THE EFFECT OF SULFUR DIOXIDE ON THE DEGRADATION OF THE TART CHERRY ANTHOCYANIN BY TYROSINASE

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

Louis P. Goodman

1963

AN ABSTRACT

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This study was carried out using both red tart cherry (Prunus cerasus L. var. Montmorency) juice and the chromatographically and electrophoretically purified mecocyanin pigment isolated from this juice. The degradation of the pigment was measured spectrophotometrically. The rate of the degradation of the mecocyanin pigment by tyrosinase, in a citrate-phosphate buffer, at pH 6.5, decreased rapidly as the concentration of sulfur dioxide was increased. The half life of the mecocyanin pigment, degradation at pH 6.5, with no sulfur dioxide added, was 40 seconds. There was almost no decolorization at the level of 8 ppm sulfur dioxide during the two-minute period of observation.

Cherry juice, at pH 6.5, exhibited the same trend as the mecocyanin pigment, but 25 ppm of sulfur dioxide were required to inhibit the reaction for the 75 minutes of observation. The half life of the cherry juice anthocyanin, at pH 6.5, with no added sulfur dioxide was 8 minutes.

At lower pH levels than 6.5, less sulfur dioxide was required to inhibit the decolorization reaction both in the purified pigment system and in the cherry juice.

Preincubation of enzyme and sulfur dioxide at pH 6.5, before addition of the purified pigment or the juice, further decreased the subsequent rate of decolorization. Again, a higher concentration of sulfur dioxide in the preincubation enzyme-sulfur dioxide mixture was required for the juice than for the pure pigment.

Experiments conducted with model systems using sulfur dioxide to inhibit the enzymic oxidation of tyrosine and catechol showed that the rate of oxidation by tyrosinase of both tyrosine and catechol decreased as the concentration of sulfur dioxide was increased. Complete inhibition of both the catechol and tyrosine oxidation was obtained with 4 ppm sulfur dioxide. If the enzyme and the sulfur dioxide were preincubated, lower levels of sulfur dioxide were required for inhibition of the reaction than without preincubation.

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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

1963

Approved: Pericles Markakis

IN MEMORY

of

my mother Helen Goodman

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. Pericles Markakis for his guidance, aid, and encouragement during the research for this project and in the preparation of this manuscript. He is deeply indebted to the following people: to Dr. Clifford L. Bedford for his beneficial suggestions and advice; to Dr. Donald H. Dewey for his help in the preparation of this manuscript; to Mr. Chung-Yen Peng for his assistance in the necessary techniques used in this experiment; and to Dr. Clarence H. Suelter, of the Department of Biochemistry, for the use of his equipment.

The author also expresses his appreciation to the United States Public Health Service for the financial support of this project.

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INTRODUCTION

Enzymatic degradation of the anthocyanin pigments found in red tart cherries (Prunus cerasus, L. Var. Montmorency) is one of the major causes of loss in color of the fruit. The discoloration, known as "scald" in the cherry industry, is exhibited on the fruit by the appearance of a light colored area on the cherry skin. In severe scald these areas turn brown. Van Buren et al. (27) showed that the anthocyanin pigment, in the areas of the cherry which exhibited scald, was actually decolorized by an enzyme present in the cherry. An enzyme specifically attacking anthocyanins has not yet been found. However, anthocyanins are polyphenols and polyphenolases are widely distributed in the plant kingdom without being very substrate-specific. Therefore, the enzyme chosen for this work was a commercial mushroom polyphenolase which Peng (20) showed decolorized the purified anthocyanin pigment of cherries. Bedford (13) has subsequently shown that polyphenolase activity is present in red tart cherries.

Sulfur dioxide (SO_2) has been widely used in the food industry both as a microbial inhibitor and for the preservation of color in fruits (1,5,7,10,12,13,16,26,28,29). It is also used extensively in the Maraschino cherry industry for bleaching the natural cherry pigments (4).

The purpose of this study has been to investigate the effects of sulfur dioxide on the degradation of the tart cherry anthocyanin pigments by a mushroom tyrosinase.

REVIEW OF THE LITERATURE

Enzymatic Degradation of Anthocyanins

The enzymatic degradation of anthocyanin pigments from various fruits and flowers had been studied by a number of workers. In 1917, Nagai (18), working with the anthocyanin from chrysanthemum, showed that the plant itself contained an oxidase which decolorized the anthocyanin of the flower at room temperature. The same result was produced by dilute solutions of hydrogen peroxide. Boiled plant juice did not exhibit this decolorization action.

Continuing his work, in 1921 Nagai (19) showed that the red anthocyanin pigments of scarlet Papaver were destroyed by a peroxidase obtained from soybean seedling hypocotyls and rootlets.

The decolorization of blackberry anthocyanins was studied by Huang (8) in 1955, who used crude enzyme extracts from Aspergilli. He found that the decolorization was rapid at 30°C. over a pH ranging from 3.0 to 4.5. The anthocyanins were hydrolyzed by the enzyme to anthocyanidin and sugar followed by a spontaneous transformation of the aglycone to colorless derivatives. This enzyme acted as a glycosidase.

In 1956, Huang (9) studied the kinetics of the decolorization of chrysanthemum anthocyanin by a fungal

anthocyanase at 30°C. and pH 3.0. An apparent first order rate constant for the enzymic hydrolysis of the glucoside was shown.

An enzyme extracted from the leaves of Coleus hybridus by Bayer and Wegmann (2),in 1957, was capable of degrading the anthocyanin of red roses to yellow colored products. The enzyme, which they called cyaninoxidase, had an optimum activity at the pH range of 7.0 to 7.5. Catechol and oxygen were necessary for the enzymic reaction to occur.

Van Buren et al. (27), in 1959, reported the presence of an oxidizing enzyme isolated from Montmorency cherries. Using the cyanidin 3-rhamnoglucoside of cherries as a substrate, they found that this anthocyanin was decolorized by the enzyme upon the addition of catechol.

Scheiner (24), in 1961, showed that a crude enzyme preparation obtained from cherries decolorized the cherry anthocyanins. He found that catechol oxidase activity and anthocyanin decolorizing activity followed each other closely during the purification and that catechol was oxidized by all the preparations that decolorized anthocyanins. Purified preparations of the enzyme had little decolorizing effect on the anthocyanin unless catechol or some other o-dihydroxyphenol compound was present in the reaction mixture. p-Benzoquinone and o-benzoquinone, the first oxidation products of the catechol-catechol oxidase system, decolorized the anthocyanins non-enzymatically.

Peng (20), in 1962, used a commercial preparation of mushroom tyrosinase and showed that it decolorized the meco-cyanin 3-gentiobioside anthocyanin pigment from cherries when catechol was added.

The Use of Sulfur Dioxide to Inactivate Enzymes of Fruits

The enzyme-catalyzed oxidative browning of fruit and fruit products was reviewed by Joslyn and Ponting (14) in 1951. This review was continued by Joslyn and Braverman (15) in 1954. More recently (1959), Ponting used spectro-photometric methods to show the effect of SO_2 on the poly-phenol oxidase activity when different concentrations of SO_2 were added to a buffered catechol solution. The enzyme was added last. Even one part per million (ppm) SO_2 caused a significant drop (20%) in activity. Ten ppm SO_2 inactivated the enzyme almost completely. His studies with apple juice containing added catechol showed that if five ppm of SO_2 were added to the apple juice after oxidation had proceeded long enough to form quinones, the SO_2 reacted instantaneously with the quinones to lighten the color of the juice as was shown by a drop in optical density. It had no effect on the activity of the enzyme, presumably because the SO_2 had been oxidized before it had a chance to react with the enzyme. From these two experiments, Ponting concluded that polyphenol oxidase was very sensitive to SO_2, but to be most effective, the SO_2 had to react with the enzyme before any enzymatic oxidation occurred.

Diemair et al. (6), in 1960, found that there was no direct relationship between enzymatic activity in grape juice used for wine and the amount of SO_2 required to inactivate it. They showed that the inactivation was reversible and was not connected with the oxidation of H_2SO_3 to H_2SO_4. Preincubation of SO_2 and enzyme decreased the activity of the enzyme as measured by the purpurogallin test.

Sastry et al. (23), in 1961, added peroxidase obtained from the fruit of the custard apple to various quantities of potassium metabisulphite and let the two incubate for ten minutes. After the addition of guaiacol and hydrogen peroxide, a gradual decrease in the development of browning was noted with increasing SO_2 concentrations. A concentration of 350 ppm of SO_2 completely inhibited both color production and enzymic activity. The purpurogallin test was used to determine enzymic activity.

METHODS AND MATERIALS

Preparation of Purified Anthocyanin

Frozen, pitted Montmorency cherries were partially defrosted and placed in boiling 95 per cent (v/v) ethanol in such a proportion as to achieve a 70 to 75 per cent (v/v) final ethanol concentration. The mixture was boiled for five minutes and allowed to cool. This treatment extracted the anthocyanins, inactivated the enzymes, and precipitated the pectins.

The mixture was filtered through a milk filter and the filtrate was concentrated under reduced pressure in a rotatory flash evaporator thermostated at 37°C.

