Properties of Polyphenolases Causing Discoloration of Sweet Potatoes during Processing

JETT C. ARTHUR, JR., and T. A. McLEMORE

Southern Regional Research Laboratory, New Orleans 19, La.

During dehydration of sweet potatoes, injury to the plant tissue, by heat or mechanical handling used in processing, results in activation of the polyphenolases and discoloration of the product. In determining the mechanism of this enzymatic discoloration, the presence of monophenolase, catecholase, and cytochrome c oxidase activities in various extracts prepared from the sweet potato was demonstrated by the differential effects of inhibitors, electron donors, and mediators. The in vivo discoloration of the tissue of the sweet potato may be caused, when the balance between the rate of oxidation (and subsequent reduction by ascorbic acid) of naturally occurring chlorogenic acid is affected by accelerating the rate of oxidation or decreasing the available quantity of reduced ascorbic acid during raw storage or processing.

THE IMPORTANCE OF COPPER OXIDASES in plant respiration is not clearly understood. Upon injury of the plant tissue the uptake of oxygen is greatly accelerated, indicating that one of the roles of the copper oxidases may be as protective enzymes to form tanninlike products as protective coats for the injured tissue. The acceleration of the oxidation leads to the reaction inactivation of the oxidases and to the formation of products that discolor the tissues (*2–4, 7, 9–11, 15*).

During the dehydration of sweet potatoes, the injury to the plant tissue, by the heat and the mechanical handling used in processing, results in activation and/or acceleration of the activity of the copper oxidases, mono- and polyphenolases, and leads to the discoloration, greening or browning, of the product. From a practical point of view, the sweet potatoes may be preheated to a point just below the "activation" temperature of the oxidase, and then the temperature of the surface layers of the sweet potato rapidly increased to an "inactivation" temperature of the oxidase during peeling operations. After dicing, the product may be steamed to inactivate the portion of the oxidase found in deeper layers (*14*). The effectiveness of this procedure depends primarily on the condition of the raw material at the time of processing (*1*) and on the rapidity with which the necessary operations can be performed.

The purposes of this investigation were to determine some of the properties of the mono- and polyphenolases which cause the discoloration of sweet potatoes during processing, to evaluate the relative mono- and polyphenolase activities in different extracts, and to indicate the presence of cytochrome c oxidase in the sweet potato.

Methods

The enzymatic activities were followed in a respirometer at 25° C. by the rate of increase in oxygen uptake. The flasks contained: buffer, 0.3 ml. of 0.5*M* phosphate, pH 6.7 or as indicated; electron donor, 3.0 mg. of hydroquinone, 3.0 mg. of ascorbic acid, or 20 mg. of catechol; mediator of the oxidation of the donor, 0.1 mg. of catechol, 0.1 mg. of chlorogenic acid, or 0.3 mg. of cytochrome c; enzyme extract and distilled water to give a total volume of 2.8 ml. A carbon dioxide absorber, filter paper wet with 0.2 ml. of 10% potassium hydroxide, was placed in the center well. After temperature equilibrium had been reached, electron donor and/or mediator of the oxidation of the donor were added from the side arm at zero time (*5, 6*).

Materials

Sweet potatoes of the Unit I Puerto Rico variety were obtained directly from a grower. They were washed and peeled. The peelings and cortex were removed, and 150 grams of these materials were blended with 300 grams of ice and sodium barbiturate equivalent to a concentration of 0.05*M* for 5 minutes. The temperature of the homogenate was maintained at less than 5° C. This homogenate was filtered through cheesecloth and clarified at 500 × gravity for 15 minutes. The pulp was discarded. The supernatant was designated as extract A.

Extract B was made by centrifuging 100 ml. of extract A at 16,000 × gravity for 15 minutes. The supernatant was discarded, and one sixth of the sediment was dispersed in 10 ml. of water by means of a hand homogenizer.

Extract C was made by dispersing two thirds of the sediment from extract B in 80 ml. of 0.01*M* sodium barbiturate and by centrifuging at 16,000 × gravity for 15 minutes. The supernatant was discarded, and the washing operation was repeated. The sediment from the second washing was dispersed in 20 ml. of 0.01*M* sodium barbiturate. These methods are similar to those used by Goddard and Holden (*6*) in preparing extracts of white potato.

