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FINAL REPORT

PROJECT No. I-537-82

Prevention of Browning in Fruits and Vegetable Foods

V. Kahn, J.R. Whitaker

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Names of Investigators:

Principal Investigator: Varda Kahn

Cooperating Investigator(s): John R. Whitaker

Name and Address of Affiliated Institutions:

Principal Institution: The Volcani Center, Agricultural Research Organization,

Institute for Technol. & Storage of Agricultural Products, P.O. Box 6, Bet-Dagan 50-250
ISRAEL

Cooperating Institution(s): University of California, Davis, CA 95616, U.S.A.

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Signatures:

Principal Investigator

Varda Kahn

Institution's Authorizing Official

Allen G. Marr, Dean
Graduate Studies & Research

G. Gelsenstein

Cooperating Investigator

John R. Whitaker 6/1/87

Allen Marr

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II. ABSTRACT

The objectives of the research project were to explore for new, more effective and safe means, as well as to improve existing methods, to prevent enzymatic browning in fruits and vegetables via control of polyphenol oxidase activity. In the present research, the investigators have shown that H_2O_2 at low concentrations increases polyphenol oxidase activity by shortening the lag period for hydroxylation of monophenols, but at higher H_2O_2 concentrations inactivates polyphenol oxidase, especially under anaerobic conditions. Tropolone and methimazole are potent inhibitors of mushroom polyphenol oxidase with K_i of 4×10^{-7} M and 4.6×10^{-6} M, respectively, making them potentially valuable inhibitors in preventing browning. Tiron has been shown to be a substrate for both polyphenol oxidase and peroxidase. It could be invaluable in elucidating some of the steps in polyphenol oxidase action since different spectral intermediates (at least three) are observed in the reaction and the polymerized products are soluble, unlike the melanins formed from most substrates.

Detailed studies of the effect of ascorbic acid in the presence of copper ions has demonstrated that there is a specificity for histidine residues. The best evidence indicates that the copper ion functions to bind ascorbic acid, the imidazole side chain of histidine and O_2 . In the presence of O_2 , it appears a free radical, semidehydroascorbic acid, is formed which destroys the histidine residue. This is important since the two copper ions/active site are probably coordinated to histidine residues.

III. REPORT

A. INTRODUCTION

The enzyme polyphenol oxidase is found in most plant and animal tissues, as well as in several fungi. In plants, the enzyme is thought to function by forming a 'scab' over wounds caused by insect bites and other physical damage. On wounding, polyphenol oxidase and the monophenols and *o*-diphenols are brought into contact, together with O₂ to produce melanin. The insoluble melanin seals off the wound, preventing loss of fluids and microbial infestation.

During the harvesting of fruits and vegetables and during storage there is damage to the plant tissue via bruising and abrasion. Intentional disruption of cellular organization by peeling, slicing, pressing, and homogenization provide the ideal conditions for polyphenol oxidase action. Methods for preventing the enzymatic browning have been sought for more than one hundred years. The difficulty of preventing browning, in large part, is because we do not yet understand the complete mechanism of action of the enzyme.

Polyphenol oxidase (E.C. 1.14.18.1; diphenol:O₂ oxidoreductase; also called tyrosinase, phenolase and catecholase) is known to catalyze two types of reactions, where AH₂ is a hydrogen donor:

The *o*-benzoquinone is very reactive and polymerizes with itself or with other phenols to form melanins, the insoluble brown pigments responsible for the undesirable effect of the enzyme. The colored pigments are usually brown but may be yellow, red, blue or green when condensation with other phenols occurs. The browning is undesirable because of the off color formed. But the browning reaction also causes change in flavor and decrease in nutritional quality via condensation of the benzoquinone with the ε-amino group of lysyl residues of proteins causing them to become insoluble, as well as modified.

It may be possible to remove or reduce the levels of polyphenol oxidase in plants by recombinant DNA techniques sometime in the future. Until that time, we must depend upon normal breeding selection for minimizing the concentration of this enzyme and via better understanding of its mechanism of action in order to prevent its action. It is in this latter area that we feel most qualified to contribute toward solving one of the major problems of food production, storage and transportation.

B. OBJECTIVES AND SIGNIFICANCE OF THE PRESENT RESEARCH

The objective of this research project has been to explore for new, effective and safe ways, as well as to improve existing methods, to inhibit polyphenol oxidase activity. We proposed to do this by searching for better synthetic inhibitors of polyphenol oxidase as well as through a better understanding of how some presently used inhibitors, such as ascorbic acid, sodium bisulfite and thiol compounds, function in direct inactivation of the enzyme. We also proposed to study the destruction of the active site via k_{cat} type inhibitors, preferably by finding photosensitive competitive inhibitors of the enzyme.

We have been partly successful in achieving our objectives as will be pointed out below. As summarized in the abstract more potent inhibitors, tropolone and methimazole, have been studied, as well as the direct mechanism of ascorbic acid inactivation of polyphenol oxidase. The three-year period has been an exceptionally productive one for the two laboratories and we believe we have advanced our understanding of what is required for inactivation of the enzyme significantly.

C. RESEARCH REPORT

This report will be presented under the following different sections. Because of the diversity of research and different stages of completeness, it is impossible to attempt to present it as a 'single paper'.

