only 1/4 to 1/2 of that observed in regular process peas. To determine the cause of this difference, a lot of No. 3 sieve-size peas was divided into two portions. One portion was processed by the regular method, the other by the Blair process (*2*). Pyrrolidonecarboxylic acid content was found to be 28 mg. in the regular process peas, and 21 mg. in the Blair process peas. Thus, 25% less PCA was present in the lot processed by the Blair method. Evidently this loss resulted from the leaching of substantial amounts of free glutamine from the peas during the soaking and blanching steps (in sodium carbonate and calcium hydroxide solutions, respectively) of the Blair method.

Other factors, such as variety, also may affect the PCA level in peas. Samples of Blair and regular process peas were obtained from the 1958 pack of a processor (Canned Food, Inc., Waupun, Wis.) who used a special variety of peas for the Blair process. The regular pack, All Sweet type, contained 11 mg. while the special variety processed by the Blair method contained 19 mg. of PCA.

With products processed at a high temperature and containing low acid content, the apparent increase in PCA in the brine, as shown in Table II, was due to the gradual diffusion of PCA from the plant tissue. The rate of diffusion differed somewhat depending on the plant product and the storage temperature.

Conclusion

Low acid foods that normally are processed at high temperatures will contain PCA in an amount proportional to the glutamine content of the raw stock, and stoichiometrically related to the amount of glutamine decomposed. The probability of eliminating PCA seems remote, unless cultural practices, raw product storage conditions, and varieties are selected to minimize the free glutamine content in the raw product. High acid foods that normally require processing temperatures below 212° F., and short sterilization times will have only a part of the initial free glutamine converted to PCA during the processing operation. Conversion of the remaining glutamine will occur during storage, but the rate of conversion will be dependent on the storage temperature.

Acknowledgment

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Literature Cited

(1) Archibald, R. M., *Chem. Revs.* **37,** 161 (1945).
(2) Blair, J. S., Ayers, T. B., *Ind. Eng. Chem.* **35,** 85 (1943).
(3) Bulen, W. A., Varner, J. E., Burrell, R. C., *Anal. Chem.* **24,** 187 (1952).
(4) Carangal, A. R., Jr., Alban, E. K., Varner, J. E., Burrell, R. C., *Plant Physiol.* **29,** 355 (1954).
(5) Dame, C., Jr., Marsh, G. L., Chichester, C. O., *Food Research* **24,** 20 (1959).
(6) Foreman, F. W., *Biochem. J.* **8,** 481 (1914).
(7) Hartmann, R. G., Hilling, F., *J. Assoc. Agr. Chemists* **17,** 522 (1934).
(8) Lee, F. A., Shallenberger, R. S., *Food Research* **24,** 68 (1959).
(9) Martin, A. J. P., Synge, R. L. M., *Biochem. J.* **35,** 1358 (1944).
(10) Nelson, E. F., *Am. Med., New Ser.* **23** (II), 812 (1928).
(11) Olcott, H. S., *J. Biol. Chem.* **153,** 71 (1944).
(12) Pucher, G. W., Vickery, H. B., *Ind. Eng. Chem.* **12,** 27 (1940).
(13) Rice, A. C., Pederson, C. S., *Food Research* **19,** 106 (1954).
(14) Shallenberger, R. S., Moyer, J. C., J. AGR. FOOD CHEM. **6,** 604 (1958).
(15) Shallenberger, R. S., Pallesen, H. R., Moyer, J. C., *Food Technol.* **13,** 92 (1959).
(16) Wilson, H., Cannan, R. K., *J. Biol. Chem.* **119,** 309 (1937).
(17) Zamir, A., Lichtenstein, N., *Anal. Chim. Acta* **12,** 577 (1955).

