THE DEGRADATION, IN MODEL SYSTEMS, OF THE ANTHOCYANIN OF THE MARSHALL STRAWBERRY

by

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INTRODUCTION

The color of a food product is a most important factor in determining consumer acceptance. Often this is the only factor which can influence a consumer's choice when the product is on display. Poor practice in processing and handling generally results in an inferior product, as a consequence of undesirable changes in color, flavor and texture. Thus, color, to a certain extent, reflects the quality of the product. Hence to the food processor, retention of a good color during processing and maintenance of this color during storage is of prime concern.

Retention of a good color becomes a real problem in those heat processed foods in which the pigment is an anthocyanin. For example in the production of strawberry preserves by conventional procedures, pigment losses of at least 30% and up to 70% or more are observed (14). In such strawberry products the initial loss through heat processing is followed by further degradation on storage. Hence improved processing techniques and storage procedures, which would result in improved color retention in strawberry products, would be of real value to the food industry.

In order to obtain some understanding of the nature of the degradation of the pigment in strawberry products, several workers have studied the degradation of the anthocyanin of strawberries in model systems. Such investigations, although not duplicating exactly the conditions found in a strawberry product, enable the investigator to isolate the factors associated with the pigment degradation. In this manner the relative importance of these factors may be evaluated and some indication of the mechanism of the reaction obtained.

It has thus been demonstrated that the three most important factors which determine the rate of pigment degradation in strawberry products are pH, temperature and the presence of oxygen (13 and 16). Ascorbic acid has a marked effect and sugars also contribute to the degradation of the pigment (16).

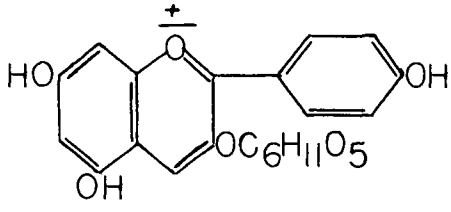
Since sugars are present at such high concentration in many strawberry products and also since there is a variety of sugars available for use in the preparation of those products, it was considered advantageous to study the effects of sugars on the rate of pigment breakdown. Previous studies by Meschter (16) and Mackinney, Lukton and Chichester (14) have been carried out at a temperature in the range of 35-40° C. A temperature of 90° C. was chosen for the present studies, approximating

processing temperatures rather than storage temperatures. It has been suggested that sugars are active in the form of their degradation products in accelerating the rate of pigment degradation and amino acids accelerate this effect.

The studies reported in this thesis have been designed to investigate the effect of different sugars on the rate of pigment degradation at 90° C. incorporating such variables as pH sugar concentration and amino acid interaction. An effort has been made to determine the relative importance of the factors associated with the degradation of the pigment of strawberries.

LITERATURE SURVEY

The flavonoid pigments which have been identified in the strawberry are:- pelargonidin-3-glucoside (I), cyanidin-3-glucoside (II), kaempferol (III), and quercetin (IV).



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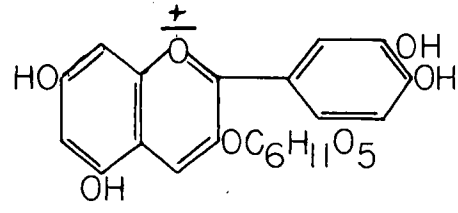

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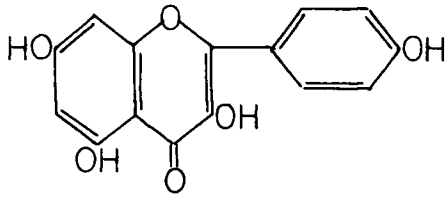

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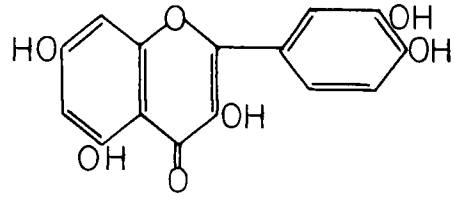


IV

Robinson and Robinson (20) first demonstrated the presence of pelargonidin-3-glucoside in strawberries. This fact was confirmed by Sondheimer and Kertesz (25) who also described a procedure for isolating the pigment in a pure form from strawberry juice. The concentration of pelargonidin-3-glucoside in fresh strawberries was found to be of the order of 20-30mg per 100g of

strawberries. A pelargonidin-3-galactoside has also been detected in the fruit of the wild strawberry (21). Lukton, Chichester and Mackinney (12) identified a second anthocyanin in strawberries as cyanidin-3-glucoside. The relative proportions of these two anthocyanins in the wild strawberry (Fragaria vesca) and in a cultivated strawberry has been determined by Sondheimer and Karash (28). Pelargonidin-3-glucoside and cyanidin-3-glucoside were found to be present in a 1:1 ratio in the wild berry and a 20:1 ratio in the cultivated berry. The flavonols quercetin and kaempferol, which are related to the anthocyanidins cyanidin and pelargonidin, have been isolated from strawberries by Williams and Wender (30). No quantitative data concerning the relative or absolute concentrations of these flavonols are available at the present.

Most of the earlier studies on the degradation of the color of strawberry juice are listed in a paper by Pratt, Balkcom, Powers and Mills (19). These authors along with the earlier reports emphasize the influence of ascorbic acid on the rate of pigment degradation. In the past five years there has been a marked increase in the amount of work done with model systems which have been designed to isolate the different factors associated with the degradation of the anthocyanin with a view to obtaining an understanding of the mechanism of the degradation reaction.

Hydrogen peroxide has a very marked effect on anthocyanins. The kinetics of the reaction between pelargonidin-3-glucoside and hydrogren peroxide have been studied in model systems by Sondheimer and Kertesz (26), who differentiate between a noncatalysed and an iron-catalysed reaction. The latter gives the more rapid degradation of the pigment. An even faster rate was observed in the presence of hydrogen peroxide, ferrous ion and ascorbic acid. In this case it is suggested that the ascorbic acid is active in retaining the iron in the ferrous state.

The effect of ascorbic acid on the rate of degradation of pelargonidin-3-glucoside has also been studied in model systems. Sondheimer and Kertesz (27) have demonstrated that the accelerating effect on the rate of pigment breakdown is associated with the oxidation of the ascorbic acid. It is suggested that the hydrogen peroxide produced in the oxidative breakdown of ascorbic acid is responsible for the pigment degradation. Dehydroascorbic acid is also active but not to the same extent as ascorbic acid (16). Degradation of pelargonidin-3-glucoside in the presence of ascorbic acid and glycine was found to be greater than in the presence of either ascorbic acid or glycine alone (14). This would indicate that the interaction of ascorbic acid and

glycine was responsible for this increased activity. Investigations on the stoichiometric aspects of the reaction between ascorbic acid and pelargonidin-3-glucoside in the presence of nitrogen by Markakis, Livingston and Fellers (15) showed that 1 mole of pigment was destroyed for approximately 50 moles of ascorbic acid. These workers attempted to reduce the pigment degradation in strawberry juice by treatment of the juice with ascorbic acid oxidase. A small effect was observed but it was insignificant compared with that produced by deaeration.

Meschter (16) has observed the rate of pigment degradation in the presence of different sugars at 38° C. Sugars such as arabinose, levulose and sorbose which are relatively labile, produced a higher rate of pigment degradation than more stable sugars such as maltose and sorbitol. Sugar degradation products, 5-hydroxymethylfurfural and furfural also increased the rate of degradation. Further work in model systems by Mackinney, Lukton and Chichester (14) showed fructose to be more active than sucrose. These workers also demonstrated an added effect in a sugar-amino acid system which was presumably due to the interaction of the sugar and amino acid. Decereau, Livingston and Fellers (7) have made some observations on the rate of loss of pigment in strawberry jellies. Contrary to expectation replacement

of sucrose by fructose increased the half-life of the pigment. Some recent work by Aref, Sidwell and Litwiller (1) indicated that sugars could have some effect on the color of frozen strawberries.

The rate studies reported to date have generally been carried out in citrate buffers in the range of pH 3.00-3.40 which approximates the pH of strawberry juice. The effect of pH on the spectral characteristics of anthocyanins has been interpreted by Sondheimer (23) as being due to the effect of pH on the equilibrium between a colorless pseudo-base form, ROH and a colored benzopyrylium salt, R^+.

$$R^+ + 2H_2O \rightleftharpoons ROH + H_3O^+$$

With pelargonidin-3-glucoside the pK for this reaction was shown to be 2.98 at 25° C. (23). The same value was also obtained at 45° C. (13). The pigment has been shown to be more stable at lower pH (13). Lukton, Chichester and Mackinney (13) have shown that for oxidative degradation of pelargonidin-3-glucoside a linear relation was obtained between the rate constant and the concentration of the ionic form of the pigment. It was suggested that under these conditions the rate of pigment degradation was a function of the concentration of the pseudo-base form of the pigment. pH had little effect on

the rate of pigment degradation in an atmosphere of nitrogen. In strawberry syrup the minimum rate of pigment breakdown was observed at pH 1.80.

Most of these studies in model systems have been carried out at temperatures in the range of 30-40° C. Meschter has shown that an increase in temperature increases the rate of pigment degradation (16). Decoreau et al. have approached this variable from the processing angle (7). The pigment losses were observed in strawberry jellies given different heat treatments. A comparison of equivalent processes at different temperatures indicated that a "high-temperature short-time" process resulted in lower pigment loss for a given process.

