The Polyphenol of *Dioscorea alata* (Yam) Tubers Associated with Oxidative Browning

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The cut surfaces of some but not all yams (*Dioscorea* spp.) turn brown when exposed to air. The substances responsible for browning were extracted from cooked yams with ethanol. Chromatographic characteristics, color reactions, and uv light-absorption spectra showed the main substance to be a catecholamine. Two more weakly oxidizable substances were sometimes found, both leucoanthocyanidins. Oxidizable substances occurring in extracts or on chromatograms were most easily revealed by treatment with macerated tissue from nonbrowning tuber, which resulted in rapid browning.

The browning of fruits and vegetables, especially after harvest, storage, or wounding, commonly reduces their attractiveness and may be associated with astringency and off flavors (Bate-Smith, 1954). The most common substances associated with enzymatic browning are the cinnamic acids, especially caffeic and chlorogenic acids, tyrosine, which is oxidized to a melanin-like pigment, and the catechins and leucoanthocyanidins.

Browning of cut surfaces of yams (*Dioscorea* spp.) is often seen. As commonly observed, tubers of any variety can tend to darken; tubers of some varieties are more sensitive to browning than others; browning tendency differs among tubers of a given variety; and in different parts of a single tuber, browning is associated with wounding of the tissue, age of the tissue, and prolonged storage time. In addition, browning of the cut surface can be eliminated or reduced by exclusion of oxygen from the surface or by treatment of the surface with an antioxidant such as ascorbic acid. Also, catechin and caffeic acid applied in methanolic solution to the cut surface of any yam resulted in a rapid characteristic color change due to the oxidation of these substances. This suggests that browning in yam is associated with polyphenolic oxidation. Furthermore, tubers that are very sensitive to browning also often produce large quantities of a gummy substance when cooked. Thus, the substrate involved in oxidation can be polymerized rapidly. The production of colored, gummy polymers is very characteristic of the catechins and the leucoanthocyanidins.

Browning in the Chinese yam, *Dioscorea batatas*, has been attributed to the oxidation of dopamine 4-(2-aminoethyl)pyrocatechol under the control of an enzyme (Imakawa, 1967). The substance is not present in significant quantities in newly harvested yams, but increases in quantity with tuber aging. Storage at low temperatures reduces browning tendency. Tono (1970) isolated not only dopamine, but a catecholamine from the same yam species. In further studies of browning, Satoh and Tanabe (1971) found that maleic hydrazide (1,2-dihydro-3,6-pyridazinedione) applied to the foliage of the growing yam plant reduced the browning reaction of the tuber.

MATERIALS AND METHODS

From a large collection of varieties of *D. alata* L., some were selected whose tubers often browned when cut and exposed to air (Table I). One variety in which browning is not common was selected as a source of oxidation-free tissue.

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In a test for the presence of oxidizable substances, tubers were cut and exposed to air 60 min before use. Those that turned brown rapidly were considered to contain oxidizable substances.

A wide variety of solvents and extraction techniques were used during preliminary studies, and the following technique was developed. The tuber was peeled and cut into cubes about 2 cm long. The cubes were cooked in boiling water about 10 min or until soft. They were then placed on blotting paper to remove excess water, and were blended with 100 ml of ethyl acetate or ether.

The residue left after ethyl acetate or ether extraction was reextracted with 100 ml of 95% ethanol and filtered. The extract was evaporated in a warm water bath with hot air. The aqueous residue was then treated with an equal quantity of ethyl acetate and separated into ethyl acetate and aqueous fractions. All fractions were partly evaporated and spotted on filter paper. The spots were treated with a 1:1 mixture of macerated yam tuber tissue and water. These crude enzyme preparations were used to detect readily oxidizable polyphenols.

Only the aqueous fraction contained readily oxidizable substances. This fraction was used for chromatography on Whatman no. 81 chromatography paper impregnated with silica gel. Components were separated with three different solvent systems: the upper phase of a 1:1 mixture of normal butanol and 2 *N* HCl; a 6:4:1 mixture of toluene, ethyl formate, and formic acid; and a 1:1:3 mixture of benzene, acetic acid, and methanol. Chromatograms were developed twice, and discrete bands resulted.

After drying at room temperature, the chromatograms were inspected under short-wave and long-wave uv light, before and after treatment with NH_3 vapor. The colors and their relative intensities were recorded. Chromatograms were treated with macerated yam tissue from the variety resistant to oxidation, and bands that turned brown were recorded. Spontaneous oxidation and oxidation at 80°C also were observed. Other chromatograms were sprayed with a 1:4 mixture of concentrated HCl and ethanol saturated with vanillin, to reveal catechols and leucoanthocyanidins. Dried chromatograms were placed in a sealed glass jar with a small amount of formaldehyde. After 12 hr, the chromatograms were observed under uv light before and after heating at 80°C, for detection of catecholamines.

