BROWNING OF PARSNIPS

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
MIKLOS S. KALDY
1969

ABSTRACT

BROWNING OF PARSNIPS

by Miklos S. Kaldy

Parsnips, Pastinaca sativa L., develop a browning coloration upon storage. The active principle for this browning was extracted from parsnips with water. 3, 4-dihydroxyphenylalanine (DOPA), catechol and chlorogenic acid were found to form colored products upon incubation with the extract. The optimum pH for the browning reaction with DOPA as substrate was 8.5 The Michaelis constant for the reaction with catechol was 1.7×10^{-2}M. The active principle of the water extract was heat resistant as it required 120 minutes at 100°C for complete inactivation. Gel electrophoresis indicated that the browning agent of the extract is a protein.

Keeping the parsnips in a 0.48% $NaHSO_3$ solution prevented the browning. Temperatures near 0°C delayed the browning considerably.

BROWNING OF PARSNIPS

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science

1969

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. Pericles Markakis for his guidance throughout this study and for his aid in preparing this manuscript. He is also indebted to Dr. David R. Dilley and Dr. Walter M. Urbain for their advice and help in preparation of this manuscript.

The author feels deeply grateful to the Canada Department of Agriculture, Research Branch, for the educational leave and financial assistance granted to him during his studies at this university.

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INTRODUCTION

Parsnips, the fusiform root of the biennial herb, Pastinaca sativa, L, was Europe's main vegetable before the introduction of potatoes. It came to the New World with the European settlers; and although today parsnips are not a staple for most people, it is a vegetable well-liked for its spicy flavor and characteristic aroma.

Parsnips are white when they are harvested but they turn light brown soon after. The brownish color became accepted by the farmers and home gardeners. The sophisticated supermarket buyer, however, appears to prefer the light colored parsnips. Such selective consumer acceptance naturally penalizes growers who cannot supply parsnips with the right color.

Mechanical or physical injury incurred during harvesting, cleaning and packaging of certain fruits and vegetables may produce changes in their color. But parsnips need experience no injury to undergo a color change. Once harvested and exposed to air, the white color of the parsnips turns brown within a few hours. While immediate cooling after harvest slows the process down, parsnips harvested in warm weather may begin browning even while the roots are still in the soil.

Many factors may influence the degree of browning, such as soil type, parsnip variety and storage conditions. But there must be one primary factor that initiates the browning. The objective of this research was to determine the nature of this browning, study the conditions under which the discoloration develops and explore ways of preventing it.

REVIEW OF THE LITERATURE

It is a common observation that many fruits and vegetables develop a brown coloration after harvesting or when cut or bruised. In most instances the browning of fresh fruits and vegetables is undesirable.

The literature distinguishes enzymatic from non-enzymatic browning reactions. While the latter fall into four broad classes: (1) the reaction of aldehydes and ketones, such as the reducing sugars, with amino compounds; (2) caramelization of polyhydroxycarbonyl compounds, such as sugars; (3) oxidation of ascorbic acid; and (4) reactions of metals with tannis (Meyer, 1960; Stadtman, 1948), enzymatic browning is an oxidative reaction requiring the presence of an enzyme, a substrate and oxygen. Lacking any one of these components, the mechanism is incomplete and the reaction will not proceed (Ponting, 1960). Cantaloupe and tomato do not discolor, for instance, because they lack both enzyme and substrate in any significant amounts (Ponting, 1960). The enzyme involved in this reaction is known as tyrosinase, catecholase, phenolase, polyphenoloxidase, cresolase and phenoloxidase. The name adapted by the International Union of Biochemistry is o-diphenol: O_2 oxidoreductase (E.c.1.10.3.1) (Dixon and

Webb, 1964). The phenolic compounds most commonly oxidized by this enzyme include catechol, 3,4-dihydroxyphenylalanine (DOPA), tyrosine, caffeic acid, and chlorogenic acid. Phenoloxidase purified from mushrooms (Mason, 1956; Kertesz and Zito, 1957) was found to have one atom of copper per molecule of enzyme. Removing the copper inactivated the enzyme; the inactivated enzyme may regain its activity when exposed to copper ions (Reed, 1966).

The mode of phenoloxidase activity has been studied extensively (Dawson and Magee, 1955; Dressler and Dawson, 1960a; 1960b), and multiple forms of plant phenoloxidase identified (Constantinides, 1966). Researchers have particularly sought to inhibit or prevent the browning reaction catalyzed by phenoloxidase (Guadagni et al., 1949; Reyes, 1960; Scott et al., 1960; Goodman and Markakis, 1965; Joslyn and Braverman, 1954; Ponting, 1960), by eliminating the substrate or oxygen, or by inactivating the enzyme. While the enzyme can be inactivated by heat, of course, the most commonly used inhibitor is sulfite in either the SO_2 or $NaHSO_3$ form. Embs and Markakis (1965) studied the mechanism of inhibition of the phenoloxidase browning reaction. Ascorbic acid also prevents the completion of the browning reaction, by reducing the o-quinone before it polymerizes (Hope, 1961; Meyer, 1964). And sodium chloride has reportedly inhibited enzymatic browning (Reed 1966).

Chubey studied the oxidative browning of carrot and found that storage duration increased the susceptibility to browning but browning was not affected by storage temperature.

Existing investigations on parsnips pertain to cultural and storage practices (Thompson and Kelly, 1957; Bleasdale and Thompson, 1966; U.S.D.A., 1968); apparently no one has yet studied the browning in parsnips.

MATERIALS AND METHODS

Parsnips

Parsnips of the Harris Model variety were obtained from L. Campbell and Sons Company, Almont, Michigan, in the fall of 1968 at harvest time. The roots were washed, placed in plastic containers containing water and transported to the laboratory in East Lansing where they were stored under different conditions. (See Storage Studies.)

Preparation of Extracts

In order to test the possible enzymatic nature of the browning of parsnips, tissue extracts with three different solvents were prepared. Parsnips stored at 1°C for 3 to 4 months in polyethylene bags cut into cubes, approximately 3/8 of an inch, and disintegrated in a Waring blender for one minute at high speed, with an equal weight of demineralized water, or 0.1 M phosphate buffer pH 6.5, or 1% Polyclar L (soluble polyvinylpyrrolidone supplied by General Aniline and Film Corp.).

Two additional extracts were also prepared. In one of them 20 g of peel were disintegrated with 100 ml of demineralized water and in the other 20 g of tissue without peel was disintegrated with 100 ml of demineralized water.

The slurry was centrifuged at 39,000 x g for 20 minutes in a Sorvall Superspeed RC-2 refrigerated centrifuge. The supernatant solution was first filtered through a double layer of No. 42 Whatman filter paper, and then through 0.45 μ and 0.30 μ millipore filters. All filtrations were done at 4°C. To each 100 ml of freshly prepared extract 0.1 ml of a thymol solution (1% thymol in toluene) were added to prevent microbial growth.

Spectrophotometric Studies

Spectrophotometric measurements were made with a Bausch and Lomb Spectronic 505 and a Beckman DU spectrophotometer at room temperature. The following reaction mixture was prepared for spectrophotometric measurements: 1 ml 0.5 M phosphate buffer, of the desired pH (6.5-9.0), 1 ml substrate, 1 ml clear parsnip extract, 0.1 ml 1% ethylenediaminetetraacetate (EDTA) and 0.1 ml of 0.5% gelatin (purified pigskin). Four substrate solutions were used, each at 0.33×10^{-2}M final concentration: catechol, chlorogenic acid, 3,4-dihydroxyphenylalanine (DOPA) and L-tyrosine. For the heat inactivation tests the extract was placed in boiling water for various periods of time. In the spectrophotometric tests the blank contained the same solutions with the samples, but no enzyme; the volume was made up with water. Readings of the absorbance were made at intervals of time up to 10 hours depending on the velocity of the reaction.

Gel Electropheresis Study

Disc electrophoresis (Davis, 1964; Ornstein, 1964; Constantinides, 1966), using polyacrylamide gel, was employed to separate the proteins present in the parsnip extract. Stock solutions were prepared as follows:*

A. To 1N 48 ml HCl, add 36.3 g tris (hydroxymethyl) aminomethane (TRIS), 0.23 ml N, N, N', N'-tetramethylethylenediamine (TEMED), and H_2O to make 100 ml (pH 8.8-9.0).

B. To 60.0 g acrylamide and 0.4 g N, N'-methylenebisacrylamide (BIS) add H_2O to make 135 ml.

C. To 0.14 g ammonium persulfate add H_2O to make 100 ml of catalyst solution.

D. To 6.0 g TRIS and 28.8 g glycine, add H_2O to make 1 liter (pH 8.3). This buffer solution must then be diluted 1/10 before use.

E. To 1 g aniline blue black add 7% acetic acid to make 200 ml of protein stain.

F. 7% glacial acetic acid was prepared for destaining.

The working solution was made from 1.0 part A, 1.4 parts B, and 2.1 parts H_2O. To form the gel, the working solution is combined with the catalyst (C) in 1:1 ratio. Gel tubes, 3 x 1/4 in. I.D., were cleaned in acid solution,

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*All reagents used were from Eastman Chemical Co., Rochester 3, New York.

rinsed in a Kodak Photo-Flo solution (diluted 200:1), dried and filled with gel solution to a height of 15/8 in. from the bottom. One drop of water was placed on top of the gel solution to ensure a flat surface on the gel as it solidified. When the gel had set, the drop of water was removed and one inch of buffer solution (D) was placed on top of the gel.

