5

13.11

BARD

United States - Israel Binational Agricultural Research and Development Fund

FINAL REPORT

PROJECT No. I-223-80

Prevention of Browning in Fruits and Vegetable Foods

V. Kahn, J.R. Whitaker

1984

630.72
BAR
2nd copy

2033 2

Standard BARD Cover Page for Scientific Reports

Date January 30, 1984

BARD
P.O. Box 6
Bet Dagan, ISRAEL

BARD Project No. I-223-80

Title of Research Project: "Prevention of Browning in Fruits and Vegetable Foods"

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,
Inst. for Tech. & Storage of Agricultural Products, P.O.B. 6, Bet-Dagen (20-500)
ISRAEL

Cooperating Institution(s): University of California, Davis, CA 95616

Project's starting date: December 1, 1980

Type of Report: 1st Annual _____ 2nd Annual _____ Final X

Signatures:

Principal Investigator

Varda Kahn

Institution's Authorizing Official

Yuval Eshkol

Cooperating Investigator

John R. Whitaker

1) Food - Preservation.

630.72 : 664.8
BAR

2nd copy

TABLE OF CONTENTS

I.	Standard BARD Cover Page for Scientific Reports	i
II.	Abstract	1
III.	Report	2
	A. Introduction	2
	B. Objectives and Significance of the Present Research	3
	C. Organization of Report	3
	D. Multiple Effects of Hydrogen Peroxide on the Activity of Avocado Polyphenol Oxidase	5
	E. Evidence Against Superoxide Involvement in Tyrosine Hydroxylation by Mushroom Tyrosinase	10
	F. Avocado Mesocarp; Browning Potential, Carotenoid Content, Polyphenol Oxidase, Catalase and Peroxidase Activities: Comparison Between Six Avocado Cultivars	19
	G. Inhibition of Mushroom Tyrosinase Activity by Methimazole	22
	H. Effect of Proteins, Protein Hydrolyzates and Amino Acids on the α -Dihydroxyphenolase Activity of Mushroom Tyrosinase	24
	I. Inhibitor of Tyrosinase in Extracts from <u>Ceratocystis</u> <u>fibriata</u>	26
	J. Relationship Between Structure of Polyphenol Oxidase and Prevention of Browning	29
	K. Immunoglobulin (IgG) Affinity Chromatography of Mushroom Polyphenol Oxidase	32
	L. Effect of Ascorbic Acid, Sodium Bisulfite and Thiol Compounds on Mushroom Polyphenol Oxidase	36
	M. Inactivation of Mushroom Polyphenol Oxidase by Specific Modification of Histidine by Ascorbic Acid and Cupric Ions	40
	N. Modification of Histidine in Proteins by Treatment with Ascorbic Acid in the Presence of Cupric Ions	44
	O. k_{cat} Inactivation of Mushroom Polyphenol Oxidase	47
IV.	Description of Cooperation	50
V.	Benefit to Agriculture	51
VI.	List of Publications	52

1970
 1971
 1972

II. ABSTRACT

Added H_2O_2 increases the rate of tyrosine hydroxylation and slightly increases the rate of DOPA oxidation at low concentrations. At higher concentrations, H_2O_2 decreases both activities. The increase in rate of hydroxylation of tyrosine in the presence of H_2O_2 does not appear to involve superoxide ions. Rather, the H_2O_2 appears to facilitate the oxidation serving as a partial replacement for O_2 or as a reductant. Carotenoids naturally present in fruits do not appear to protect against PPO activity. Methimazole and some amino acids (less effective) are inhibitors of PPO. Ceratocystis fimbriata, a fungus, contains low molecular peptide inhibitors of PPO. The structures are being studied.

Ascorbic acid, sodium bisulfite and thiols have a direct effect on PPO, causing quite a rapid loss of activity in the absence of substrates. Ascorbic acid is apparently not the effective agent; it is either dehydroascorbic acid or semidehydroascorbic acid radical. Incubation of ascorbic acid alone or with Cu^{2+} markedly increases its effectiveness in inactivating PPO. Ascorbic acid- Cu^{2+} treatment of PPO leads to loss of 3 of the 4 histidine residues per PPO subunit, without fragmenting the peptide chain.

The reaction inactivation of PPO by substrates has been examined in detail. Reaction inactivation occurs at least as rapidly in the presence of ascorbic acid as in its absence. In the presence of ascorbic acid ≤ 0.04 moles of ^{14}C -labelled product is covalently attached to PPO while in the absence of ascorbic acid 85 moles of ^{14}C -labelled product is covalently bound, presumably by non specific interaction with the protein. These data, and others, including effect of ascorbic acid alone, indicate that inactivation of PPO occurs by a free radical mechanism at the active site of the enzyme, causing the loss of histidine and probably copper.

