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Received for review February 25, 1979. Accepted May 12, 1980. Paper No. 6268 of the journal series of the North Carolina Agricultural Research Service, Raleigh, N.C. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, North Carolina State University, or Brigham Young University, nor does it imply approval to the exclusion of other products that may be suitable.

# Effect of Substrate Levels and Polyphenol Oxidase Activity on Darkening in Sweet Potato Cultivars

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Homogenization of sweet potato in the presence of oxygen causes differing degrees of brown discoloration to occur, depending upon the cultivar. When browning occurs in processed sweet potato, it is a serious quality defect. In order to better understand browning, five sweet potato cultivars produced in two crop years were evaluated by relating browning to phenolic content, polyphenolase activity, and ascorbic acid levels. Browning was found to be significantly correlated only to phenolic content. Considerable within-year and year-to-year cultivar variation was observed in browning potential.

Although sweet potatoes do not discolor when cut or sliced as do certain fruits like peaches (Guadagni et al., 1949) or apples (Harel et al., 1966), they do darken or brown when heat processed (Scott et al., 1944). Scott et al. (1944) demonstrated that discoloration occurred when the outer portion of sweet potato roots was subjected to elevated temperatures not high enough to denature enzymes, but sufficient to disrupt cellular organization and, thus, cause polyphenol oxidase to react with "tannins". Since their report, the polyphenol oxidase (PPO; *o*-diphenol:$O_2$ oxidoreductase, EC 1.10.3.1) of sweet potato has been studied by several groups and shown to consist of soluble and particulate fractions specific for *o*-diphenols, especially chlorogenic acid and its isomers (Eiger and Dawson, 1949; Arthur and McLemore, 1956; Hyodo and Uritani, 1965).

Efforts to avoid processing-induced darkening have centered on producing cultivars with low discoloration potential. However, some research has been conducted on using pre-peel heating, longer lye-peeling time, and/or additives (Scott and Kattan, 1957; Twigg et al., 1974) to prevent darkening. In order to produce sweet potato cultivars of low discoloration potential, it has been suggested that plant breeding programs screen selections for PPO activity (Jones, 1972) and reject those selections which are high in the enzyme. High PPO activity is generally associated with sweet potatoes which darken during processing and, thus, are poorly accepted by consumers (Scott et al., 1944; Scott and Kattan, 1957).

Research on the enzymatic browning of many fruits and vegetables has shown that the potential for discoloration is related to phenolic levels, PPO activity, or a combination of both. In white potatoes (Mondy et al., 1967), peaches (Guadagni et al., 1949), and bananas (Weaver and Charley, 1974), the degree of darkening or browning is highly correlated with phenolic content but poorly related with PPO activity. Since the development of the PPO test for darkening potential (Scott and Kattan, 1957), very little data have been forthcoming concerning the relationship among PPO, substrate, and darkening potential. Walter et al. (1979) reported that, in the seven cultivars studied, the only phenolics present were chlorogenic acid and its isomers. These phenolics are effectively oxidized by sweet potato PPO. The purpose of our study was to determine the role native phenolics play in the enzymatic discoloration of sweet potato cultivars.

## MATERIALS AND METHODS

"Centennial", "Jewel", "Australian Canner", and "Pelican Processor" sweet potato cultivars were obtained at harvest from the North Carolina Agricultural Research Service, Central Crops Research Station at Clayton, NC. The roots were cured and stored as currently recommended (Covington et al., 1976). "Porto Rico 198", obtained from the Horticultural Crops Research Station at Clinton, NC, was handled in the same manner as the other cultivars.