The parsnip extract was diluted with 3 volumes of water. Then sucrose was added (2% of the diluted extract) to increase the specific gravity so as to prevent diffusion into the buffer above the gel, and 0.3 ml of this diluted extract was injected just above the top of the gel with a syringe. In the test for substrate specificity, catechol, chlorogenic acid, DOPA and tyrosine in 2×10^{-3}M concentration was used instead of the staining solution. In this process the gels were immersed in the substrate and left there until bands developed. To approximately 6 ml of substrate 0.1 ml of ethyl alcohol (95%) were added to facilitate the development of the band (Constantinides, 1966). The gels were then washed in water and stored in 30% ethyl alcohol.

Electrophoresis was carried out at 4°C. The current for the protein separation was obtained from a Heathkit Variable Voltage Regulated Power Supply Model PS-3.*

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*Manufactured by the Heath Co., Benton Harbor, Michigan.

The current was maintained at 4 milliamperes per tube, with a total running time of 2 hours.

Storage Studies

In an exploratory experiment regarding the effect of storage conditions on the condition of parsnips the following samples were prepared. Groups of 3 roots were immersed in 0.0%, 0.02%, 0.08%, 0.16%, 0.48%, and 0.81% $NaHSO_3$ solutions for 2, 5, and 10 minutes. After the soaking the roots were dried with absorbant paper and stored in punctured polyethylene bags at 1°C and 4°C, R.H. 85%.

Groups of 3 parsnips were also stored at 1°C and 4°C in jars filled with bisulfite solutions of the same concentrations with those used in the soaking tests. Parsnips were also stored at 1°C in polyethylene bags from which the air had been evacuated by a mechanical pump.

A number of parsnips were stored in perforated polyethylene bags at 1° and 4°C. The observation on storage lasted for a total period of 6 months.

RESULTS AND DISCUSSION

A. Spectrophotometric Studies

Determination of Maximum Absorption

When a water extract of a whole parsnip was incubated with solutions of three different phenolic substrates and the absorption spectrum of the reaction mixture was taken in the 505 Bausch and Lomb spectrophotometer, the following absorption maxima were observed:

catechol	500 mμ
3,4-dihydroxyphenylalanine (DOPA)	420 mμ
chlorogenic acid	420 mμ

Comparison of Substrates

Using the maximum absorption wavelength values, each substrate was tested at pH 6.5 with enzyme extract for activity on a Beckman DU spectrophotometer (Fig. 1). The activities expressed in absorbance units per hour (ΔA/hr) are as follows:

Substrate	Concentration	Activity (ΔA/hr)
dopa	$.33 \times 10^{-2}$M	.114
catechol	$.33 \times 10^{-2}$M	.072
chlorogenic acid	$.33 \times 10^{-2}$M	.052
tyrosine (at 420 mμ)	$.33 \times 10^{-2}$M	.000

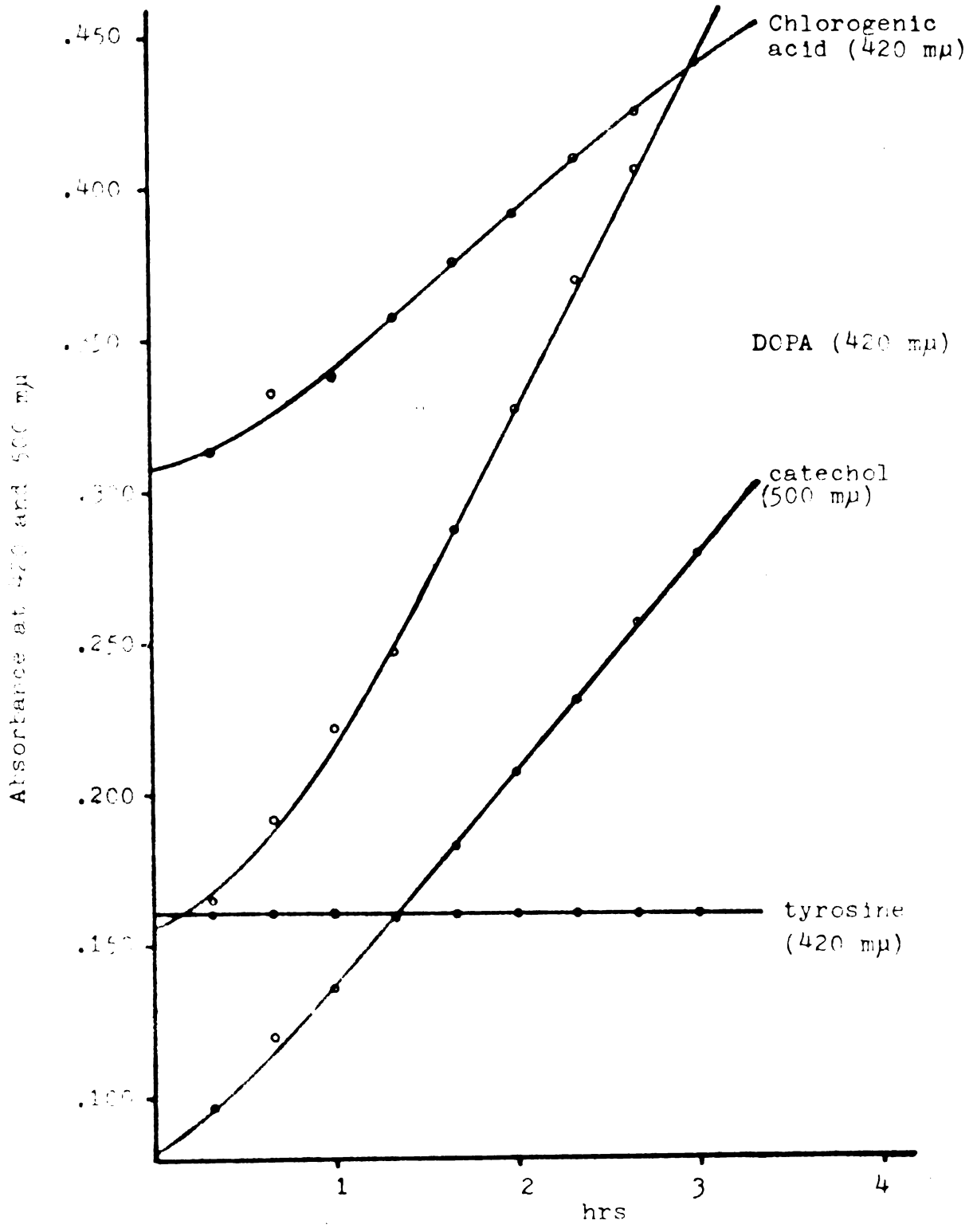


Fig. 1 Absorbance changes of phenolic compounds (1.33×10^{-2} M) by a water extract of parsnip.

Comparison of Extracting Liquids

Enzyme samples extracted with water, 1% Polyclar L, and 0.1 M phosphate buffer pH 6.5 were tested for phenoloxidase activity using DOPA as a substrate (Fig. 2). Activities of .038 ΔA_{420}/hr. with water, .037 ΔA_{420}/hr. with 1% Polyclar L, and .033 ΔA_{420}/hr. with the 0.1 M buffer were obtained. Since the water extract had the highest enzymic activity, water was used for extraction in subsequent experiments.

Distribution of Enzyme in the Parsnips

When the water extracts from the whole parsnip, the peel and the peeled part of it were compared for enzymic activity in terms of absorbance (ΔA_{420}) changes in a DOPA solution (0.33×10^{-2}M) the following activity were obtained (Fig. 3):

Peel:	0.490 ΔA_{420}/hr/g
Peeled part:	0.170 ΔA_{420}/hr/g
Whole:	0.180 ΔA_{420}/hr/g

Enzyme Stability at Room Temperature

Enzyme extracts stored in a cold room (4°C) or at room temperature (22°C) for 24 hours showed no difference in activity (Fig. 4). In both cases the relative enzyme activity was .082 ΔA_{420}/hr.