The concentrated anthocyanin extract was applied to a 2 x 10 cm. column of Dowex 50W-X8 (100 to 200 mesh, H^+ form) resin which retained the pigments along with other basic components of the extract. The column was eluted with 50 to 100 ml. of 0.2 HCl in methanol. The eluate was concentrated in vacuo and applied as a narrow band to Whatman 3 MM paper. The paper was placed in a chromatography cabinet and irrigated ascendingly with 1 N acetic acid for 25 to 35 minutes. At the end of this time the two Montmorency cherry anthocyanins appeared as well separated zones. These zones were cut out and eluted separately with methanol containing a trace of concentrated hydrochloric acid.

The eluate of the pigment with the higher R_f value (mecocyanin pigment) was concentrated in vacuo and further purified by zone electrophoresis. A Reco Model E apparatus was used for this purpose. Whatman cellulose powder, standard grade, was made into a past with 1 N acetic acid solution. A filter paper long enough to connect the two electrode vessels, containing 1 N acetic acid, was placed over the plate of the apparatus. The cellulose paste was then spread evenly over the paper. Pigment was applied as a narrow band on the paste at five places across the electric field and 700 volts of direct current, approximately 50 milliamperes, were applied for about six hours. At the end of this time the pigment had moved 2 to 3 cm. The colored zones of the cellulose paste were removed and eluted with methanol. The eluate was concentrated in vacuo and the dry pigment was dissolved in water.

Preparation of Cherry Juice

Frozen, pitted Montmorency cherries were partially defrosted and pressed through a cheesecloth to extract the juice. The juice was heated in 1-1/2 x 15 cm. test tubes for 3 to 4 minutes in boiling water, with constant shaking, to inactivate the enzymes. It was cooled and filtered through No. 2 Whatman filter paper using a Buchner funnel and suction. It was then diluted with demineralized water using approximately one part water to three parts juice.

Preparation of Enzyme

The tyrosinase preparation used in this study was obtained from Worthington Biochemical Corp., Freehold, New Jersey. Its activity was found to be 417 units per mg. of dried enzyme. A unit of tyrosinase activity equals an increase in absorbance of 0.001 per minute under the specified conditions of the assay.

Yasunobu (30) distinguished between a true tyrosinase and polyhenoloxidase in that the former catalyzed the oxidation of both mono and diphenols while the latter only catalyzed the oxidation of o-diphenols. The enzyme used in this study of pigment degradation was a true tyrosinase which exhibited a low substrate specifity.

The stock solution consisted of 10 mg. of enzyme diluted in 10 ml. of water. No subsequent dilutions were made for the work on the purified pigment and cherry juice. It was necessary, however, to make dilutions of the stock solution for experiments involving the inhibition of tyrosinase by sulfur dioxide using catechol and tyrosine as substrates.

Preparation of Sulfur Dioxide

SO_2 was generated from a 5 per cent sodium bisulfite solution by adding concentrated sulfuric acid. The gas was bubbled into a flask containing boiled distilled water which had been cooled. Boiling removed air dissolved in the water, while cooling increased the solubility of SO_2. The stock

solution contained approximately 1700 ppm SO_2. Due to oxidation of the sulfurous acid when the container was opened, the stock solution had to be titrated with 0.02 N iodine, using starch as an indicator, every four or five days to determine the exact SO_2 concentration. The required dilutions were made from the stock solution.

Other Reagents

Three buffer solutions were employed. The reaction mixture contained a buffer consisting of equal volumes of 0.1 M citric and 0.1 M phosphoric acids adjusted to the desired pH level with sodium hydroxide at the time of preparing the reaction mixture.

The two other buffers employed were 1.0 M citric acid solution adjusted to pH 2.0 and 7.0, respectively, with sodium hydroxide. These were used in the determination of anthocyanin concentration by means of differential color development, as will be explained later (p. 12).

A 0.1 M solution of potassium cyanide was used to stop the enzymic reaction at the necessary time intervals during the reaction.

A 0.2 mM catechol solution was used to accelerate the enzymic reaction causing pigment degradation as shown by Peng (20).

Mecocyanin and Cherry Juice

Enzyme, catechol, and SO_2 were allowed to react in a citrate-phosphate buffer with either the purified

mecocyanin pigment, or cherry juice and the progress of the reaction was followed colorimetrically in a Beckman D U Spectrophotometer. Two factors affecting the reaction were studied: SO_2 concentration and pH. Temperature, enzyme concentration, and catechol concentration were kept constant. The pigment concentration in the reaction mixture differed within $\pm$ 15 per cent, but it was found that these differences did not affect the degradation rate of the pigment. In the case of cherry juice, 1.0 ml. of water was added instead of catechol, since the juice contained enough phenolic compounds to act as substrates for the enzymic reaction.

The reaction was carried out in a 30 ml. beaker. The mixture consisted of the following: 3.0 ml. of the citric-phosphoric acid mixture, 1.0 ml. of 0.2 mM catechol, and 1.0 ml. of the mecocyanin solution or cherry juice. The pH was adjusted to the desired level by adding 4 N sodium hydroxide solution from a graduated 1.0 ml. pipette and noting the quantity of base added. At this point 1.0 ml. of SO_2 solution was added followed by distilled water to make a total volume of 6.8 ml.

A 0.5 ml. aliquot of the reaction mixture was transferred to a test tube containing 2.5 ml. of the citrate buffer at pH 2.0 and 0.1 ml. of 0.1 M potassium cyanide, and another 0.5 ml. of the reaction mixture to a tube containing 2.5 ml. of the citrate buffer at pH 7, and 0.1 ml. of the potassium cyanide solution. These tubes served for determining the anthocyanin concentration at zero time. Following the removal

of these 0.5 ml. aliquots, 0.2 ml. of the enzyme solution was added to the remaining 5.8 ml. of the mixture and a stop watch was started. At various time intervals, two 0.5 ml. aliquots of the reaction mixture were transferred to tubes containing the citrate-cyanide solutions.

After a 15-minute development time, at room temperature (22 to 25°C.), the contents of these tubes were transferred to one cm. matched cuvettes for absorbancy measurements in a Beckman D U Spectrophotometer at the wavelength of 520 mμ for the mecocyanin pigment and 518 mμ for the cherry juice. The pH 2 buffer was used as a blank. The concentration of the mecocyanin and the cherry juice were expressed in absorbancy difference between pH 2 and pH 7.

Sondheimer and Kertesz (25), working with strawberry products, showed that the difference in absorbancy at two pH levels was a more accurate measurement of the anthocyanin concentration than the absorbancy at one pH when interferring substances were present. The main source of interference in the system presently used came from browning products. The absorbancy at 520 mμ of these brown products did not change with pH and cancelled out when the difference of absorbance at two pH levels was calculated for the reaction system.

The zero time reading was multiplied by a factor of 0.966 to correct for the dilution of the reaction mixture by the addition of enzyme, after the removal of the two 0.5 ml.

aliquots. All subsequent values were then subtracted from the corrected zero value to give absorbancy units of destroyed pigment.

Preincubation of Tyrosinase and Sulfur Dioxide using the Mecocyanin Pigment and Cherry Juice

The preincubation of tyrosinase and SO_2 was carried out in a 5 ml. beaker, with constant stirring, for 30 minutes. To 0.8 ml. of an enzyme solution, containing 1.0 mg. tyrosinase/ml., 4.0 ml. of a 13.2 ppm SO_2 solution was added. The final concentration of SO_2 in this mixture was 11 ppm.

During the preincubation period the reaction mixture was prepared by adding 1.0 ml. of the purified mecocyanin pigment, 1.0 ml. of 0.2 mM catechol, and 3.0 ml. of the citrate-phosphate buffer to a 30 ml. beaker. The pH was adjusted with 3 N sodium hydroxide to 6.5, noting the amount added, and distilled water was added to make a total volume of 5.8 ml.

From the above reaction mixture, two 0.5 ml. aliquots were pipetted into the pH 2 and pH 7 developing tubes, previously described. At zero time, 1.2 ml. of the enzyme-SO_2 mixture was pipetted into the remaining 4.8 ml. of the reaction mixture and a timer was started. At 15 minute intervals, two 0.5 ml. aliquots were pipetted into the pH 2 and pH 7 developing tubes. The readings were taken after a 15 minute developing time, measuring the absorbancy at 520 mμ in a Beckman DU Spectrophotometer. The final concentration

of SO_2 in the reaction mixture was 2.5 ppm.

Preincubation experiments with cherry juice were carried out in the exact manner as indicated above, with the exception that the SO_2 concentrations were changed to 11.7 ppm in the enzyme-SO_2 mixture and 2.9 ppm in the final reaction mixture for the first experiment and to 16.8 ppm and 3.2 ppm for the second experiment.

The zero time reading was multiplied by a factor of 0.8 to correct for the dilution of the reaction mixture by the addition of the 1.2 ml. of enzyme-SO_2 mixture.

