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Influence of Peroxidase and Polyphenol Oxidase on the Color of Puree from Machine-harvested Strawberries[1]

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Abstract. **Horseradish peroxidase (PO) and mushroom polyphenol oxidase (PPO) were added to strawberry purees from 'Cardinal' and Arkansas breeding-line 5344 to determine their influence on color during 48 hours at 30°C. Neither enzyme affected strawberry puree color or phenolic content. PO activity was reduced to near zero 24 hours after addition and PPO activity was undetectable 1 hour after addition to puree. Aeration did not affect anthocyanin and flavonoid concentrations, but increased discoloration and nonflavonoid concentration. Strawberry purees containing 50% immature plus 50% ripe fruits were poorer in color and had higher levels of flavonoids. As holding time at 30°C increased, puree color decreased.**

Once-over machine harvesters for strawberries (9, 10, 11) led to interest in utilizing immature (green) strawberries in puree products. However, the strawberry processing industry is concerned about the influences of these fruits on product color and color stability. Previous research (17, 18, 19) indicated that color of strawberry puree and jam was reduced in proportion to the amount of immature fruit used, but that they had no influence on anthocyanin stability in puree or jam.

Immature strawberries contain higher levels of peroxidase (E.C. 1.11.1.7) and polyphenol oxidase (E.C. 1.14.18.1) activity, phenolic compounds, and chlorophyll than strawberries at inception to overripe (19); and peroxidase (PO) and polyphenol oxidase (PPO) degrade anthocyanins (3, 5, 13, 14, 16). Most of these studies used a model system approach (5, 14) or juice (3) instead of puree (16) to determine enzymatic color loss. The purpose of this study was to determine the interactive effects of peroxidase and polyphenol oxidase × aeration × cultivar × percentage of ripe fruit on the color stability of puree from machine-harvested strawberries.

Materials and Methods

Plant material and experimental design. 'Cardinal' and Arkansas breeding-line 5344 (A-5344) were harvested with a University of Arkansas-Blueberry Equipment, Inc. strawberry harvester (11) from a 3-year-old strawberry planting at the Main Experiment Station, Fayetteville, Ark. 'Cardinal' and 'A-5344' were used since both of these cultivars have traits suited to machine harvesting and good raw product quality (11, 18). Fruits were cleaned and sorted into large (mostly ripe) and small (immature, mostly green) categories on the University of Arkansas in-plant cleaning-line (11). The sorted fruits were frozen in 13.5 kg (30-lb.) tins which were later transported without thawing by air to the Irrigated Agriculture Research and Extension Center at Prosser, Wash. The fruit was kept at −15°C until needed.

Berries were decapped, thawed for 3 hr at 20°C, and blended in a commercial stainless-steel Waring blender for 5–10 min at high speed. Purees of the immature and ripe berries were combined to create mixtures of 50 and 100% ripe fruit.

Enzyme treatments consisted of peroxidase (PO), polyphenol oxidase (PPO), a combination of both enzymes (PO + PPO), and no enzyme (control) added to the puree. Sufficient horseradish peroxidase (United States Biochemical Corporation) and mushroom polyphenol oxidase (tyrosinase, United States Biochemical Corporation) in distilled water were added to achieve an inoculation level of 1000 units and 10 units of activity/gram fresh weight (gfw) strawberry puree, respectively.

Purees were kept in a water bath at 30°C for 0, 24, and 48 hr, during which puree was either not aerated or air was bubbled through the puree at the rate of 9 liters/hr. Test tube openings were covered with Parafilm to reduce moisture loss. The study was a factorial design with 4 enzyme treatments × 2 aeration treatments × 2 cultivars × 2 maturities × 3 holding times.

To determine if the enzymes were active when added to the puree, PO and PPO activities were determined on frozen fruit and after puree was inoculated with both enzymes and held at 30°C for 1 and 24 hr without aeration. Crude enzyme extracts were prepared as described by Spayd and Morris (19).

The influence of pH on crude strawberry PO and PPO extracts and commercial horseradish PO and mushroom PPO was determined using 0.1M sodium acetate buffer (pH 3.0 to 7.0) in the assay mixtures. As a blank, a portion of the extracts was held in boiling water for 3 min and enzyme activity was determined. Extracts from the immature fruits of both cultivars were used since they had the highest enzyme activity levels.

