Prevention of Enzymatic Darkening in Frozen Sweet Potatoes [*Ipomoea batatas* (L.) Lam.] by Water Blanching: Relationship among Darkening, Phenols, and Polyphenol Oxidase Activity

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Enzymatic darkening in sweet potato [*Ipomoea batatas* (L.) Lam.] is a result of phenol oxidation catalyzed by polyphenol oxidase (PPO). Water blanching prevents darkening in frozen sweet potatoes by significantly decreasing the PPO activity but does not reduce phenol levels. The effect of curing on darkening was indirect and cultivar dependent. Compared to Centennial, the cultivar Jewel contained lower phenols but higher PPO activity. Generally, a blanch treatment at 100 °C for 3 min or at 94 °C for 5 min is required to produce products with minimal darkening. The results suggest that the phenol concentration should be used as an indicator for the potential enzymatic darkening in green sweet potatoes, whereas the residual PPO activity is a better predictor of darkening in the blanched or processed products.

INTRODUCTION

Enzymatic darkening in some fruits and vegetables during postharvest and processing is a serious quality defect and is usually undesirable because of the unpleasant appearance and concomitant development of off-flavor and off-color (Walter and Purcell, 1980; Sciancalepore and Longone, 1984). It is generally agreed that the enzyme system mainly responsible for the darkening in fruits and vegetables is polyphenol oxidase (PPO) (Arthur and McLemore, 1956; Lee et al., 1990). However, the darkening potential of sweet potatoes has been reported to be directly related to the phenol levels (Walter and Purcell, 1980), the PPO activity (Scott et al., 1944), or a combination of both (Walter and Schadel, 1981). Therefore, it is obvious that the actual relationship among darkening, phenol concentration, and PPO activity has not been well-defined.

Studies to avoid or minimize enzymatic darkening have been conducted on the areas of breeding for low darkening prone cultivars (Jones, 1972), using longer lye-peeling times (Scott and Kattan, 1957) or additives (Twigg et al., 1974; Molnar-Perl and Friedman, 1990). Water blanching, as an effective means to inactivate the enzymes and stabilize the frozen vegetables against off-flavor development and nutrition loss, has been long and widely used (Williams et al., 1986; Halpin and Lee, 1987). It might also provide a new approach to investigate the enzymatic darkening in sweet potatoes. Unfortunately, there is no information available on the effect of water blanching on the enzymatic darkening in frozen sweet potatoes.

The present paper investigated the effects of various time/temperature combinations on darkening, phenol concentration, and PPO activity in two sweet potato cultivars with two storage treatments. The individual roles of these factors involved in the enzymatic darkening and the actual relationship among them have been defined. The potential applications of the results have also been discussed.

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MATERIALS AND METHODS

Sweet Potatoes. Green (freshly harvested) and cured (stored at 30 °C and 90% relative humidity for 7 days) (SCS, 1980) sweet potato roots from two cultivars, Centennial and Jewel, were obtained in 22-kg lots (replications) from the Pontotoc branch of the Mississippi Agricultural and Forestry Experiment Station (MAFES), MS, during the 1987 season. Roots from each lot (three lots per cultivar) were washed and peeled in an abrasive peeler. The peeled sweet potato roots were sliced cross-sectionally into 1.0 cm thick pieces using a semiautomatic slicer (Model A20, Hobart Corp., Troy, OH). The slices were collected in cool water (20 °C) to prevent oxidation prior to being blanched.

Blanching Treatment. Subsamples of the sliced product were blanched in a stainless steel jacketed kettle (Model D10, Groen Manufacturing Co., Chicago, IL) at different time/temperature combinations, using water as the heat-transfer medium. Two blanching temperatures, 94 (commercial blanching temperature) and 100 °C (boiling water temperature), and three blanching times, 1, 3, or 5 min, were used as the treatment variables. Each treatment combination was repeated three times (three lots). After blanching, the slices were cooled to 20 °C with flowing cold water and were individually quick frozen in a sharp freezer -23 °C for 2 h. The slices were then packed in 3.7-mil polyethylene bags and stored in a -18 °C freezer.