Oxygen has a marked effect on the rate of degradation of pelargonidin-3-glucoside. The paper by Lukton et al. (13) gives an extensive survey of the differences observed in the oxidative and non-oxidative breakdown of the pigment. The rate of breakdown was much faster in the presence of oxygen; an insoluble brown precipitate and a soluble brown material were formed. In the presence of nitrogen only small amounts of a red-brown precipitate were formed for equivalent pigment losses compared with the breakdown in oxygen. In these studies the anthocyanidin has not been detected and hence it does not appear that hydrolysis of the glucoside bond is a

prerequisite for pigment degradation (13). However, it has been shown that pelargonidin decomposes at a much faster rate than pelargonidin-3-glucoside (13).

In summary we can list the factors which have been shown to be active in accelerating the rate of pigment breakdown: temperature, oxygen, pH, ascorbic acid, hydrogen peroxide and sugars apparently active in the form of their degradation products. Apart from the importance of the pseudo-base form in oxidative degradation we have no knowledge of the mechanism of these breakdown reactions.

EXPERIMENTAL PROCEDURE

I. Preparation of Pelargonidin-3-glucoside

Sondheimer and Kertesz (25) have described a method for the isolation of pelargonidin-3-glucoside from strawberry juice and this procedure proved to be very satisfactory for the preparation of a pure sample of the pigment. n-Butyl alcohol was used to extract the pigment from the strawberry juice and after concentration under vacuum a crude product was precipitated by the addition of anhydrous ethyl ether. The crude product was converted to the picrate which was purified by recrystallization from hot dilute picric acid. The recrystallization was repeated four times. The anthocyanin chloride was precipitated with anhydrous ethyl ether from a solution of the picrate in 5% alcoholic hydrogen chloride. A yield of approximately 700mg of the pelargonidin-3-glucoside was obtained from 30 pounds of strawberries.

Paper chromatograms of the pigment using a solvent system composed of equal volumes of n-butanol and 27% acetic acid showed only one spot, the Rf of which corresponded to that reported in the literature (8, p.477). A comparison of the spectrum of this preparation and that reported by Sondheimer and Kertesz (25) for pelargonidin-3-glucoside indicated close agreement in the wave lengths of maximum absorption.

Preparation of 5-Hydroxymethyl-2-furaldehyde

A small quantity of this compound was prepared by the method described by Haworth and Jones (10).

II. Analytical Procedures

A. Identification and Estimation of the Free Amino Acids in Strawberry Juice

These experiments were carried out on a sample of juice obtained from Marshall strawberries. The berries were frozen and held at 0° F. After thawing the juice was expressed through cheesecloth, a clear sample being obtained by further filtration through a sintered glass filter.

Hackman and Lazarus (9) have described a procedure for the isolation of amino acids using one dimensional paper chromatography and a series of solvent systems. With an n-butanol/acetic acid/water system (77:6:17, v/v) and running the chromatogram twice in the one direction, the amino acids, leucine, phenylalanine, valine, tyrosine, methionine and alanine may be separated. A phenol/water system (74:26, water:buffer v/v) buffered with borate buffer at pH 10.0 and using paper also buffered at pH 10.0 gave an excellent separation of cysteic acid, aspartic acid, serine, glycine, threonine and alanine. Histidine, proline and hydroxyproline were separated by using 60%

acetone as the solvent. Arginine and lysine were separated by first running the chromatogram in acetone buffered with phosphate buffer at pH 7.0. After drying the chromatogram was run in the same direction using 80% phenol as the solvent. Whatman No. 1 filter paper was used in all these experiments.

When running one dimensional chromatograms it is possible to run standard acids as well as the unknown on the one paper. However, because of the amount of extraneous material in strawberry juice and its effect on the Rf of the amino acids, direct comparisons with these standards was not always possible. The presence of a particular amino acid was confirmed by the following procedure. Two aliquots of strawberry juice were applied to the same chromatogram and an additional aliquot of a standard amino acid was superimposed on one of the juice spots. After the chromatogram was developed, comparison of the distribution of the spots from these two samples indicated whether or not the amino acid in question was present.

The presence of asparagine and glutamine was ascertained by the comparison of the aspartic acid and glutamic acid spots from hydrolysed and non-hydrolysed samples. To hydrolyse asparagine and glutamine the juice was made 2 normal with respect to hydrochloric acid and

then heated on a boiling water bath for 3 hours. The hydrolysed sample was evaporated to dryness under vacuum and then placed in a desiccator with solid sodium hydroxide. The residue was taken up in the required amount of water and the chromatograms run.

Before chromatographic analysis cysteine and cystine were converted to cysteic acid. The aliquot of juice after being applied to the paper was treated with 30% hydrogen peroxide and ammonium molybdate, as described by Block, Durrum and Zweig (4, p.94). Cysteic acid was identified in the buffered phenol system. No differentiation was made between cysteine and cystine.

When the amino acids had been identified and a satisfactory separation achieved, those present in larger amounts were estimated quantitatively. The method described by Moore and Stein(17) was adapted for this purpose. A standard curve was obtained by adding 0, 10, 30, and 40 µl aliquots of 2 x 10^{-3}M leucine (258mg/liter) to pieces of washed Whatman No. 1 filter paper (3 x 2 cm). Each of these was placed in a test tube to which was added 0.5ml of water and 2ml of ninhydrin reagent. The ninhydrin reagent was prepared by adding 0.80g of stannous chloride dihydrate dissolved in 500cc of citrate buffer at pH 5 to 20g of ninhydrin in 500cc of methyl cellosolve. After sealing the tubes with aluminum caps the color was

developed by heating on a boiling water bath for 20 min. Five ml of a mixture of n-propyl alcohol and water (1:1) was added and the absorbance measured at 570 mμ using a Beckman Model D.U. spectrophotometer. A straight line relation between absorbance and concentration was obtained over this concentration range.

The amino acids were estimated by chromatographing a sample of the juice sufficient to give a concentration falling within the range of the standard curve. This was achieved by running 3 or 4 aliquots of equal size 1cm apart and also an indicator aliquot on the same paper. After the chromatogram had been developed the latter was cut from the chromatogram, the color developed with ninhydrin and then the spots on this strip were used to position those to be cut out for analysis. These strips together with blanks of equal size were washed several times with ethyl ether to remove the solvent and then the color developed in the same manner as that described for the standard curve. Moore and Stein (17) give factors which relate the color yield of different amino acids to that of leucine, hence only one standard curve was required. All unknown samples were run in triplicate.

B. Estimation of Pelargonidin-3-glucoside

The method described by Sondheimer and Kertesz (24) was used for the estimation of pelargonidin-3-glucoside.

This procedure is based on the linear relation between concentration and the differences in absorption between samples at pH 3.4 and pH 2.0 at 500mμ. Using a Beckman Model D.U. spectrophotometer and 0.1M citrate buffer a linear relation between pigment concentration and difference in absorbance at these two pH values was obtained in the concentration range of 0-3mg per 100ml.

In actual practice aliquots of the reaction mixture at pH 3.40 were withdrawn and added to an aliquot of citrate buffer which were adjusted so that dilution to the required concentration range resulted in a pH of 2.0 and 3.40. On dilution of a sample to pH 2.0 it was necessary to allow at least an hour for the sample to come to equilibrium. Failure to do this resulted in considerable error in absorbance measurements at this pH. This effect was not observed in samples made up to pH 3.40. In systems where no interfering colored products were produced the absorbance measurements at pH 3.40 were used to determine the pigment concentration. Absorbancy measurements at 440mμ indicated the production of interfering pigments.

C. Measurement of the pK of the Equilibrium between the Benzopyrylium Salt and the Pseudo Base Form of Pelargonidin-3-glucoside

The pK of this reaction at 86° was determined using

the method described by Sondheimer (23). Pelargonidin-3-glucoside was dissolved in solutions of hydrochloric acid, the pH of which varied from pH 1.0 to 3.0. The absorbance of these solutions was measured at room temperature and at 86° C. The measurements at 86° C. were obtained by using a "Thermospacer" (2, p.53) attachment with the Beckman Model D.U. spectrophotometer. The pK of this reaction was then calculated from these measurements as described in the above mentioned paper.

III. Rate Studies

Three 100ml three-necked Standard Taper round-bottom flasks fitted with air inlet tubes, condensers and thermometers, were mounted in a constant temperature bath. The rate of air flow was controlled for each individual flask at 160-170 c.c./min. measured at standard temperature and pressure. In this manner the reaction mixture was saturated with air and kept well stirred. The temperature of the heating bath was adjusted to give a temperature of 90.0 ± 0.2° C. in the reaction flask.

In carrying out a rate study a 40ml solution containing all the required components except the citrate buffer and pigment was allowed to come up to temperature (approximately 10 min.). At this time 5ml of citrate buffer and 5ml of distilled water containing 10mg of

pigment were added. The buffer was prepared in such a manner that a 1:10 dilution resulted in 0.1M citrate buffer of pH 3.40. The final volume of solution was 50ml and the reaction mixture was 0.427 millimolar with respect to the pigment. As soon as the reaction mixture came up to 90° C. an aliquot was withdrawn and the pigment concentration of this aliquot was taken as that at zero time. At definite time intervals aliquots were withdrawn and the concentration of pelargonidin-3-glucoside determined. Where reaction rates were measured in the presence of nitrogen, water pump nitrogen was used and the solutions were flushed with nitrogen before the rate studies commenced. In systems containing ascorbic acid or amino acids these components were added along with the buffer and pigment. The reaction was followed until the original pigment concentration had been reduced to at least one half and in all cases a minimum of seven readings was taken.

RESULTS

I. The Free Amino Acids of Strawberry Juice

The degradation of pelargonidin-3-glucoside in a sugar or ascorbic acid system has been found to be further accelerated by the presence of glycine (14). This effect is probably due to the production of an increased amount of active degradation products as a result of the interaction of the amino acid with the sugar or ascorbic acid. For a better understanding of the extent of this reaction in strawberry juice samples a knowledge of the free amino acids of strawberry juice would be necessary. The only references in the literature to amino acids in strawberries are those by Casimir and Jakovlev (6) and Casimir (5). These studies were made on samples of strawberry products after acid hydrolysis and hence do not represent the free amino acids in strawberry juice.