Enzyme assays. PO activity was measured as the change in absorbance at 460 nm using a substrate mixture of 0.01 M o-dianisidine-0.03% H_2O_2 in 0.1 M sodium phosphate buffer (pH 5.4) (19). PPO activity was measured as the change in absorbance at 420 nm using an assay mixture of 8.5 ml of 0.1 M sodium acetate buffer (pH 5.4), 1.0 ml of 0.3 M catechol, and 0.5 ml of enzyme extract. The reaction mixture was equilibrated at 35 ± 1°C for 5 min prior to the addition of the enzyme extract. Enzyme activity was calculated as the change (Δ) in absorbance/min either per gfw or mg protein. One unit of enzyme activity equals 0.001 Absorbance units/min. Protein concentration was

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Table 1. Main effect F-test values for color measurements of strawberry puree.

Main effect	Color measurements						
		Hunter CDM		Phenols		Color intensity	Lack of discoloration
	TAcy	a	Hue	Flavonoid	Nonflavonoid		
Enzyme	0.8	7.1**	1.3	0.9	1.3	0.1	0.1
Air	2.7	32.9**	4.7*	0.8	13.0**	0.1	19.0**
Cultivar	576.1**	0.8	3024.9**	51.7**	0.5	2430.5**	224.9**
% Ripe	1717.7**	664.7**	6199.7**	143.7**	0.1	1240.1**	123.8**
Time	110.7**	1594.2**	41.9**	16.2**	89.4**	216.3**	1256.3**

*,**Significant F values at 5% (*), 1% (**) levels.

determined as described by Bradford (2). Bovine serum albumin was used for the standard curve.

Color and phenol concentration. To determine total anthocyanin (TAcy) concentration, 2 g of puree were extracted with EtOH-HCl (pH 1.0) for 1 hr, and filtered through facial tissue. Absorbance of the filtrate at 520 and 430 nm was determined using a Beckman DU Spectrophotometer equipped with a Gilford Modernization System which was standardized with distilled water. TAcy concentration was calculated as absorbance at 520 nm × dilution factor. Using the EtOH-HCl extracts, total phenols were determined by the method of Singleton and Rossi (15) and flavonoid and nonflavonoid phenols were determined by the method of Kramling and Singleton (7).

Color was determined on a Hunter Color and Color Difference Meter (CDM) which was standardized by a pink plaque ("L" = 65.9, "a" = 20.0, and "b" = 10.1). Hue was calculated as the angle whose tangent equals b/a (6). Color intensity and lack of discoloration were rated by the senior author on a scale of 1 to 10, with 10 = excellent and 5 = acceptable.

Results and Discussion

In general, cultivar, percentage of ripe fruit, and time had greater influences than enzyme and aeration on color parameters of strawberry puree (Table 1). Enzymes had no effect on color or phenols of strawberry puree except for CDM "a," which was slightly but significantly decreased by both enzymes (Table 2). This decrease was discounted since visually the color was not affected by enzyme.

Although oxygen has a detrimental effect on anthocyanins (1, 8), aeration of puree did not significantly affect TAcy concentration and color intensity (Tables 1 and 2). Discoloration slightly increased (lower lack of discoloration) and nonflavonoids and CDM "a" color slightly decreased. The lack of significant differences in color between purees by aeration was probably the result of incorporation of air into the puree during blending.

As shown in previous studies (11, 17, 18, 19), 'Cardinal' had better color (higher TAcy, color intensity, and lack of discoloration and lower hue value) than A-5344. 'Cardinal' had a

Table 2. Main effects of enzyme, air, cultivar, percentage ripe, and time on the color of machine-harvested strawberries.

Main effect	TAcy (Abs/gfw)	Hunter CDM		Phenols		Color intensity[z]	Lack of discoloration
		a	Hue	Flavonoid (mg/10 g)	Nonflavonoid (mg/10 g)		
Enzyme							
None	22.6a[y]	22.5a	21.6a	7.5a	17.9a	6.2a	6.9a
PO	22.1a	22.1b	21.6a	7.6a	17.4a	6.2a	6.9a
PPO	21.8a	22.0b	21.5a	7.6a	17.8a	6.2a	6.9a
PO + PPO	22.0a	22.2b	21.6a	7.9a	17.8a	6.2a	6.9a
Air							
Not aerated	22.4a	22.5a	21.7a	7.6a	18.1a	6.2a	7.1a
Aerated	21.8a	22.0b	21.5b	7.7a	17.4b	6.2a	6.8b
Cultivar							
Cardinal	26.4a	22.3a	30.1b	7.0b	17.8a	7.7a	7.6a
A-5344	17.9b	22.2a	23.1a	8.3a	17.7a	4.8b	6.3b
Ripe (%)							
100	29.4a	23.3a	19.5b	6.5b	17.8a	7.3a	7.4a
50	14.8b	21.2b	23.7a	8.8a	17.7a	5.2b	6.5b
Time at 30°C							
0 hr	25.7a	25.4a	21.9a	8.4a	19.5a	7.0a	9.9a
24 hr	21.1b	21.4b	21.4b	7.3b	16.8b	6.1b	6.2b
48 hr	19.6c	19.9c	21.5b	7.3b	16.9b	5.5c	4.8c

