Table III. Analysis of Variance

Data on enzyme activity and ascorbic acid content as affected by cooking parsnips by four different methods to various internal temperatures (ten replicates)

Constituent	Temp., ° C.	Source of Variation	Degrees of Freedom	F	Significance
Ascorbase	25–40	Cooking method, *C*	3	4.91	At 0.01 level
		Replication within *C*	36		
		Temperature, *T*	2	31.04	At 0.01 level
		T × *C*	6	2.91	At 0.05 level
		Residual	72		
		Total	119		
Ascorbase	45–55	*C*	3	1.93	Not significant
		Replication within *C*	36		
		T	2	7.81	At 0.01 level
		T × *C*	6	1.62	Not significant
		Residual	72		
		Total	119		
Peroxidase	25–60	*C*	3	23.54	At 0.01 level
		Replication within *C*	36		
		T	6	20.46	At 0.01 level
		T × *C*	18	1.77	Not significant
		Residual	216		
		Total	279		
Ascorbic acid	20, 25	*C*	3	2.73	Not significant
		Replication within *C*	36		
		T	1	27.29	At 0.01 level
		T × *C*	3	1.88	Not significant
		Residual	36		
		Total	79		
Ascorbic acid	25–90	*C*	3	3.08	At 0.05 level
		Replication within *C*	36		
		T	8	17.47	At 0.01 level
		T × *C*	24	2.08	At 0.01 level
		Residual	288		
		Total	359		

Table IV. Retention of Ascorbic Acid in Potatoes and Parsnips at 55°, 85°, and 90° C.

Cooking Method	Potatoes, %			Parsnips, %	
	55° C.	85° C.	90° C.	55° C.	90° C.
Pressure-cooked	75	73	..	47	30
Steamed	90	..	..	35	22
Boiled	85	..	..	31	31
Baked	89	..	108	25	35
Av.	85	..	..	35	30

for parsnips ranged from 25% for baked parsnips to 47% for pressure-cooked parsnips, whereas the range was from 75% for pressure-cooked potatoes to 90% for steamed potatoes. Preparation of the vegetables for cooking differed in that the tip and stem ends of the parsnips were removed, whereas in all cases the potatoes were cooked in their skins. However, the difference in average vitamin retention for the two vegetables could not be explained on the basis of leaching, as baked parsnips retained less ascorbic acid than did boiled parsnips cooked to 55° C.

While ascorbic acid losses in potatoes and parsnips cooked to an internal temperature of 55° C. were considerable, losses of the vitamin over the range 55° to 90° C. were slight. Retentions of ascorbic acid in parsnips cooked to 90° C. are reported in Table IV. Comparable retentions in potatoes are available only for pressure-cooked and for baked potatoes. Comparison of retentions for parsnips at 55° and at 90° C. shows that a small decrease in ascorbic acid content resulted from pressure-cooking and steaming between 55° and 90° C., boiling caused no change, and baking caused an apparent gain. These data are further indication that leaching was not the reason for low ascorbic acid retentions in parsnips; and they also indicate that both potatoes and parsnips, when baked, show between 55° and 90° C., an apparent gain in ascorbic acid content of 19 and 10%, respectively.

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Received for review April 1, 1953. Accepted June 19, 1953. Data presented by Johnnie H. Watts to the Faculty of the Division of the Biological Sciences of the University of Chicago in partial fulfillment of the requirements for the degree of doctor of philosophy.

FRUIT COLOR LOSS

Effects of Carbohydrates and Other Factors On Strawberry Products

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STRAWBERRY PRESERVES represent about 26% of all fruit preserve flavors, or about 12% of the total production of all fruit spreads, and consequently the largest volume of a single variety of fruit spread in the country.