Tyrosine-Tyrosinase-Sulfur Dioxide

These experiments were conducted with a Beckman D U Spectrophotometer equipped with a Gilford cuvette changer and automatic recorder. The reactions were carried out in one cm. matched cuvettes. Two reactions and a blank were run simultaneously. All three cuvettes contained 1.0 ml. of 1.0 mM tyrosine, and 1.0 ml. of 0.5 M phosphate buffer, pH 6.5. The blank (first) cuvette contained 0.9 ml. of SO_2 solution and 0.1 ml. of water. The second cuvette contained 0.9 ml. of distilled water, while the third cuvette contained 0.9 ml. of SO_2 solution of the same concentration as the blank. To the second and third cuvettes, 0.1 ml. of enzyme solution, containing 0.5 mg. tyrosinase/ml. were added simultaneously. The recorder was started and the reaction was followed at the wavelength of 280 mμ.

Catechol-Tyrosinase-Sulfur Dioxide

These reactions were carried out in one cm. matched cuvettes. Two reactions and a blank were run simultaneously. The blank (first) cuvette contained 1.0 ml. of 0.5 M phosphate buffer, pH 6.5, 1.1 ml. of water, and 0.9 ml. of SO_2. The second cuvette contained 1.0 ml. of buffer, 1.0 ml. of catechol, and 0.9 ml. of SO_2 solution of the desired concentration. To the second and third cuvettes 0.1 ml. of enzyme solution, containing 0.1 mg. enzyme/ml. were added simultaneously at zero time. The recorder was started and the reaction was followed at the wavelength of 420 mμ. Various SO_2 concentrations were employed, using 0.2 mM catechol as a substrate.

Preincubation Experiments with Tyrosinase and Sulfur Dioxide Using Tyrosine and Catechol

For these experiments 1.8 ml. of SO_2 solution of the desired concentration was allowed to incubate in a 5 ml. beaker with 0.2 ml. of enzyme solution, containing 0.5 mg. tyrosinase/ml. for 30 minutes, with constant stirring. After the allotted time, 1.0 ml. of the mixture was transferred to a cuvette containing 1.0 ml. of 0.5 M phosphate buffer, pH 6.5, and 1.0 ml. of 1 mM tyrosine. The reaction was followed at the wavelength of 280 mμ. These results were compared with the no preincubation experiments.

Preincubation experiments were also conducted using 0.2 mM catechol instead of tyrosine as the substrate. In this case the enzyme solution was diluted to give 0.01 mg.

enzyme/ml. water. The reaction was followed at the wavelength of 420 mμ. These results were also compared with the no preincubation experiments.

RESULTS AND DISCUSSION

Mecocyanin Pigment

The results on the effect of SO_2 concentration on the rate of mecocyanin degradation by tyrosinase at various pH levels are presented in Appendix Tables I, II, and III, and they are graphically summarized in Figure 1. Presented in tabular form are the absorbancy readings at the two pH levels used for the estimation of anthocyanin concentration, their difference corrected for the dilution by the enzyme addition, and the amounts of pigment destroyed in absorbancy units. From these data graphs were drawn (not shown) to determine the initial rate of pigment destruction per minute, in absorbancy units. These rates were then plotted in Figure 1 as the rate of mecocyanin decolorization versus SO_2 concentration in ppm.

The rate of mecocyanin decolorization, at pH 6.5, was highest with no SO_2 present. The reaction rate decreased slightly with 2 ppm SO_2. At a concentration of 4 ppm SO_2, the decolorization rate decreased to approximately one-third the rate as compared to the no SO_2 level. Very little decolorization occurred at the 6 and 8 ppm SO_2 levels.

At pH 5.5, the highest rate of mecocyanin degradation occurred with no SO_2 present. Using a concentration of 4

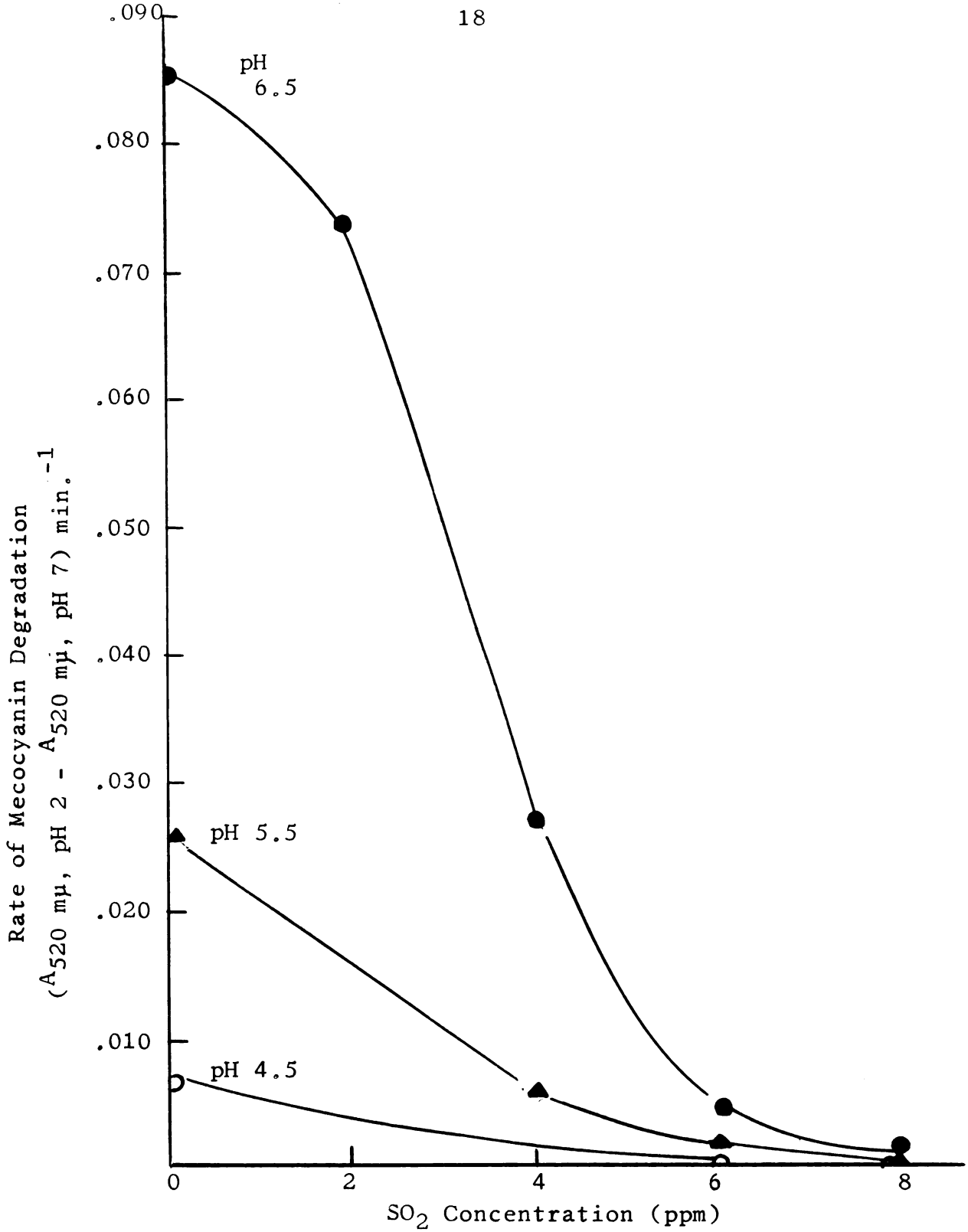

Figure 1. Effect of various levels of SO_2 concentration on the rate of mecocyanin degradation by tyrosinase.

ppm SO_2 the reaction rate decreased to approximately one-quarter of the rate at the no SO_2 level. A level of 6 ppm SO_2 showed a very low decolorization rate, while 8 ppm SO_2 stopped the reaction completely for the period of two minutes during which the reaction was followed.

The reaction was completely inhibited by 6 ppm SO_2 at pH 4.5.

While the concentration of SO_2 played an important role in determining the rate of mecocyanin degradation at any one pH level, it is evident from Figure 1 that pH also played an important role in determining the amount of enzymic decolorization of the pigment. Less SO_2 was needed to decrease the decolorization rate at the lower pH levels.

Tyrosinase has been shown to have an optimum pH range of 5.0 to 7.8 (30). Yasunobu (30) showed that the variation of activity with pH is due to a change of ionization of the enzyme and not the substrate (catechol), since the substrate did not ionize in the pH range studied (pH 5 to 7.8).

Ingraham (11) pointed out that at pH levels much below 4.0, the tyrosinase enzyme was not active. This work is in agreement with Ingraham's.

To show that SO_2 at the concentration level of 10 ppm could inhibit the enzymic destruction of the mecocyanin pigment, at the optimum pH 6.5, for longer periods than two minutes, the reaction was followed for 75 minutes. No decolorization was observed during this extended period (Table I).

Preincubation experiments of enzyme and SO_2 showed that 11 ppm of SO_2 in the enzyme-SO_2 mixture was adequate to inhibit the subsequent mecocyanin decolorization, at pH 6.5, even though the concentration of SO_2 in the reaction mixture was only 2.5 ppm (Table IV). The reaction was followed for one hour. From this it can be concluded that the enzyme was quite sensitive to SO_2.

Comparing the results of the no preincubation experiment at pH 6.5, and 2.5 ppm of SO_2, shown in Figure 1, with the results of the preincubation experiment at pH 6.5 and 2.5 ppm SO_2 shown in Table IV, it is apparent that there was no decolorization of the mecocyanin pigment with preincubation while there was a decolorization rate of 0.063 absorbancy units per minute with the no preincubation experiments.