Determination of Darkening. Darkening of frozen sweet potato slices was determined visually by an expert panel as described by Ma et al. (1989, 1990). Five sample slices from each treatment were taken, thawed at room temperature for 4 h, and rated by six trained panelists on a 7.0-point rating scale with 1.0 being totally dark, 4.0 containing some dark spots, and 7.0 having no dark spots. A visual color score of 5.5 or higher was considered to be an acceptable product as to appearance (Ma et al., 1989, 1990). All samples from each replication were evaluated at the same time. The panelists' scores were averaged and expressed as the final score for each of the experimental observations. All treatments were carried out in triplicate.

Determination of Phenol Concentration. Five frozen sweet potato slices from each treatment were randomly selected and thawed at room temperature (25 °C) for 1 h. A 30-g portion of each sample was weighed and extracted with an ethanol-water mixture (80:20 v/v) (Walter and Purcell, 1979; Amiot et al., 1986). The results from the average of three independent determinations were expressed as milligrams per 100 g of wet sample.

Preparation of Enzyme Extract. A crude enzyme extract was prepared by taking 3 g of sweet potato sample from each treatment in the same manner as for the phenol determination. The sample was ground in 9.0 mL of citrate-phosphate buffer

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Table I. Effect of Blanching Treatments on Darkening in Frozen Sweet Potato Slices

blanching treatment		darkness rating[a]			
		cv. Centennial		cv. Jewel	
temp, °C	time, min	green	cured	green	cured
control[b]		3.6aB	2.2aA	4.4aB	4.0aB
94	1	3.1bAB	2.6aB	4.4aB	4.3aB
	3	6.5cB	3.5bA	5.5bB	5.2bB
	5	6.6cA	6.1cA	6.3cA	5.3bA
100	1	4.3bAB	5.7cB	3.8aA	5.6bB
	3	6.2cA	6.2cA	6.5cA	5.6bA
	5	6.6cA	5.9cA	6.1abA	6.3cA

[a] Darkening was rated on a scale of 1.0–7.0 with 1.0 being totally discolored, 4.0 being moderately discolored, and 7.0 being absent of discoloration. (abc) Means within column not followed by same letter differ ($P < 0.05$). (AB) Means within row not followed by same letter differ ($P < 0.05$). [b] Control is the unblanched frozen samples. Standard errors are 0.36 for the control and 0.53 for all others. All data are the average of three independent experiments.

(pH 4.5) by mortar with pestle on ice. The homogenate was centrifuged at 13000*g* at 4 °C for 15 min. The supernatant was decanted and stored at 4 °C until assayed.

PPO Assay. The enzyme activity was assayed by using catechol (Sigma Chemical Co., St. Louis, MO) as the substrate (Wisserman and Lee, 1980; Coseteng and Lee, 1987) with modifications. The enzyme activity was calculated on the basis of the slope of the linear portion of the curve plotted with ΔA_{420} against time (up to 3 min). One unit of enzyme activity was defined as $0.001\Delta A_{420}$ min^{-1} (g of wet weight)$^{-1}$. Residual PPO activity was expressed as a ratio of treated sample vs its control.

Statistical Analysis. A factorial in a completely randomized design with three replications was used. There were two cultivars (Jewel and Centennial), two storage treatments (green and cured), two blanching temperatures (100 and 94 °C), and three blanching times (1, 3, and 5 min). The data were analyzed using the SAS GLM program (SAS, 1985). To determine whether any of the variables were correlated, the CORR procedure (SAS, 1985) was used to calculate Pearson's correlation coefficients (r) (Steel and Torrie, 1980).

RESULTS

Effect of Blanching on Darkening. The effectiveness of various blanching treatments based on darkness rating is shown in Table I. The controls, sliced from green roots, showed less darkening than those from cured roots for both cultivars, with Centennial being darker than Jewel. Except for those blanched at 94 °C for 1 min, blanched products were less dark than their respective controls, indicating that properly controlled blanching can improve product appearance. Blanching at 100 °C for 3 min or more resulted in products with minimum darkening, and there were no significant ($P > 0.05$) differences beyond 3 min. However, at 94 °C, decreased darkening in sweet potatoes is time dependent (up to 5 min). A 3-min blanch would produce marginally acceptable products from green roots, while 5 min was required for the cured ones. Both cultivars required a minimum blanching of 3 min at 100 °C or 5 min at 94 °C to produce acceptable products (darkness rating higher than 5.5).