When strawberry preserves are made by modern vacuum pan methods, the finished product is a bright red or bright maroon-red, depending on the variety of strawberry used. As the preserve is stored at room temperature in a ware-

Pigment extracted from strawberry juice with 1-butanol was used to study effects of various materials on the loss of color. The data show that temperature and pH value have a great effect on the rate of pigment loss. Salt concentration affects the rate of pigment loss in strawberry juice. The effect seems to be related to the presence of sugars, as a similar effect is not observed in the reaction with extracted pigment when no sugars are present. Both ascorbic acid and dehydroascorbic acid change the magnitude of the rate of pigment loss. Both furfural and hydroxymethylfurfural increase the rate of pigment loss, and because these compounds are typical of sugar degradation products, it is strongly suggested that sugar deterioration products react with the pigment of strawberry preserves. Materials present in strawberry juice itself (probably sugars) apparently have an appreciable effect on rate of pigment loss.

house or on a grocery shelf, the pleasing red color diminishes, so that within six months the product has lost so much of its appealing color as to become deep maroon-brown and be unsalable. The author's approach to this problem has been based on measurement of kinetics of the pigment system in various environments in the presence of different sweetening agents.

The first step in this investigation was to isolate the pigment from strawberry juice by the method described by Sondheimer and Kertesz (*11*). This method involved the saturation of the juice with salt and extraction with 1-butanol, then concentration of the butanol extract under vacuum and in a nitrogen atmosphere. The anthocyanin concentrate was then taken up with hydrochloric acid in anhydrous methanol, precipitated with ether, dissolved in 0.01% hydrochloric acid, and saturated with picric acid. The anthocyanin picrate crystallized out of this solution upon storage at 0° C. as lustrous reddish bronze prisms which were further purified by recrystallization.

The purified anthocyanin chloride, believed to be pelargonidin 3-monoglucoside, obtained by this procedure, had external characteristics which were identical with the product prepared by Sondheimer and Kertesz.

However, the chromatogram on paper of this purified material [by ascending technique using 4 parts of 1-butanol–1 part of glacial acetic acid–5 parts of water as the solvent] showed only one red-orange spot, while the filtered, water-soluble portion of the pigment just prior to the picration step showed not only the same red-orange spot but also a purple spot which is perhaps an isomer of pelargonidin 3-monoglucoside (*2*) just below and not imposed upon the larger, more intense red-orange pelargonidin spot. (The study of the nature of this purple spot has not been completed.) It seemed evident, therefore, that the purification by crystallization as the picrate had left behind a portion of the pigment normal to the original juice. For this reason the basic coloring material, wherever extracted pigment was used, was the water-soluble portion of the ether-precipitated concentrate of the 1-butanol extract of strawberry juice (St B). This material has not been further purified by picration and crystallization. It contains essentially all the anthocyanin coloring pigments in strawberries free from the natural sugars. It was found, however, that the reactions of the water-soluble portion and the recrystallized material were not significantly different.

The natural strawberry material was prepared from a good commercial grade of frozen Blakemore strawberry juice. Its history showed that it had never been pasteurized, was pressed on stainless steel equipment, and had not been otherwise abused. The frozen juice was slowly thawed and the early free-run juice was collected at a soluble solids content of 22% (St J).

This made it possible to conduct experiments allowing more than 2 to 1 dilution for the incorporation of other reacting material and buffer solutions, and still maintain the strawberry ingredients in concentration normal to the original natural juice.

The color of some of this concentrated strawberry juice was destroyed by autoclaving at 121° C. (250° F.) for 1 hour. This material (St JD) was brown colored, slightly turbid, and identical with the concentrate (St J), except for the damage done by the heat treatment.

Some of the concentrate was diluted back to single-strength strawberry juice and saturated at room temperature with sucrose. This mixture (St S) closely simulates the composition of a finished strawberry preserve.

In all cases, the storage temperature for this study was 38° C. (100° F.) and the pH value was maintained at 3.0 ± 0.03. All juice and buffer solutions stored at 38° C. were saturated with thymol for prevention of fermentation and mold growth. Thymol has been found to be clearly inert as far as the color loss is concerned. Buffer solution in all cases refers to Sorensen's citrate buffer, adjusted to pH 3.0 with hydrochloric acid, and saturated with thymol. The importance of buffer concentration is shown below.

The nitrogen content of all the materials added to the strawberry juice was reported by a consulting analytical laboratory to contain a trace or no nitrogen as determined by the Dumas method.

The ascorbic acid content of the frozen concentrated juice was very low—less than 1 mg. per 100 grams as determined by potentiometric titration. Normal ascorbic acid content of fresh strawberries is about 50 to 100 mg. per 100 grams (*3*). Ascorbic acid may have been lost during the freezing and thawing operations involved in the concentration procedure.