The extracts were stored under oil in a refrigerator at about 4° C. Usually fresh extracts were made for each series of tests.

All chemicals were C.P. grade, with the exception of catechol, in which the C.P.

Table I. Evaluation of Extracts from Sweet Potatoes for Oxidase Activity[a]

Oxidase Extract	Catechol	Hydroquinone	Cytochrome c	Time, Minutes 1	3	5	13	15
				Oxygen Uptake, μl.				
A	−	+	−	4	12	22	51	65
	[b]	+	−	15	29	45	97	111
	+	−	−	13	32	47	...	112
B	−	+	−	0	0	0	...	11
	+	−	−	13	32	51	...	152
	[b]	+	−	8	21	38	...	130

Oxidase Extract	Catechol	Hydroquinone	Cytochrome c	Time, Minutes 10	20	30	40
A′[c]	[b]	+	−	94	173	...	...
	−	+	[b]	1	3	3	3
B′[c]	[b]	+	−	60	113	128	142
	−	+	[b]	9	22	30	40
C′[c]	[b]	+	−	25	53	70	81
	−	+	[b]	8	18	29	36
	−	+	−	0	0	0	0

[a] + or −. Presence or absence of chemical in electron donor concentration—i.e., catechol, 20 mg., or hydroquinone, 3 mg.
[b] Presence of chemical as mediator of oxidation of electron donor—i.e., catechol, 0.1 mg., or cytochrome c, 0.3 mg.
[c] A′, B′, C′. Extracts prepared and tested during a different season than A, B extracts.

grade was further purified by crystallizing from five times its weight of toluene. Fresh solutions of the reagents were prepared as required.

Results

An evaluation of the oxidase extracts, prepared from sweet potatoes by a method similar to that used by Goddard and Holden (*6*), is given in Table I. It is observed that crude extract A contained both monophenolase and *o*-dihydroxyphenolase activities. Kertesz has indicated that the monophenolase and *o*-dihydroxyphenolase activities may be due to a single enzyme, with traces of cupric ion and naturally occurring electron mediators participating in the oxidation of the monophenols (*8*). Extract A was active with substrates of hydroquinone, catechol, or hydroquinone containing a trace of catechol. However, when the last two substrates were used, the activity of the oxidase was apparently greater than when hydroquinone alone was used. The addition of cytochrome c as a mediator of the oxidation to the hydroquinone substrate did not increase the oxygen uptake.

Extracts B and C (less water-soluble than A and more tightly bound to the particulate matter) contain practically no monophenolase activity, as demonstrated by the absence of activity with aqueous hydroquinone as a substrate. However, B and C contain *o*-dihydroxyphenolase and cytochrome c oxidase activities, as demonstrated by oxygen uptake with aqueous hydroquinone containing a trace of catechol or cytochrome c. If present, cytochrome c oxidase is almost always associated with the particulate fractions of the cells. When both mediators of the oxidation of hydroquinone were added simultaneously, an almost additive effect was obtained. Webster (*16*) has also reported the presence of cytochrome c oxidase in the sweet potato.

In order to show that residual catecholase activity cannot account for the cytochrome c oxidase activity, the catecholase activity was selectively inhibited by addition of 10 mg. of phenylthiourea, as shown in Table II. Catecholase activity is inhibited practically 100% on the addition of phenylthiourea; the cytochrome c oxidase activity is not significantly affected. The differential effect of 0.05 mg. of potassium cyanide, on the two activities, also shown in Table II, is additional evidence for the presence of two oxidases.

The effect of pH on the activity of sweet potato oxidase contained in extract A is shown in Table III. The initial rate of oxygen uptake (first 5 minutes) is relatively constant over the range of pH 3 to 7.

The effect of concentration of electron donor on the activity of sweet potato oxidase contained in extract B is shown in Table IV. It is observed that the initial rate of oxygen uptake (first 5 minutes) is relatively constant over the range of concentration from 2 to 4 mg. of hydroquinone.

The effect of concentration of mediator of the oxidation of hydroquinone on the activity of sweet potato oxidase contained in extract B is shown in Table IV. It is observed that the initial rate of oxygen uptake (first 5 minutes) is relatively constant for 0.1 and 0.3 mg. of catechol.