Using the methods described earlier (see page 12) the following amino acids were identified:-

Aspartic acid	Glutamine	Cystine and/or Cysteine
Glutamic acid	Asparagine	Leucine and/or Isoleucine
Alanine	Threonine	Valine
Serine	Arginine	

Asparagine, glutamine, alanine, aspartic acid and glutamic acid were found to be present in the largest amounts.

The presence of these acids was confirmed by superimposing known acids on samples of the juice and chromatographing. Some additional observations also confirm the presence of several of the acids identified. Asparagine gives a characteristic brown color with ninhydrin reagent. Hydrolysis of the juice sample removes the spots due to asparagine and glutamine at the same time producing an increase in the size of the spots of aspartic acid and glutamic acid. With chromatograms run in the butanol/acetic acid/water system aspartic acid gives with the ninhydrin reagent a characteristic blue-green color which turns purple on standing. With a juice sample it proved to be difficult to separate serine from glutamic acid with the buffered phenol system. The presence of serine was determined by first chromatographing a sample of juice in the butanol system. After drying, the lower portions of the chromatogram were cut out and eluted onto a buffered filter paper and run in the phenol system. The presence of serine was established in this manner.

The concentrations of the amino acids present in the largest amounts were determined and the results obtained are tabulated in Table I.

TABLE I

Concentration of Free Amino Acids in Strawberry Juice

Amino Acid	Concentration mg/100ml
Asparagine	59
Glutamine	15
Alanine	12
Glutamic Acid	8
Aspartic Acid	3

Each value represents the mean of three estimations. The accuracy of the measurements was from 5-10%, which was adequate to give the order of concentration. The other free amino acids, serine, threonine, arginine, valine, cystine and/or cysteine, and leucine and/or isoleucine were present in concentrations of less than 2mg per 100ml of juice.

II. Degradation Studies on Pelargonidin-3-glucoside

A. Pigment Degradation in the Presence of Sugars and Their Derivatives

The degradation of pelargonidin-3-glucoside was observed in 0.5M solutions of the monosaccharides and 0.25M solutions of the disaccharides. These concentrations approximate the soluble solids content of strawberry

juice. A pH of 3.40 was maintained with 0.1M citrate buffer and the reactions were followed in the presence of air and nitrogen.

For first order kinetics the characteristic relation is:-

$$\ln \frac{a}{a-x} = k_1 t$$

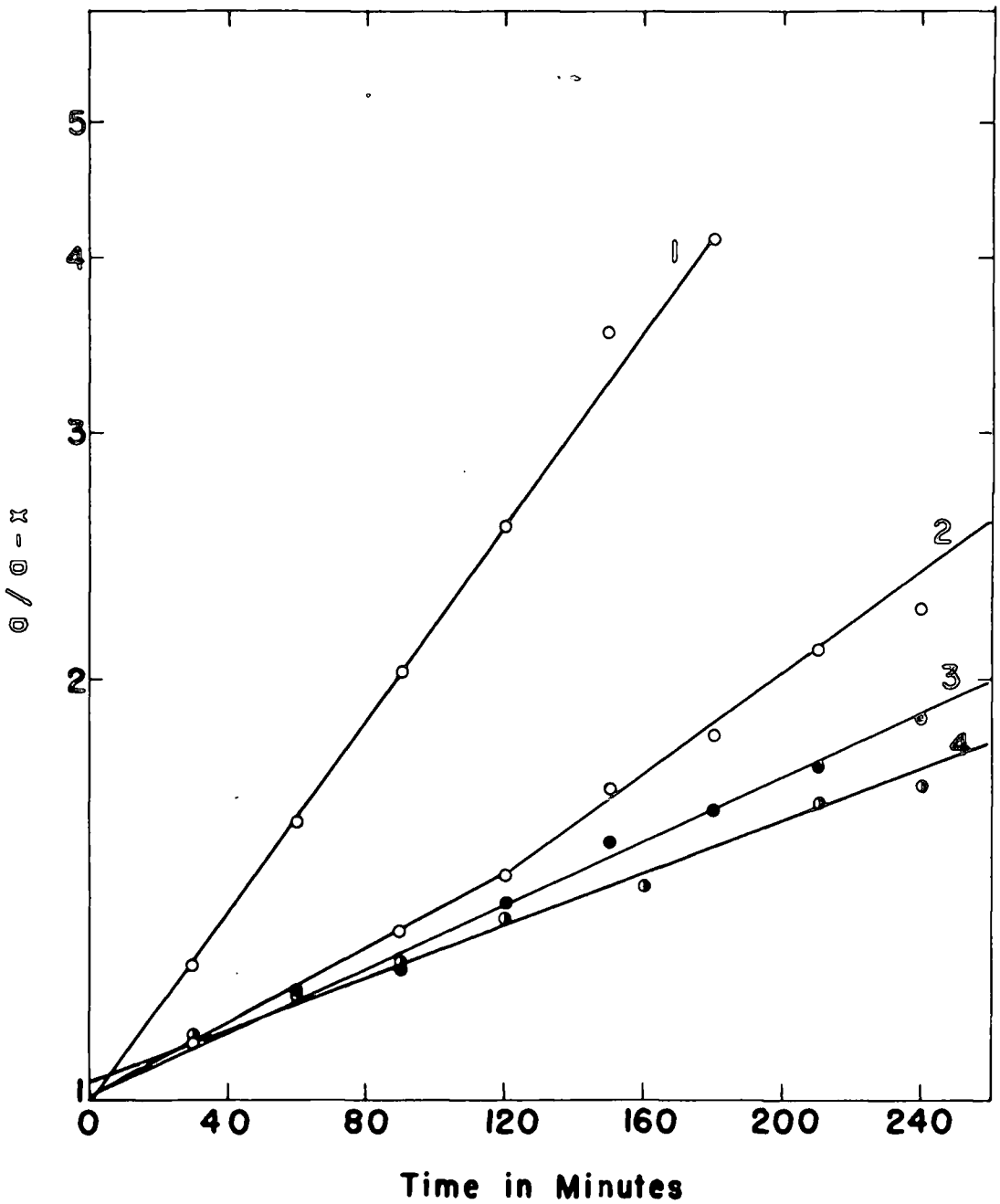
where a = initial concentration of reactant

x = fraction of reactant destroyed in time "t"

k_1 = first order rate constant

For a system which follows first order kinetics a plot of $\ln \frac{a}{a-x}$ against time gives a straight line passing through the origin. The slope of this line is equal to the first order rate constant. The results from the rate studies were analysed in this fashion and it was found that in most cases the rate of disappearance of pelargonidin-3-glucoside followed first order kinetics. The type of rate curves obtained are given in Figure 1; $\frac{a}{a-x}$ is plotted on a logarithmic scale against time. The rate of pigment degradation in different systems can thus be expressed by the first order rate constant. In sucrose systems first order kinetics were not attained until the reaction had proceeded for approximately 120 minutes. First order rate constants quoted for sucrose systems refer to this portion of the rate curve.

Figure 1



Reaction Rate Curves for the Degradation of Pelargonidin-3-glucoside in the Presence of Sugars. (1) 0.5M Fructose, (2) 0.25M Sucrose, (3) 0.5M Glucose, (4) 0.5M Gluconic Acid.

In Table II the sugars used and the rate constants, both in air and nitrogen, are listed. The percent reduction in rate constant observed in the presence of nitrogen is also tabulated. In the presence of air the rate of pigment degradation in 0.25M sucrose is approximately half that observed in the 0.5M fructose system. This would suggest that the lag observed is due to the hydrolysis of the sucrose.

TABLE II

First Order Rate Constants for the Degradation of Pelargonidin-3-glucoside in Different Sugar Solutions

Sugar	First Order Rate Constant Min.$^{-1}$ x 10^3		Percent Reduction
	Air	Nitrogen	
Glucose	2.60	1.93	26.0
Fructose	7.91	2.80	65.0
Sucrose	3.96	2.88	27.0
Sorbitol	2.04	2.16	0.0
Gluconic Acid	2.14	2.15	0.0
Glucuronic Acid	14.70	10.00	32.0
Maltose	2.66	2.07	22.0
Lactose	3.50	2.32	34.0
Buffer	2.02	1.77	12.5

From these results it is seen that sugars such as fructose and glucuronic acid, which are known to degrade quite rapidly at low pH, have a pronounced effect on the rate of pigment degradation. Glucuronic acid decomposes to furfural and fructose to 5-hydroxymethylfurfural (18, p.69-71). Sorbitol and gluconic acid, which are stable under the conditions of these experiments, show degradation rates comparable with that observed in the buffer system.

In the presence of nitrogen, marked reductions in the rate of pigment degradation are observed. However, differences in the rate of pigment degradation are still observed with the sugars used. The relative reduction observed in rate constant was not the same for all sugars used. The rate of pigment breakdown in the gluconic acid and sorbitol systems was virtually the same in air as it was in nitrogen.

B. Effect of Sugar-Amino Acid Interaction on the Rate of Degradation of Pelargonidin-3-glucoside

Experiments conducted by Mackinney, Lukton and Chichester (14) indicated that pelargonidin-3-glucoside was decomposed more rapidly in a sugar solution containing 1% glycine than in a pure sugar or glycine solution. A 1% glycine solution would be somewhat more concentrated

than one would expect in strawberry juice. In the studies carried out in this laboratory the effect of sugar-amino acid interaction on the rate of pigment degradation has been observed at amino acid concentrations of the same order as that expected in strawberry juice. For each amino acid used the rate of pigment degradation was observed in a pure amino acid system, amino acid plus 0.5M glucose and amino acid plus 0.25M sucrose. In each case the amino acid concentration was 2.5×10^{-3}M and all systems were buffered to pH 3.40 with 0.1M citrate. The amino acids used were representative of the different functional types found in strawberries.