The copper content of the natural juice was found to be about 1 p.p.m. This is approximately the normal amount in natural strawberries and indicates trace or no contamination of the juice by copper in processing.

The raw material, then, consisted of:

1. St B. Water-soluble portion of the ether-precipitated butanol-extracted pigment of strawberry juice.
2. St J. A representative sample of commercial strawberry juice, concentrated by freezing.
3. St JD. The same juice as in (2) damaged by high temperature processing.
4. St S. Single-strength strawberry juice made up to 67% solids with sucrose.

The changes in anthocyanin concentration were followed by a modification of the method of Sondheimer and Kertesz (*9*), which is based on the measurement of absorbance (optical density) at 500 mμ of solutions of the anthocyanin at pH 3.4 and 2.0. The anthocyanin concentration is proportional to the absorbance difference. Figure 1 shows the spectra of solutions of anthocyanin pigment of the same concentration at different pH values.

The authors have found, however,

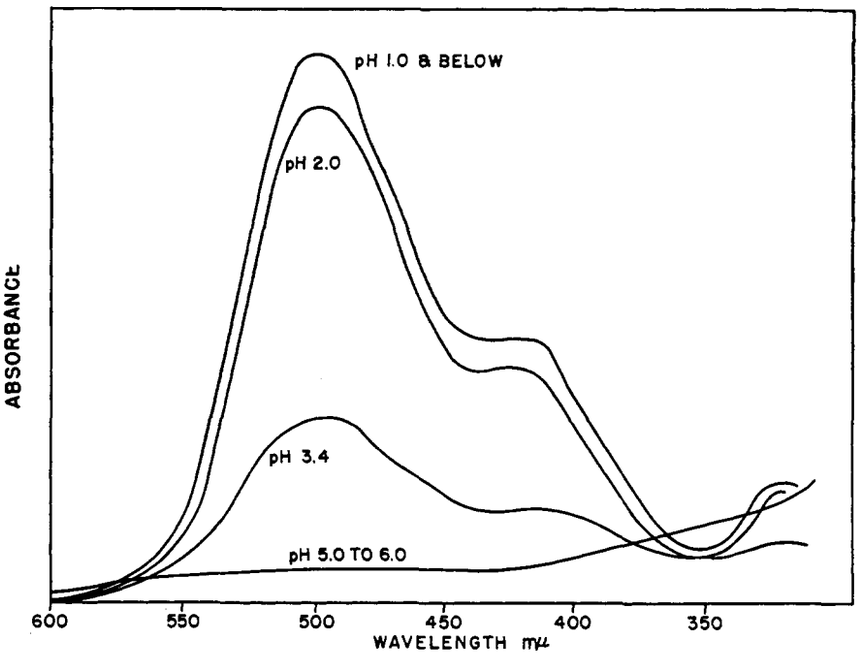


Figure 1. Change in spectra of buffered solution of strawberry juice pigment concentrate with change in pH at constant concentration

that at pH 1.0 the absorbance reaches a maximum, and a pH value of less than 1.0 does not further increase the absorbance measurably at its 500 mμ peak. At 5.4 or at any pH values between 5.0 and 6.0, the absorbance of anthocyanin at 500 mμ is at an indistinguishable minimum. Therefore, the critical control of pH is eliminated, and test papers can be used to determine the broad range of pH in which absorbance difference accurately reflects anthocyanin content. The factor for conversion for absorbance difference, pH 2.0 to 3.4 to pH 1.0 to 5.4, is 1.7. This method is believed to be more accurate, less tedious, and considerably faster than the original method, as it eliminates the need for precise pH measurement.

Anthocyanin content is not the sole factor in determining the acceptability of a strawberry preserve. Mackinney has pointed out in reports to the National Preservers Association that the pigment content may drop to as low as 10% and still be acceptable, as long as browning development is low and brightness remains high. Even if pigment content is high, its value may be overshadowed by a high browning rate. Browning increases in rate four times as fast as pigment loss with increasing temperature. Therefore a preserve stored at 10° C. will be much more acceptable when half of its pigment has been lost than one of the same pigment content that has been stored at 20° C., because browning will be much lower in the lower temperature sample. Anthocyanin content alone has been used as a measure of color or pigment stability in this paper, even though some samples of higher pigment content were less acceptable visually than others, as the dark brown color prevented realization of the high pigment content. A method of color measurement for evaluating visual acceptability may have to include a measure of brightness, turbidity, and dominant wave length rather than pigment content alone.