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SWEET POTATO DEHYDRATION

Interactions between Copper Ions and Sweet Potato Polyphenolase Oxidized Substrates

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THE POLYPHENOLASE OF THE SWEET POTATO is often dormant or inactive in the potato tissue, becoming active on injury of the plant tissue or on separation from the plant tissue. During the dehydration of sweet potatoes, the activation of the enzyme leads to the oxidation of natural substrates which discolor the potato tissue, unless certain practical procedures, which can minimize this discoloration, are followed (*1*).

The activation of the enzyme also leads to its reaction inactivation. Probably one of the causes of the phenomenon of reaction inactivation is the decrease in the effective concentration of the enzyme by the removal or binding of its metal prosthetic group, copper (*4*), during oxidation of the substrate. Joselow and Dawson (*7*), using radioactive copper-64 and relatively large quantities of enzyme, investigated the exchange reaction between the copper of ascorbic acid oxidase and ionic copper. They reported a significant amount of exchange when the enzyme was actively catalyzing the oxidation of ascorbic acid in an aerobic system. In a previous communication from this laboratory, using ion exchange methods, radioactive copper-64, and catalytic quantities of tyrosinase, free copper ions formed complexes with the oxidation products of the substrates (*2*).

This report presents experimental data, obtained with sweet potato polyphenolase and radioactive copper, which indicate the effects of reaction conditions on the formation of copper complexes with oxidation products of the substrates and possibly ionic copper exchange with enzymatic copper.

Materials

Sweet potatoes of the Unit I Porto Rico variety were obtained directly from a grower. The peelings and cortex were

The formation of copper complexes with sweet potato polyphenolase and oxidized catechol was determined, using copper-64 and an ion exchange method. An increase in enzyme or cupric ion concentration increased the apparent amount of cupric ion complexed and/or exchanged. An increase in oxidation time—oxidized substrate concentration—also increased the apparent amount of cupric ion interacting with the components of the solution. A change in experimental conditions—the pH of the solution at the time of addition of cation exchanger—affected the results quantitatively; however, the same qualitative results were obtained. These data may indicate a possible way of decreasing the effective enzyme concentration during polyphenolase catalyzed oxidations.

removed and polyphenolase extracts were prepared from them by methods similar to those used by Goddard and Holden (*5*) in preparing extracts from white potatoes and as previously applied by Arthur and McLemore in preparing other samples of sweet potato polyphenolase. Specific data, on the preparation of the polyphenolase extracts evaluated, are given in the results section.

The copper-64 (half life 12.88 hours; β-0.57, 0.65; γ-1.34 m.e.v.) was obtained as copper wire, of initial specific activity 300 mc. per gram. About 0.64 gram of copper was dissolved in 5 ml. of concentrated nitric acid, then 3 ml. of concentrated sulfuric acid were added, and the mixture was heated until sulfur trioxide was evolved. The acidified copper-64 sulfate solution was diluted with copper-free distilled water to yield a stock solution.

The other chemicals used were chemically pure or reagent grade.

Methods

The activity of the polyphenolase was measured manometrically (Warburg apparatus) at 25° C. and pH 6.7 by determining the rate of oxygen uptake. The flasks, of 17- to 18-ml. capacity, contained two side arms and a center well. The enzyme preparation and copper-64 sulfate solutions were put in separate side arms. The center well contained a filter paper wick wetted with 0.2 ml. of 10% potassium hydroxide to absorb the carbon dioxide which might be released. Phosphate buffer (0.3 ml. of 0.5*M*) and substrate solution (optimum amount previously determined) were placed in the main reservoir of the flask, sufficient copper-free distilled water being added to bring the total liquid volume to 3 ml. (*2*).

During the oxidation of different substrates by polyphenolase, copper-64 sulfate solution was added. After a predetermined time, an ion exchange material, phosphorylated cotton fabric (*6*), was introduced to remove the copper ions from the solution. The copper-64 complexed and/or exchanged was determined by drying an aliquot of the solution (which had been extracted with the ion exchange material by shaking the material with the solution for 1 hour, either at pH 6.7 or 2.0 as indicated) and measuring its radioactivity in a gas flow counter. This method has been used by Arthur and McLemore (*2*).