The study was conducted on roots produced in 1977 and 1978. Ascorbic acid was measured on the 1977 crop only. Extent of browning, PPO activity, and phenol levels were measured for both crop years. The analyses for each

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Table I. PPO Activity,[a] Phenol[b] and Vitamin C[c] Content, and Darkening[d] among Sweet Potato Cultivars[e] Harvested in 1977 and 1978

| cultivar | PPO[f] | | phenol[f] | | darkening[f] | | vitamin C, |
|---|---|---|---|---|---|---|---|
| | 1977 | 1978 | 1977 | 1978 | 1977 | 1978 | 1977 |
| "Jewel" | 4.2 | 5.0 | 16.3 | 13.8 | 0.126 | 0.130 | 23.3 |
| "Centennial" | 2.7 | 2.4 | 32.9 | 36.5 | 0.278 | 0.289 | 25.1 |
| "Porto Rico" | 2.8** | 6.4 | 40.2 | 29.6 | 0.275 | 0.285 | 19.2 |
| "Australian Canner" | 8.0 | 6.0 | 144.0* | 33.8 | 0.668* | 0.335 | 26.7 |
| "Pelican Processor" | 51.6** | 28.0 | 76.5** | 20.4 | 0.449* | 0.205 | 21.1 |
| LSD[g] | 6.23 | 2.6 | 49.3 | 18.8 | 0.152 | 0.052 | 7.93 |

[a] $\Delta A_{450nm}$ $min^{-1}$ (g of sweet potatoes)$^{-1}$. [b] Milligrams of phenol/100 g of sweet potatoes (as chlorogenic acid). [c] Milligrams of vitamin C/100 g of sweet potatoes, 1977 only. [d] $A_{450}$ of filtered sweet potato homogenate. [e] Each value is the mean of four replicate analyses. [f] Significant within-cultivar differences between 1977 and 1978 where indicated by * ($P < 0.05$) and ** ($P < 0.01$). [g] LSD values apply to vertical columns under which they are located.

cultivar were replicated four times. Each replicate contained two roots.

**Sampling Procedure.** Roots were washed, hand-peeled, and halved longitudinally. Slices (ca. 3 mm thick) were cut, discolored areas were excised, and the slices were diced. Samples were removed as follows: (1) 10 g for PPO activity and degree of darkening, (2) 20 g for phenol assay, (3) 50 g for ascorbic acid measurement.

**Measurement of Degree of Darkening and PPO Activity.** The 10-g sample was homogenized with 50 mL of cold, buffered 0.05 M phosphate, pH 6.3, 0.15 M NaCl. The homogenate was held at 5 °C for at least 2 h, centrifuged at 40000*g* for 10 min, and filtered through a 0.2-$\mu$m filter. The filtrate was kept in an ice bath. A portion was allowed to warm to room temperature, and its absorbance at 450 nm ($A_{450}$) was measured and called the degree of darkening. Preliminary studies showed that there was no change in $A_{450}$ value for 4 h provided the original homogenate was held at 5 °C for 2 h before centrifugation.

In order to measure PPO activity, the sample filtrate was diluted with buffer as necessary so that when 0.5 mL of substrate (1 mg/mL caffeic acid) was added to 0.6 mL of filtrate, the $A_{450}$ did not exceed 0.2 absorbance unit within a 7-min reaction period (1-cm light path). The curvette was rapidly inverted and inserted into the sample compartment of a Gilford Model 250 spectrophotometer. The sample $A_{450}$ was recorded at 20-s intervals for 7 min vs. a 1.1-mL blank containing no substrate. The net $A_{450}$ was tabulated and plotted against time, and the slope was calculated for the linear portion of the curve (to 3.0 min). The PPO activity was expressed as $\Delta A_{450}$ $min^{-1}$ (g of tissue)$^{-1}$ (fresh weight). Each sample was assayed at two enzyme levels, and the results were averaged.

**Phenol Measurement.** The 20 g of diced sweet potatoes was homogenized with 70 mL of 95% ethanol, quantitatively transferred to a 100-mL volumetric flask, and held at 5 °C until analyzed. For the analysis, the ethanol extract was diluted to volume and thoroughly mixed. Aliquots were removed and evaporated to dryness, and the residues were taken up in 0.1 M phosphate buffer (pH 6.35). These mixtures were then filtered with a 0.2-$\mu$m filter, and the absorbance of the filtrate at 323 nm ($A_{323}$) was measured. The filtrates were then shaken with Dowex 1-X8 ($Cl^-$, 200–400 mesh) for 30 min and allowed to settle. The $A_{323}$ was measured. The phenol content was calculated from the loss in $A_{323}$ and expressed as mg/100 g fresh weight as chlorogenic acid (Walter and Purcell, 1979). For the 1977 crop year, the phenolic levels were also determined by the high-pressure liquid chromatography (LC) method previously reported (Walter et al., 1979).