The determination of color change in strawberry products is not a clear-cut, ideally accurate process. In all measurements of color loss in solutions employing only extracted pigment as the color source, reaction rates were clear cut and reproducible. Where natural sugars and the complex materials found in all natural products were present, it was necessary to rely on trends and orders of magnitude to evaluate the importance of additives in changing the reaction rates. For this reason, changes of less than 5% in magnitude are in general insufficient evidence of important effect and are not reported here.

In considering the possible means by which strawberry color is deteriorated, investigation reveals five significant factors:

1. Effect of temperature
2. Effect of pH value
3. Effect of salt concentration
4. Effect of ascorbic and dehydroascorbic acid
5. Effect of sugars and sugar destruction products

Effect of Temperature

The most important factor in changing the kinetics of the degradation of color in strawberry products is temperature. The preserver can alter this factor to a limited degree in his choice of manufacturing and storage procedures. The rate of color deterioration increases in proportion to the log of the temperature. Figure 2 shows the relationship graphically.

At room temperature (20° C.), the half life of the color of strawberry preserves is about 1300 hours. In the summertime, the grocery shelf in the front of the corner grocery store may reach 38° C., at which temperature the half life drops to 240 hours. It is possible to increase the life of the color in the finished preserves six or seven times over room temperature storage by storing under egg storage conditions (4° C.), where the half life is extended to 6000 to 8000 hours (250 to 320 days).

Effect of pH Value

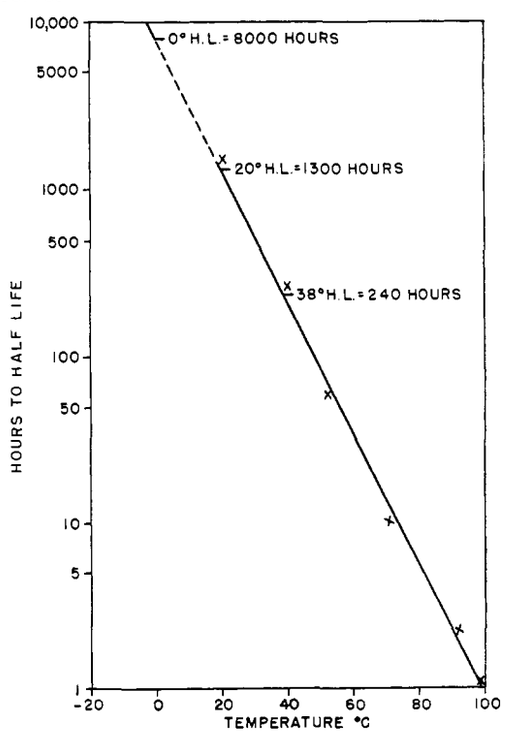
The rate of anthocyanin degradation is greatly affected by pH value. Increasing acidity has a protective effect on the stability of the pigment. Figure 3 shows the relative rate of pigment loss in buffered solutions at various pH values.

It is obvious that pH must be accurately controlled when effects of added ingredients are to be studied. Sorensen's citrate buffer solution was used throughout.

Effect of Buffer Salt Concentration

The importance of maintaining constant buffer salt concentration is graph-

Figure 2. Effect of temperature on half life of color in strawberry preserves



ically illustrated in Figure 4, which shows an increase in rate of pigment loss when increments of sodium citrate were added to strawberry juice (St J). However, this detrimental effect was not observed when sodium citrate was added to extracted pigment (St B) solutions, as shown in Figure 5. A protective effect of the citrate ion on color loss in the iron-catalyzed anthocyanin–hydrogen peroxide reaction has been shown by Sondheimer and Kertesz (*10*). A similar mechanism may account for the increased stability of the color with increments of sodium citrate in the sugar-free color extract. In the strawberry juice, however, any slight protective effec shown by the citrate ion is overpowered by the effect of the sodium ion on sugar destruction observed by Moyer (*6, 7*). The products formed by this degradation may thus be more reactive with the pigment.