In determining the amount of copper complexed, an alternative procedure was to prepare, in the same proportions, larger volumes of reactants (10 to 20 ml.) in a flask and while shaking to add the copper-64 sulfate solution, ion exchange fabric, or other reagents at the desired time.

The copper contents of the polyphenolase extracts were determined by a spectrophotometric method, involving the reaction of cupric ion with sodium diethyldithiocarbamate (*3*).

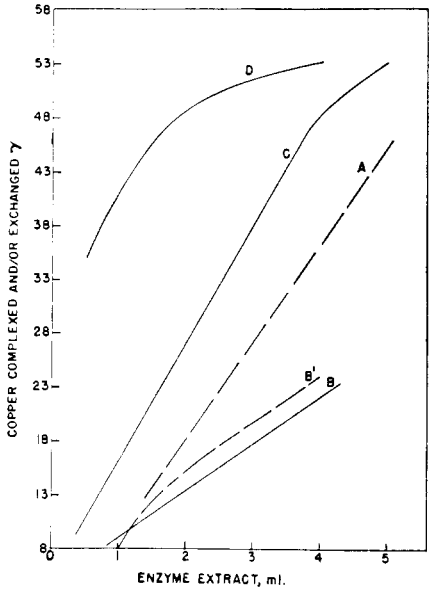

Figure 1. Effect of relative enzyme concentration and of time of addition of cupric ion on the amount of cupric ion complexed and/or exchanged

- A. Copper content of enzyme
- B. Cupric ion added to resting enzyme
- B'. Calculated cupric ion added to resting enzyme, assuming free exchange
- C. Cupric ion added at zero time to oxidizing enzyme-cotechol solution
- D. Cupric ion added at 10 hours to oxidizing enzyme-catechol

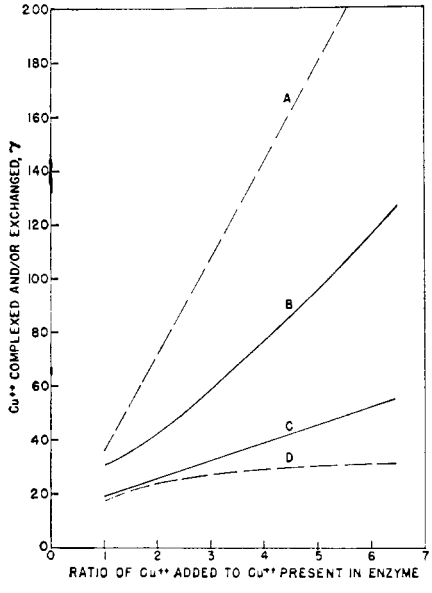

Figure 2. Effect of amount of cupric ion added on the amount of cupric ion complexed and/or exchanged

- A. Cupric ion added to resting enzyme or to oxidizing enzyme-catechol solution
- B. Cupric ion complexed and/or exchanged in oxidizing solution
- C. Cupric ion complexed and/or exchanged in resting enzyme solution
- D. Calculated cupric ion exchanged by resting enzyme, ossuming free exchange

Results and Discussion

The effects of relative enzyme concentration and of time of addition of cupric ion on the amount of cupric ion complexed and/or exchanged are shown in Figure 1. The enzyme extract was prepared by centrifuging the crude juice at 16,000 × gravity for 30 minutes and then dialyzing the centrifugate against distilled water at 4° C. for 18 hours. The copper content of the extract was about 9 γ per ml. About 76 γ of copper-64 were added in each test. The copper-64 was added at the time indicated and was left in contact with the solution for 1 hour. Then at pH 6.7, the ion exchange fabric was inserted and the free cupric ion was removed from the solution. The calculated curve was drawn on the