In these systems also, the rate of decomposition of pelargonidin-3-glucoside followed first order kinetics. The rate curve for sucrose systems was similar to that observed previously. In Table III, the first order rate constants are tabulated for the different systems studied.

The results indicate that arginine and cystine may have some effect on the rate of degradation of pelargonidin-3-glucoside in a buffer system. Even at this pH where amino acid-sugar interaction is relatively low, the presence of amino acids in the glucose and sucrose systems produced an increase in the rate of pigment degradation. There is some question in the arginine-glucose system whether the increase is due to the

arginine or arginine-glucose interaction. The increase in rate constant over that observed in a pure glucose system is only slightly larger than the increase in rate constant of the arginine-buffer system above that of a pure buffer system. Cystine proved to be the most active of the amino acids tested.

TABLE III

First Order Rate Constants for the Degradation of Pelargonidin-3-glucoside in the Presence of Sugar-Amino Acid Systems

	First Order Rate Constant ($Min^{-1} \times 10^{3}$)		
Amino Acid	Buffer	Glucose	Sucrose
Asparagine	1.96	3.09	4.24
Alanine	2.09	2.86	4.49
Serine	2.05	2.92	4.54
Arginine	2.36	3.04	4.90
Cystine[a]	2.53	3.58	4.96
No Amino Acid	2.02	2.60	3.96

[a] Concentration 1.25×10^{-3} M

In some preliminary experiments the amino acids were heated in a 0.5M glucose solution buffered at pH 3.40. The ultra-violet spectra of these systems showed a maximum absorbancy at 285 mμ. This maximum is characteristic of 5-hydroxymethylfurfural. Arginine and cystine were also

the most active in these systems. It would appear that the extent to which amino acid-sugar interaction affects the rate of pigment degradation is dependent on the extent of sugar degradation as measured by the appearance of 5-hydroxymethylfurfural.

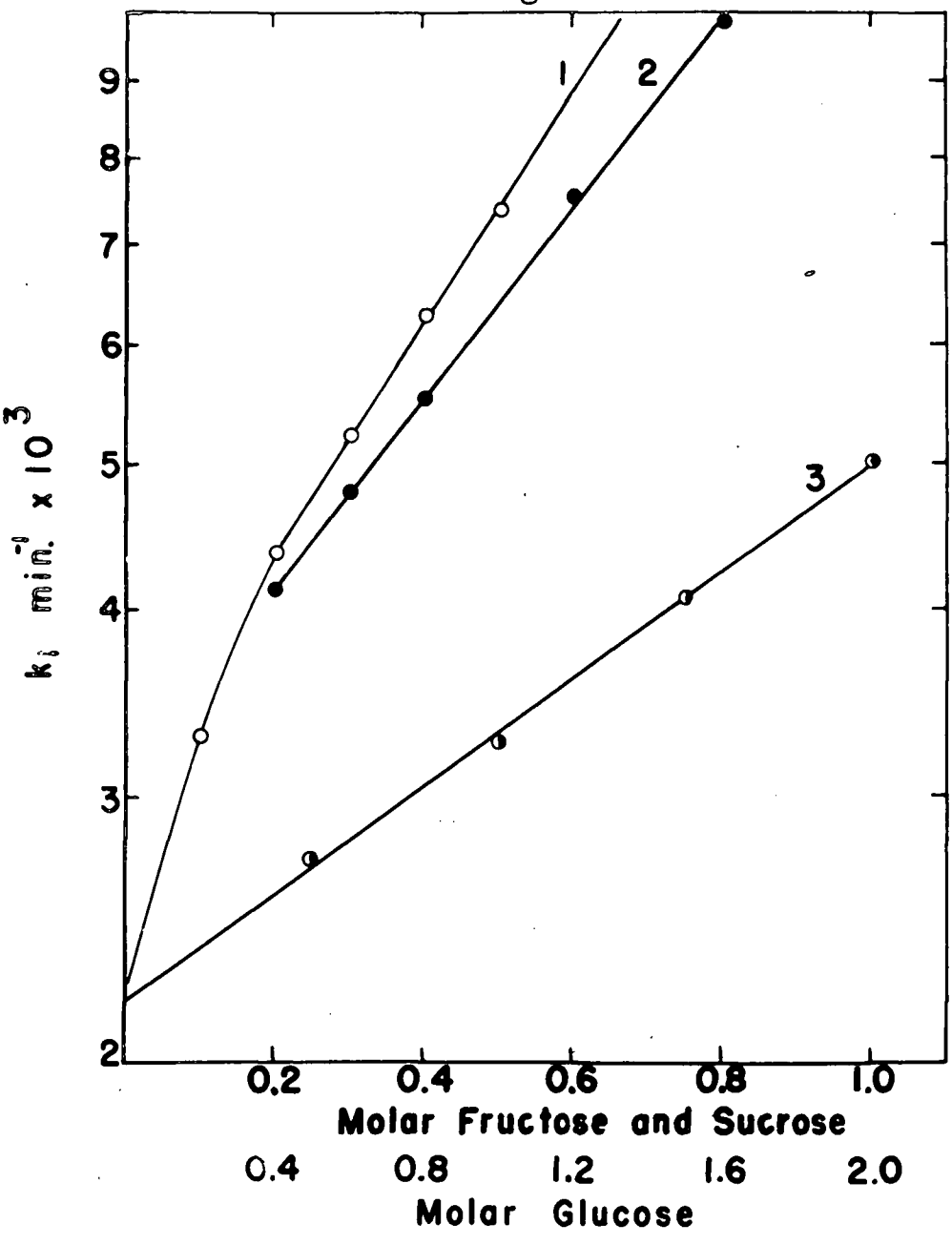
C. Effect of Variation in Sugar Concentration on the Rate of Degradation of Pelargonidin-3-glucoside

The rate of degradation of pelargonidin-3-glucoside was observed in varying concentrations of glucose, sucrose and fructose at pH 3.40. In all cases an increase in the sugar concentration resulted in an increase in the rate of pigment degradation at pH 3.40 and 90° C.

In Figure 2 the logarithm of the first order rate constant is plotted against the sugar concentration. Over most of the concentration range investigated a linear relation was observed. The reason for this relation is not apparent. With the glucose systems extrapolation to zero concentration gives a value for the rate constant of 2.2×10^{-3} min^{-1} which corresponds with that already observed. In the fructose and sucrose systems extrapolation to the ordinate does not correspond to the rate constant observed in the buffer system previously.

An indication of the stability of the pigment in different sugar solutions can be obtained from Table IV.

Figure 2



Effect of Variation in Sugar Concentration on the Rate of Degradation of Pelargonidin-3-glucoside. (1) Fructose, (2) Sucrose, (3) Glucose.

In this table the time for the initial concentration of the pigment to be reduced by one half is given.

TABLE IV

Times for Reduction of Initial Pigment Concentration by One Half in Different Sugar Solutions

Glucose		Fructose		Sucrose	
Concen. (Moles/l)	Time (Min.)	Concen. (Moles/l)	Time (Min.)	Concen. (Moles/l)	Time (Min.)
0.5	254	0.1	210	0.2	200
1.0	213	0.2	159	0.3	188
1.5	170	0.3	132	0.4	166
2.0	138	0.4	110	0.6	138
		0.5	93	0.8	125

D. The Effect of pH on the Rate of Degradation of Pelargonidin-3-glucoside in Sugar Solutions

It has been shown that pelargonidin-3-glucoside is more stable at lower pH (13), but on the other hand it is known that the rate of degradation of both glucose and fructose to 5-hydroxymethylfurfural increases as the pH decreases (22). Also it has been demonstrated that 5-hydroxymethylfurfural is active in accelerating the degradation of this pigment. Thus two apparently counteracting variables are in existence in this system. In some preliminary experiments ultra-violet spectra of

glucose and fructose solutions heated at pH 3.40 indicated the presence of 5-hydroxymethylfurfural. In these experiments the rate of degradation of pelargonidin-3-glucoside was followed in 0.1M citrate buffer and 0.5M glucose and 0.1M fructose solutions also buffered with 0.1M citrate. The pH of these systems was varied from 3.95 to 1.80 and the reactions were carried out in the presence of air.

In order to interpret the results it was necessary to obtain an estimate of the pK of the reaction

$$R^+ + 2H_2O \rightleftharpoons ROH + H_3O^+$$

where R^+ represents the colored benzopyrylium form of the anthocyanin and ROH the pseudo base form. A value of 2.98 for the pK of this reaction at 25° C. and 45° C. has been reported (23 and 13). No values for higher temperatures have been reported. Using the procedure outlined (see page 16) a value of 2.95 at a temperature of 86° C. was obtained. This value is within the range of 2.98 ± 0.06 reported by Sondheimer (23). Hence in interpreting these results from experiments carried out at 90° C. it would seem that a pK of 2.98 would be a valid approximation.