[z]Color intensity (intensity of red color) and lack of discoloration were rated on a scale of 1-10 with 10 = excellent and 5 = acceptable.

[y]Means within main effects within columns pooled across other main effects and 2 replications. Mean separation within main effects within columns by Duncan's multiple range test, 5% level.

Table 3. Peroxidase activity in puree of machine-harvested strawberries.

	Peroxidase activity (1 unit = 0.001 absorbance/min-gfw)		
		After enzyme added	
	Frozen	1 hr	24 hr
Cardinal			
100% ripe	244bc[z]	330b	1e
50% ripe	654a	671a	3de
A-5344			
100% ripe	107cde	224bc	54cde
50% ripe	206bcd	323b	6de

[z]Mean separation by Duncan's multiple range test, 5% level.

lower flavonoid concentration than A-5344, while there was no difference in nonflavonoid concentration.

Of the experimental variables, percentage of ripe fruit and holding time had the greatest influences on puree color (Table 1). Puree containing 50% immature fruit had lower TAcy, CDM "a," color intensity, and lack of discoloration and a higher hue value and flavonoid concentration (Table 2). This agreed with previous research (20). As expected, puree color decreased with increased time at 30°C. Hue slightly improved (decreased) after holding for 24 hr. Flavonoid and nonflavonoid concentrations decreased from initial levels after holding for 24 hr.

Enzyme activity. Prior to thawing and blending, PO activity ranged from 107 to 654 units (Table 3). Within a cultivar, the puree containing 50% immature fruit had the highest PO activity. One hr after the addition of 1,000 units of PO/g of puree, PO activity in crude enzyme extracts from the puree was similar to that found in comparable extracts from frozen fruit. After 24 hr at 30°C, PO activity was considerably decreased from 1-hr levels.

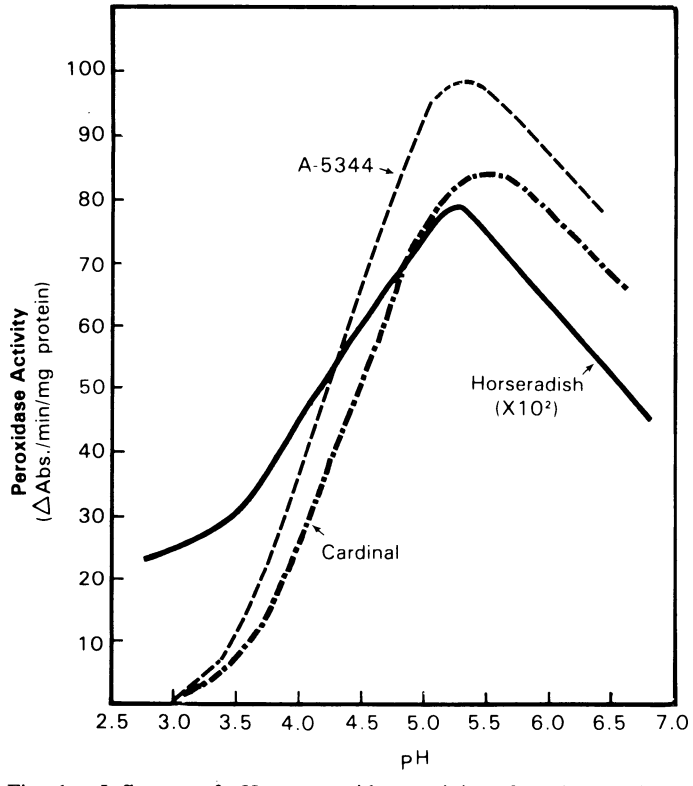

Fig. 1. Influence of pH on peroxidase activity of crude strawberry enzyme extracts and horseradish peroxidase.