Effect of Blanching on Phenol Levels. Blanching and curing treatments did not significantly change the phenol levels (Table II). The only significant difference in phenol levels was observed between cultivars. Centennial had a higher ($P < 0.05$) phenol level than Jewel. These phenol levels are in the range among those of a number of sweet potato cultivars reported by Walter and Purcell (1980).

Effect of Blanching on PPO Activity. Cultivar Jewel had much higher (3–4 times) residual PPO activity than

Table II. Effect of Blanching Treatments on Phenol Levels in Frozen Sweet Potato Slices

blanching treatment		phenols,[a] mg/100 g of sample			
		cv. Centennial		cv. Jewel	
temp, °C	time, min	green	cured	green	cured
control[b]		33.91Aa	34.56Aa	51.80Aa	77.86Ba
94	1	37.07Aa	40.48Aa	63.19Ba	46.51Ab
	3	47.64Aa	41.56Aa	67.31Ba	59.75Bab
	5	47.22Aa	47.08Aa	66.31Ba	68.79Ba
100	1	39.91Aa	37.48Aa	60.09Ba	64.49Ba
	3	48.41Aa	38.69Aa	63.16Ba	63.68Ba
	5	38.66Aa	41.72Aa	62.14Ba	56.92Bab

[a] Phenols were expressed as milligrams per 100 g of wet tissue sample. (ab) Means within column not followed by same letter differ ($P < 0.05$). (AB) Means within row not followed by same letter differ ($P < 0.05$). [b] Control is the same as in Table I. All data are the average of three independent experiments.

Table III. Effect of Blanching on Residual PPO Activity in Frozen Sweet Potato Slices

blanching treatment		residual PPO act.,[a] %			
		cv. Centennial		cv. Jewel	
temp, °C	time, min	green	cured	green	cured
control[b]		100bc (310)	100a (310)	100ab (1125)	100a (1410)
94	1	116ab	115a	118a	117a
	3	30c	44a	15bc	6b
	5	18c	23a	5c	3b
100	1	68c	95a	21bc	12b
	3	11c	23a	10c	4b
	5	7c	15a	5c	3b

[a] Residual PPO activity is expressed as a percentage of each respective control. One unit of PPO activity equals $0.001\Delta A_{420}$ min^{-1} (g wet sample)$^{-1}$. Numbers in parentheses indicate the actual PPO activity. (abc) Means within column not followed by same letter differ ($P < 0.05$). [b] Control is the same as in Table I. All data are the average of three independent experiments.

Centennial (Table III). Curing treatment increased PPO activity by 25% in Jewel, but no increase was detected in Centennial. Upon blanching, residual PPO activity decreased dramatically in a time-dependent manner. Curing increased the heat resistance of PPO in Centennial but decreased the heat resistance in Jewel. Thus, Centennial needs more heat energy to achieve the same degree of inactivation of PPO (Table III). It was noted that the residual PPO activity in the slices blanched for 1 min at 94 °C was higher than in their raw counterparts, meaning that the heat given to them was not enough to inactivate the enzyme and the increase in activity may be explained by heat activation. It was already stated that a minimum blanching at 100 °C for 3 min or at 94 °C for 5 min was required to produce acceptable products (Table I) in which a 70–97% reduction in the PPO activity was shown (Table III). Since blanching did not change the phenol concentration, the inhibition of darkening in frozen sweet potatoes was mainly contributed by inactivation of the PPO.