Chromatograms were cut into distinct bands, which were eluted from the paper with methanol. The uv absorption spectra were recorded with a Beckman Acta CIII spectrophotometer, before and after treatment with sodium ethoxide.

The eluted substances in methanol were boiled with an equal volume of 2 *N* HCl for 1 hr. To the resulting

Table I. Species and Varieties of Yams Used for the Study, Their Recent Origins, and a Few Notes

Species	Variety	Recent geographical source	Note
D. alata	15539 Suidie	Ivory Coast	Usually oxidation free
D. alata	15567 unnamed	Ghana	Principal variety used, variable in oxidation tendency
D. alata	15547 Alowinrin	Nigeria	Used for comparison
D. alata	15573 unnamed	Nigeria	Used for comparison
D. alata	15575 Suave	Nigeria	Used for comparison
D. rotundata	15654 Pape	Ghana	Used for comparison

Table II. Bands of Substances Found in Extracts of Yam Tubers, Their R_f Values in One Solvent System,[a] and Their Colors

Band no.	R_f	Color on paper	Color under uv light		
			Long wave	Short wave	Long wave plus NH_3
1	0.00	Tan	Absorbent[b]	Absorbent	Absorbent
2	0.02	None	Faint violet	Faint violet	Violet
3	0.15	None	Greenish grey	Grey	Dark blue
4	0.28	None	Greenish grey	Grey	Dark blue
5	0.35	None	Light blue	Blue	Light blue
6	0.52	None	Absorbent	Absorbent	Absorbent
7	0.60	None	Blue	Blue	Blue
8	0.65	None	Absorbent	Absorbent	Violet[c]
9	0.74	None	Yellow	Orange	Blue
10	0.80	None	Yellow	Yellow	White
11	0.88	None	Light blue	Light blue	Light blue
12	0.90	None	Blue	Blue	Yellow
13	0.98	Tan	Absorbent	Absorbent	Absorbent

[a] Toluene–ethyl formate–formic acid, 6:4:1. [b] In this table absorbent means that the compound absorbed uv without fluorescing and therefore appeared dark. [c] Purple in visible light.

mixture, equal quantities of ethyl acetate and water were added. The mixture was separated into aqueous and ethyl acetate fractions. The uv absorption peaks were noted and compared to those of the original compounds. The fractions were then evaporated and separated chromatographically by the previous solvent systems.

From different yam varieties, tubers that oxidized readily were compared for their substances. Oxidizable and nonoxidizable parts of the same tuber also were compared.

RESULTS

Extraction. For enzyme actions to stop, tuber pieces were boiled before extraction; boiled pieces were not subject to spontaneous polyphenolic oxidation. The gums in the tubers of yams made aqueous extractions impossible and methanolic and ethanolic extractions difficult. Ethyl acetate extracted carotenoid pigments and nonoxidizable substances. The advantage of preliminary extractions with ethyl acetate is that it coagulates the gums and facilitates later extractions with ethanol. Used to reextract the coagulated residue after ethyl acetate extraction, the ethanol contained the bulk of the oxidizable substances. After evaporation of the alcohol and phase separation with ethyl acetate, the aqueous fraction contained all and the ethyl acetate none of the oxidizable substances. They were obtained only from regions of tubers that oxidized rapidly.

Enzyme Preparations. All parts of all tubers tested, whether of *D. alata* or *D. rotundata* Poir. and whether from the oxidizable or nonoxidizable part of the yam, could change the color of caffeic acid, chlorogenic acid, catechol, and the oxidizable substance from tubers, when these substances or solutions of them were placed on the freshly cut tuber. On the other hand, these reactions did not occur on freshly cut cooked tuber. The reaction could be detected on well-dried chromatograms before and after chromatographic separation of components, by application of a mixture of macerated tuber and water. Color changes were inhibited when air was replaced with nitrogen, or when air was excluded by covering surfaces with cellophane.

Chromatography. Up to 13 substances were obtained on typical chromatograms (Table II). These substances were separated best with the solvent system of toluene, ethyl formate, and formic acid. However, to clean up the main compounds and obtain them in reasonable purity, we had to rechromatograph them several times with the other solvent systems.

Some bands absorbed uv light and were recognized as dark streaks. The main substance (band 6) that could be oxidized by macerated yam tissue was light absorbent under long-wave uv light.

As shown in Tables II and III, bands 1 and 3 were either slightly colored or readily oxidized to light tan. On heating of the chromatogram, band 6 was weakly oxidized (Table III); it was readily oxidized by macerated yam tissues to a dirty brown. Occasionally, bands 1 and 13 were faintly oxidized to tan, if not already tan.