1. Inactivation of Mushroom Tyrosinase by Hydrogen Peroxide.
Andrawis Andrawis and Varda Kahn
Phytochemistry 24, 397-405 (1985)
2. Inhibition of Mushroom Tyrosinase by Tropolone.
Varda Kahn and Andrawis Andrawis
Phytochemistry 24, 905-908 (1985)
3. Tropolone as a Substrate for Horseradish Peroxidase.
Varda Kahn and Andrawis Andrawis
Phytochemistry 24, 909-913 (1985)
4. Tropolone - A Compound That Can Aid in Differentiating Between Tyrosinase and Peroxidase.
Varda Kahn
Phytochemistry 24, 915-920 (1985)
5. Effect of Methimazole on the Activity of Mushroom Tyrosinase.
Andrawis Andrawis and Varda Kahn
Biochem. J. 235, 91-96 (1986)
6. Multiple Effect of Hydroxylamine on Mushroom Tyrosinase.
Varda Kahn and Andrawis Andrawis
Phytochemistry 25, 333-337 (1986)

7. Tiron as a Substrate for Mushroom Tyrosinase.
Varda Kahn and R.W. Miller
Accepted by **Phytochemistry** 1987
8. Interaction of Tiron with Mushroom Tyrosinase, with Horse Radish Peroxidase and with Non-Enzymatic Oxidation Systems.
Varda Kahn
(In preparation)
9. Effect of Tiron on Monohydroxy- and o-Dihydroxyphenolase Activities of Mushroom Tyrosinase.
Varda Kahn
(In preparation)
10. Partial Characterization of the Yellow Product(s) Formed When Tiron is Oxidized by Mushroom Tyrosinase.
Varda Kahn
(In preparation)
11. Tiron as a Substrate for Horseradish Peroxidase (HRP).
Varda Kahn
(In preparation)
12. Non-Enzymatic Oxidation of Tiron to a Yellow Product(s).
Varda Kahn
(In preparation)
13. Relationship Between Structure of Polyphenol Oxidase and Prevention of Browning.
Avi Golan-Goldhirsh and John R. Whitaker
IN: **Nutritional and Toxicological Aspects of Food Safety**, M. Friedman, ed., Plenum Press, New York and London, 1984, pp. 437-456.
14. Modification of Proteins by Polyphenol Oxidase and Peroxidase and Their Products.
Günter Matheis and John R. Whitaker
J. Food Biochem. 8, 137-162 (1984)
15. k_{cat} Inhibition of Mushroom Polyphenol Oxidase.
Avi Golan-Goldhirsh and John R. Whitaker
J. Mol Catalysis 32, 141-147 (1985)
16. Effect of Ascorbic Acid, Sodium Bisulfite and Thiol Compounds on Mushroom Polyphenol Oxidase.
Avi Golan-Goldhirsh and John R. Whitaker
J. Agric. Food Chem. 32, 1003-1009 (1984)

17. Immunoglobulin (IgG) Affinity Chromatography of Mushroom Polyphenol Oxidase.
Avi Golan-Goldhirsh, Varda Kahn and John R. Whitaker
Israel J. Bot. (In press)
18. Effect of Ascorbic Acid and Copper on Proteins and Other Polymers.
Avi Golan-Goldhirsh, David T. Osuga, Andi O. Chen and John R. Whitaker
To be published in American Chemical Society Monograph on Archaeological Chemistry, 1988.

the rate of inactivation; in fact, it appears that CuSO_4 has a small preventive effect. NaHSO_3 rapidly reduces Cu^{2+} to Cu^+ . Since Cu^+ salts are generally insoluble, in contrast to Cu^{2+} salts, we wonder if this may be important in the inactivation. So far, we have lots of questions but few answers about the direct effect of NaHSO_3 .

IV. DESCRIPTION OF COOPERATION

The research proposal was developed collaboratively by the Principal Investigator and the Cooperating Investigator and direct responsibility for the identified research areas planned in advance. The investigators exchanged letters numerous times and manuscripts in the course of the investigation. Dr. Kahn spent November 19 to December 26, 1985 in Dr. Whitaker's laboratory. During that time, they spent many hours together discussing research manuscripts and research yet to be done on the project, sharing ideas and hypotheses. The two investigators have worked together in preparing the final report.

Dr. Kahn also became acquainted with Dr. Whitaker's laboratory associates, with both learning new techniques from each other.

V. BENEFIT TO AGRICULTURE

Polyphenol oxidase is responsible for up to 50% loss of fresh fruits in many developing countries and a substantial loss in the developed countries. It is also important in the juice and wine industries, fresh salad bars of restaurants, etc. Minimization of polyphenol oxidase activity is via breeding programs where other desirable characteristics may be compromised, spraying of fruits on the trees, control of climatic and soil conditions, harvesting green, minimization of bruising during harvesting, controlled atmosphere storage, through use of chemical inhibitors such as ascorbic acid, NaHSO_3 , and citric acid, and by heat treatment.

Ascorbic acid, NaHSO_3 , and citric acid are not totally satisfactory inhibitors. Prevention of browning is transitory and when these compounds are destroyed via polyphenol oxidase action, the food materials brown. Recently, NaHSO_3 usage has been curtailed for use in fresh salads in restaurants because persons with respiratory problems are particularly susceptible to SO_2 . In addition, NaHSO_3 affects the taste of some foods, including wines.

In this research, the investigators have shown there are some better inhibitors, including the possible k_{cat} -type inhibition where the enzyme literally inactivates itself via the catalytic mechanism. The researchers have shown that ascorbic acid, previously thought to prevent browning primarily by its reducing ability, can actually inactivate polyphenol oxidase directly, probably by a free radical intermediate.

Tropolone has been shown to be a potent inhibitor for polyphenol oxidase and a substrate for peroxidase; therefore, peroxidase can be easily determined

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in the presence of polyphenol oxidase using tropolone.

The investigators' increased knowledge of polyphenol oxidase has been used by the food industries in their respective countries, saving several millions of dollars in losses normally due to polyphenol oxidase activity.

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