The question of other substances in the reaction mixture, competing for the SO_2 with the enzyme, or reacting with it, now becomes evident. The proposed mechanism for the destruction of anthocyanin pigment was that tyrosinase with oxygen oxidased the catechol to o-benzoquinone, and the quinone destroyed the pigment non-enzymatically (24). Ponting (21) has shown in his work on apple juice that if SO_2 was added after some o-benzoquinone had formed, the SO_2 was readily oxidized by the o-benzoquinone and browning resulted. Extending this reasoning, if SO_2 is present from the beginning of the reaction in very small quantities, o-benzoquinone may be formed and oxidize the SO_2 before the latter has the chance to inactivate the enzyme. Larger quantities of SO_2,

however, may inactivate the enzyme even if part of the former had been oxidized by any o-benzoquinone that may have been formed.

Cherry Juice

The results of experiments with cherry juice are given in Tables V, VI, VII, VIII, and Figure 2. Before complete inhibition of the enzyme resulted in cherry juice, the SO_2 concentration had to be tripled over that used in the pure mecocyanin experiments. This was probably due to sugars and other carbonyl compounds present which combined with SO_2 (7). Since it is only the free SO_2 which inactivates the enzyme, more SO_2 was required to compensate for that SO_2 bound by the sugars and other carbonyl compounds.

The highest rate of anthocyanin decolorization occurred at pH 6.5 with no SO_2 present. At a level of 5 ppm of SO_2 the decolorization rate was decreased to approximately one-half of the rate at the no SO_2 level. Concentrations of 15 and 20 ppm SO_2 further decreased the decolorization rate, and 25 ppm SO_2 stopped the reaction completely for the 75 minutes of observation.

The results at pH 5.5 were not very different from those obtained at pH 6.5. A possible explanation for the similarity of these data is that there is a broad pH optimum range for the tyrosinase oxidation of the juice substrates and pH 5.5 and 6.5 are within this range.

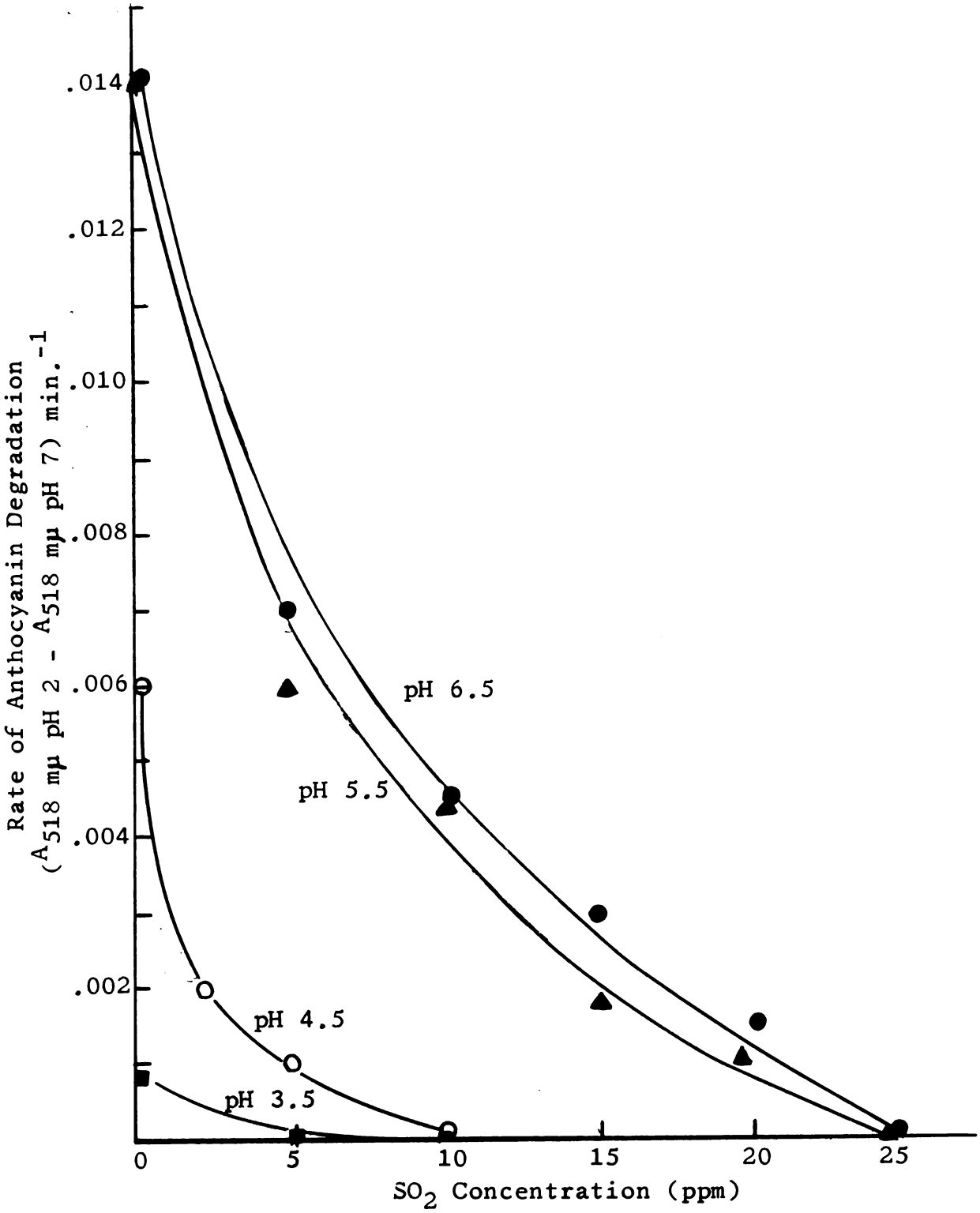

Figure 2. Effect of various levels of SO_2 concentration on the rate of cherry juice anthocyanin degradation by tyrosinase.

At pH 4.5, the highest rate of anthocyanin decolorization was obtained with no SO_2 present. At a level of 2 ppm SO_2 the decolorization rate decreased to one-third the rate of the no SO_2 level. A concentration of 5 ppm SO_2 decreased the rate even further, while 10 ppm SO_2 almost completely inhibited the degradation when the reaction was followed for 75 minutes.

At pH 3.5 duplicate results were difficult to obtain since the decolorization reaction was very slow. An average of four runs were taken to obtain the results of the no SO_2 level. A concentration of 5 ppm SO_2 almost completely stopped the reaction when followed for 75 minutes.

What is meant by complete inhibition holds true only for the time of observation. In cases where 25 to 30 ppm SO_2 were added to the juice, at pH 6.5, complete inhibition of the degradation reaction was observed for the 75 minutes of observation. However, when 30 ppm SO_2 was added to the juice, at pH 6.5, and the reaction was followed for six hours, a small but noticeable decolorization rate of 0.004 absorbancy units per hour was obtained after the second hour of observation (Table V). The no SO_2 sample at pH 6.5 showed a rate of 0.014 absorbancy units per minute during the ten minutes of observation.

During the above experiments, two blanks were also followed for six hours. One blank contained the usual reaction mixture containing SO_2, except that water was added in place of enzyme. This blank showed that no decolorization

took place due to the direct effect of SO_2 on the pigment even after six hours of observation. This may appear contrary to the common experience of SO_2 bleaching of fruits pigmented with anthocyanins. It appears, therefore, that a certain minimum quantity of SO_2 is necessary for the bleaching effect to occur and the concentrations of SO_2 used in this work were below this minimum. The second blank contained no SO_2 and no enzyme. Water replaced the latter two substances. This blank showed that there was no decolorization due to non-enzymatic degradation of the juice during the six hours of observation (Table 5).

Preincubation experiments with enzyme and SO_2 for the cherry juice, showed that 11.7 ppm of SO_2 in the enzyme-SO_2 mixture did not stop the subsequent reaction in the final reaction mixture which contained 2.9 ppm SO_2. This was in contrast to the results obtained with the mecocyanin pigment. It took 16.8 ppm SO_2, in the enzyme-SO_2 mixture, to stop the reaction in the final mixture which contained 3.2 ppm SO_2, when the reaction was followed for 75 minutes. These results indicated that even though the enzyme was preincubated with SO_2 for 30 minutes, the final reaction depended upon the substances present in the reaction mixture. Since the cherry juice contained sugars and other carbonyl compounds, it may have been these substances which "pulled" the SO_2 away from the enzyme. Results appear in Table IX.

Tyrosine-Tyrosinase-Sulfur Dioxide

Results of these experiments are presented in Figure 3. At an SO_2 concentration of 3.6 ppm, the reaction was completely stopped when followed for 75 minutes. At lower levels, there was a lag period followed by a small increase in the reaction, and finally the reaction proceeded as with the no SO_2 sample. The straight line portions of the graph then began to level off (this portion not shown) and became parallel to the time axis. The reaction rates were almost the same for the 0.9 and 1.8 ppm SO_2 levels. Lag phases of the graph may be explained as was previously stated for the mecocyanin pigment. If there was not an excess of SO_2 to completely inhibit the enzyme, then some DOPA quinone was formed which oxidized the SO_2 during the lag phase.