**Ascorbic Acid Measurement.** The 50-g sample of diced sweet potatoes was immediately homogenized with 50 mL of deionized, distilled water and 5.26 g of oxalic acid. The homogenate was flushed with nitrogen, capped, and placed in −10 °C storage until analyzed. Ascorbic acid was measured by the fluorometric AOAC procedure (AOAC, 1975).

**Effect of NaCl and Polyvinylpyrrolidone on PPO Activity.** Sodium chloride (NaCl) is an inhibitor of PPO in some fruit (Luh and Pithakpol, 1972; Halim and Montgomery, 1978). The extraction procedure described above included NaCl; thus, the following experiment was performed to assess the effect of NaCl at the levels used in this study upon sweet potato PPO. Duplicate 10-g samples of "Jewel" sweet potato tissue were homogenized in 50 mL of cold phosphate buffer (as described earlier). The buffer for one sample contained 0.15 M NaCl and that for the other sample contained none. The PPO activities of the two solutions were then measured and compared.

Rupture of cellular membranes when tissue is disrupted exposes native phenolics to the action of oxidases and the quinones formed by that action inhibit enzyme activity, including that of PPO. To avoid quinone formation, some workers perform cellular disruption in the presence of a phenol scavenger such as polyvinylpyrrolidone (PVP; Flurkey and Jen, 1978; Halim and Montgomery, 1978). In order to measure the effect of PVP on sweet potato PPO activity, samples of "Jewel", "Centennial", and "Porto Rico 198" were ground in the presence or absence of 1.0 g of purified PVP (Andersen and Todd, 1968), and the PPO activities were measured.

## RESULTS AND DISCUSSION

**Polyphenol Oxidase Activities.** PPO activities of the cultivars examined ranged from 2.4 to 51.6 units, but most were clustered between 2.4 and 8.0 units (Table I). Except for "Pelican Processor" (PP) and "Porto Rico 198" (PR), each cultivar had about the same PPO activity in both 1977 and 1978.

Preliminary tests were carried out with roots from the 1977 crop to determine if either NaCl or endogenous phenols could inhibit PPO activity, as has been reported in pears (Halim and Montgomery, 1978). The PPO activity of a "Jewel" tissue homogenate prepared in phosphate buffer containing NaCl was 30% greater than that of a tissue homogenate prepared without NaCl. However, when tissue was extracted with phosphate buffer and then adjusted to 0.15 M NaCl just before PPO assay, the resulting solution had about 50% less activity than an extract which contained NaCl at the time of homogenization. An explanation of this inconsistency is that when NaCl is used in the extraction buffer, it results in the solubilization of more enzyme than when the homogenization is performed with buffer alone. Thus, although NaCl did inhibit PPO from sweet potatoes, it also caused more of the total enzyme to be solubilized. Since NaCl is inhibitory, care was

taken to assure that levels of NaCl used were identical for all of the analyses.

The effect of PVP on PPO activity was studied for "Jewel", "Centennial", and "Porto Rico" cultivars. The tissue from each cultivar was homogenized with and without PVP, and the activities were measured. The mean PPO activity of 5.5 units ($\Delta A_{450nm}$ $min^{-1}$ (g of sweet potato)$^{-1}$) for untreated homogenate was significantly greater ($P < 0.01$) than the mean activity of 3.5 units for PVP-treated homogenate, indicating that the PVP treatment caused the PPO activity to decrease. This decrease in enzyme activities is somewhat surprising since PVP has been used in many cases to complex phenols in an attempt to prevent their oxidation by PPO and thus prevent loss of enzymatic activity. A thorough study of PVP–protein interaction has not been done, but it is likely that such an interaction exists in light of the inhibition of PPO by PVP which is reported herein.