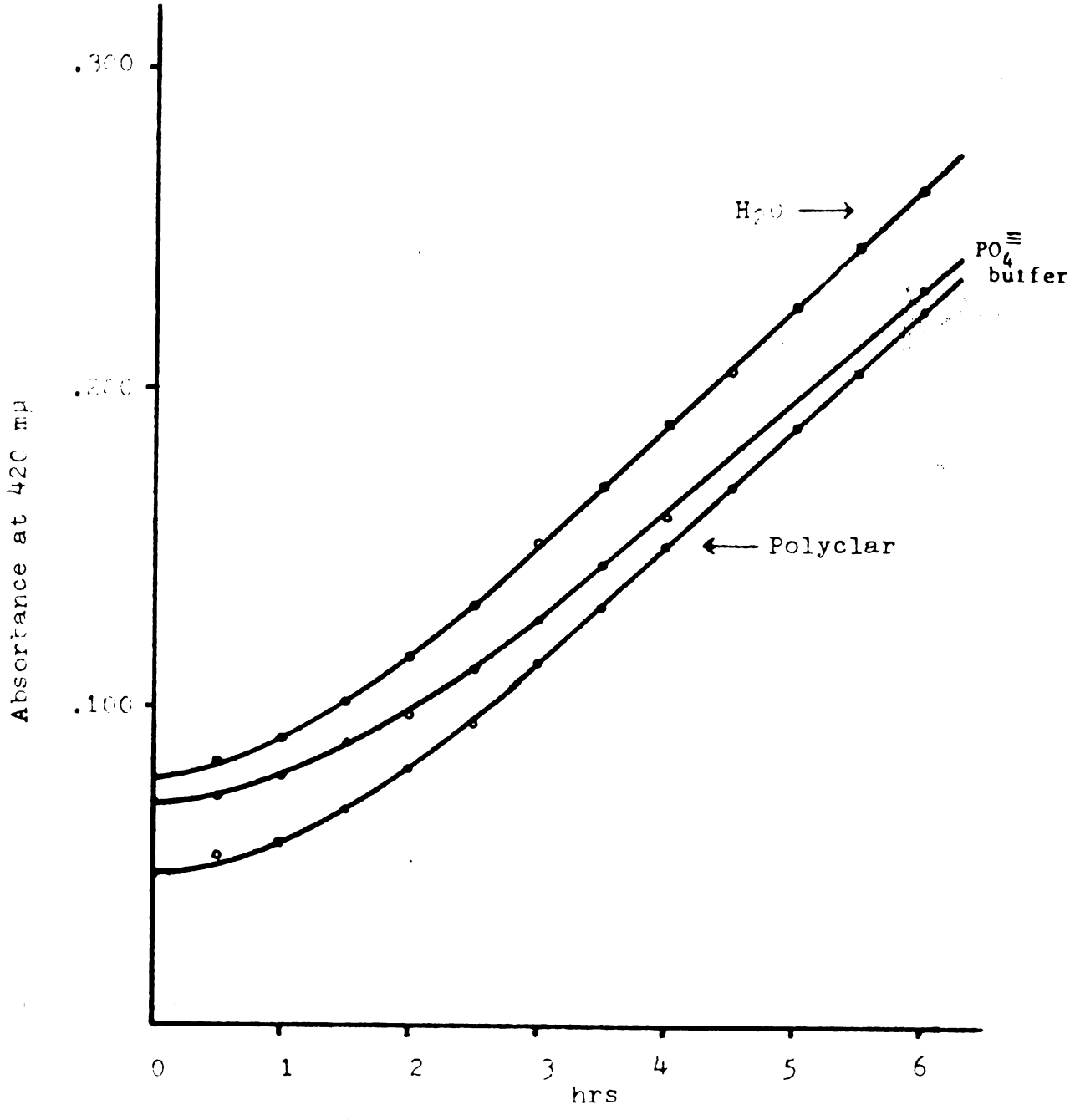


Fig. 2. Absorbance changes of DOPA (0.33×10^{-2}M) by 3 different parsnip extracts; water, 0.5M phosphate buffer pH 6.5, 1% Polyclar L.

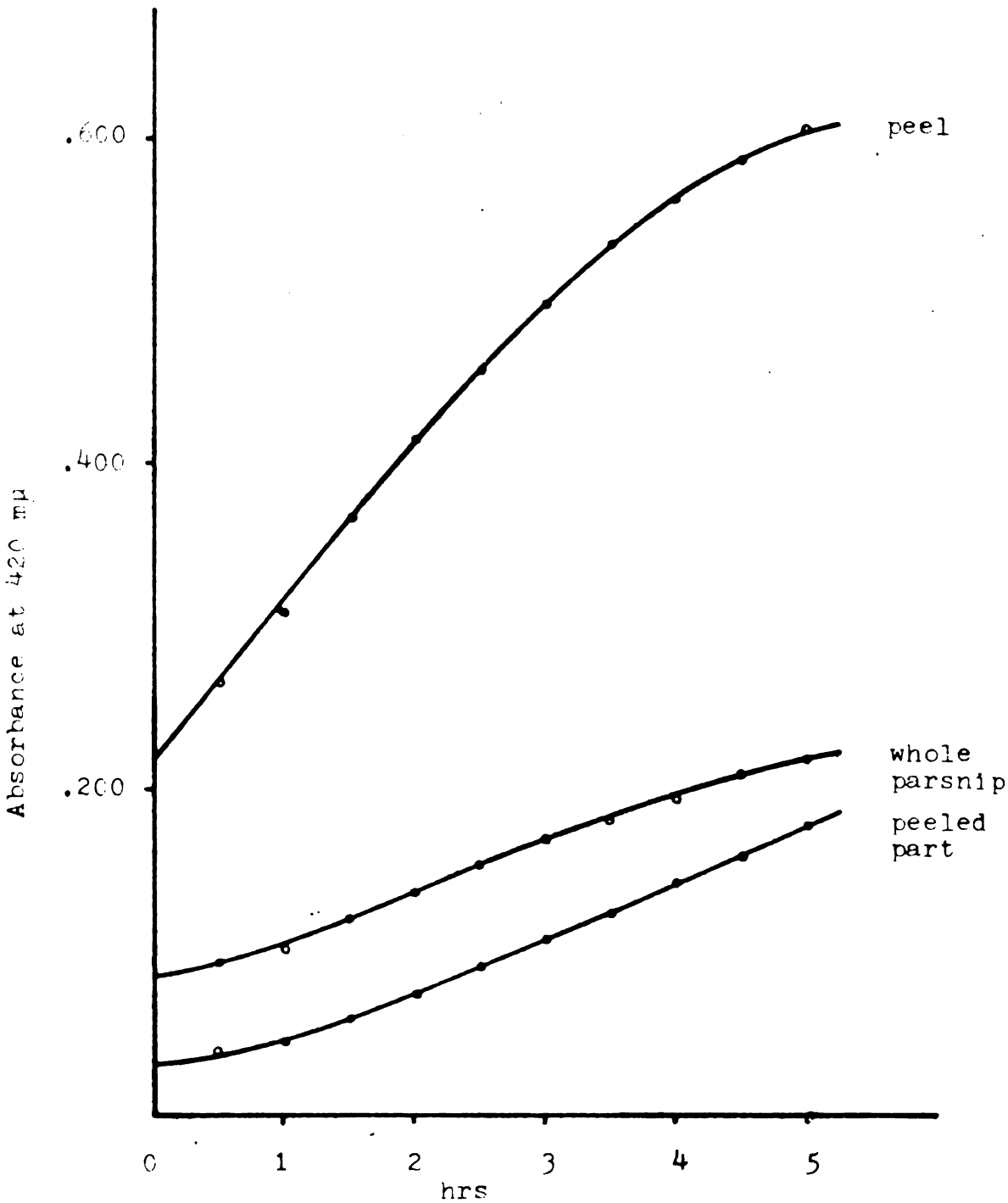


Fig. 3. Absorbance changes of DOPA (0.33×10^{-2} M) by water extracts of the whole parsnip, the peel and the peeled part of it.