Effect of Ascorbic and Dehydroascorbic Acids

The effect of ascorbic acid on color loss in strawberry products has been studied by Sondheimer and Kertesz (*10*). This reaction is catalyzed by the presence of iron or copper. Although they make it clear that the presence of hydrogen peroxide in strawberry products has not been demonstrated, Sondheimer and Kertesz have suggested that the hydrogen peroxide formed when ascorbic acid is oxidized to dehydroascorbic acid may play a role in the degradation of pelargonidin 3-monoglucoside. Indeed, this may well account for some of the kinetic difference in the reaction between ascorbic acid and the pigment and dehydroascorbic acid and the pigment. However, the relatively high velocity of pigment destruction with dehydroascorbic acid suggests that a much more complex mechanism is involved.

The dehydroascorbic acid used in this experiment was prepared by the Research Laboratories of the Hoffmann-La Roche Co., Nutley, N. J., by the process of Pecherer (*8*) and is reported to be 95 to 100% pure. The impurities may include some ascorbic acid and inert oxidation products which result during processing. Even if the maximum impurities consisted entirely of ascorbic acid, the importance of the effect of dehydroascorbic acid on the pigment system would not be significantly changed.

The relationship between ascorbic acid and dehydroascorbic acid on the rate of strawberry pigment loss in water-soluble pigment (St B) is given graphically in Figure 6.

The susceptibility of ascorbic acid to oxidation has long been known, but it is surprising to note its stability when strawberry preserves are made by vacuum processing methods.

A sample of strawberries contained 39 mg. (360 p.p.m.) of ascorbic acid per 100 grams as determined by potentiometric titration. After the preserves were sterilized and cooled, 82% of the original ascorbic acid content still remained. After the preserve had been stored for 2 weeks at 38° C., 52% of the ascorbic acid and 41% of the original anthocyanin content remained.

It is obvious that the mechanism of ascorbic acid attack on the strawberry pigment is of very practical interest, as

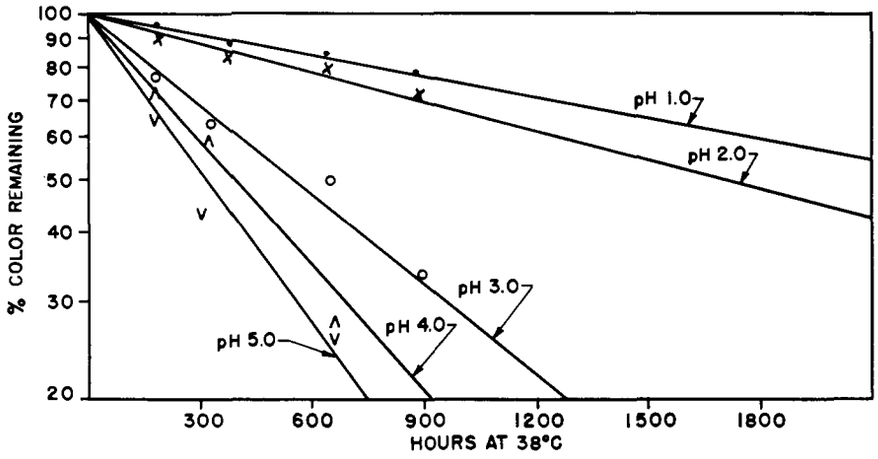


Figure 3. Effect of change in pH on rate of color loss in buffered solutions of strawberry juice concentrate

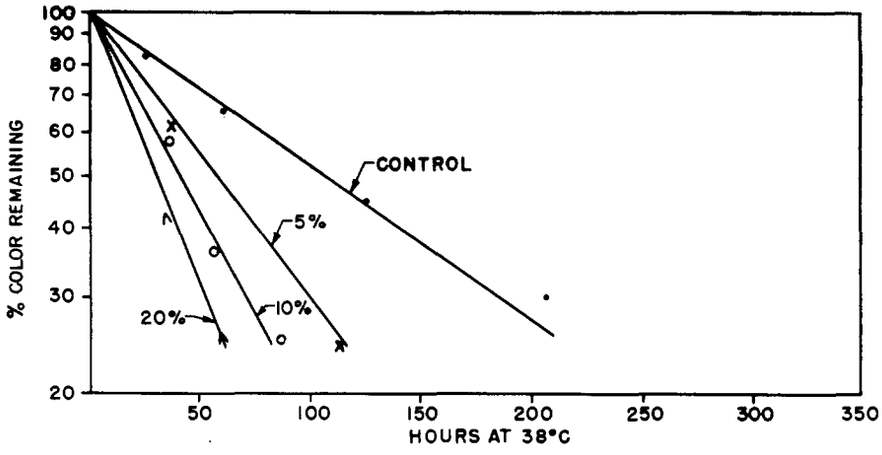


Figure 4. Effect of sodium citrate on rate of color loss in frozen and thawed strawberry juice