When enzyme and SO_2 were preincubated with 5.4 ppm SO_2 in the enzyme-SO_2 mixture, and 1.8 ppm SO_2 in the final reaction mixture, no reaction took place when followed for 40 minutes.

Catechol-Tyrosinase-Sulfur Dioxide

Results of these experiments appear in Figure 4. At an SO_2 concentration of 4 ppm, the reaction was completely inhibited when followed for 30 minutes. At lower concentrations a lag phase was noticed and then the reaction proceeded. In contrast to the experiment with tyrosine, the reaction rates were not the same for all levels of SO_2 concentrations.

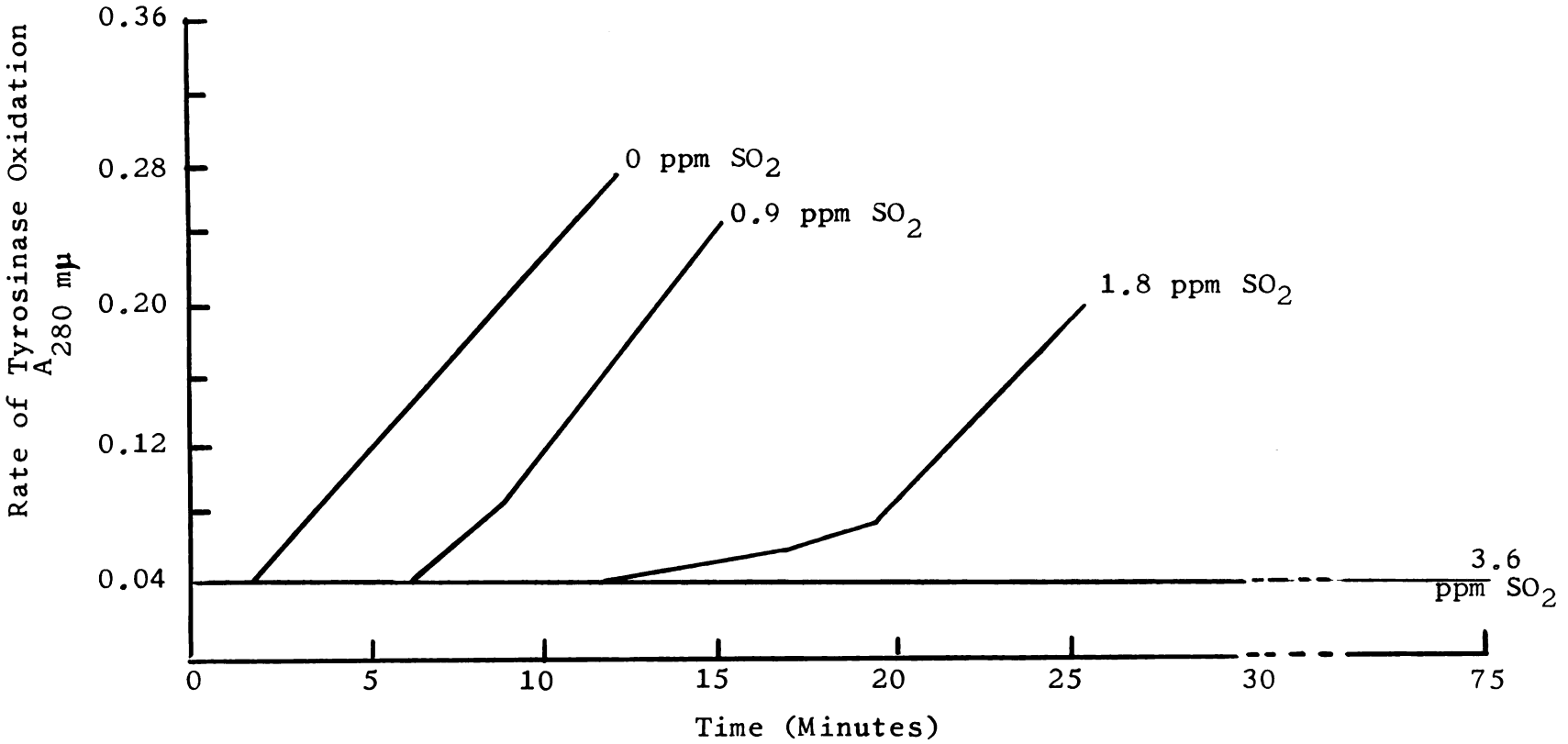

Figure 3. Effect of various levels of SO_2 concentration on the rate of catechol oxidation by tyrosinase (1 mM tyrosinase, 0.5 mg. tyrosinase/ml., 0, 0.9, 1.8, and 3.6 ppm SO_2, 20°C.). (Redrawn from automatic recordings.)

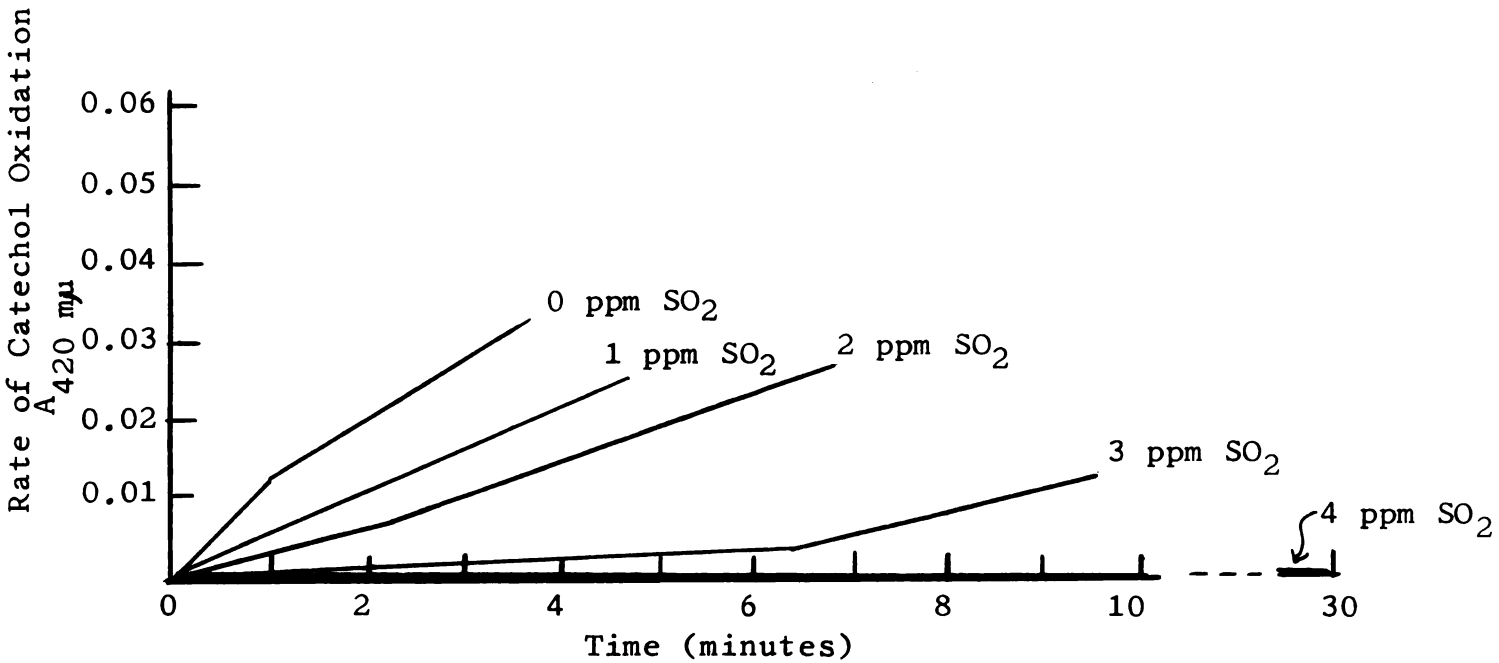

Figure 4. Effect of various levels of SO_2 concentration on the rate of catechol oxidation by tyrosinase (0.2 mM catechol, 0.01 mg. tyrosinase/ml., 0, 1, 2, 3, and 4 ppm SO_2, 25°C.). (Redrawn from automatic recordings.)

Preincubation of enzyme and SO_2 with 5.4 ppm SO_2 in the enzyme-SO_2 mixture, and 1.8 ppm in the final reaction mixture gave no reaction when followed for 30 minutes.

SUMMARY AND CONCLUSIONS

The effect of various concentrations of SO_2 on the enzymic decolorization of the purified mecocyanin pigment was studied at various pH levels. At pH 6.5, which was in the optimum range of the reaction, the rate of enzymic decolorization of mecocyanin was decreased rapidly as the concentration of SO_2 was increased to 8 ppm. The decolorization rates at the lower pH levels of 5.5 and 4.5 with no SO_2 added, were less than the rate at pH 6.5. The same trend of decreased mecocyanin decolorization as the concentration of SO_2 was increased to 8 ppm was noticed for the lower pH levels. Preincubation of SO_2 with enzyme further decreased the degradation rate of the mecocyanin pigment in a model system.