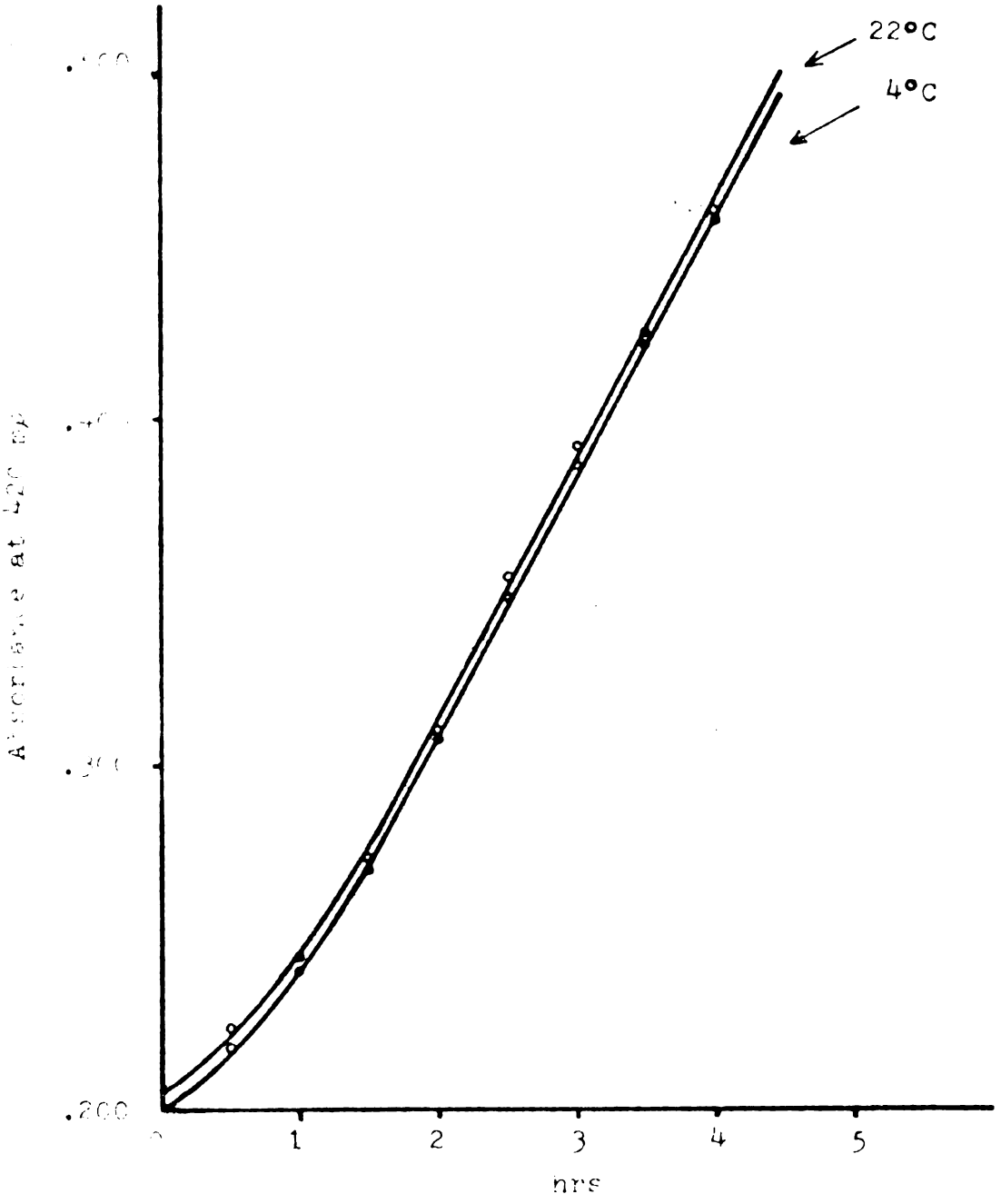


FIG. 4. Absorbance changes of DOPA (0.33×10^{-2} M) by water extracts of parsnip kept at 4°C and 22°C for 24 hours.

Activation of Tyrosine

When tested with tyrosine (Fig. 1), the enzyme showed no activity. It is known, however, that monophenols, such as tyrosine, may be oxidized by phenoloxidase if a reducing agent is present in the reaction mixture (Bright et al., 1963). Additions of 0.05 ml of 0.1 M ascorbic acid or 0.05 ml of 1 x 10^{-2}M DOPA to the 3 ml reaction mixture indicated the triggering action to a very small extent: ascorbic acid raised the activity from .0 to .004 ΔA_{420}/hr. and DOPA from .0 to .014 ΔA_{420}/hr. (Fig 5). Since a similar amount of DOPA in a reaction mixture contained all other reagents but tyrosine had an activity of .008 ΔA_{420}/hr., the net activity of the enzyme with tyrosine as substrate can be assumed to be .006 ΔA_{420}/hr.

Effect of EDTA

Since the enzyme extract used in this study was not purified, EDTA was added to the test solution for the purpose of chelating metal ions which might inhibit the phenoloxidase activity of the extract. Tests with and without EDTA, showed that 0.1 ml of 1% EDTA in 3 ml reaction mixture slightly enhanced the enzyme activity (Fig. 6): .063 ΔA_{420}/hr. with EDTA and .060 ΔA_{420}/hr. without.

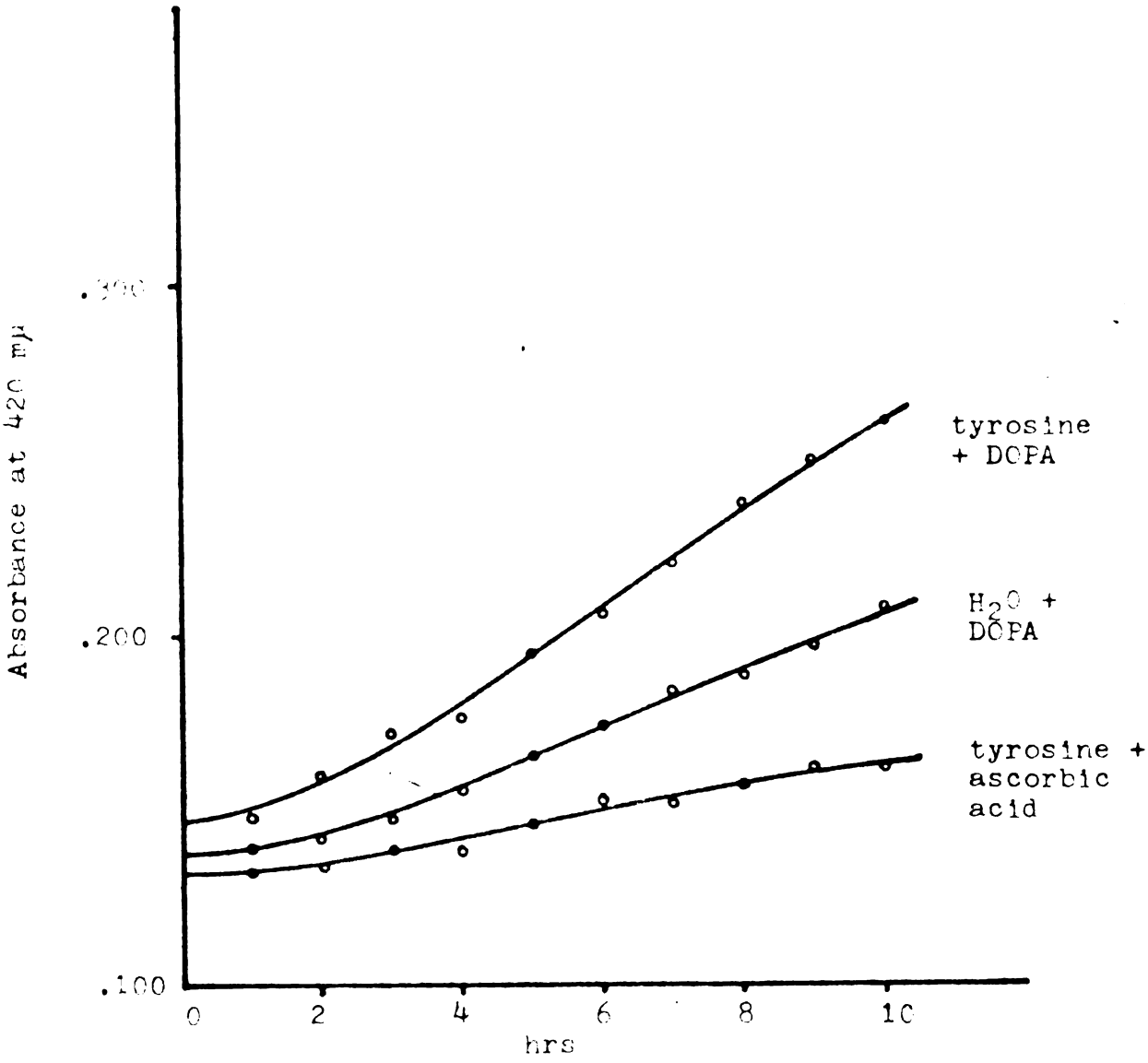


Fig. 5. Effect of ascorbic acid (0.0017M) and DOPA (0.00017M) on the oxidation of tyrosine by parsnip extract.