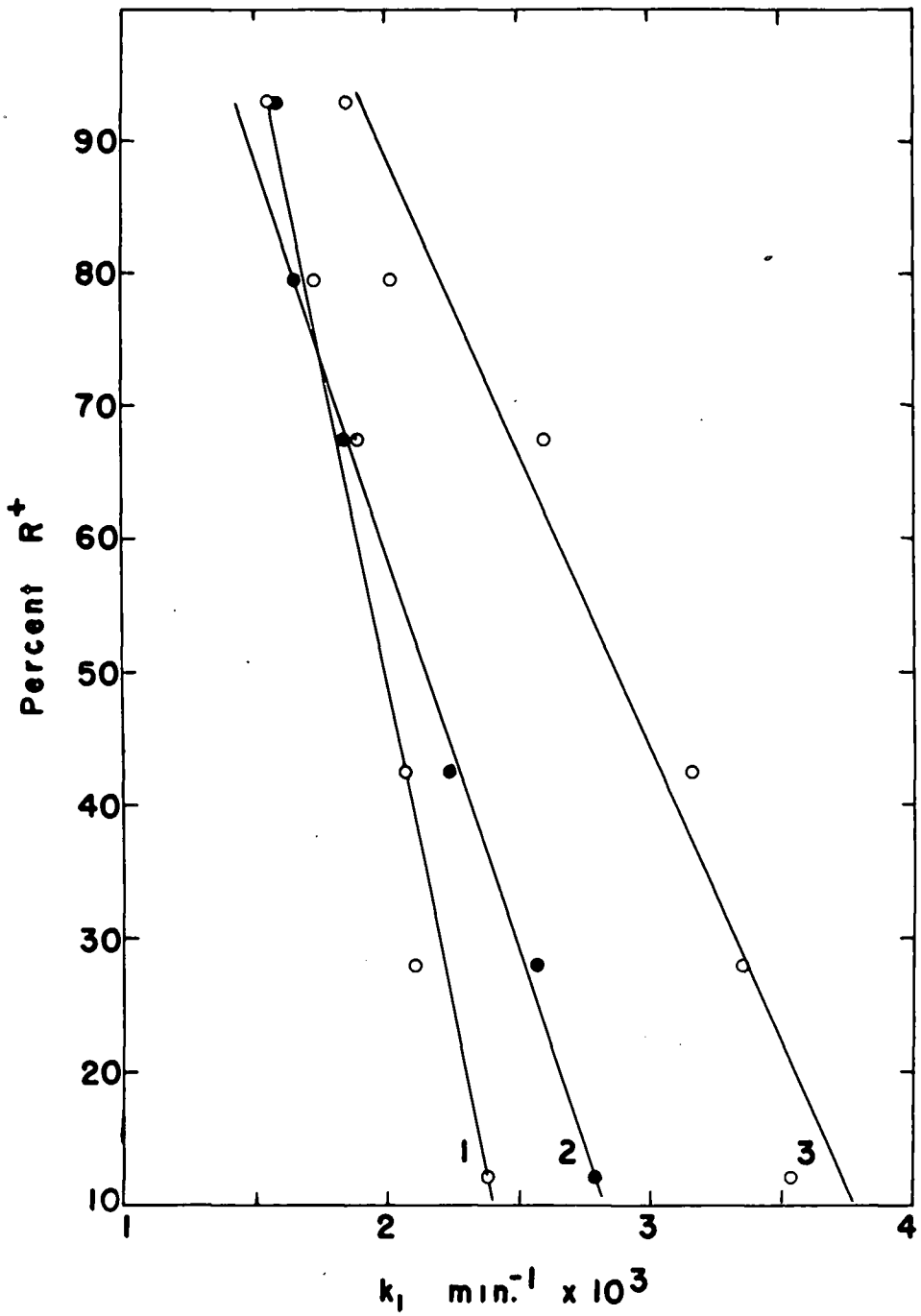
The citrate buffer maintained the pH of the systems within 0.05 of a pH unit. The rate of pigment loss followed first order kinetics in all the systems studied

in this series of experiments. The results are tabulated in Figure 3 where the rate constants are plotted against the percentage of the anthocyanin present as the benzopyrylium form. The linear relation observed in the three systems studied implies that, over the pH range investigated, the degradation of the anthocyanin is dependent on the proportion of anthocyanin present as the pseudo base. Lukton, Chichester and Mackinney (13) observed this effect in buffer systems as well as in juice samples at a temperature of 40° C. The results from the fructose system are not as consistent as in the other two systems but the general trend is the same. As the pH decreases the differences in rate constants for the three systems also decreases.

E. The Effect of Some Sugar Degradation Products on the Rate of Degradation of Pelargonidin-3-glucoside

1. Furfural and 5-Hydroxymethylfurfural. The increase in pigment degradation in the presence of furfural and 5-hydroxymethylfurfural has been described by Meschter (16) and Markakis, Livingston and Fellers (15). These studies were carried out at 38° C. and 40° C. respectively. Meschter's results indicate that, at equivalent concentrations, furfural is considerably more active than 5-hydroxymethylfurfural at 38° C. The

Figure 3



Effect of pH on the Rate of Degradation of Pelargonidin-3-glucoside in 0.1M Citrate Buffer, 0.5M Glucose and 0.1M Fructose. (1) Citrate Buffer, (2) Glucose, (3) Fructose.

concentrations, however, are quoted in parts per million and a rough interpretation of these results on the basis of equivalent molar concentrations indicates only slight differences in activity between these two compounds at this temperature.

The effect of furfural and 5-hydroxymethylfurfural on the rate of degradation of pelargonidin-3-glucoside at 90° C. and pH 3.40 was followed. The reactions were carried out both in the presence of air and in the presence of nitrogen. The furfural was distilled under a vacuum in an atmosphere of nitrogen before use. The first order rate constants obtained are listed in Table V.

TABLE V

First Order Rate Constants for the Degradation of Pelargonidin-3-glucoside in the Presence of Furfural and 5-Hydroxymethylfurfural at pH 3.40

	First Order Rate Constant ($Min^{-1} \times 10^3$)			
	Furfural		5-Hydroxymethylfurfural	
Concentration	Air	Nitrogen	Air	Nitrogen
0.01M	2.88	1.87	-	-
0.05M	4.66	2.44	17.8	7.29

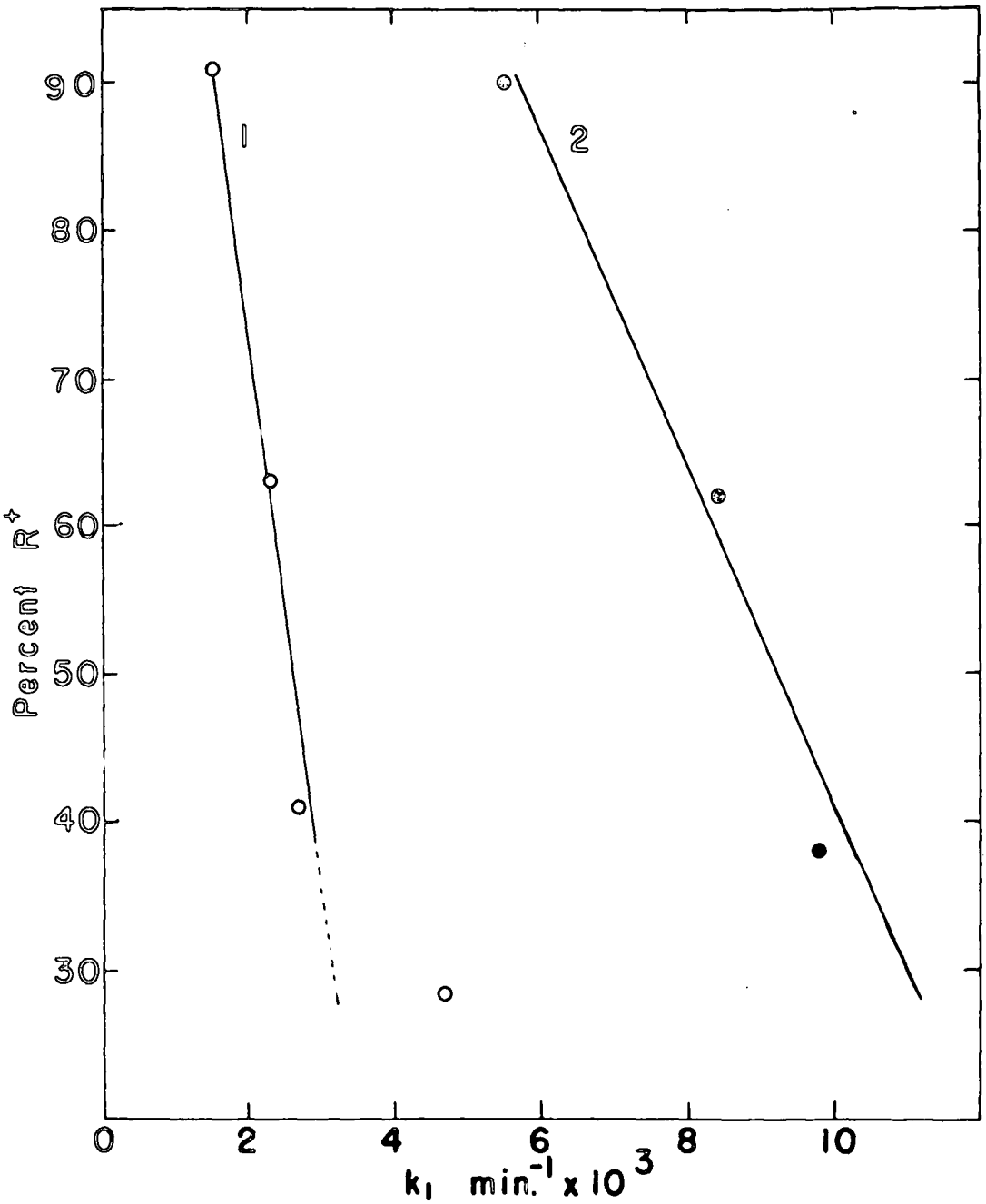
Degradation rates were also observed in 0.1M furfural but first order kinetics were not observed in either air or nitrogen. A considerable reduction in the

rate of pigment degradation was observed when the reaction was carried out in the presence of nitrogen rather than air. The reaction rate, however, was not reduced to that observed in a buffer system in the presence of nitrogen. Either there was a residual effect from the small amount of oxygen that could be present or these compounds do have some effect in the absence of oxygen.