In studying the effects of various concentrations of SO_2 on the enzymic decolorization of the cherry juice at various pH levels, the same trend was noticed as with the purified mecocyanin pigment. However, the concentration of SO_2 had to be increased to 25 ppm before inhibition resulted during the period of observation. More SO_2 was required for the cherry juice presumably because of the reaction of SO_2 with carbonyl compounds of the juice.

If the enzyme and SO_2 were preincubated, lower levels of SO_2 had to be added to the juice in order to obtain inhibition similar to that of no preincubation experiments.

The enzymic oxidation of tyrosine by tyrosinase was shown to proceed rapidly with no SO_2 present. At a concentration of 3.6 ppm SO_2, the reaction was completely inhibited for the time period during which the reaction was followed. Lower levels of SO_2 inhibited the reaction for a short period of time, and then the reaction proceeded at the same rate as the no SO_2 reaction. Preincubation of enzyme and SO_2 showed that the subsequent enzymic oxidation of tyrosine was completely inhibited for the time period during which the reaction was followed.

Experiments with catechol showed that a concentration of 4 ppm SO_2 completely stopped the enzymic oxidation of catechol for the time period observed. Lower levels of SO_2 inhibited the reaction for a short period of time and then the reaction proceeded at different rates depending upon the concentration of SO_2 used. Preincubation of enzyme and SO_2 showed that the subsequent enzymic oxidation of catechol was completely inhibited for the time period observed.

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APPENDIX

TABLE I

EFFECT OF SULFUR DIOXIDE CONCENTRATION ON THE RATE OF MECOCYANIN DEGRADATION BY TYROSINASE AT pH 6.5

Time (Min.)	A_{520} mμ (pH 2)	A_{520} mμ (pH 7)	A_{520} mμ Difference (pH 2 - pH 7)	Mecocyanin Degrad. in A_{520} Units	A_{520} mμ (pH 2)	A_{520} mμ (pH 7)	A_{520} mμ Difference (pH 2 - pH 7)	Mecocyanin Degrad. in A_{520} Units
			No SO_2					
0	0.149	0.026	0.118*	0 .000	0.138	0.014	0.119*	0.000
1/2	0.099	0.028	0.071	0.047	0.096	0.019	0.077	0.042
1	0.064	0.027	0.037	0.081	0.053	0.020	0.033	0.086
2	0.044	0.026	0.018	0.100	0.028	0.019	0.009	0.110
			2 ppm SO_2					
0	0.137	0.009	0.124*	0.000	0.165	0.026	0.134*	0.000
1/2	0.098	0.011	0.087	0.037	0.124	0.028	0.096	0.038
1	0.073	0.011	0.062	0.062	0.086	0.029	0.057	0.077
2	0.044	0.010	0.034	0.090	0.053	0.029	0.024	0.110

*Corrected Value

TABLE I (continued)

Time (Min.)	$A_{520\ m\mu}$ (pH 2)	$A_{520\ m\mu}$ (pH 7)	$A_{520\ m\mu}$ Difference (pH 2 - pH 7)	Mecocyanin Degrad. in A_{520} Units	$A_{520\ m\mu}$ (pH 2)	$A_{520\ m\mu}$ (pH 7)	$A_{520\ m\mu}$ Difference (pH 2 - pH 7)	Mecocyanin Degrad. in A_{520} Units
			4 ppm SO_2					
0	0.156	0.029	0.123*	0.000	0.163	0.029	0.129*	0.000
1/2	0.135	0.028	0.107	0.016	0.144	0.029	0.115	0.014
1	0.129	0.031	0.098	0.025	0.131	0.029	0.102	0.027
2	0.106	0.027	0.079	0.044	0.094	0.029	0.065	0.064
			6 ppm SO_2					
0	0.169	0.026	0.137*	0.000	0.151	0.024	0.122*	0.000
1/2	0.159	0.026	0.133	0.004	0.142	0.024	0.118	0.004
1	0.159	0.027	0.132	0.005	0.145	0.026	0.119	0.003
2	0.153	0.026	0.127	0.010	0.139	0.025	0.114	0.008
			8 ppm SO_2					
0	0.166	0.029	0.132*	0.000	0.158	0.024	0.129*	0.000
1/2	0.156	0.027	0.129	0.003	0.152	0.027	0.125	0.004
1	0.159	0.027	0.132	0	0.155	0.027	0.128	0.001
2	0.154	0.024	0.130	0.002	0.154	0.028	0.126	0.003

*Corrected Values

TABLE I (continued)

Time (Min.)	$A_{520\ m\mu}$ (pH 2)	$A_{520\ m\mu}$ (pH 7)	$A_{520\ m\mu}$ Difference (pH 2 - pH 7)	Mecocyanin Degrad. in A_{520} Units	$A_{520\ m\mu}$ (pH 2)	$A_{520\ m\mu}$ (pH 7)	$A_{520\ m\mu}$ Difference (pH 2 - pH 7)	Mecocyanin Degrad. in A_{520} Units
			10 ppm SO_2					
0	0.179	0.029	0.145*	0.000				
2	0.177	0.031	0.146	-0.001				
5	0.179	0.032	0.147	-0.002				
10	0.182	0.039	0.143	+0.002				
30	0.184	0.036	0.148	+0.003				
75	0.193	0.042	0.151	-0.006				

*Corrected Values

TABLE II

EFFECT OF SULFUR DIOXIDE CONCENTRATION ON THE RATE OF MECOCYANIN DEGRADATION BY TYROSINASE AT pH 5.5

Time (Min.)	$A_{520\ m\mu}$ (pH 2)	$A_{520\ m\mu}$ (pH 7)	$A_{520\ m\mu}$ Difference (pH 2 - 7 pH)	Mecocyanin Degrad. in A Units	$A_{520\ m\mu}$ (pH 2)	$A_{520\ m\mu}$ (pH 7)	$A_{520\ m\mu}$ Difference (pH 2 - 7 pH)	Mecocyanin Degrad. in A Units 520 mu
				No SO_2				
0	0.169	0.022	0.142*	0.000	0.168	0.023	0.140*	0.000
1/2	0.159	0.029	0.130	0.012	0.154	0.027	0.127	0.013
1	0.141	0.026	0.115	0.027	0.140	0.026	0.114	0.026
2	0.112	0.026	0.086	0.056	0.112	0.026	0.086	0.054
				4 ppm SO_2				
0	0.178	0.026	0.147*	0.000	0.167	0.025	0.137*	0.000
1/2	0.170	0.027	0.143	0.004	0.161	0.026	0.135	0.002
1	0.169	0.027	0.142	0.005	0.159	0.027	0.132	0.005
2	0.162	0.027	0.135	0.012	0.151	0.027	0.124	0.013

*Corrected Value

TABLE II (continued)

Time (Min.)	$A_{520\ m\mu}$ (pH 2)	$A_{520\ m\mu}$ (pH 7)	$A_{520\ m\mu}$ Difference (pH 2 - pH 7)	Mecocyanin Degrad. in ^{A}Units	$A_{520\ m\mu}$ (pH 2)	$A_{520\ m\mu}$ (pH 7)	$A_{520\ mu}$ Difference (pH 2 - 7 pH)	Mecocyanin Degrad. in ^{A} Units 520 mμ
				6 ppm SO_2				
0	0.174	0.025	0.144*	0.000	0.166	0.022	0.139*	0.000
1/2	0.168	0.026	0.142	0.002	0.159	0.023	0.136	0.003
1	0.169	0.028	0.141	0.003	0.162	0.024	0.138	0.001
2	0.165	0.027	0.138	0.006	0.156	0.002	0.134	0.005
				8 ppm SO_2				
0	0.174	0.024	0.145*	0.000	0.170	0.022	0.143*	0.000
1/2	0.171	0.027	0.144	0.001	0.165	0.026	0.139	0.004
1	0.171	0.026	0.145	0	0.167	0.026	0.141	0.002
2	0.169	0.026	0.143	0.002	0.165	0.025	0.140	0.003

*Corrected Value

TABLE III

EFFECT OF SULFUR DIOXIDE CONCENTRATION ON THE RATE OF MECOCYANIN DEGRADATION BY TYROSINASE AT pH 4.5

Time (Min.)	$A_{520\ m\mu}$ (pH 2)	$A_{520\ m\mu}$ (pH 7)	$A_{520\ m\mu}$ Difference (pH 2 - pH 7)	Mecocyanin Degrad. in A_{520} Units	$A_{520\ m\mu}$ (pH 2)	$A_{520\ m\mu}$ (pH 7)	$A_{520\ m\mu}$ Difference (pH 2 - pH 7)	Mecocyanin Degrad. in A_{520} Units
				No SO_2				
0	0.190	0.024	0.161*	0.000	0.192	0.026	0.161*	0.000
2-1/2	0.169	0.028	0.141	0.020	0.173	0.031	0.142	0.019
5	0.150	0.029	0.121	0.040	0.156	0.032	0.124	0.037
7	0.137	0.029	0.108	0.053	0.139	0.029	0.110	0.051
				4 ppm SO_2				
0	0.153	0.022	0.126*	0.000	0.148	0.024	0.117*	0.000
5	0.143	0.024	0.119	0.007	0.130	0.022	0.108	0.009
10	0.123	0.024	0.099	0.027	0.113	0.023	0.090	0.027
15	0.106	0.023	0.083	0.043	0.095	0.023	0.072	0.045