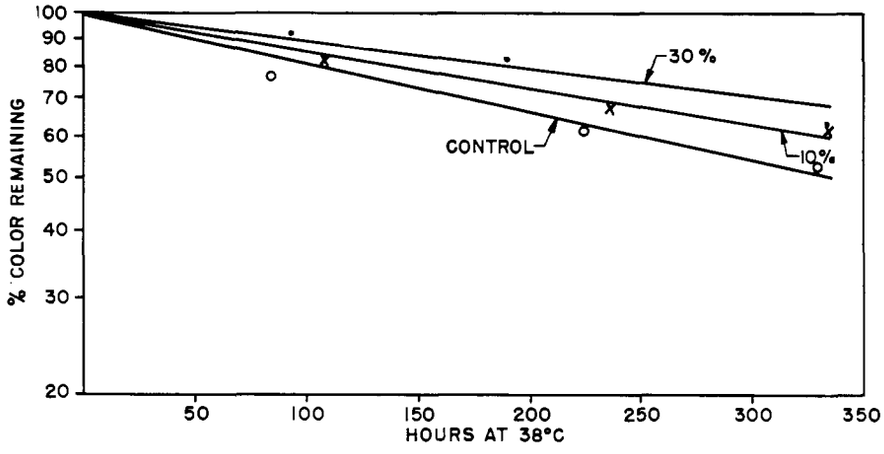


Figure 5. Effect of sodium citrate on rate of color loss in buffered solutions of strawberry juice

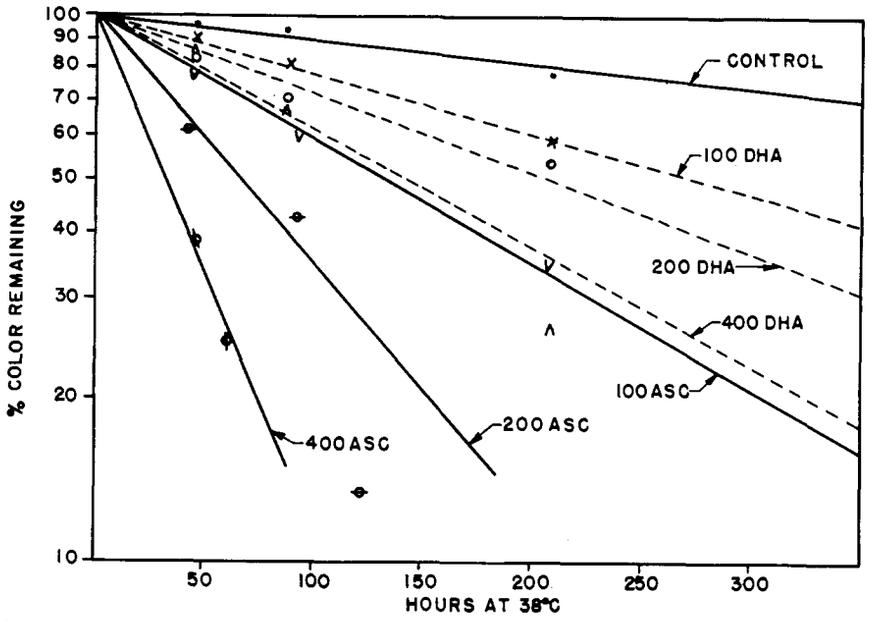


Figure 6. Effect of ascorbic and dehydroascorbic acids on rate of colcr loss in buffered solutions

Units, p.p.m.

the preserver can do nothing about the original ascorbic acid content of his raw material. This also emphasizes the importance of the amount of metallic contamination in the strawberry preserve. Iron and copper in themselves are not important contributors to the pigment loss mechanism, except in so far as they hasten the destruction of the ascorbic acid. These destruction products in turn attack the pigment at an increased rate. If only ascorbic acid, and not its degradation products, were responsible for the rapid loss of color, as the ascorbic acid in the pigment medium was destroyed, the rate of loss of remaining pigment would be expected to decrease. Such a change in rate is not significant and apparently the destruction products of ascorbic acid also react with the color. Table I shows the relationship between rate of pigment loss in single-strength strawberry juice and the metallic ion content with and without ascorbic acid.