In a previous report, it was shown that a considerable quantity of ascorbic acid is present in the raw sweet potato and that the retention of ascorbic acid is dependent, in part, on the conditions of storage of the raw sweet potato. It was further reported, from a practical point of view, that the control of discoloration and of losses of raw materials during processing by preheating was less effective when the raw sweet potatoes were stored at 50° F. than when stored at 60° or 70° F. for various periods of time. The loss of ascorbic acid during raw storage was much greater for products stored at 50° F. than at other temperatures (*1*).

Other workers have reported the presence of chlorogenic acid and caffeic acid, a hydrolysis product of chlorogenic acid (both compounds containing an *o*-dihydroxyphenolic ring), in sweet potatoes (*12*).

Based on information from the literature and on the results presented, it is

Table II. Inhibition of Sweet Potato Oxidases by Addition of Phenylthiourea and Potassium Cyanide to Extract C′[a]

Test	Catechol	Cytochrome c	KCN	Phenylthiourea	Time, Minutes 10	20	30	40	50
					Oxygen Uptake, μl.				
A	[b]	−	−	−	68	87	119	135	151
B	[b]	−	+	−	4	9	17	32	34
C	[b]	−	−	+	0	3	...	...	...
D	−	[b]	−	−	11	24	38	55	64
E	−	[b]	+	−	0	0	0	0	3
F	−	[b]	−	+	7	26	41	51	60

[a] Electron donor, hydroquinone, 3 mg.
+ or −. Presence or absence of chemical.
[b] Presence of mediator of oxidation of hydroquinone, catechol, 0.1 mg., or cytochrome c, 0.3 mg.
Inhibitor concentration, KCN, 0.05 mg., or phenylthiourea, 10 mg.

Table III. Effect of pH on Activity of Sweet Potato Oxidase Contained in Extract A[a]

Time, Minutes	Oxygen Uptake, μl.				
	2	3	4	5	7
5	1	52	58	58	54
10	3	87	105	98	97
15	6	96	115	110	114
20	7	98	117	114	120
25	9	99	122	117	125

[a] Electron donor, hydroquinone, 3 mg.; mediator of oxidation of hydroquinone, catechol, 0.1 mg.

Table IV. Effect of Concentration of Electron Donor or Mediator on Activity of Sweet Potato Oxidase Contained in Extract B

Electron Donor or Mediator, Mg.	Time, Minutes 5	10	15	20	25
	Oxygen Uptake, μl.				
Hydroquinone (donor)	Catechol, 0.1 Mg. (Mediator)				
0	0	...	...	...	...
1	27	56	72	83	92
2	38	73	101	123	139
3	42	82	116	142	173
4	38	81	114	145	172
6	34	75	108	142	179
9	24	63	98	132	167
Catechol (Mediator)	Hydroquinone, 3 Mg. (Donor)				
0	2	0	0	...	...
0.02	12	29	44	78	...
0.05	22	51	79	102	126
0.1	42	82	116	142	173
0.3	46	92	132	160	...

suggested that the in vivo substrate for the mono- and polyphenolases may be ascorbic acid, having chlorogenic acid as a mediator of the oxidation of ascorbic acid. The chlorogenic acid is enzymatically oxidized to its *o*-quinone. This *o*-quinone is chemically reduced by the ascorbic acid to yield chlorogenic acid and dehydroascorbic acid. As long as the initial oxidation of chlorogenic acid is relatively slow and sufficient reduced ascorbic acid is present, discoloration does not occur or occurs very slowly. When this balance is disturbed by either "activation" of the mono- and/or polyphenolases or by decreasing the quantity of reduced ascorbic acid by raw storage losses or processing, the *o*-quinones of the chlorogenic acid or similar type compounds are not reduced but polymerize to yield products that discolor the tissue of the sweet potato. A similar mechanism, involving ascorbic acid, to account for the in vivo discoloration has also been proposed for white potatoes (*13*).

In Table V, the in vitro oxidation of ascorbic acid by the sweet potato oxidase contained in extract B in the presence of a mediator of the oxidation of ascorbic acid of catechol or chlorogenic acid is demonstrated.