*Corrected Values

TABLE III (continued)

Time (Min.)	A_{520} mμ (pH 2)	A_{520} mμ (pH 7)	A_{520} mμ Difference (pH 2 - pH 7)	Mecocyanin Degrad. in A_{520} Units	A_{520} mμ (pH 2)	A_{520} mμ (pH 7)	A_{520} mμ Difference (pH 2 - pH 7)	Mecocyanin Degrad. in A_{520} Units
				6 ppm SO_2				
0	0.157	0.022	0.130*	0 .000	0.154	0.19	0.130*	0.000
5	0.151	0.022	0.129	0.001	0.149	0.21	0.128	0.002
10	0.147	0.021	0.126	0.004	0.144	0.21	0.123	0.007
15	0.142	0.022	0.120	0.010	0.143	0.21	0.122	0.008
				8 ppm SO_2				
0	0.149	0.023	0.121*	0.000	0.173	0.026	0.142*	0.000
2-1/2	0.147	0.024	0.123	-0.002	0.173	0.028	0.145	-0.003
5	0.144	0.023	0.121	0.000	0.171	0.029	0.142	0.000
7	0.143	0.023	0.120	0.001	0.170	0.027	0.143	-0.003

*Corrected Value

TABLE IV

EFFECT OF PREINCUBATION OF SULFUR DIOXIDE AND ENZYME ON THE RATE OF MECOCYANIN DEGRADATION BY TYROSINASE AT pH 6.4
(SO_2 and enzyme preincubated for 30 minutes)

Concentration of SO_2 in Reaction Mixture --------- 2.5 ppm

Concentration of SO_2 in Enzyme-SO_2 Mixture ------- 11.0 ppm

Time (Min.)	A_{520} mμ (pH 2)	A_{520} mμ (pH 7)	A_{520} mμ Difference (pH 2 - pH 7)	Mecocyanin Degrad. in A_{520} Units	A_{520} mμ (pH 2)	A_{520} mμ (pH 7)	A_{520} mμ Difference (pH 2 - pH 7)	Mecocyanin Degrad. in A_{520} Units
0	0.221	0.032	0.152*	0.000	0.232	0.038	0.155*	0.000
15	0.186	0.031	0.155	-0.003	0.195	0.031	0.164	-0.009
30	0.182	0.032	0.150	0.002	0.193	0.032	0.161	-0.011
60	0.178	0.035	0.143	0.009	0.192	0.035	0.157	-0.002

*Corrected Value

TABLE V

EFFECT OF SULFUR DIOXIDE CONCENTRATION ON THE RATE OF ANTHOCYANIN DEGRADATION BY TYROSINASE AT pH 6.5

Time (Min.)	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units
				No SO_2				
0	0.206	0.039	0.161*	0.000	0.209	0.036	0.167*	0.000
1/2	0.194	0.044	0.150	0.011	--	--	--	--
1	0.190	0.044	0.146	0.015	0.191	0.038	0.153	0.014
2	0.179	0.046	0.133	0.028	0.181	0.041	0.140	0.027
4	--	--	--	--	0.165	0.044	0.121	0.046
5	0.154	0.049	0.105	0.056	--	--	--	--
8	--	--	--	--	0.139	0.049	0.090	0.077
10	0.124	0.057	0.067	0.094	0.126	0.053	0.073	0.094
				5 ppm SO_2				
0	0.225	0.035	0.184*	0.000	0.225	0.037	0.182*	0.000
2	0.219	0.041	0.178	0.006	0.223	0.044	0.179	0.003
3	0.215	0.042	0.173	0.011	0.216	0.044	0.172	0.010
4	0.205	0.044	0.161	0.023	0.211	0.044	0.167	0.015
5	0.200	0.044	0.156	0.028	0.207	0.046	0.161	0.021

*Corrected Value

TABLE V (continued)

Time (Min.)	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units
				10 ppm SO_2				
0	0.219	0.034	0.179*	0.000	0.220	0.030	0.184*	0.000
10	0.205	0.043	0.162	0.017	0.208	0.041	0.167	0.017
15	0.184	0.046	0.138	0.041	0.186	0.043	0.143	0.041
20	0.169	0.049	0.120	0.059	0.170	0.045	0.125	0.059
25	0.147	0.052	0.095	0.084	--	--	--	--
				15 ppm SO_2				
0	0.201	0.041	0.155*	0.000	0.196	0.038	0.153*	0.000
15	0.206	0.056	0.150	0.005	0.201	0.050	0.151	0.002
30	0.170	0.060	0.110	0.045	0.169	0.056	0.113	0.045
45	0.126	0.065	0.061	0.094	0.120	0.064	0.056	0.094
60	0.092	0.074	0.018	0.137	0.089	0.075	0.014	0.137
75	0.078	0.097	-0.029	0.184	0.077	0.087	0.010	0.163
				20 ppm SO_2				
0	0.191	0.037	0.149*	0.000	0.190	0.035	0.150*	0.000
15	0.196	0.047	0.149	0	0.195	0.047	0.148	0.002
30	0.193	0.052	0.141	0.008	0.196	0.051	0.145	0.005
45	0.185	0.057	0.128	0.021	0.181	0.049	0.132	0.018
60	0.164	0.060	0.100	0.049	0.162	0.056	0.106	0.044
75	0.146	0.067	0.079	0.070	0.144	0.062	0.082	0.068

*Corrected Values

TABLE V (continued)

Time (Min.)	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units
			25 ppm SO_2					
0	0.196	0.030	0.160*	0.000	0.200	0.029	0.165*	0.000
15	0.199	0.039	0.160	0.000	0.200	0.037	0.163	0.002
30	0.203	0.046	0.157	0.003	0.206	0.043	0.163	0.002
45	0.209	0.051	0.158	0.002	0.209	0.047	0.162	0.003
60	0.217	0.055	0.162	-0.002	0.217	0.051	0.166	-0.001
75	0.225	0.060	0.165	-0.005	0.218	0.056	0.162	0.003
			30 ppm SO_2					
(Hrs.)								
0	0.181	0.026	0.150*	0.000	0.180	0.023	0.149*	0.000
2	0.189	0.042	0.147	0.003	0.189	0.040	0.149	0.000
3	0.189	0.046	0.143	0.007	0.189	0.044	0.145	0.004
4	0.184	0.048	0.136	0.014	0.188	0.047	0.141	0.008
5	0.181	0.051	0.130	0.020	0.191	0.049	0.142	0.007
6	0.181	0.052	0.029	0.021	0.187	0.050	0.137	0.012
	30 ppm SO_2, no enzyme added				No enzyme and no SO_2 added			
0	0.188	0.023	0.159*	0.000	0.194	0.037	0.152*	0.000
2	0.189	0.032	0.157	0.002	0.184	0.028	0.156	-0.004
3	0.195	0.036	0.159	0.000	0.186	0.028	0.158	-0.006
4	0.197	0.037	0.160	-0.001	0.186	0.029	0.157	-0.005
5	0.197	0.038	0.159	0.000	0.183	0.029	0.154	0.002
6	0.201	0.043	0.158	0.001	0.185	0.030	0.155	-0.003

*Corrected Value

TABLE VI

EFFECT OF SULFUR DIOXIDE CONCENTRATION ON THE RATE OF ANTHOCYANIN DEGRADATION BY TYROSINASE AT pH 5.5

Time (Min.)	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units
			No SO_2					
0	0.219	0.040	0.173*	0.000	0.219	0.051	0.162*	0.000
1/2	0.209	0.042	0.167	0.006	0.206	0.051	0.155	0.007
1	0.204	0.044	0.160	0.013	0.198	0.052	0.146	0.016
2	0.192	0.046	0.146	0.027	0.185	0.051	0.134	0.028
4	0.172	0.047	0.125	0.048	0.169	0.053	0.116	0.046
5	--	--	--	--	0.160	0.052	0.108	0.054
			5 ppm SO_2					
0	0.238	0.031	0.200*	0.000	0.236	0.032	0.197*	0.000
3	0.226	0.041	0.185	0.015	0.225	0.040	0.185	0.012
6	0.204	0.043	0.161	0.039	0.205	0.044	0.161	0.036
9	0.191	0.044	0.147	0.053	0.186	0.044	0.142	0.055
12	0.172	0.045	0.127	0.073	--	--	--	--

*Corrected Value

TABLE VI (continued)

Time (Min.)	$A_{518\ m\mu}$ (pH 2.0	$A_{518\ m\mu}$ (pH 7)	$A_{518\ m\mu}$ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units	$A_{518\ m\mu}$ (pH 2)	$A_{518\ m\mu}$ (pH 7)	$A_{518\ m\mu}$ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units
				10 ppm SO_2				
0	0.231	0.027	0.197*	0.000	0.235	0.031	0.197*	0.000
5	0.226	0.039	0.187	0.010	--	--	--	--
10	0.214	0.041	0.173	0.024	0.215	0.042	0.173	0.024
15	0.195	0.042	0.153	0.044	0.192	0.043	0.149	0.048
20	0.173	0.044	0.129	0.068	0.171	0.043	0.128	0.069
25	--	--	--	--	0.149	0.044	0.105	0.092
				15 ppm SO_2				
0	0.196	0.022	0.168*	0.000	0.201	0.023	0.172*	0.000
15	0.196	0.036	0.160	0.008	0.201	0.039	0.162	0.010
30	0.169	0.036	0.133	0.035	0.171	0.037	0.134	0.038
45	0.124	0.039	0.085	0.083	0.133	0.039	0.094	0.078
60	0.094	0.045	0.049	0.119	0.106	0.042	0.064	0.108
75	0.075	0.052	0.023	0.145	0.089	0.048	0.041	0.131