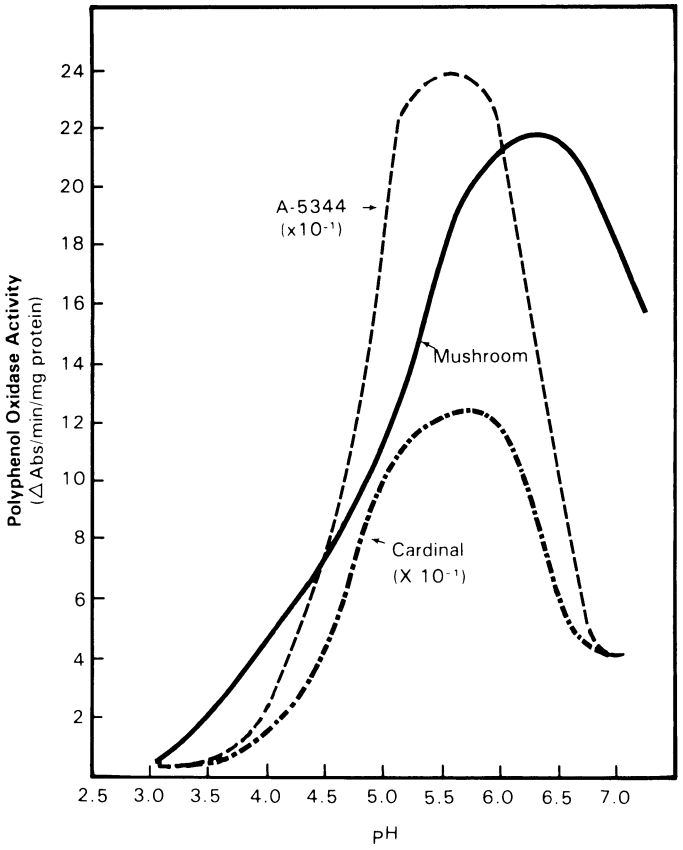

Fig. 2. Influence of pH on polyphenol oxidase activity of crude strawberry enzyme extracts and mushoom polyphenol oxidase.

No PPO activity was found in the frozen fruit or in the purees to which 10 units of PPO activity/g were added. In a previous report (19), an average PPO activity of 1.03 units in fruit from these same 2 cultivars after 5 months of frozen storage was reported.

Fig. 1 and 2 show pH curves for horseradish and crude strawberry PO activities and for mushroom and crude strawberry PPO activities, respectively. At pH 3.0, horseradish PO activity was readily detectable, while there was no activity in the strawberry extracts. Activity rapidly increased from about pH 3.5 to 5.0 and maximum activity levels were reached between pH 5.0 and 6.5. Similar activity curves were obtained for PPO (Fig. 2). Mushroom PPO was active within the pH range of strawberries (3.0 to 4.0), but maximum activity was between pH 6.0 to 6.5. Strawberry PPO extracts were essentially inactive below pH 3.5 and reached maximal activity between pH 5.3 and 5.8. Using mushroom polyphenol oxidase, Goodman and Markakis (4) reported decreased rates of anthocyanin degradation as pH decreased.

The fruit used in this study was machine-harvested and handled as it would be under commercial conditions, while in the initial study (19) the fruit was hand-stripped to simulate once-over harvest, individually quick-frozen, and stored under a nitrogen atmosphere. In these studies, maintenance of PPO activity in the fruit was difficult, especially after pureeing. Cash and Sistrunk (3) reported rapid loss of color in aerated strawberry

juice when 3 to 5% of the original PPO activity (using strawberry PPO) of the fruit was added back. Addition of 5 g of mushroom polyphenol oxidase per 100 g strawberry puree caused more rapid color loss than controls when puree was held at 50°C (16). Wrolstad, et al. (21) attributed higher anthocyanin concentrations in juice from blanched vs. unblanched strawberries to inactivation of PPO. Pederson and Beattie (12) showed that hot pressing of strawberries yielded juice with better color than cold pressing.

Results from this study indicated that color loss in strawberry puree is not significantly affected by mushroom PPO or horseradish PO when the enzyme activity level added was within the range found naturally in the 2 strawberry cultivars tested. Low pH and possibly inhibitors reduced the effectiveness of PPO and PO in degrading anthocyanins in the strawberry purees. Under the time-temperature conditions in this study, it is doubtful that PO and PPO contribute to color loss in strawberry purees. The effect of exogenous PO and PPO on processed product color was not determined. However, addition of immature fruits, which contain higher levels of PO and PPO than ripe fruits, to ripe strawberry puree did not affect color stability of strawberry jam during 1 year of storage at 2, 25, or 35°C (18).

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