III. REPORT

A. INTRODUCTION

Browning reactions in plant tissues can be divided into two categories: (a) "functional browning", which is associated with the normal life cycle of plants (formation of seed coats, cuticles of ripe fruits, and spores and hyphae of edible fungi) and (b) "adventitious browning" associated with rapid changes in the color of the tissue as a result of mechanical or physiological injury. The present research proposal was concerned with the latter.

Browning in raw fruits and vegetables is caused mainly by the enzymatic oxidation of phenols to α -quinones and their subsequent non-enzymatic polymerization to brown pigments and reaction with other phenols, proteins and other amines to give variously colored products. The non-enzymatic browning reaction, the Maillard reaction, involving the reaction of amino groups (primarily of lysine of the proteins) with reducing sugars (for example, glucose) is of secondary importance in most raw fruits and vegetables. The enzymes initiating the sequence of reactions associated with enzymatic browning are known as polyphenol oxidases (E.C. 1.14.18.1; diphenol: O_2 oxidoreductase; polyphenol oxidase (PPO)) as well as by the terms tyrosinases, phenolases and catecholases.

PPO catalyzes two distinct reactions, in which molecular oxygen is the hydrogen acceptor and phenols act as hydrogen donors:

where AH_2 represents a reducing agent. These two reactions are catalyzed by the monophenolase (cresolase, tyrosinase) and the dihydroxyphenolase (catecholase) activities of the PPO complex, respectively. The α -quinones produced by PPO subsequently undergo nonenzymatic oxidative polymerization to yield dark-brown pigments.

PPO plays a beneficial role in some food processes (tea and cocoa fermentation, coffee, beer) where brown colors are traditionally desired by the consumer. On the other hand, in the case of fresh and processed frozen and dehydrated vegetables and fruits, PPO activity causes unacceptable discolored food appearance often accompanied by unacceptable off-flavors.

The browning of fruits and vegetables (prior to or after processing) presents a serious problem in the food industry not only because it causes undesirable changes in color and flavor but also because it leads to reduction of nutritional quality of raw materials as well as to considerable economic losses to the producer. An important investigative problem of food scientists is the conditions affecting this reaction in order to understand the mode of action of PPO so as to find effective ways to eliminate its undesirable effects.

The enzymatic browning reaction catalyzed by polyphenol oxidase is a major nutritional, economic and sensory factor in the provision of cheap, wholesome and plentiful fruits and vegetables throughout the year. Major research interest of long duration has been devoted to the question of the prevention of enzymatic browning. Such research has primarily resulted in the selection of

certain varieties and changes in handling practices to minimize the browning reaction. These methods are limited in prevention of browning and may result in selection of varieties which are less productive and less nutritional.

We believe it important to move rapidly ahead, using some of the advances of modern biochemistry and food technology, to control this reaction so as to maximize the production and storage of a plentiful, nutritious food supply. This we have attempted to do in this research project.

B. OBJECTIVES AND SIGNIFICANCE OF THE PRESENT RESEARCH

The objectives of this research were to investigate several potentially important methods for the control and prevention of enzymatic browning in plant tissues important for food use. These methods in and of themselves must not produce toxic effects or off-flavors in the treated food and must be appropriate for use in the sequence between harvesting and heat processing in order to minimize the nutritional, economic and sensory losses associated with undesirable browning reactions.

Our research included investigation of the prevention of browning by:

- a. Use of naturally-occurring proteinaceous and non-proteinaceous inhibitors of polyphenol oxidase extracted from unripe fruit and from selected fungi.
- b. Use of antioxidants and synergists to "trap" or remove the benzoquinone from the reaction as formed by polyphenol oxidase.
- c. Use of naturally-occurring non substrate phenols or other compounds to inhibit polyphenol oxidase or to "trap" the benzoquinone formed so that it cannot polymerize to the brown pigment, melanin.
- d. Use of superoxide dismutase to decompose the superoxide radical anion thought to be formed in polyphenol oxidation reactions and the use of carotene and similar pigments to quench singlet oxygen formation by the superoxide dismutase.

C. ORGANIZATION OF REPORT

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

- D. Multiple Effects of Hydrogen Peroxide on the Activity of Avocado Polyphenol Oxidase
Varda Kahn
Phytochemistry 22, 2155-2159 (1983)
- E. Evidence Against Superoxide Involvement in Tyrosine Hydroxylation by Mushroom Tyrosinase
Varda Kahn, Avi Golan-Goldhirsh and John R. Whitaker
Phytochemistry 22, 1883-1893 (1983)
- F. Avocado Mesocarp; Browning Potential, Carotenoid Content, Polyphenol Oxidase, Catalase and Peroxidase Activities: Comparison Between Six Avocado Cultivars
Osnat Sharon-Raber and Varda Kahn