*Corrected Value

TABLE VI (continued)

Time (Min.)	$A_{518\ m\mu}$ (pH 2)	$A_{518\ m\mu}$ (pH 7)	$A_{518\ m\mu}$ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units	$A_{518\ m\mu}$ (pH 2)	$A_{518\ m\mu}$ (pH 7)	$A_{518\ m\mu}$ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units
				20 ppm SO_2				
0	0.225	0.026	0.192*	0.000	0.233	0.026	0.200	0.000
45	0.231	0.044	0.187	0.005	0.230	0.041	0.189	0.011
60	0.225	0.038	0.187	0.005	0.212	0.042	0.170	0.030
75	0.204	0.041	0.163	0.029	0.192	0.042	0.150	0.050
				25 ppm SO_2				
0	0.192	0.022	0.166*	0.000	0.189	0.021	0.162*	0.000
5	0.190	0.027	0.163	0.003	0.190	0.026	0.164	-0.002
15	0.191	0.033	0.158	0.008	0.196	0.033	0.163	-0.001
30	0.200	0.039	0.161	0.005	0.204	0.039	0.165	-0.003
45	0.204	0.043	0.161	0.005	0.204	0.045	0.159	0.003
60	0.212	0.046	0.166	0.000	0.211	0.047	0.164	-0.002

*Corrected Value

TABLE VII

EFFECT OF SULFUR DIOXIDE CONCENTRATION ON THE RATE OF ANTHOCYANIN DEGRADATION OF TYROSINASE AT pH 4.5

Time (Min.)	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units
			No SO_2					
0	0.179	0.024	0.150*	0.000	0.181	0.025	0.151*	0.000
1	0.171	0.028	0.143	0.007	0.172	0.027	0.145	0.006
4	0.152	0.030	0.122	0.028	0.156	0.030	0.126	0.025
6	0.145	0.031	0.114	0.036	0.148	0.031	0.117	0.034
10	0.130	0.032	0.098	0.052	0.134	0.032	0.102	0.049
15	0.121	0.033	0.088	0.062	0.126	0.034	0.092	0.059
			2 ppm SO_2					
0	0.177	0.022	0.150*	0.000	0.179	0.026	0.148*	0.000
1	0.173	0.029	0.144	0.006	0.174	0.031	0.143	0.005
4	0.170	0.027	0.143	0.007	0.172	0.031	0.141	0.007
6	0.166	0.029	0.137	0.013	0.164	0.030	0.134	0.014
10	0.158	0.029	0.129	0.021	0.157	0.032	0.125	0.023
15	0.147	0.029	0.118	0.032	0.149	0.031	0.118	0.030

*Corrected Value

TABLE VII (continued)

Time (Min.)	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units
			5 ppm SO_2					
0	0.173	0.025	0.144*	0.000	0.176	0.025	0.146*	0.000
15	0.172	0.032	0.140	0.004	0.171	0.032	0.139	0.007
30	0.160	0.031	0.129	0.015	0.154	0.031	0.123	0.023
45	0.149	0.032	0.117	0.027	0.141	0.033	0.108	0.038
60	0.139	0.036	0.103	0.041	0.132	0.035	0.097	0.049
75	0.138	0.039	0.099	0.045	0.127	0.037	0.090	0.056
			10 ppm SO_2					
0	0.181	0.026	0.150*	0.000	0.176	0.026	0.145*	0.000
15	0.179	0.042	0.137	0.008	0.173	0.043	0.130	0.015
30	0.179	0.041	0.138	0.007	0.178	0.041	0.137	0.008
45	0.184	0.042	0.142	0.008	0.177	0.044	0.133	0.012
60	0.186	0.042	0.144	0.006	0.179	0.042	0.137	0.008
75	0.189	0.045	0.144	0.006	0.183	0.049	0.134	0.011
(Hrs.)			10 ppm SO_2					
0	0.188	0.025	0.158*	0.000	0.188	0.024	0.158*	0.000
2	0.189	0.044	0.145	0.013	0.186	0.039	0.147	0.011
3	0.189	0.049	0.140	0.018	0.185	0.036	0.149	0.009
4	0.190	0.045	0.145	0.013	0.186	0.035	0.151	0.007
5	0.191	0.049	0.142	0.016	0.187	0.036	0.151	0.007
6	0.195	0.049	0.146	0.012	0.189	0.036	0.153	0.005

*Corrected Value

TABLE VIII

EFFECT OF SULFUR DIOXIDE CONCENTRATION ON THE RATE OF ANTHOCYANIN DEGRADATION BY TYROSINASE AT pH 3.5

Time (Min.)	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units
				No SO_2				
0	0.199	0.030	0.163*	0.000	0.204	0.034	0.164*	0.000
1	0.195	0.032	0.163	0.000	0.197	0.036	0.161	0.003
4	0.194	0.032	0.162	0.001	0.194	0.036	0.158	0.006
6	0.192	0.031	0.161	0.002	0.194	0.035	0.159	0.005
10	0.188	0.033	0.155	0.008	0.191	0.036	0.155	0.009
15	0.186	0.034	0.152	0.011	0.191	0.036	0.155	0.009
				No SO_2				
0	0.197	0.030	0.161*	0.000	0.194	0.024	0.164*	0.000
1	0.191	0.032	0.159	0.002	0.189	0.032	0.157	0.007
4	0.189	0.032	0.157	0.004	0.187	0.032	0.155	0.009
6	0.188	0.033	0.155	0.006	0.186	0.032	0.154	0.010
10	0.185	0.034	0.151	0.010	0.185	0.033	0.152	0.012
15	0.184	0.034	0.150	0.011	0.182	0.033	0.149	0.015

*Corrected Value

TABLE VIII (continued)

Time (Min.)	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units
				5 ppm SO_2				
0	0.191	0.029	0.157*	0.000	0.196	0.031	0.159*	0.000
15	0.187	0.037	0.150	0.007	0.189	0.034	0.155	0.004
30	0.189	0.034	0.155	0.002	0.189	0.035	0.154	0.005
45	0.186	0.034	0.152	0.005	0.186	0.035	0.151	0.008
60	0.186	0.037	0.149	0.008	0.189	0.036	0.153	0.006
75	0.187	0.038	0.149	0.008	0.186	0.036	0.150	0.009
				10 ppm SO_2				
0	0.191	0.032	0.154*	0.000	0.193	0.032	0.156*	0.000
15	0.191	0.041	0.150	0.004	0.194	0.042	0.152	0.004
30	0.191	0.042	0.149	0.005	0.194	0.043	0.151	0.005
45	0.201	0.044	0.157	-0.003	0.201	0.043	0.158	-0.002
60	0.202	0.044	0.158	-0.004	0.201	0.043	0.158	-0.002
75	0.211	0.049	0.162	-0.008	0.207	0.047	0.160	-0.004

*Corrected Value

TABLE IX

EFFECT OF PREINCUBATION OF SULFUR DIOXIDE AND ENZYME ON THE RATE OF ANTHOCYANIN DEGRADATION BY TYROSINASE AT pH 6.5
(SO_2 and enzyme preincubated for 30 minutes)

Concentration of SO_2 in reaction mixture ---------- 2.9 ppm

Concentration of SO_2 in enzyme-SO_2 mixture ---------- 11.7 ppm

Time (Min.)	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units	A_{518} mμ (pH 2)	A_{518} mμ (pH 7)	A_{518} mμ Difference (pH 2 - pH 7)	Anthocyanin Degrad. in A_{518} Units
0	0.210	0.036	0.139*	0.000	0.212	0.040	0.138*	0.000
15	0.162	0.034	0.128	0.011	0.163	0.037	0.126	0.012
30	0.152	0.036	0.116	0.023	0.153	0.037	0.116	0.022
45	0.146	0.038	0.108	0.031	0.148	0.039	0.109	0.029
60	0.137	0.039	0.098	0.041	0.144	0.042	0.102	0.036
75	0.136	0.043	0.093	0.046	0.142	0.042	0.100	0.038

Concentration of SO_2 in Reaction Mixture ---------- 3.2 ppm
Concentration of SO_2 in Enzyme-SO_2 Mixture ---------- 16.8 ppm

0	0.211	0.042	0.135*	0.000	0.209	0.046	0.130*	0.000
15	0.172	0.035	0.137	-0.002	0.175	0.037	0.138	-0.008
30	0.172	0.035	0.137	-0.002	0.172	0.039	0.133	-0.003
45	0.173	0.037	0.136	-0.001	0.181	0.039	0.142	0.012
60	0.172	0.039	0.133	0.002	0.175	0.037	0.138	-0.008
75	0.181	0.039	0.142	-0.007	0.178	0.039	0.139	-0.009

*Corrected Value