e]	107[c]	110[c]	112[c]	88[cd]	76[d]	70[d]
Ester + Tween 60	19[d]	8[f]	0[g]	88[de]	61[f]	50[e]	72[d]	23[e]	20[e]
Ascorbic acid	96[c]	48[e]	15[f]	100[cd]	74[ef]	24[f]	96[cd]	30[e]	11[e]

[a] Esters and ascorbic acid at concentration of 1.14 mM in juice. Ratio of emulsifying agent/ester = 1:1 on weight basis.

[b] Based on change in percent reflectance at 440 nm.

[c-g] Means of duplicate trials; means within a column, followed by different superscripts, are significantly different by the Bonferroni LSD test ($p<0.05$).

persions in pH 7 phosphate buffer, to be used as dips to control browning in apple, and the HLB of the emulsifying agent (El-Atawy and Sapers, 1987). The discrepancy may be related to the much greater solubility of AP in the buffer compared to the highly acidic SAJ system. Unlike AP dispersions, AL and AD were stabilized by both lipophilic and hydrophilic emulsifying agents.

Effects of emulsifying agents on inhibition of browning

Without added emulsifying agents, the 6-fatty acid esters of AA (1.14 mM) inhibited browning in Granny Smith juice for at least 6 hr (Table 2). AP failed between 6 and 24 hr while AL and AD were still effective after 24 hr (data not shown). The addition of EC-25 had little effect on browning inhibition by the esters. Santone 8–1–0 significantly decreased the effectiveness of AP and AD. Tween 60 (as well as the other Tweens tested) greatly decreased the ability of the three esters to inhibit browning. The adverse effect of the Tweens also was seen at the lower ester concentration before these treatments failed and in samples where the ratio of emulsifying agent to ester was decreased from 1:1 to 1:2; samples with a ratio of 2:1 tended to become cloudy and were not tested for effects on browning inhibiton. In the absence of the fatty acid esters, Granny Smith juice containing Tween 60 at the same concentration used with AP (1:1 ratio) browned faster than the control juice (data not shown).

These results lead us to the unexpected conclusion that improving the stability of the fatty acid ester dispersions by adding emulsifying agents does not improve the effectiveness of these browning inhibitors and may have an adverse effect on their performance. Previously, we reported that dips prepared with Tweens or with AL or AD tended to induce browning in treated apple plugs, presumably due to leakage of polyphenol oxidase (PPO) and its substrates from disrupted cell membranes (Sapers et al., 1989). The new data suggest that the detergent action of these emulsifying agents and esters may have a direct effect on PPO activity in apple juice. The juice used in these experiments may have contained significant quantities of membrane- or organelle-bound PPO. The ability of detergents such as Tween 80 to solubilize or even activate bound PPO is well-known (Mayer and Harel, 1979; Vamos-Vigyazo, 1981). Thus, improvements in the performance of the fatty acid esters of AA as browning inhibitors in juices would have to be based on approaches other than the preparation of more stable dispersions with emulsifying agents, since such efforts are likely to be self-limiting.

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Ms received 3/6/89; accepted 3/27/89.