Summary

The presence of monophenolase, catecholase, and cytochrome c oxidase activities in various extracts prepared from the sweet potato was demonstrated. It was shown that the more water-soluble fraction of the phenolase contained both monophenolase and catecholase activities and that the fraction of the oxidases more tightly bound to the particulate matter contained catecholase and cytochrome c oxidase activities. The presence of two distinctly different oxidases was demonstrated by the differential effects of inhibitors (phenylthiourea and potassium cyanide) on their activities. The effects of pH and concentrations of electron donor and/or mediator of the oxidation of the donor on the activities were shown.

It is suggested that the in vivo discoloration of the tissue of the sweet potato is caused when the balance between the rate of oxidation (and subsequent reduction by ascorbic acid) of chlorogenic acid is affected by acceleration of the rate of oxidation, or by decrease of the available quantity of reduced ascorbic acid during raw storage or processing, leading to the formation of colored products.

Literature Cited

(1) Arthur, J. C., Jr., McLemore, T. A., Miller, J. C., Jones, L. G., Sistrunk, W. A., J. AGR. FOOD CHEM. **3**, 151–5 (1955).

(2) Dawson, C. R., in "Symposium on Copper Metabolism," W. D. McElroy and B. Glass, eds., pp. 18–47, Johns Hopkins Press, Baltimore, 1950.

(3) Dawson, C. R., Mallette, M. F., *Advances in Protein Chem.* **2**, 179–248 (1945).

(4) Dawson, C. R., Tarpley, W. B., in "The Enzymes," J. B. Sumner and K. Myrback, eds., vol. **II**, Pt. 1, pp. 454–98, Academic Press, New York, 1951.

(5) Eiger, I. Z., Dawson, C. R., *Arch. Biochem.* **21**, 194–209 (1949).

(6) Goddard, D. R., Holden, C., *Ibid.*, **27**, 41–7 (1950).

(7) Joselow, M., "Copper of Ascorbic Acid Oxidase," dissertation, Columbia University, 1949.

(8) Kertesz, D., *Biochem. Biophys. Acta* **9**, 170–9 (1952).

(9) Mallette, M. F., in "Symposium on Copper Metabolism," W. D. McElroy and B. Glass, eds., pp. 48–75, Johns Hopkins Press, Baltimore, 1950.

(10) Nelson, J. M., *Ibid.*, pp. 76–88.

(11) Nelson, J. M., Dawson, C. R., *Advances in Enzymol.* **4**, 99–152 (1944).

(12) Rudkin, G. O., Nelson, J. M., *J. Am. Chem. Soc.* **69**, 1470–5 (1947).

(13) Schwimmer, S., J. AGR. FOOD CHEM. **1**, 1063–9 (1953).

(14) Scott, L. E., Appleman, C. O., Wilson, M., Maryland Agr. Expt. Sta., Tech. Bull. **A33**, 11–26 (1944).

(15) Walter, E. M., Nelson, J. M., "Enzyme Studies in Plant Respiration," dissertation, Columbia University, 1947; Edwards Bros., Inc., Ann Arbor, Mich., 1947.

(16) Webster, G. C., *Am. J. Botany* **39**, 739–45 (1952).

Received for review September 8, 1955. Accepted February 13, 1956.

Table V. Oxidation of Ascorbic Acid in Presence of a Mediator of Oxidation by Sweet Potato Oxidase Contained in Extract B[a]

Ascorbic Acid	Catechol	Chlorogenic Acid	Time, Minutes 2	4	6	8	10	12	14	16	30
			Oxygen Uptake, μl.								
−	+	−	17	31	41	52	55	59	63	63	71
+	−	−	1	0	0	1	1	1	1	2	1
+	[b]	−	2	5	9	14	17	19	23	25	36
−	[b]	−	5	3	8	10	8	8	13	13	15
+	−	[b]	14	22	38	48	57	68	80	90	139
−	−	[b]	2	3	5	7	5	7	7	7	13

[a] + or −. Presence of chemical in electron donor concentration—i.e., catechol, 20 mg., or ascorbic acid, 3 mg.

[b] Presence of chemical mediator for oxidation of electron donor—i.e., catechol or chlorogenic acid, 0.1 mg.