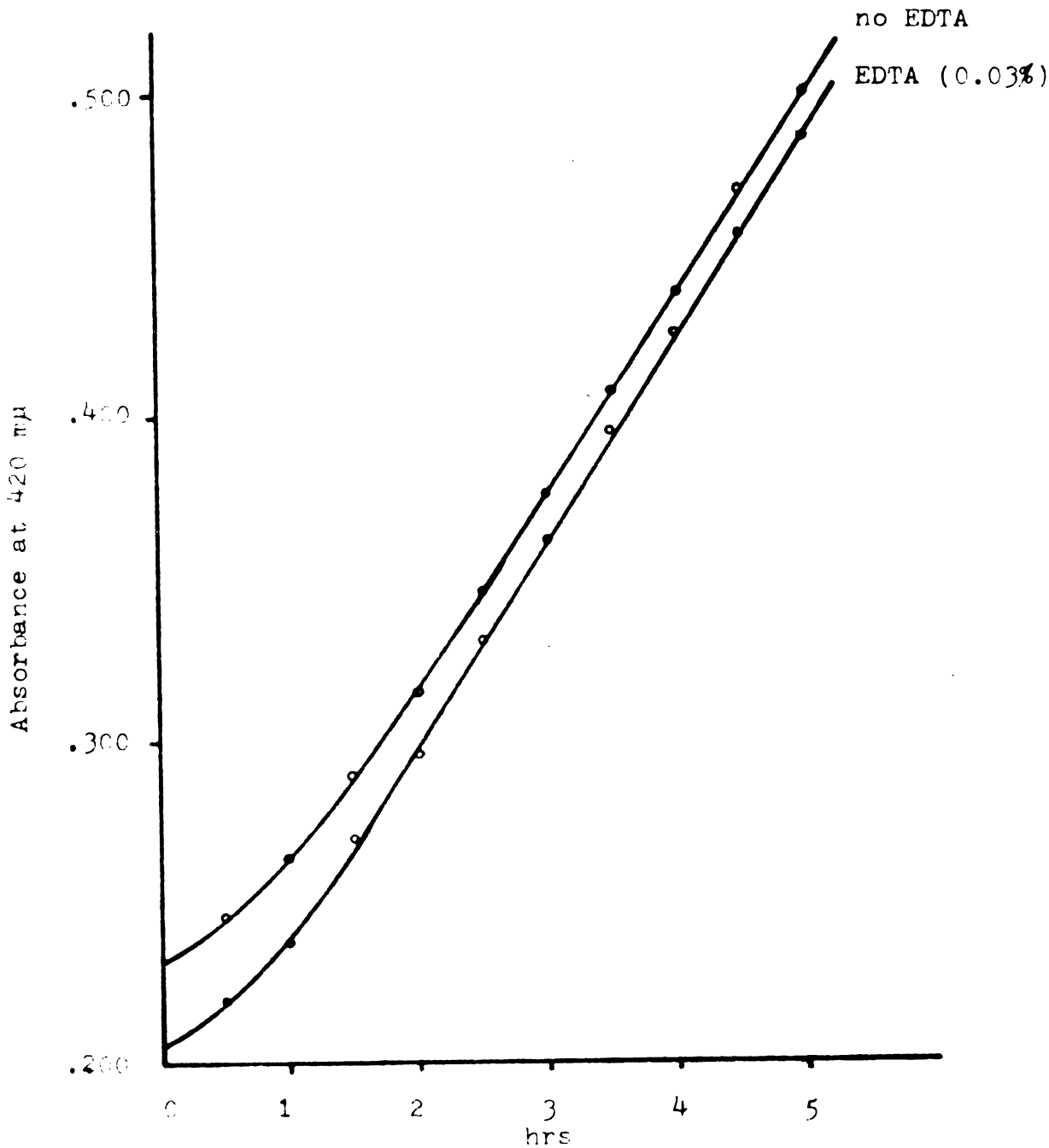


Fig. 7. Effect of EDTA (0.03%) on the oxidation of DCPA (7.33×10^{-2} M) by parsnip extract.

Effect of pH on Enzyme Activity

Preliminary experiments with DOPA suggested that the optimum pH for phenoloxidase in parsnips is in the higher pH regions. But since DOPA autoxidizes quite rapidly at higher pH values, cathechol was used as the substrate in these tests. Blanks, as in previous measurements, contained all components of the test solution except the enzyme. In addition, 0.5 ml 0.5% gelatin was used in the test solution for stabilizing the enzyme (Dawson and Magee, 1955). Test solutions remained the same in content, except for adding 0.5 ml enzyme and 0.5 ml gelatin (instead of the usual 1 ml enzyme extract). The optimum pH for the enzyme was found to be 8.5 (Fig. 7).

Effect of Substrate Concentration

Six concentrations, .42, .83, 1.33, 1.67, 2.50, and 3.33×10^{-3}M of catechol as substrate were used in order to determine the Michaelis constant of the reaction. The data were plotted according to the Lineweaver-Burk, Hofstee, and Woolf methods (Christensen and Palmer, 1967) and are illustrated in Figures 8, 9 and 10. From these graphs the following K_m values were obtained:

1.67×10^{-2}M	Lineweaver-Burk Plot
1.70×10^{-2}M	Hofstee Plot
1.72×10^{-2}M	Woolf Plot

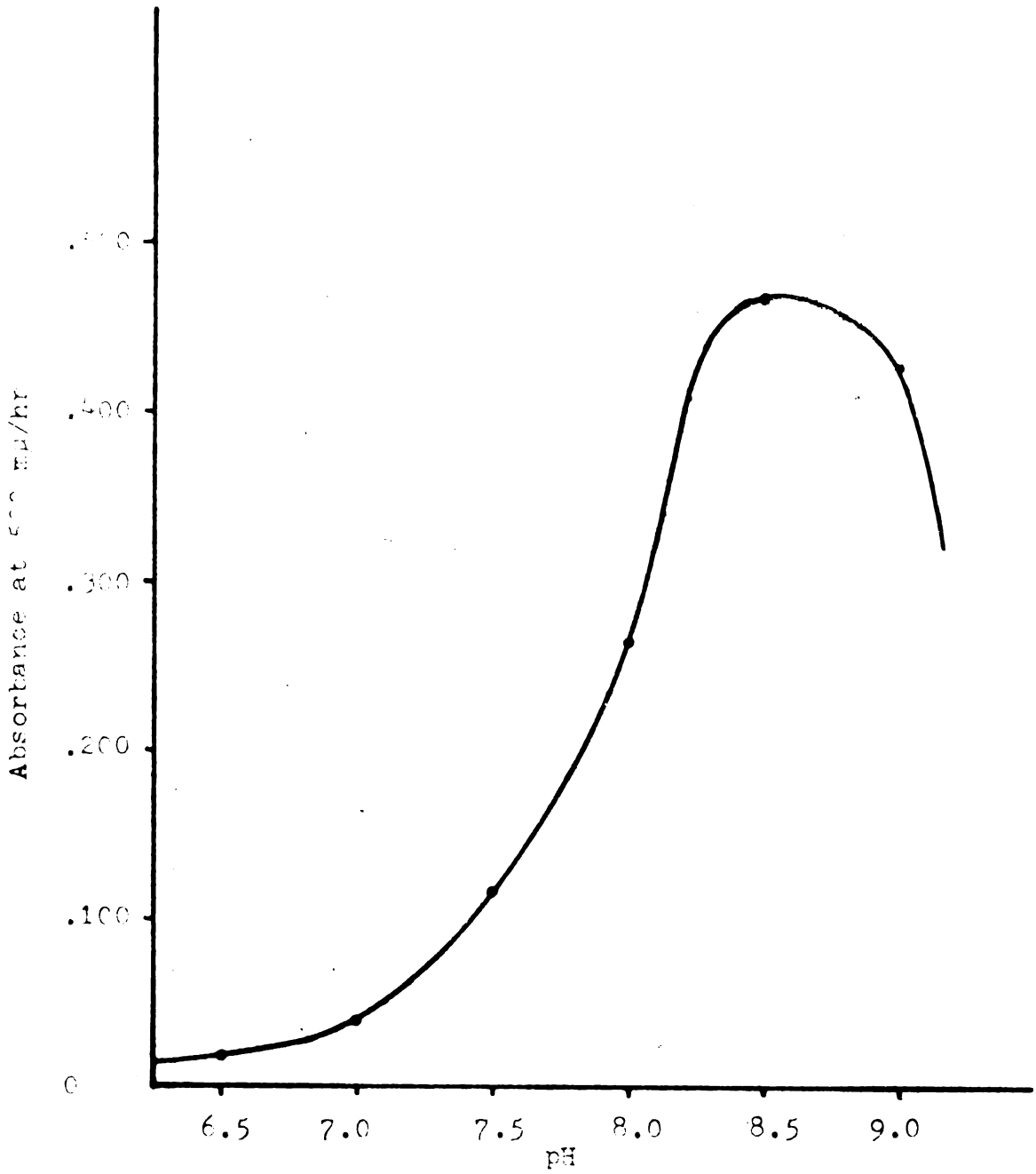


Fig. 7. Effect of pH on the oxidation of catechol (0.16×10^{-2}M) by parsnip extract.