When PPO oxidizes phenolics, the resulting quinones react with each other or with other cellular constituents to form brown-colored polymers with broad, nonspecific absorbance. The same reaction pathway is followed when fruits and vegetables are mechanically injured. In the canning of sweet potatoes, heat from the lye peeling steps is believed to damage the tissue sufficiently to allow the PPO enzymes to react with phenolic substrates. Qualitative observations indicated that those cultivars which exhibit the greatest discoloration after lye peeling also have the highest degree of darkening when the raw tissue is homogenized. It is not possible to objectively quantitate discoloration by reflectance measurements because of large differences in carotene levels among the cultivars studied.

Preliminary tests indicated that if homogenized sweet potatoes are held at 5 °C for at least 2 h before centrifugation, the darkening, or $A_{450}$, does not change for at least 6 h. This indicated that the enzymatic reaction had progressed to completion. Further, it was found that no change in PPO activity could be detected in the homogenate or the centrifuged, filtered homogenate during 6 h of storage at 5 °C.

Phenols of "Jewel" and "Centennial" were present at similar levels for 1977 and 1978 (Table I), while "Porto Rico 198" (PR) phenolics declined slightly but not significantly for both years. "Australian Canner" (AC) and PP phenolic levels decreased signficantly during the same period. Darkening in "Jewel", "Centennial", and PR was similar in 1977 and 1978, but darkening in AC and PP was about twice as great in 1977 as in 1978. The data in Table I illustrate the large amount of variability in PPO activity, phenolic content and darkening values which occurred for some of the cultivars during the 2 years of this study. Considerable variability was also observed within cultivar replicates, as illustrated by the relatively high LSD values (Table I). The causative factor(s) for this variation is presently unknown.

Phenols for the 1977 crop year were measured by both spectrophotometric (Walter and Purcell, 1979) and LC procedures (Walter et al., 1979). For the cultivars studied, the phenol × cultivar relationship as measured by the spectrophotometric method was highly correlated ($r$ = 0.99) with the phenolic × cultivar interaction obtained by the LC method. Thus, 1978 analyses were performed by the more rapid spectrophotometric procedure. For both years and for all cultivars, only chlorogenic acid (CA) and its isomers were present.

Ascorbic acid (AA) analysis was performed to find out if this inhibitor of PPO (Halim and Montgomery, 1978) played a role in darkening of sweet potatoes. It is the practice of some fruit processing operators to add AA to stop browning during precanning or prefreezing stages. Consequently, the levels of endogenous AA were measured to examine their effect upon darkening. From a comparison of the endogenous AA levels and darkening values for the 1977 crop year, we concluded that AA levels had little or no effect on darkening; consequently, the AA concentrations for crop year 1978 were not measured. A plausible explanation for the apparent lack of inhibition by AA is that the ascorbate "inhibits" darkening by reducing the quinones back to the phenolic level before they can produce brown pigments. In the process, AA is oxidized. When all of the AA has been consumed in this way, then the PPO–phenol reaction proceeds beyond the quinone stage to equilibrium, as is the case in this study.

Table II. Relationships among PPO, Phenols, Ascorbic Acid, and Darkening[a]

| | darkening | phenols | ascorbic acid |
|---|---|---|---|
| PPO | 0.248 | 0.226 | 0.190 |
| darkening | | 0.951[b] | 0.286 |

[a] Linear correlation coefficients from 20 observations in 1977 and 20 observations in 1978. [b] $P < 0.01$, others nonsignificant at $P < 0.1$.

**Variable Interactions.** In order to determine whether any of the variables were statistically interrelated, simple correlation coefficients were calculated by treating the analytical data from each replicate as a separate observation (Table II). The only statistically significant correlation was between phenol concentration and darkening ($r$ = 0.95, $P < 0.01$ level), which indicates that at the substrate–enzyme levels investigated, the substrate level was the major contributing factor to darkening.

Review of other research concerning the relationship between darkening, PPO activity and substrate levels showed that with some varieties of the same commodity, the enzyme activity is more likely related to darkening than substrate levels (Jen and Kahler, 1974). With other varieties, substrate level and darkening are highly related (Guadagni et al., 954). In potatoes (Mondy et al., 1967), apples (Harel et al., 1966), and bananas (Weaver and Charley, 1974), darkening was highly correlated with substrate content and poorly correlated with PPO activity. For avocado, it has been reported that both PPO activity and substrate levels are highly correlated to darkening (Golan et al., 1977). There seems to be a very critical relationship among darkening, enzyme activity and substrate levels which governs the relative importance of enzyme and substrate to the darkening process.