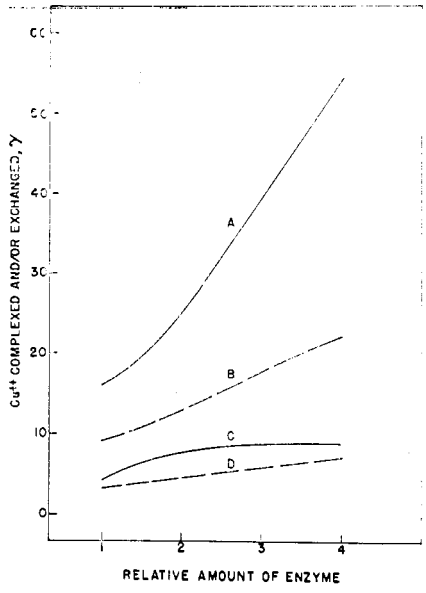

Figure 3. Effect of relative enzyme concentration and of pH during free cupric ion removal on the apparent amount of cupric ion complexed and/or exchanged

- A. Cupric ion added at zero time to oxidizing enzyme-catechol solution
- B. Cupric ion added to resting enzyme, free cupric ion removal at pH 6.7
- C. Same as A
- D. Same as B, except free cupric ion removal at pH 2.0

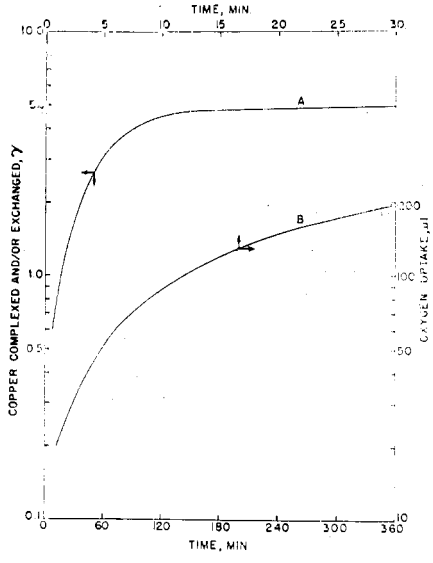

Figure 4. Relationship between time of oxidation and amount of cupric ion complexed and/or exchanged

- A. Cupric ion complexed and/or exchanged
- B. Activity of enzyme extract, free cupric ion removal at pH 2.0

assumption that the ion exchange fabric could not remove the copper prosthetic group of the enzyme. Knowing the quantities of copper present in the enzyme and of tagged copper ions added, the proportionate fraction of the tagged ions possibly exchanging with the enzymatic copper was calculated.

The effects of the amount of cupric ion added on the amount of cupric ion complexed and/or exchanged are shown in Figure 2. In making these tests, the free cupric ion was removed as previously indicated.

Table I. Effect of Relative Amount of Cupric Ion Added[a] and of Substrate on Amount of Cupric Ion Complexed and/or Exchanged

Cu^{++} Added, γ	O_2 Uptake at 1 Min., μl.	Cu^{++} Complexed and/or Exchanged, γ
38[b]	..	19
38[c]	29	31
38[d]	4	19
38[e]	15	20
76[b]	..	31
76[c]	29	60

[a] Cupric ion present in enzyme, 36 γ.
[b] No substrate.
[c] Catechol present in substrate concentration.
[d] Hydroquinone present in substrate concentration.
[e] Hydroquinone, plus trace of catechol, present in substrate concentration.

The effects of the relative enzyme concentration and of pH, during free cupric ion removal, on the apparent amount of cupric ion complexed and/or exchanged are shown in Figure 3. About 76 γ of cupric-64 ion were added in each test.

The relationship between time of oxidation and amount of cupric ion complexed and/or exchanged is shown in Figure 4.

The effects of relative amount of cupric ion added and of substrate on the amount of cupric ion complexed and/or exchanged are shown in Table I. When catechol was used as a substrate, the amount of cupric ion interacting with the components of the oxidizing solution was greater than that interacting when substrates of hydroquinone or hydroquinone plus a trace of catechol were used. An increase in cupric ion added, resulted in an increased interaction.