Relationship among Darkening, Phenolic Concentration, and PPO Activity. To determine whether any of the variables were statistically interrelated, Pearson's correlation coefficients (r) were calculated by treating the analytical data from each triplicate as a separate observation (Table IV). In controls, the only statistically significant negative correlation to darkening was phenol level, which indicates that at the substrate–enzyme levels investigated the substrate level was the major contributing factor to darkening. However, when the slices were blanched, residual PPO activity and not phenol concen-

Table IV. Pearson's Correlation Coefficients (r) between Darkening and Phenols and PPO Activity in Frozen Sweet Potatoes

		Pearson's correlation coefficient (r)[a]			
		control[b]		blanched[c]	
cultivar	storage	phenols	PPO act.	phenols	PPO act.
Jewel	green	−0.99*	−0.81	0.64	−0.57
	cured	−0.93*	−0.10	−0.02	−0.82*
Centennial	green	−0.97*	−0.39	0.50	−0.91*
	cured	−0.99*	−0.23	0.78	−0.46

[a] Pearson's correlation coefficients (r) were calculated by using CORR procedure as described under Materials and Methods. *$P <$ 0.05, others nonsignificant at $P < 0.05$. [b] Control is the same as in Table I. [c] Products were blanched at 100 °C for 3 min.

tration was significantly and negatively correlated with darkening. Although these correlations were not sufficiently high for predictive purpose in all cases, they did indicate that residual PPO activity was an important factor in darkening of blanched sweet potatoes. Other factors, such as cultivar and curing treatment, may contribute to the low correlations in the blanched slices.

DISCUSSION

Enzymatic darkening in sweet potatoes has been extensively studied (Arthur and McLemore, 1956; Twigg et al., 1974; Walter and Purcell, 1980). However, a close review of the literature reveals that a conclusive relationship among phenol level, PPO activity, and darkening in sweet potatoes has not been achieved. Also, no reference was available using water blanching to prevent the enzymatic darkening to produce commercially acceptable frozen sweet potato products. More importantly, most previous studies, if not all, did not examine the individual variables independently, which is critical to make a correct conclusion. Blanching partially inactivates PPO to certain low levels, provides a totally new approach to study the enzymatic darkening, and made it possible to investigate the individual roles of phenol and PPO in enzymatic darkening independently.

To understand how phenols and PPO activity contribute to the darkening and why blanching can prevent darkening, the phenolic content and PPO activity in each sample were determined and correlated to darkening. Results show that both phenols and PPO activity are critical to darkening situations. For example, in unblanched controls, the darkening is more dependent on the original amount of substrate available than on PPO activity, because PPO enzyme is present in such high amounts relative to substrate levels that at equilibrium most of the substrate has been oxidized to brown pigments (Walter and Purcell, 1980). This explained that in raw roots Centennial was darker than Jewel, since the former contains higher phenol levels. In contrast, in the blanched samples, PPO activity decreased dramatically while substrate concentration did not change. In this situation, PPO became the limiting factor; thus, the degree of darkening mainly depends on the residual PPO activity in the samples. This can be proved by the fact that as long as the residual PPO activity decreased to a certain degree, longer blanching time will not further improve the product color (Tables I and III). It can also be expected that when PPO in sweet potatoes is completely inactivated, no enzymatic darkening can occur.

Cultivar difference in darkening was noted as reported in previous studies (Porter et al., 1976; Walter and Purcell, 1980). Centennial contains higher phenols but lower PPO activity than Jewel, which resulted in Centennial being darker. For example, curing increased PPO activity in Jewel but not in Centennial. Curing also increased the heat resistance of PPO in Centennial but decreased the heat resistance in Jewel. These differences may be explained by the genetic influence such as different localization of phenols and PPO among cultivars (Schadel and Walter, 1981; Walter and Schadel, 1981). However, additional factors, such as cultural practices, length and temperature of storage, and processing technique, may also affect darkening.

In summary, prevention of enzymatic darkening in frozen sweet potatoes by blanching is the result of the dramatic reduction of the PPO activity but not a reduction in phenol levels. The darkening potential was correlated to phenol levels in the green roots but to residual PPO activity in the blanched or processed products. For both cultivars investigated, a blanch treatment at 100 °C for 3 min or 94 °C for 5 min would produce products with minimal darkening.

ABBREVIATIONS USED

PPO, polyphenol oxidase.

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