In sweet potatoes, it appears that genetic selection for minimal browning should be based on a low level of substrate rather than low PPO activity (Table II). From a kinetic viewpoint, the evidence indicates that the PPO enzyme is present in such high amounts relative to substrate levels that at equilibrium most of the substrate has been oxidized to brown pigment, and that the amount of browning is more dependent on the original amount of substrate available than on PPO activity.

Since browning seems to be a direct function of phenol concentration in sweet potatoes, the present practice of dipping a slice into 0.04 M catechol solution and subjectively rating the severity of darkening (Scott and Kattan, 1957) in terms of PPO activity would appear to be a poor predictor of the tendency for a selection to darken during processing. On the basis of the results of our study, several alternative screening methods can be suggested. The first of these is the hot water dip procedure of Scott and Kattan

(1957), which provides a qualitative estimate of darkening tendency based on the color change produced by the PPO–phenol interaction in a water-heated tissue slice. The second procedure is to quantify the phenol content of selections and establish a darkening scale based on these values. A third screening method is to measure the darkening of tissue homogenates of the selections of interest and establish a scale based directly on darkening values. The last two methods appear to be of potentially more use because they provide quantitative rather than qualitative comparisons among selections. Studies of factors such as harvest maturity, rate of fertilization, water application, etc. should provide information on the year-to-year and within-cultivar variability of PPO activity, phenol content, and darkening tendency, thus eliminating these concerns and allowing the unqualified use of either of the latter two screening methods.

ACKNOWLEDGMENT

The authors express their appreciation to W. W. Collins of the Department of Horticultural Science at North Carolina State University for providing samples of "Australian Canner", "Pelican Processor", and "Porto Rico 198". The authors also thank F. G. Giesbrecht of the Department of Statistics for advice concerning statistical treatment of the data.

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Received for review January 11, 1980. Accepted April 10, 1980. Paper No. 6245 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or North Carolina State University, nor does it imply approval to the exclusion of other products that may be suitable. Presented at the 178th National Meeting of the American Chemical Society, Division of Agricultural and Food Chemistry, Washington, DC, Sept 1979.

# Determination of Protein in Tannin–Protein Precipitates

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The amount of protein precipitated by tannin under a variety of conditions is measured by using radioiodinated protein. The protein is mixed with purified tannin, finely ground plant tissue, or an unpurified plant extract; the mixture is centrifuged to remove the insoluble tannin–protein complex. An aliquot of the supernatant layer is counted to calculate the amount of protein precipitated. Complex formation is dependent on the pH and solvent composition. Tannin specific activity is useful for comparing tannin from various sources and for monitoring tannin purification. It is defined here as the ratio between the amount of protein precipitated and the amount of oxidizable material present.

Both the environmental role (Swain, 1979) and the nutritional effects (Price and Butler, 1980) of tannin might be better understood if adequate analyticaly techniques were available for quantitative study of tannin–protein interactions. With the protein precipitation assay we described previously (Hagerman and Butler, 1978) only precipitated tannin is measured. We have now modified our method so that the amount of protein precipitated is determined. By use of this new protein binding method in conjunction with our previous method (Hagerman and Butler, 1978), both components in the precipitated tannin–protein complex can be determined.

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## METHODS

All chemicals were reagent grade. Condensed tannin was purified from sorghum [*Sorghum bicolor* (L.) Moench, variety BR 54] as described in the accompanying paper (Hagerman and Butler, 1980). Tannic acid was from Sigma Chemical Co. Methanol solutions of purified tannin or crude methanol extracts of grain were used within 24 h of preparation. For assays of unextracted grain, sorghum was ground in a electric hand-held coffee mill (Krups KM-75), Germany) and used within 3 days. Protein concentration was determined with the biuret assay (Layne, 1957), and radioactivity was measured with a Beckman Gamma 300 $\gamma$ counter.

Bovine serum albumin (BSA) (Sigma Chemical Co.; fatty acid free, prepared from fraction V albumin) was labeled with iodine-125 by the Chloramine-T method

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