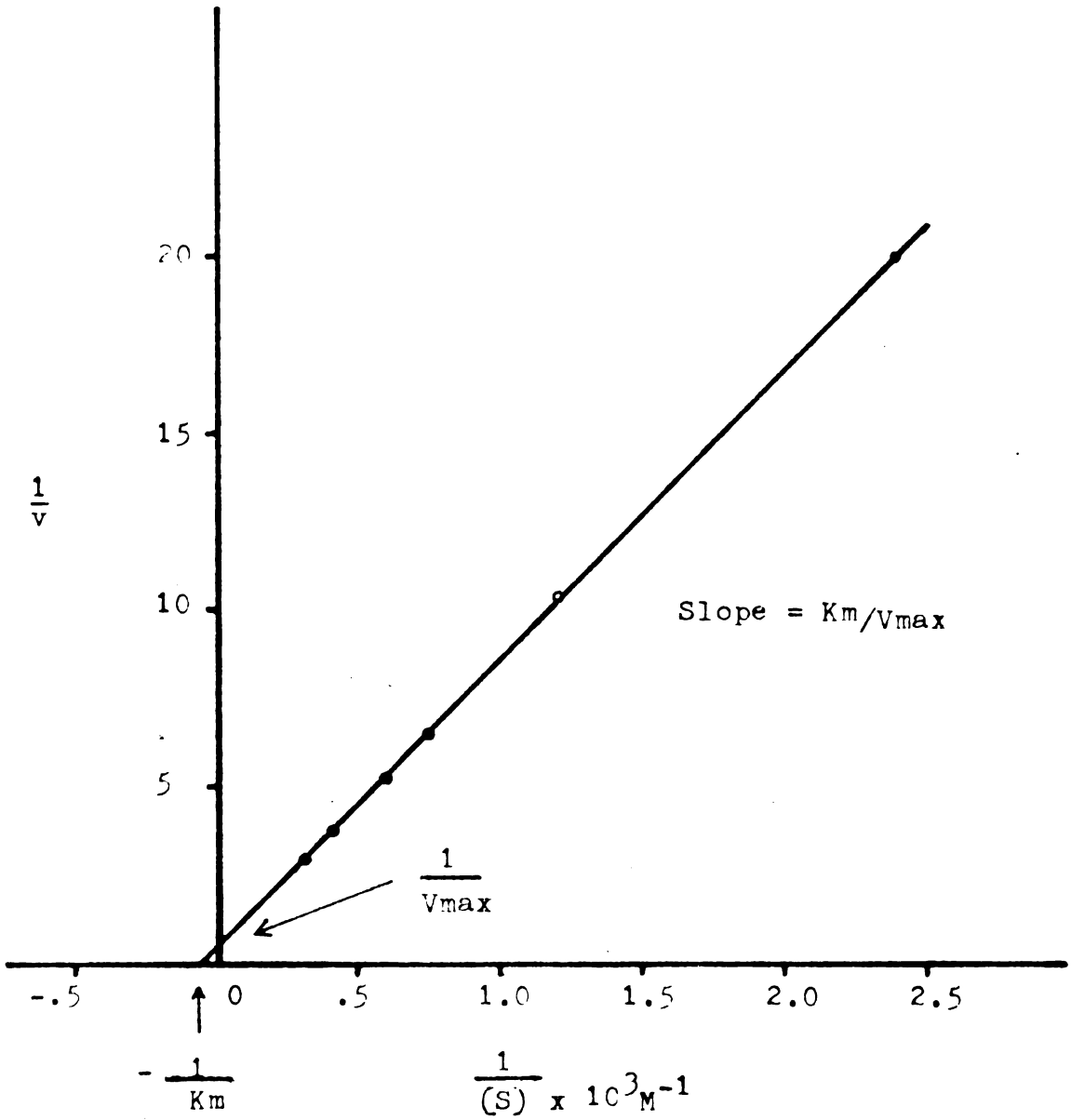


Fig. 8. Lineweaver-Burk plot for the catechol-parsnip extract reaction.

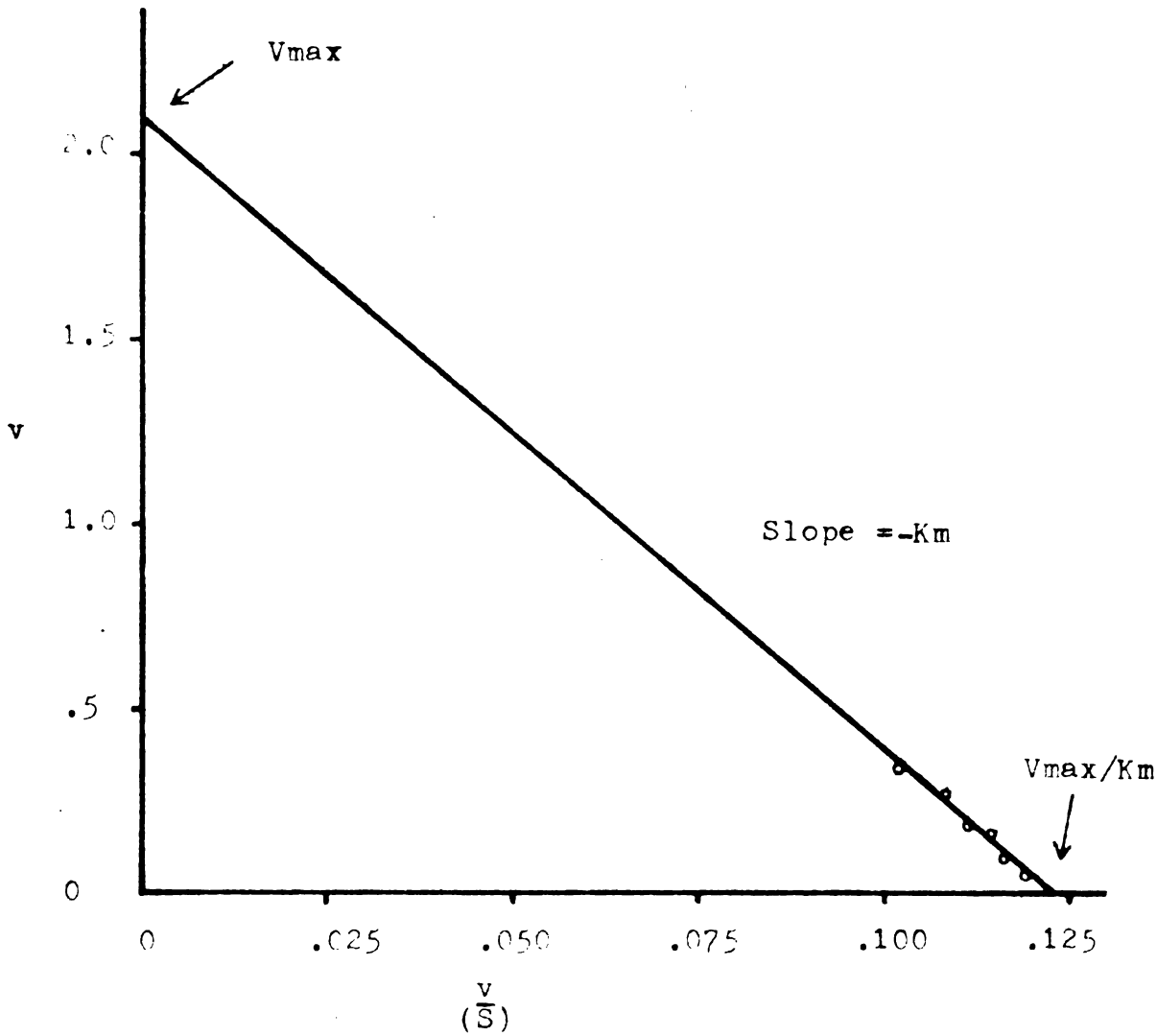


Fig. 9. Hofstee plot for the catechol-parsnip extract reaction.

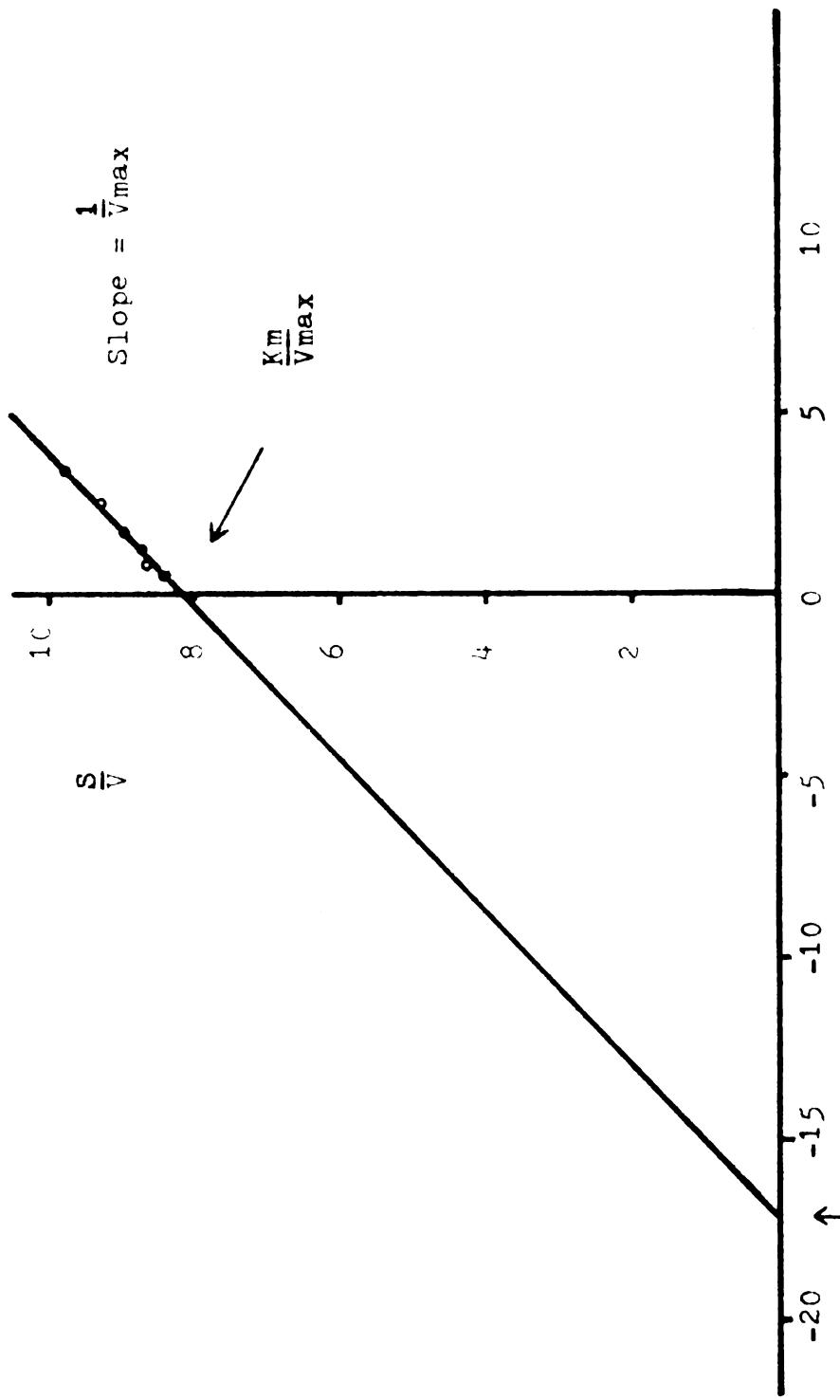


Fig. 10. Woolf plot for the catechol-parsnip extract reaction.