The rate of pigment degradation was also observed in 0.05M solutions of furfural and 5-hydroxymethylfurfural at different pH values. As in previous experiments 0.1M citrate buffer was used. First order kinetics were observed and the rate constants are plotted against the percentage of the anthocyanin present as the benzopyrylium salt (Figure 4). The values obtained at the lower pH region suggest a linear relation but the rate constants observed at pH 3.40 (Table V) were not consistent with this relation.

Both furfural and 5-hydroxymethylfurfural produced a marked effect on the rate of destruction of pelargonidin-3-glucoside. The magnitude of this effect was considerably reduced by carrying out the reaction in the presence of nitrogen or reducing the pH. 5-Hydroxymethylfurfural was consistently more reactive than furfural at equivalent concentrations. These experiments, carried out in systems of varying pH, would suggest that the pseudo-base

Figure 4



Effect of pH on the Rate of Degradation of Pelargonidin-3-glucoside in the Presence of Furfural and 5-Hydroxymethylfurfural.
(1) Furfural, (2) 5-Hydroxymethylfurfural.

form of the anthocyanin was the active form in these systems also. It cannot be concluded from these studies that the pigment reacts directly with either of these two compounds. Both are very labile, particularly in the presence of oxygen and the degradation of the pigment could possibly be due to the presence of some oxidative product derived from these aldehydes. However, in a system in which either of these two compounds was produced in some fashion, one could expect a significant rate of pigment degradation. Thus the degradation of sugars in the presence of acid or amino acids which result in the production of these types of compounds produce an increase in the rate of pigment breakdown. It is possible that the effect of ascorbic acid could be due in part to its breakdown to furfural.

2. Levulinic Acid and Formic Acid. In acid medium 5-hydroxymethylfurfural is degraded to levulinic acid and formic acid. The kinetics of this reaction have been studied by Teunissen (29). Because of the possibility that either formic or levulinic acid was the reactive specie in pigment breakdown, the effect of levulinic acid and formic acid on the breakdown of pelargonidin-3-glucoside was observed. The reactions were carried out in 0.1M citrate buffer at pH 3.40 and at a temperature of 90° C. The first order rate constants obtained are given

in Table VI.

TABLE VI

First Order Rate Constants for the Degradation of Pelargonidin-3-glucoside in the Presence of Levulinic Acid and Formic Acid

	First Order Rate Constant ($Min^{-1} \times 10^3$)	
Concentration	Formic Acid	Levulinic Acid
0.10M	3.15	3.68
0.05M	2.55	2.75
0.01M	2.25	2.40

The rate of pigment degradation in these systems, although greater than that observed in a buffer system of the same pH, was much slower than that observed in equivalent concentrations of 5-hydroxymethylfurfural. Thus it is apparent that the accelerating effect of 5-hydroxymethylfurfural is not dependent on its breakdown to levulinic acid and formic acid. It might be added that in the breakdown of this compound, other substances besides these two acids are formed. A certain amount of resinous material is produced.

F. The Relative Effect of Factors Active in the Degradation of Pelargonidin-3-glucoside

In strawberry juice the constituents which appear to

be most active in determining the rate of pigment degradation would appear to be ascorbic acid, sugars, and amino acids. A sample of strawberry juice obtained from frozen Marshall strawberries was analysed for pigment concentration, ascorbic acid, soluble solids and pH. The results are summarized in Table VII.

TABLE VII

Analysis of Strawberry Juice Sample

Soluble Solids	10.0%
Ascorbic Acid	20 mg/100ml
Pigment	22 mg/100ml
pH	3.35

The rate of degradation of pelargonidin-3-glucoside in a sample of this juice adjusted to pH 3.40 with citric acid was compared with the rate of degradation of this pigment in the following systems:-

1. 0.5M glucose
2. 0.5M glucose plus amino acids
3. 0.5M glucose plus ascorbic acid
4. 0.5M glucose plus ascorbic acid plus amino acids

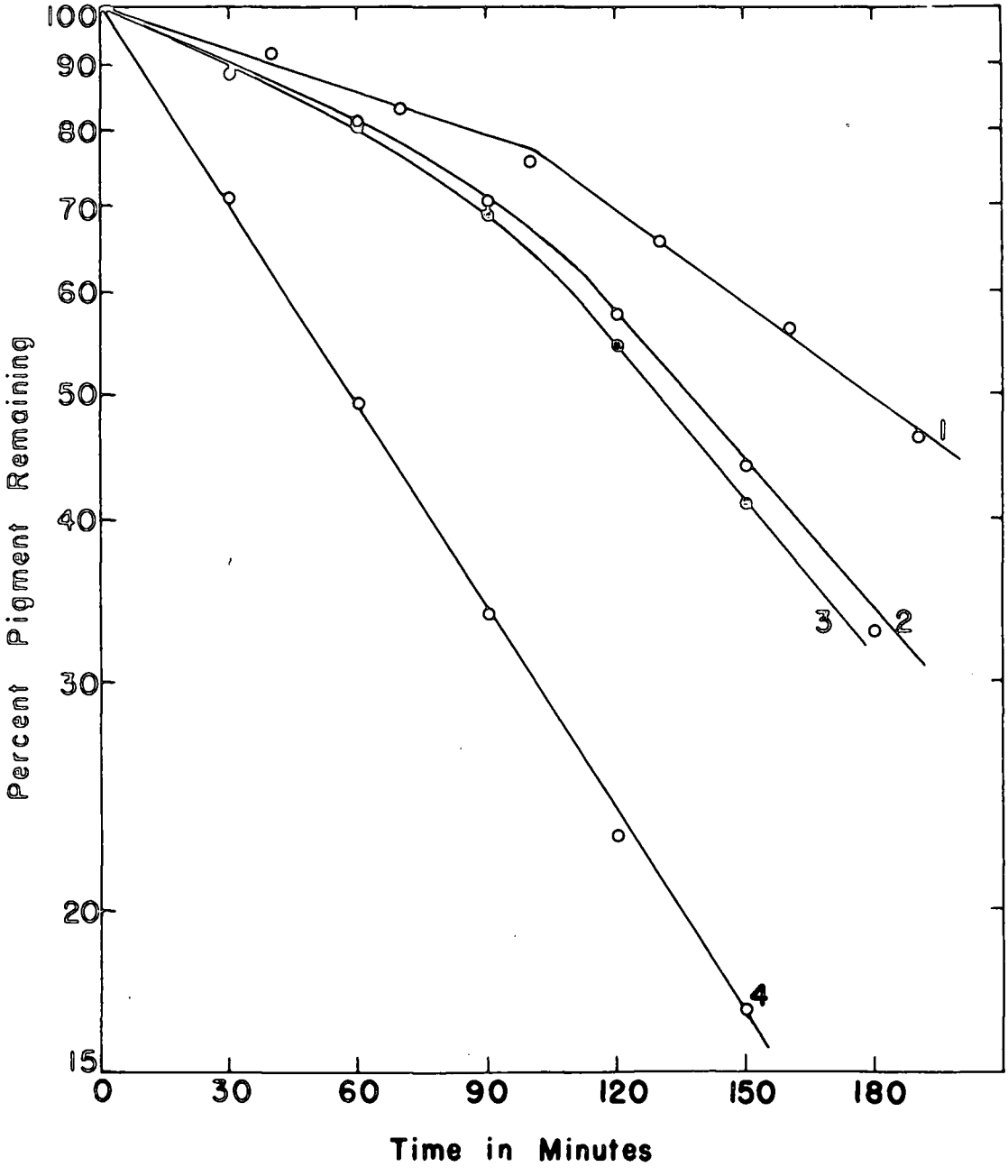
These experiments were repeated substituting 0.25M sucrose for the glucose. The concentration of ascorbic

acid was 20mg per 100ml (Table VII) and the amino acids consisted of a mixture of the five most abundant acids found free in strawberry juice present in the concentrations given in Table I (see page 21).

The rate curves for the sucrose systems are given in Figure 5. Both glucose and sucrose systems give a more rapid rate of pigment degradation than that observed in a buffer system (see Table I, page 21), sucrose being more active over a three-hour period. The addition of the amino acids to the sugars resulted in an additional increase in the rate of pigment degradation. In the sugar plus ascorbic acid system there was little difference between the glucose and sucrose systems, a marked increase in the rate of pigment breakdown being observed on addition of ascorbic acid. Further addition of the amino acids to the sugar-amino acids produced a small increase in the rate of pigment degradation. It was interesting to note that in the latter system after an initial lag period which was not observed in the juice sample, the rate of pigment degradation approaches that observed in strawberry juice. This would indicate that these three factors account for the greater part of the pigment breakdown in strawberry juice.

The juice sample showed first order kinetics for the degradation of the pigment. The fact that no lag was

Figure 5



Comparison of the Rate of Degradation of Pelargonidin-3-glucoside in Composite Systems with That Observed in a Strawberry Juice Sample.

1. Sucrose and Amino Acids
2. Sucrose and Ascorbic Acid
3. Sucrose, Amino Acids and Ascorbic Acid
4. Strawberry Juice

observed in the juice sample may be explained by the fact that the active principles were already present in the sample but with the synthetic sample a short time was necessary before these factors were produced at a rate sufficient to equal the rate of pigment breakdown in the juice. The ultra-violet spectrum of an ether extract of strawberry juice showed a maximum at 283mμ. This corresponded to the maximum absorption of 5-hydroxymethylfurfural.