The vanillin–HCl reaction is considered to be specific for catechins and leucoanthocyanidins. Spraying with this reagent produced a strong pink color only in bands 1 and 13 (Table III). Treatment of chromatograms with formaldehyde gas followed by heating produced a brilliant yellow-green fluorescence in long-wave uv in band 6 and brilliant bright blue in band 7. The development of brilliant fluorescence is reported to be associated with catecholamines (Bjorklund et al., 1969).

Uv Absorption. Spectral measurements of the strongest substances were made before and after treatment of the methanolic solution with a few drops of sodium methoxide in methanol. Bands 1, 6, 7, and 13 absorbed light in the region 275–283 nm, the region of absorption

Table III. Oxidation and Color Reactions of the Main Substances Extracted from Yam Tuber with Ethanol

Band no.	Oxidation			Reaction to		
	Spontaneous	At 80°C	With macerated yam tissue	Vanillin-HCl	Formalin + uv	Hydrolysis
1	Yes	No more	Weak	Pink	Absorbent	Pink
2	None	None	None	None[a]	Yellowish	Yellow
3	None	None	None	None	Bluish	Lt. yellow
4	None	None	None	None	Bluish	Lt. yellow
5	Weak	None	None	None	Blue	None
6	None	Weak	Strong	None	Brilliant yellow green	None
7	None	Very weak	None	None	Brilliant bright blue	None
8	None	None	None	None	Absorbent	None
9	None	None	None	None		None
10	None	None	None	None	Yellow	None
11	None	None	None	None	Violet	None
12	None	None	None	None	Violet	None
13	Yes	No more	Weak	Strong pink	Absorbent	Pink

[a] Blackish when heated.

Table IV. Uv Absorption Spectra of the Principal Substances Obtained from Yam Tubers by Ethanolic Extraction, and Their Derivatives after Hydrolysis

Band no.	Treatment	Fractions in which found	Main uv absorption peaks, nm		Suggested substance
			In methanol	With NaOMe	
1	None	Water	281	No change	Leucoanthocyanidin
2	None	Water	211	No change	
3	None	Water	263	Destroyed	
4	None	Water	263	Destroyed	
5	None	Water	210	No change	
6	None	Water	283	296	Catecholamine
7	None	Water	285	300	Catecholamine
11	None	Water	211	No change	
12	None	Water	324		Cinnamic acid
13	None	Water	282	292	Leucoanthocyanidin
1	Hydrolyzed	Water			
		Ethyl acetate	277, 535	290	Anthocyanidin
5	Hydrolyzed	Water	276	276	
		Ethyl acetate	274	274	
12	Hydrolyzed	Water	275	275	
		Ethyl acetate	277, 535		Anthocyanidin

of catechins and leucoanthocyanidins (Table IV). The absorption spectra of these substances were shifted to a higher wavelength by the addition of sodium methoxide to the solvent with the substance.

Acid hydrolysis released a pink pigment from bands 1 and 13. The absorption peaks of 277 and 535 nm of this pigment showed that it was an anthocyaninidin, probably cyanidin. On the other hand, the substance of band 6 did not release anthocyanidin on hydrolysis, but its spectral properties were changed, and its capacity to be oxidized to a dark color was destroyed.

Species and Varietal Comparisons. Four varieties of *D. alata* and one of *D. rotundata*, the flesh of which showed a tendency to be oxidized, all contained the same main oxidizable compound (Table V) and did not contain other substances oxidized by macerated yam preparations. In all tubers, the oxidizable compound absorbed light at a maximum of 282 nm. The relative amounts of other substances varied remarkably, but no variety contained any new strong band. When chromatograms were compared from a strongly and a weakly oxidizing tuber of the same variety, the main difference was the presence of much more of the oxidizable band in the strongly oxidizable than in the weakly oxidizable tuber. The variety of *D. rotundata* appeared to contain small amounts of some substances common to the *D. alata* tubers, but also a large amount of a new substance in band 10. This compound, although not oxidizable, was found to be very acrid in taste and may account for the acridity of some varieties of this species.

Table V. Substances Found in Extracts from Five Different Yam Tubers; Strength of Band on the Chromatograms Is Rated from 0 (Absent) to 5 (Very Strong)

Band no.	15567	15547	15573	15575[a]		15654
				Low	High	
1	5	0	1	2	0	0
2	2	1	3	1	4	0
3	0	3	2	0	2	2
4	0	4	0	1	1	2
5	1	2	3	1	5	5
6	5	4	2	3	1	0
7	3	0	0	0	2	2
8	4	2	3	3	2	0
9	0	1	0	1	1	0
10	0	0	0	2	0	5
11	5	3	1	1	1	0
12	5	1	0	0	0	0
13	1	0	0	1	0	0

[a] In the first column, the sample was taken from a low oxidizable region of the tuber. In the second column, the sample was taken from a high oxidizable region.