Heat Inactivation

Studies of the time and temperature required to inactivate the enzyme sought to measure the stability of phenoloxidase in parsnip extract. Since initial trials revealed strong heat resistance, a temperature of 100°C was selected for the test. At this temperature complete inactivation was observed after 120 minutes at pH 8.0. At various intervals during the heating period, samples of the extract were tested and their activity plotted on semilogaritmic graph paper (Fig. 11). The activity declined rapidly during the first 10 minutes of heating, and decreased at a markedly slower rate upon further heating. A similar high heat resistance was observed by Jankov (1962) for the phenoloxidase of apples and plums.

B. Gel Electrophoresis Study

Figure 12 illustrates the results of the polyacrylamide gel electrophoresis of the parsnip extract. It is apparent that the bands displaying the color reaction with DOPA, cathechol and chlorogenic acid also reacted with the protein stain. No color band can be seen with tyrosine. This confirms the evidence derived from the spectrophotometric studies concerning the enzymic nature of the browning agent. One can also observe several isozymic forms of the parnship phenoloxidase.

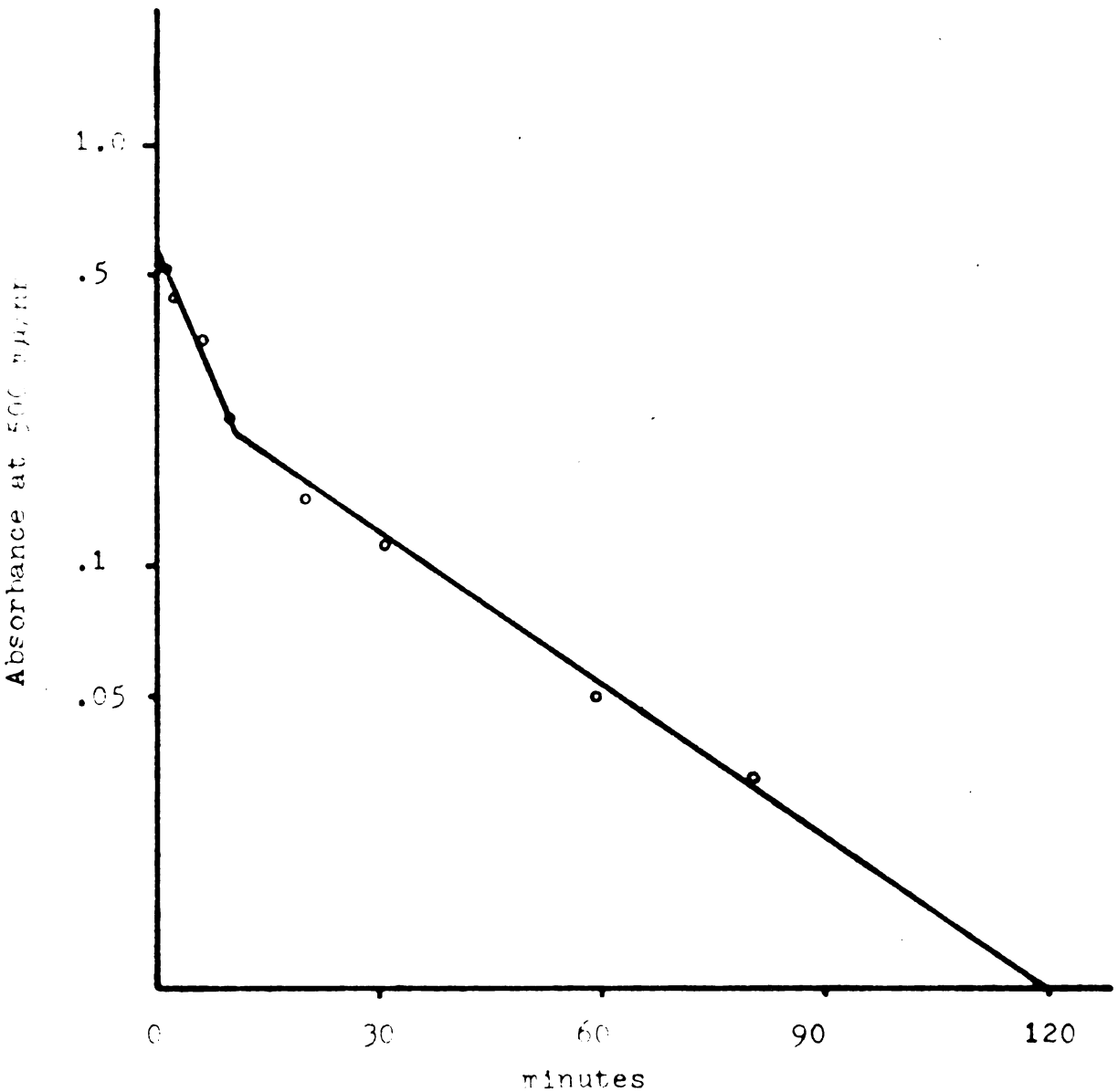


Fig. 11. Heat inactivation of the parsnip phenoloxidase.

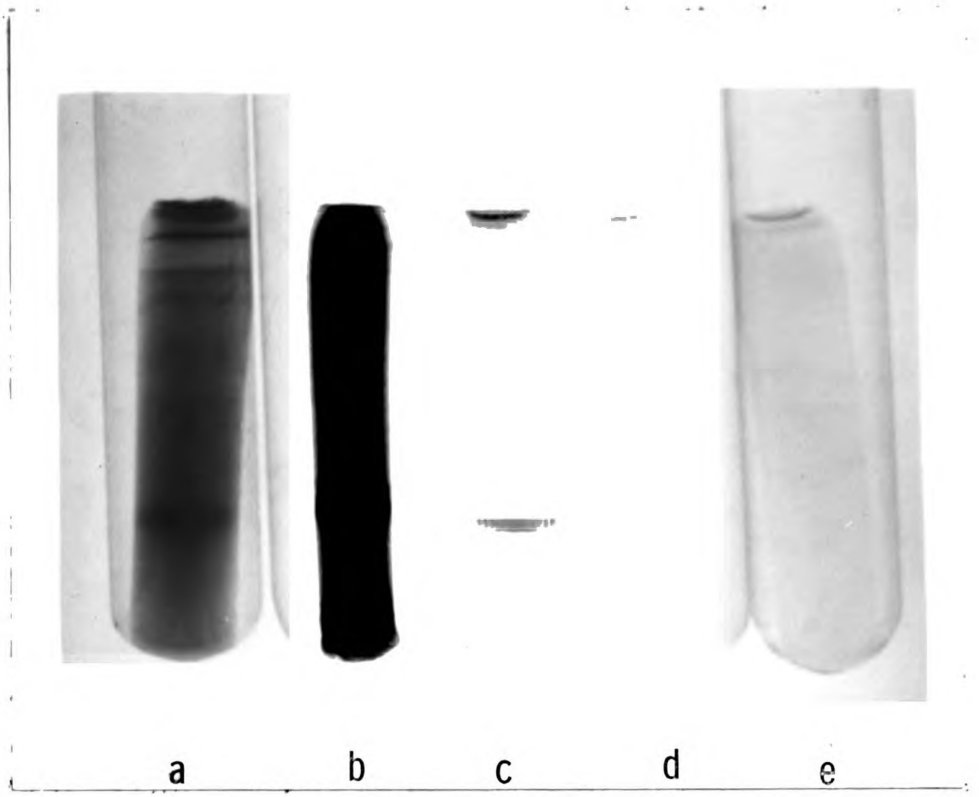


Fig. 12. Polyacrylamide gel electrophoresis of water extract of parsnip.

a. Aniline blue black staining of the proteins of the extract.

b. DOPA staining.

c. Catechol staining.

d. Chlorogenic acid staining.

e. Tyrosine staining.

C. Storage Studies

Effect of $NaHSO_3$

The storage study demonstrated that parsnips soaked in $NaHSO_3$ for 2 to 10 minutes and subsequently stored in polyethylene bags showed no inhibition of browning compared to those stored in polyethylene bags without treatment during a 4 month period of straoge at 4°C. Also, no color difference was observed between $NaHSO_3$ dipped and control parsnips stored at 1°C for 6 months. On the other hand, browning was completely inhibited in parsnips stored continuously in jars containing 0.02%, 0.08%, 0.16%, 0.48% and 0.81% $NaHSO_3$ solution (Fig. 13).

Bisulfite demonstrated some antiseptic properties for the parsnips stored in jars. Parsnips stored in 0.02% and 0.08% $NaHSO_3$ concentrations disintegrated within one month at 4°C and two months at 1°C. Concentrations of 0.16% provided one additional month of preservation at both temperatures. Parsnips stored in 0.48 and 0.81% $NaHSO_3$ concentrations (pH 4.85) kept well for four months at either temperature. After four months, molds started to grow on the surface of the solutions but not on the roots. When new $NaHSO_3$ solutions were prepared with the same concentrations of $NaHSO_3$ (0.48% and 0.81%) and the parsnips were transfered into them no deterioration signs were apparent after an additional two months of storage.

Fig. 13. Effect of $NaHSO_3$ storage on the color of parsnip.

a. Stored in 0.81% $NaHSO_3$ solution for 4 months at 0°C.

b. Stored in perforated polyethylene bag for 4 months at 1°C and 85% R.H.

Effect of Vacuum

Vacuum packed parsnips retained their white color, but the roots could not be kept under these conditions longer than 10 days because of the development of an off odor presumably due to anaerobic respiration.