The determination of the cupric ion complexed and/or exchanged in resting or oxidizing polyphenolase solutions was based primarily on the fact that at equilibrium the total metal species in solution, free metal ions, and complexed metal, was proportional to the metal on the cation exchanger and that under given experimental conditions an increase in the amount of metal remaining in solution was directly proportional to the formation of the copper complexes (*8*).

The data indicate that in resting solutions, cupric ion can complex and/or exchange with the copper of the polyphenolase preparation and that in oxidizing solutions containing a substrate of catechol, an additional amount of cupric ion can be complexed. However, in oxidizing solutions containing a substrate of hydroquinone plus a trace of catechol, no additional cupric ion was complexed in excess of that already complexed and/or exchanged with the resting enzyme.

Similarly, the interaction of carbon monoxide and cyanide with the copper prosthetic group of phenol oxidase, to inhibit its activity, has been shown (*10*). Carbon monoxide can interact with the copper of phenol oxidase by combining with the enzyme and inhibiting its activity. Cyanide can interact with the copper of phenol oxidase by splitting off the metal from the enzyme and thereby inhibiting its activity.

The interactions can probably be expressed in a manner similar to that proposed by Neurath and Schwert (*9*) for the inactivation reaction of enzymes, as follows:

$$\text{Cu-}E + \text{Cu}^{64++} \rightleftharpoons \text{Cu}^{64}\text{-}E + \text{Cu}^{++}$$

$$\text{Cu-}E + S \rightleftharpoons \text{Cu-}E\text{-}S \rightleftharpoons \text{Cu-}E + 2(x + y)P$$

$$\text{Cu-}E + \text{Cu}^{64}\text{-}E + 2xP \rightleftharpoons \text{Cu-}E\text{-}P_x + \text{Cu}^{64}\text{-}E\text{-}P_x$$

$$\text{Cu}^{64++} + \text{Cu}^{++} + 2yP \rightleftharpoons \text{Cu}^{64++}P_y + \text{Cu}^{++}P_y$$

where $\text{Cu-}E + \text{Cu}^{64}\text{-}E$ = total enzyme concentration
S = substrate concentration
$2(x + y)P$ = oxidized substrate concentration

The cation exchange material, when placed in the solution, would tend to complex with the free cupric ion in the solution, there being an equilibrium established with other complexing agents present,—e.g., enzyme, substrate, and oxidized substrate. As is shown, an increase in enzyme, cupric ion, oxidized substrate, or other changes in the experimental conditions would affect the apparent amount of cupric ion complexed and/or exchanged.

Literature Cited

(1) Arthur, J. C., Jr., McLemore, T. A., J. Agr. Food Chem. **5,** 863–7 (1957).
(2) Arthur, J. C., Jr., McLemore, T. A., *J. Am. Chem. Soc.* **78,** 4153–5 (1956).
(3) Assoc. Offic. Agr. Chemists, "Official Methods of Analysis," 8th ed., p. 110, 1955.
(4) Eiger, I. A., Dawson, C. R., *Arch. Biochem.* **21,** 194–209 (1949).
(5) Goddard, D. R., Holden, C., *Ibid.*, **27,** 41–7 (1950).
(6) Guthrie, J. D., *Ind. Eng. Chem.* **44,** 2187–9 (1952).
(7) Joselow, M., Dawson, C. R., *J. Biol. Chem.* **191,** 11–20 (1951).
(8) Martell, A. E., Calvin, M., "Chemistry of the Metal Chelate Compounds," Prentice-Hall, New York, 1952.
(9) Neurath, H., Schwert, G. W., *Chem. Revs.* **46,** 69–153 (1950).
(10) Warburg, O., "Heavy Metal Prosthetic Groups and Enzyme Action," Oxford Univ. Press, London, 1949.

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