DISCUSSION

In this preliminary study of the polyphenolic substances in yams, the class of substance responsible for oxidation was evident. Only one substance was found that could be readily oxidized to a dark color by crude enzyme preparations from yam tissue. Two other slightly oxidizable substances also occurred. On the basis of spectral properties, the main substance was shown to be a catechin.

That formaldehyde vapor followed by heat treatment caused intense fluorescence of this otherwise uv-absorbing substance suggests that it is a catecholamine. The other two substances that could be oxidized were leucoanthocyanidins, substances closely related to catechins. These were found to precipitate readily as gums and thus may influence the formation of dark gums that often are associated with oxidizable substances.

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Participation of Amadori Rearrangement Products and Carbonyl Compounds in Oxygen-Dependent Browning of Soy Sauce

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The oxygen-dependent browning of Amadori compounds was studied since it was one of the most important factors contributing to the browning of soy sauce during storage. The browning of all Amadori compounds except fructose–arginine was accelerated notably by oxygen and Fe^{2+} (40 ppm), while every mixture of a parent amino compound and sugar exhibited no browning under experimental conditions. Amadori compounds composed of aromatic or heterocyclic amino acids, such as fructose–tyrosine, fructose–phenylalanine, fructose–histidine, and fructose–tryptophan, were especially reactive in oxygen-dependent browning, and this type of browning was synergistically accelerated by the presence of Fe^{2+} and Mn^{2+} (30 ppm). On the other hand, the presence of Mn^{2+} showed an inhibitory effect on the browning of fructose–serine, fructose–glutamic acid, and fructose–leucine. The oxygen-dependent browning of fructose–glycine, fructose–lysine, fructose–β-alanine, and xylulose–glycine was not affected by Mn^{2+}. Oxygen was thought to accelerate the breakdown of Amadori compounds to liberate amino acids and glucosone. When α-hydroxycarbonyls were stored together with glycine, significant browning also occurred. However, in this case, oxygen did not contribute to browning.

Oxygen accelerates the darkening of many foodstuffs; therefore, oxygen-dependent browning has been studied by many workers using materials such as ascorbic acid (Clegg, 1964), polyphenols (Burton et al., 1963), and furfural (Dunlop et al., 1946). Soy sauce also darkens rapidly in contact with atmospheric oxygen (Figure 1). Furthermore, oxygen-dependent browning (oxidative browning; Hashiba, 1975) of soy sauce is developed remarkably in the presence of Fe^{2+} and Mn^{2+} (Hashiba et al., 1970). The oxidative browning of soy sauce is considered to have a different mechanism from those of ascorbic acid, polyphenols, and furfural because the amount of these compounds in soy sauce is very small (Omata et al., 1955). Omata et al. (1955) have reported an important participation of carbonyl compounds in the browning of soy sauce, and Kato et al. (1961) have isolated 3-deoxyglucosone (3-DG) from soy sauce as an important precursor of the browning reaction. However, the effect of oxygen on browning is obscure in these reports. Previously, the author (Hashiba, 1974) has suggested that Amadori compounds play an important role in the browning of soy sauce in the presence of oxygen. In addition, the author (Hashiba, 1975) has reported the oxidative browning of Amadori compounds, fructose–glycine (F–Gly) and fructose–diglycine (F–diGly). Because soy sauce contains many kinds of amino acids and sugars (Hashiba, 1974), many kinds of Amadori compounds must exist in soy sauce, and participate in the oxidative browning. From this point of view, the present paper deals with the examination of the browning of some Amadori compounds and compares the browning with that of carbonyl compounds which were known as highly reactive compounds in browning.

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EXPERIMENTAL SECTION

Synthesis and Isolation Procedure of Amadori Compounds. One-half mole of glucose, 0.1 mol of an amino acid, 10 g of sodium metabisulfite, and 10 ml of water were placed into a 500-ml conical flask and heated in boiling water for 1–5 hr with frequent shaking. When the mixture became yellowish brown, heating was stopped and the mixture was dissolved into about 1 l. of water and sent through a 2.5 × 45 cm column of Amberlite CG-120 (H^+). After the resin was washed with 1 l. of water, the absorbed substances were eluted with 0.1 *N* ammonia using a fraction collector. The amount of the Amadori compound was measured by a test which consisted of reduction of ferricyanide at pH 6.6 at 50°C for 20 min (Adachi's method; Adachi, 1958). The fractions reducing ferricyanide were pooled (about 400 ml) and then half was applied to preparative ion exchange chromatography (Hashiba, 1975). The fractions positive to ninhydrin, phenol–H_2SO_4 tests, and reducing ferricyanide at pH 6.6 were collected as Amadori compounds. Two such preparations obtained