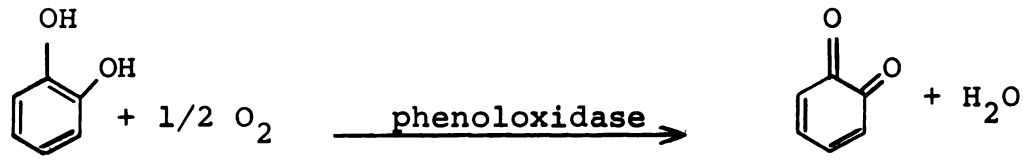
Effect of Temperature

Parsnips stored at 4°C browned sooner and developed darker color than those stored at 1°C. Better quality was maintained at the lower temperature. Parsnips stored at 4°C, whether $NaHSO_3$ treated or not, kept no longer than three months. On the other hand, all parsnips stored at 1°C in polyethylene bags remained in good conditions for 4 to 5 months.

Generally speaking, after harvesting, parsnips slowly lose their white color and turn light to medium brown. The rate at which the browning proceeds varies with the temperature and the availability of oxygen. Cooling the roots soon after harvest can delay the browning. Transporting in cold water is very effective since it also excludes the oxygen.

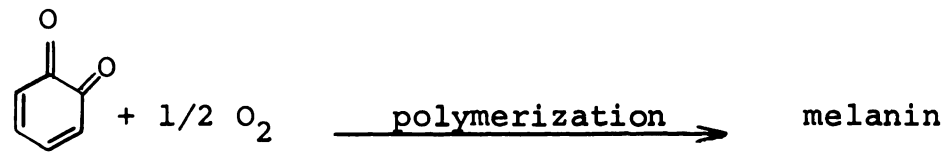
Joslyn and Ponting (1951) reported that many plant tissues displaying such general patterns of darkening contain phenolic compounds with free o-dihydroxy benzene groups, e.g. catechol, which are oxidized by molecular oxygen under catalysis by the enzyme phenoloxidase.

Nelson and Dawson (1944) proposed the following oxidation reaction for catechol, which in the presence of oxygen produces o-benzoquinone:



catechol o-benzoquinone

Upon further oxidation and condensation o-benzoquinone yields melanin, a dark-colored pigment:



o-benzoquinone

SUMMARY

1. The reaction leading to the browning of parsnips was studied chiefly in extracts of these roots. Three different solvents were compared in order to find the best extraction method: demineralized water, 0.1 M phosphate buffer at pH 6.5, and 1% Polyclar L. Demineralized water appeared to be the best of the three because it caused the greatest browning of 3,4-dihydroxyphenylalanine (DOPA) as substrate.

2. Among four possible substrates, DOPA, catechol, chlorogenic acid and tyrosine, tested for browning at the wavelength of maximum absorption of their solutions, DOPA resulted in the most rapid browning. No browning of the tyrosine solution was observed. When ascorbic acid and DOPA were used as triggering agents for the browning of tyrosine by the water extract, a slight reaction was observed.

3. The catalytic nature of the peel extract was the highest when compared to that of extract from the peeled and whole parsnips.

4. Water extracts of parsnips kept at 4°C and 22°C for 24 hours showed no difference in activity against DOPA as substrate.

5. EDTA, 0.03% in the reaction mixture, slightly enhanced the oxidation of DOPA by the parsnip extract.

6. Optimum pH for the browning of 0.16 x 10^{-2}M catechol solution was 8.5.

7. Michaelis constant for the browning reaction of catechol was found to be 1.7 x 10^{-2}M by three graphical methods.

8. Parsnip phenoloxidase showed strong heat resistance. At 100°C the activity declined rapidly during the first 10 minutes of heating, but decreased at a markedly slower rate upon further heating. Complete inactivation of the enzyme was observed after 120 minutes at pH 8.0.

9. Gel electrophoresis confirmed the enzymic nature of the browning agent in parsnips. Bands in the polyacrylamide gel which resulted in browning reactions with DOPA, catechol, and chlorogenic acid were also stained with protein stain. Tyrosine failed to react with any of the gel bands.

10. Preliminary storage studies indicated that dipping in $NaHSO_3$ solution of concentrations up to 0.81% for 10 minutes did not stop the browning of parsnips on subsequent storage in air. Keeping the parsnips immersed in 0.48% solution of $NaHSO_3$ resulted in prevention of browning for 4 months of observation. Parsnips stored in perforated polyethylene bags at 4°C browned sooner and developed darker color than those stored at 1°C. The quality was also better when parsnips were stored at the lower temperature.

REFERENCES

REFERENCES

Bleasdale, K. A. and Thompson, R. 1966. The effect of plant density and the pattern of plant arrangement on the yield of parsnips. J. hort. Sci. 41, 371.

Bright, H. J., Wood, Brian J. B., and Ingraham, Lloyd L. 1963. Copper, tyrosinase, and the kinetic stability of oxygen. Annals N.Y. Acad. Sci. 100, 965.

Christensen, H. N. and Palmer, G. A. 1967. "Enzyme Kinetics," W. B. Saunders Co., Philadelphia.

Chubey, B. B. 1966. Oxidative browning of carrots, Daucus carota. Ph.D. Thesis, University of Minnesota, St. Paul, Minnesota.

Constantinides, S. M. 1966. Multiple forms of plant phenoloxidase. Ph.D. thesis, Michigan State University, East Lansing, Michigan.

Davis, B. J. 1964. Disc electrophoresis II. Annals N.Y. Acad. Sci. 121, 404.

Dawson, C. R. and Magee, R. J. 1955. Plant tyrosinase (polyphenol oxidase). In "Methods in Enzymology," ed. Colowick, S. P. and Kaplan, N. O., p. 817. Academic Press, New York.

Dixon, M. and Webb, E. C. 1964. "Enzymes." Academic Press, New York.

Dressler, H. and Dawson, C. R. 1960a. On the nature and mode of action of the copper protein tyrosinase. I. Exchange experiments with radioactive copper and the resting enzyme. Biochim. Biophys. Acta 45, 506.

Dressler, H. and Dawson, C. R. 1960b. On the nature and mode of action of the copper protein tyrosinase. II. Exchange experiments with radioactive copper and the functioning enzyme. Biochim. Biophys. Acta 45, 515.

Embs, R. J. and Markakis, P. 1965. The mechanism of sulfite inhibition of browning caused by polyphenol oxidase. J. Food Sci. 30, 135.

Goodman, L. P. and Markakis, P. 1965. Sulfur dioxide inhibition of anthocyanin degradation by phenolase. J. Food Sci. 30, 135.

Guadagni, D. G., Sorber, D. G. and Wilbur, J. S. 1949. Enzymatic oxidation of phenolic compounds in frozen peaches. Food Technol. 3, 359.

Hope, G. W. 1961. The use of antioxidants in canning apple halves. Food Technol. 15, 548.

Joslyn, M. A. and Braverman, J. B. S. 1954. The chemistry and technology of the pretreatment and preservation of fruits and vegetable products with sulfur dioxide and sulfites. In "Advances in Food Research." ed. Mrak, E. M. and Stewart, G. F., 5 97. Academic Press, New York.

Joslyn, M. A. and Ponting, J. D. 1951. Enzyme-catalyzed oxidative browning of fruit products. In "Advances in Food Research." ed. Mrak, E. M. and Stewart, G. F., 3, 1. Academic Press, New York.

Jankov, S. I. 1962. Hitze-inaktivierung der polyphenoloxydasen in einigen fruchtsaften. Fruchtsäft-Ind. 7, 13.

Lutz, J. M. and Hardenburg, R. E. 1968. The commercial storage of fruits, vegetables, and florist and nursery stocks. Agricultural Handbook No. 66, U.S.D.A. Washington.

Mason, H. S. 1955. Comparative biochemistry of the phenolase complex. In "Advances in Enzymology," Ed. Nord, F. F., 16, 105. Interscience Publ., New York.

Meyer, L. H. 1960. "Food Chemistry." Reinhold Publ. Corp., New York.

Nelson, J. M. and Dawson, C. R. 1944. Tyrosinase. In "Advances in Enzymology," ed. Nord, F. E. and Weakman, C. H., IV. 99. Interscience Publ., New York.

Ornstein, L. 1964. Disc electrophoresis I. Annals New York. Acad. Sci. 121, 321.

Ponting, J. D. 1960. The control of enzymatic browning of fruits. In "Food Enzymes," ed. Schultz, H. W., p. 105. The AVI Publishing Co., Westport, Connecticut.

Reed, G. 1966. "Enzymes in Food Processing," Academic Press, New York.

Reyes, P. and Luh, B. S. 1960. Characteristics of browning enzymes in Fay Elberta freestone peaches. Food Technol, 14, 570.

Scott, L. E., Mattus, G. E. and Claypool, L. L. 1960. Observations on the browning reaction in bruised Bartlett pears. Proc. Amer. Soc. Hort. Sci. 75, 106.

Stadtman, E. R. 1948. Nonenzymatic browning in fruit products. In "Advances in Food Research," ed. Mrak, E. M. and Stewart, G. F., 1, 325. Academic Press, New York.

Thompson, H. C. and Kelly, Wm. C. 1957. "Vegetable Crops," McGraw-Hill Book Co., New York.