DISCUSSION

The stability of pelargonidin-3-glucoside varied considerably in the different systems studied in these experiments. A hundredfold difference in first order rate constant was observed at 90° C. The rate of degradation of pelargonidin-3-glucoside in most of the systems studied followed first order kinetics. First order kinetics for the degradation of this anthocyanin have also been observed by Lukton, Chichester and Mackinney (13) in citrate buffer systems and in strawberry juice in the presence or absence of oxygen. Markakis, Livingston and Fellers (15), however, working in citrate buffer systems also, observed first order kinetics in the presence of nitrogen but not in the presence of oxygen. It is possible that in the latter experiments the oxygen concentration became limiting since these data were obtained from sealed tube experiments. In the systems used by Lukton *et al.* (13) and in this laboratory the reaction mixture was saturated with oxygen by continually agitating with air or oxygen.

Perhaps the most striking effect in the work reported here was the increase in pigment stability associated with a decrease in pH. This increase in stability was observed even in the presence of active components such as furfural

and 5-hydroxymethylfurfural. In the presence of glucose or fructose as well as in a buffer system a linear relation was observed between the first order rate constant and the relative concentration of the anthocyanin present as the pseudo-base. Lukton et al. (loc. cit.) working with citrate buffer systems and natural strawberry juice, have observed this type of relation and suggest that the active form of the pigment is the pseudo-base form. The results obtained in this laboratory substantiate this hypothesis.

Meschter (16) has also reported on the effect of pH on the degradation of pelargonidin-3-glucoside in a concentrated sucrose system. A pH range of 0.30 to 3.18 was covered and a maximum half-life was observed at pH 1.80. At pH values lower than 1.80 the half-life decreased quite rapidly. This rather abrupt change might be explained by the fact that at the low pH values used some hydrolysis of the anthocyanin to the anthocyanidin might occur. In this case one would expect a more rapid degradation of the pigment since the work of Lukton, Chichester and Mackinney (13) has shown that pelargonidin is considerably more labile than the glucoside.

Alternatively, this effect could be explained by a marked change in the ratio of the concentration of sugar degradation products to that of the pseudo-base form of

the anthocyanin. At pH 1.80, 96% of the pelargonidin-3-glucoside is present as the benzopyrylium salt. Further decrease in pH produces only small changes in the proportions of the two forms of the anthocyanin. On the other hand with reference to a paper by Singh, Dean and Cantor (22), a comparable change in pH would produce a marked effect on the rate of degradation of sucrose. Hence even though the amount of pigment present as the pseudo-base becomes smaller a marked increase in the amount of sugar degradation products could account for the increase in the rate of pigment degradation. Under such circumstances the rate of change of the benzopyrylium salt to the pseudo-base would eventually be the limiting step.

The important part that sugars can play in the degradation of pelargonidin-3-glucoside is demonstrated by the detrimental effect of fructose and glucuronic acid in particular. Strawberry products may contain considerable quantities of fructose derived from the hydrolysis of sucrose. Although glucuronic acid would not be present in strawberry juice in significant amounts, galacturonic acid could be obtained from the hydrolysis of pectin material. Since galacturonic acid like glucuronic acid, degrades rapidly to furfural in acid medium one would obtain a comparable effect on the rate of pigment

degradation. Fructose would be of more importance since it is more likely that it would be present in concentrations sufficient to produce a significant effect.

At 90° C. none of the sugars used in these experiments exerted a protective effect on the pigment. At lower temperatures the pigment has been shown to be more stable in some sugar solutions (longer half-life) than in a buffer system (16). This could be due to differences in oxygen concentration in the different systems where sugar degradation products were not a factor.

Amino acids also have an effect on the rate of pigment degradation in the presence of sugars. The data obtained in these studies would indicate that the amino acids present in strawberry juice do not contribute to the rate of degradation of the pigment to any large extent. The low pH of strawberry juice would tend to minimize the interaction of sugars and amino acids.

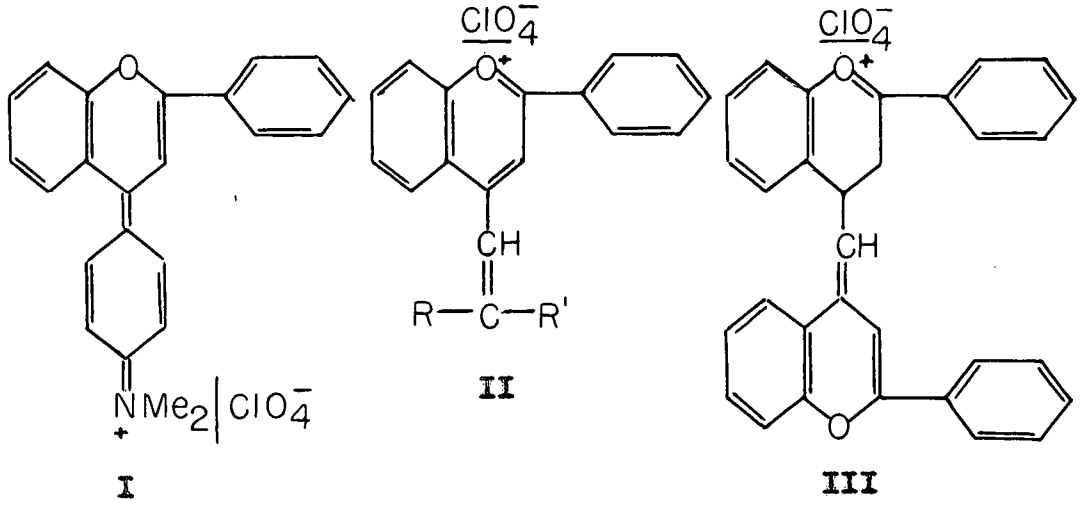
The experimental data indicate that the sugars are probably active in the form of their degradation products. Those sugars such as fructose which degrade most readily in acid medium show the most marked effect on the rate of pigment degradation. An increase in sugar concentration also results in an increase in the rate of pigment degradation. The compounds furfural and 5-hydroxymethylfurfural, which have been identified as

sugar breakdown products (18) accelerate the degradation of pelargonidin-3-glucoside. These compounds also react in a similar manner to sugars in the presence of nitrogen and to varying pH. The marked increase in the rate of pigment degradation in the presence of these two compounds suggests that they might be directly responsible for the degradation of the pigment in the presence of sugars. Since both furfural and 5-hydroxymethylfurfural are quite labile in the presence of oxygen, possibly some oxidative product might be the reactive species although typical breakdown products such as levulinic and formic acids were not effective in degrading the pigment. Another possibility is that in the presence of sugars some precursor of either furfural or 5-hydroxymethylfurfural could be the active component. At the present the exact nature of the degradation of pelargonidin-3-glucoside in the presence of sugars is not known. More information is needed both on the nature of the degradation of sugars in acid medium and the interaction of these products with anthocyanins.

The three most important factors concerned with the degradation of pelargonidin-3-glucoside are temperature, pH and the presence of oxygen. It has been demonstrated that the pH effect may be explained by the effect of hydrogen ion concentration on the equilibrium between

the pseudo-base and the flavylium salt form of the anthocyanin. However, little is known regarding the actual mechanism of the degradation reactions. Hydrogen peroxide destroys anthocyanins very rapidly, it is thought by a cleavage of the pyran ring (11, p.1321). It has been suggested that ascorbic acid owes its activity to the production of hydrogen peroxide on oxidation.

Recent publications on the reactions of flavylium salts by Wizinger and Luthiger (31) and Blackburn, Sankey and Alexander (3) indicate a possible mode of action for the degradation of pelargonidin-3-glucoside in the presence of active components such as furfural and 5-hydroxymethylfurfural. In these papers the condensation of flavylium perchlorates with dimethylaniline diarylethylenes and malonic acid to form the products I, II and III has been described



I II III

The significant aspect of these reactions is that the anthocyanin is active in the form of the pseudo-base and also the reaction does not take place in the absence of oxygen. The reaction is described as an oxidative coupling. There is a marked similarity to the conditions required for the degradation of pelargonidin-3-glucoside, where the anthocyanin reacts in the form of the pseudo-base and oxygen has a distinct effect on the rate of degradation.

These reactions were shown to be equally productive if the chloride salt was used rather than the perchlorate. Investigations on the effects of substituents on the benzpyran ring indicated that although a reaction was observed, the rate was much slower with 3- and 5- substituted flavylium salts. Since pelargonidin-3-glucoside has substituents in these two positions it might be argued that this would preclude this type of reaction as an explanation of pigment degradation. However, the extent of the reaction would be a function of the reactivity and size of the attacking group.

At this stage in our knowledge of the breakdown of pelargonidin-3-glucoside there seems to be four distinct reactions which may be active in the degradation of this anthocyanin.

1. The oxidative degradation of the anthocyanin in pure systems in the presence of oxygen results in the formation of a reddish-brown pigment and also some brown and yellowish material, as observed by Lukton *et al.* (13).

2. The degradation of anthocyanin in the absence of oxygen is quite distinct from the former case. Only small amounts of a red-brown precipitate were formed for equivalent pigment losses.

3. The degradation of the pigment in the presence of hydrogen peroxide in which a bleaching of the pigment is observed seems to be another form of pigment degradation. This is a very rapid reaction and it could be argued that it is a more extensive form of oxidative degradation of the first classification but there is no experimental evidence to support this. The importance of this degradation in natural systems has not been demonstrated.

4. This classification would cover the oxidative coupling type of reaction with such active compounds as furfurals, etc. The effect of sugars on pigment degradation might be classified in this type of reaction. One might explain the oxidative degradation of the pigment in pure systems by this type of reaction. This would require a coupling of 2 molecules of the pigment through the 4-position of each molecule. Steric considerations might preclude this type of reaction, however. In view of the

different substances shown to be active in these condensation reactions with flavylium salts, it might be expected that pelargonidin-3-glucoside may be degraded by oxidative coupling with a number of different compounds. At the present this type of reaction would seem to be the most important in food products.