- J. Food Sci.
In Press
- G. Inhibition of Mushroom Tyrosinase Activity by Methimazole
Varda Kahn
Unpublished
- H. Effect of Proteins, Protein Hydrolyzates and Amino Acids on the
 α -Dihydroxyphenolase Activity of Mushroom Tyrosinase
Varda Kahn
Unpublished
- I. Inhibitor of Tyrosinase in Extracts from Ceratocystis fimbriata
Avi Golan-Goldhirsh, John R. Whitaker and James E. De Vay
Unpublished
- J. Relationship Between Structure of Polyphenol Oxidase and Prevention of
Browning
Avi Golan-Goldhirsh, John R. Whitaker and Varda Kahn
In Press
- K. Immunoglobulin (IgG) Affinity Chromatography of Mushroom Polyphenol
Oxidase
Avi Golan-Goldhirsh, Varda Kahn and John R. Whitaker
Unpublished
- L. Effect of Ascorbic Acid, Sodium Bisulfite and Thiol Compounds on Mushroom
Polyphenol Oxidase
Avi Golan-Goldhirsh and John R. Whitaker
Submitted
- M. Inactivation of Mushroom Polyphenol Oxidase by Specific Modification of
Histidine by Ascorbic Acid and Cupric Ions
Avi Golan-Goldhirsh and John R. Whitaker
Unpublished
- N. Modification of Histidine in Proteins by Treatment with Ascorbic Acid
in the Presence of Cupric Ions
Avi Golan-Goldhirsh and John R. Whitaker
Unpublished
- O. k_{cat} Inactivation of Mushroom Polyphenol Oxidase
Avi Golan-Goldhirsh and John R. Whitaker
Unpublished

IV. DESCRIPTION OF COOPERATION

The research proposal was developed by both principal investigators in its entirety and direct responsibility for each of the research areas was agreed upon in the proposal. The principal investigators have exchanged frequent letters and both have read all manuscripts submitted for publication. They have shared materials when appropriate, as for example the antibodies developed against mushroom polyphenol oxidase produced in Davis and tested against avocado PPO at the Volcani Center.

Dr. Whitaker spent 10 days in Israel in August 1981 working with Dr. Varda Kahn and her associates on manuscripts and experimental ideas. Dr. Kahn spent one week at Davis in September 1982 where she collaborated with Dr. Whitaker and Dr. Golan-Goldhirsh on manuscripts and research ideas. In December 1983 Mr. Andreus Andreus from Dr. Kahn's laboratory visited Dr. Whitaker and Dr. Golan-Goldhirsh at UC Davis for discussions on the effect of H_2O_2 on PPO, and other aspects of PPO.

Dr. Avi Golan-Goldhirsh, who received his M.S. degree with Dr. Kahn, came to Davis to work on this project in 1981, after receiving his Ph.D. degree at the University of Alberta in 1979 and then spending two years as a postdoctoral fellow at Purdue University. Dr. Golan-Goldhirsh's expertise on avocados, his hard and persistent efforts and his personal knowledge of Dr. Kahn and the organization of the Volcani Center helped immensely in the smooth operation of this project and the interchange of ideas and information.

V. BENEFIT TO AGRICULTURE

Polyphenol oxidase is responsible for major losses in fresh fruits. Some estimates put the loss at close to 50%. Whatever the correct percentage, which will vary from country to country and fruit to fruit, the browning caused by polyphenol oxidase causes economic and nutritional losses, requires special harvesting and storage conditions to prevent losses and is responsible for considerable investment in genetic research to select varieties of fruits with less browning problems, even though other quality factors may have to be downplayed.

The most successful control of browning in fruits and vegetables is by adding sodium bisulfite (as SO_2 , metabisulfite, bisulfite). Use of this reagent is limited because of detectable taste by many persons and because asthmatic persons are extremely sensitive to it. It is likely that the regulatory agencies will either ban the use of sodium bisulfite or severely restrict its use in the near future.

For the reasons listed above, it is imperative that the mechanism of action of polyphenol oxidase be better understood in order that better control methods can be developed. We hope that the present work will contribute toward meeting these objectives.

In both countries some of these ideas are being tried by the avocado growers and processors who have an especially difficult time because of PPO. The growers and processors have benefitted already. For example, the research director of the Calavo Growers of California indicated to us that ideas developed on this project and a small grant-in-aid from Calavo Growers have already saved them more than one million dollars.

VI. LIST OF PUBLICATIONS

Kahn, V. 1983. Multiple effects of hydrogen peroxide on the activity avocado polyphenol oxidase. *Phytochemistry* 22, 2155-2159.

Kahn, V., Golan-Goldhirsh, A. and Whitaker, J.R. 1983. Evidence against superoxide involvement in tyrosine hydroxylation by mushroom tyrosinase. *Phytochemistry* 22, 1883-1893.

Sharon-Raber, O. and Kahn, V. 1983. Avocado mesocarp; browning potential, carotenoid content, polyphenol oxidase, catalase and peroxidase activities: comparison between six avocado cultivars. *J. Food Sci.* 48, 1874.

Golan-Goldhirsh, A., Whitaker, J.R. and Kahn, V. 1984. Relationship between structure of polyphenol oxidase and prevention of browning. In Press