Ascorbic acid is probably the most important constituent in strawberries relative to pigment degradation. The exact nature of the action of ascorbic acid is not known. It has been suggested that it may be due to hydrogen peroxide formed on oxidation, but this has not been demonstrated. It would seem very probable that ascorbic acid in a similar manner to sugars is active in the form of its oxidative products.

SUMMARY AND CONCLUSIONS

1. The free amino acids shown to be present in strawberry juice were aspartic acid, glutamic acid, alanine, serine, glutamine, asparagine, threonine, arginine, valine, cystine and/or cysteine and leucine and/or isoleucine. Of these, those present in the highest concentration were asparagine, glutamine, alanine, glutamic acid and aspartic acid. The concentrations of the five most abundant acids were determined.

2. In systems buffered at pH 3.40 the rate of degradation of pelargonidin-3-glucoside was observed in the presence of different sugars and sugar derivatives. Variation of the functional group indicated that the rate of pigment degradation was greatest in the presence of glucuronic acid and the ketose, fructose. Sorbitol, a sugar alcohol and gluconic acid gave the slowest rates of pigment degradation. The rate of pigment breakdown in the presence of sugars seemed to be associated with the rate at which the sugar degenerated to furfural type compounds. If the reactions were carried out in the presence of nitrogen rather than air, a marked decrease in the rate of pigment degradation was observed.

3. The presence of amino acids at concentrations of the same order as those observed in strawberry juice in

sucrose and glucose systems produced an increase in the rate of pigment degradation compared to that observed in the presence of a pure sugar or pure amino acid system. These results would also indicate that the rate of pigment degradation was associated with the rate of breakdown of the sugar.

4. At 90° C. and pH 3.40 it was found that an increase in the concentration of glucose, fructose or sucrose produced an increase in the rate of pigment degradation. A plot of the logarithm of the observed rate constants versus the sugar concentration resulted in a linear relation over the concentration range observed.

5. The rate of degradation of pelargonidin-3-glucoside was influenced to a great extent by pH. This effect may be explained by the influence of hydrogen ion concentration on the equilibrium between the pseudo-base and the flavylium salt form of the anthocyanin. Linear relations were observed between the first order rate constants and the proportion of the pigment present as the flavylium salt in glucose and fructose systems as well as the pure buffer system. The rate of degradation seemed to be dependent on the proportion of the anthocyanin present as the pseudo-base.

6. A rapid rate of degradation of pelargonidin-3-glucoside was observed in the presence of furfural and

5-hydroxymethylfurfural. Carrying out the reactions in nitrogen rather than air resulted in a considerable reduction in the rate of pigment degradation. Observations on the rate of pigment degradation in the presence of furfural and 5-hydroxymethylfurfural in systems of varying pH also indicated that the active form of the pigment was the pseudo-base form. A decrease in pH in these systems also resulted in a marked decrease in the rate of pigment destruction.

7. Levulinic acid and formic acid, degradation products of 5-hydroxymethylfurfural in acid medium also accelerate the rate of pigment degradation. However, the effect observed with these compounds was small compared to that observed with 5-hydroxymethylfurfural. This would indicate that the accelerating effect of 5-hydroxymethylfurfural was not dependent on its breakdown to these compounds.

8. A composite system containing pigment, sucrose or glucose, amino acids and ascorbic acid at concentrations similar to those observed in a sample of strawberry juice, after an initial lag period, gave a rate of pigment degradation almost equal to that observed in the natural strawberry juice sample. These factors would apparently account for the greater part of the pigment degradation in strawberry products.

9. Studies in model systems with pelargonidin-3-glucoside seem to indicate four types of degradation reaction: (1) Breakdown of the pigment in pure systems in the presence of oxygen, (2) Breakdown of the pigment in pure systems in the absence of oxygen, (3) Rapid pigment degradation observed in the presence of hydrogen peroxide, (4) Degradation of the pigment in the presence of such compounds as furfural and 5-hydroxymethylfurfural. In the latter case it is suggested that the degradation of the pigment may be due to an 'oxidative coupling' of the particular active component with the anthocyanin.

BIBLIOGRAPHY

1. Aref, A., A. P. Sidwell and E. M. Litwiller. The effects of various sweetening agents on frozen strawberries for preserve manufacture. Food Technology 10:293-297. 1956.

2. Beckman Instruments Incorporated, Fullerton, California. Beckman instruction manual, model DU spectrophotometer and accessories. 1956. 62 p.

3. Blackburn, M. et al. Reactions of flavylium salts with dimethylaniline and malonic acid. Journal of the Chemical Society 1957:1573-1576.

4. Block, Richard J., Emmett L. Durrum and Gunter Zweig. A manual of paper chromatography and paper electrophoresis. New York, Academic Press, 1956. 484 p.

5. Casimir, J. Etude des acides aminés dans les fruits en conserve. Bulletin de l'Institut Agronomique et des Stations de Recherches de Gembloux 20: 33-42. 1952.

6. Casimir, J. and G. Jakovliv. Etudes des acides aminés dansles fruits surgelés, les conserves de fruits et les confitures. Industries Agricole et Aminontaires 69:787-790. 1952.

7. Decereau, Robert V., Gideon E. Livingston and Carl R. Fellers. Color changes in strawberry jellies. Food Technology 10:125-128. 1956.

8. Geissman, T. A. Anthocyanins, chalcones, aurones, flavones and related water-soluble plant pigments. In: K. Paech and M. V. Tracey's Moderne methoden der Pflanzenanalyse. Vol. 3. Berlin, S. A. Barker, 1955. pp. 451-498.

9. Hackman, R. H. and Marian Lazarus. Quantitative analysis of amino acids using paper chromatography. Australian Journal of Biological Science 9:281-292. 1956.

10. Haworth, W. N. and W. G. M. Jones. The conversion of sucrose into furan compounds. I, 5-hydroxymethylfurfural and some derivatives. Journal of the Chemical Society 1944:667-670.

11. Link, Karl Paul. The anthocyanins and the flavones. In: Henry Gilman's Organic Chemistry an Advanced Treatise. Vol. 2. New York, John Wiley and Sons, 1947. pp. 1315-1340.

12. Lukton, A., C. O. Chichester and G. Mackinney. Characterization of a second pigment in strawberries. Nature 176:790. 1955.

13. Lukton, A., C. O. Chichester and G. Mackinney. The breakdown of strawberry anthocyanin pigment. Food Technology 10:427-432. 1956.

14. Mackinney, G., A. Lukton and C. O. Chichester. Strawberry preserves by a low temperature process. Food Technology 9:324-326. 1956.

15. Markakis, Pericles, Gideon E. Livingston and Carl R. Fellers. Quantitative aspects of strawberry pigment degradation. Food Research 22:117-130. 1957.

16. Meschter, E. Everett. Effects of carbohydrates and other factors on strawberry products. Journal of Agricultural and Food Chemistry 1:574-579. 1953.

17. Moore, Stanford and William H. Stein. Photometric ninhydrin method for use in the chromatography of amino acids. Journal of Biological Chemistry 176:367-388. 1948.

18. Pigman, William Ward and Rudolph Maximilian Goepp, Jr. Chemistry of the carbohydrates. New York, Academic Press, 1948. 748 p.

19. Pratt, Dan E. et al. Interaction of ascorbic acid, riboflavin and anthocyanin pigments. Journal of Agricultural and Food Chemistry 2:367-372. 1954.

20. Robinson, G. M. and R. Robinson. A survey of anthocyanins. II. Biochemical Journal 26: 1650-1664. 1932.

21. Robinson, Robert. Über die Synthese von Anthocyaninen. Berichte der Deutschen Chemischen Gesellschaft 67A:85-105. 1934.

22. Singh, Bhagat, G. R. Dean and Sidney M. Cantor. The role of 5-(hydroxymethyl)-furfural in the discoloration of sugar solutions. Journal of the American Chemical Society 70:517-522. 1948.

23. Sondheimer, Ernest. On the relation between spectral changes and pH of the anthocyanin pelargonidin-3-glucoside. Journal of the American Chemical Society 75:1507. 1953.

24. Sondheimer, Ernest and Z. I. Kertesz. Anthocyanin pigments. Colorimetric determination in strawberries and strawberry products. Analytical Chemistry 20:245-248. 1948.

25. Sondheimer, Ernest and Z. I. Kertesz. The anthocyanin of strawberries. Journal of the American Chemical Society 70:3476-3479. 1948.

26. Sondheimer, Ernest and Z. I. Kertesz. The kinetics of the oxidation of strawberry anthocyanin by hydrogen peroxide. Food Research 17:288-298. 1952.

27. Sondheimer, Ernest and Z. I. Kertesz. Participation of ascorbic acid in the destruction of anthocyanins in strawberry juice and model systems. Food Research 18:475-479. 1953.

28. Sondheimer, Ernest and Carole B. Karash. The major anthocyanin pigment of the wild strawberry. Nature 178:648-649. 1956.

29. Teunissen, H. P. Velocity measurements on the opening of the furane ring in hydroxymethylfurfuraldehyde. Recueil des Travaux Chimiques des Pays-Bas 49:784-826. 1930.

30. Williams, Byron L. and Simon H. Wender. The isolation of kaempferol and quercetin from strawberries (Fragaria chiloensis). Journal of the American Chemical Society 74:5919-5920. 1952.

31. Wizinger, R. and A. Luthiger. Über eine einfache Synthese von Flavyliumfarbsalzen. Helvetica Chimica Acta 36:526-530. 1953.