Although ascorbic acid, particularly in the presence of iron or copper, is capable of increasing the rate of pigment loss, the rate of loss when none of these agents is present is already of a distressing magnitude. Thus some other cause of color loss has yet to be explained.

Table I. Effect of Ascorbic Acid in Presence of Iron and Copper on Rate of Color Loss in Strawberry Juice (St J)

Ascorbic Acid, P.P.M.	*Cu or Fe, P.P.M.*	*Hours to Half Life*
	Control	320
None	Cu, 40	310
None	Fe, 40	310
400	None	190
400	Cu, 10	140

Effect of Sugars and Sugar Destruction Products

The data presented in this section attempt to show that several sugars and sugar degradation products are capable of reacting with the pigment components of strawberries to increase their rate of loss materially. Thus substances formed during the browning of sugars may cause color loss in strawberry products. It has been demonstrated that when extracted pigment is stored at various pH values, the stability of the color is greatly increased as the pH value is lowered (see Figure 3). When strawberry sirup is similarly stored, however, the stability of the pigment reaches a maximum at a pH value of 1.8. Below this value, pigment stability decreases as the pH is lowered. The high rate of browning of sugars at low pH values is well known and the data in Table II suggest that the reactivity of these sugar degradation products with the pigment is more important than the stability contributed to the pigment by the low pH value.

Table II. Effect of pH Value on Half Life of Color in Strawberry Sirup (St S)

pH	*Hours to Half Life*
0.30	240
0.65	385
1.05	415
1.55	460
1.80	480
2.10	430
2.55	330
2.88	275
3.18	210

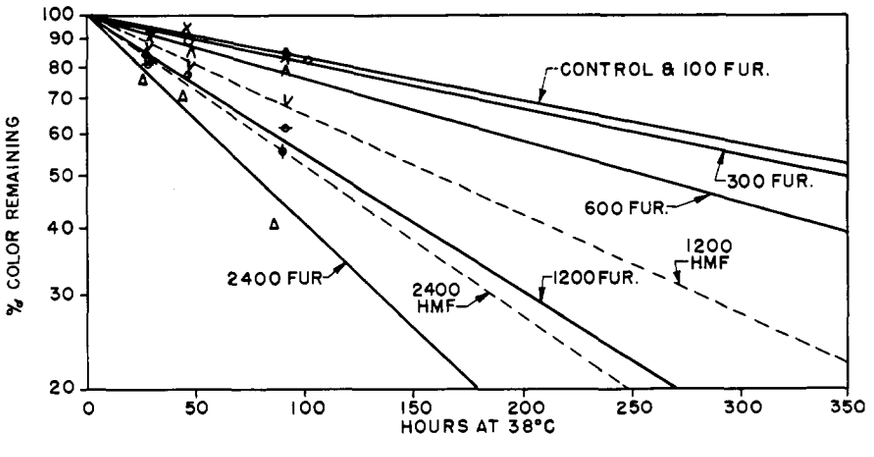
Sondheimer and Kertesz pointed out as early as 1947, in reports to the National Preservers Association, that aldehydes were capable of reacting with pelargonidin 3-monoglucoside. Formaldehyde reacts with the pigment to form a colorless solution and a purple precipitate. Furfural and hydroxymethylfurfural are known to be typical intermediates in the destruction of sugars, and the kinetics of these two products with strawberry color is shown in Figure 7.

These data illustrate the ability of some sugar degradation products to increase the rate of strawberry pigment loss. Furfural and hydroxymethylfurfural are cited only as examples typical of a great number of products which result from the breakdown of sugars.

Further evidence that the products of sugar degradation increase the rate of strawberry pigment loss is presented in Table III. Here are listed, in order of increasing anthocyanin degrading ac-

Figure 7. Effect of furfural and hydroxymethylfurfural on rate of color loss in buffered solutions

Units, p.p.m.



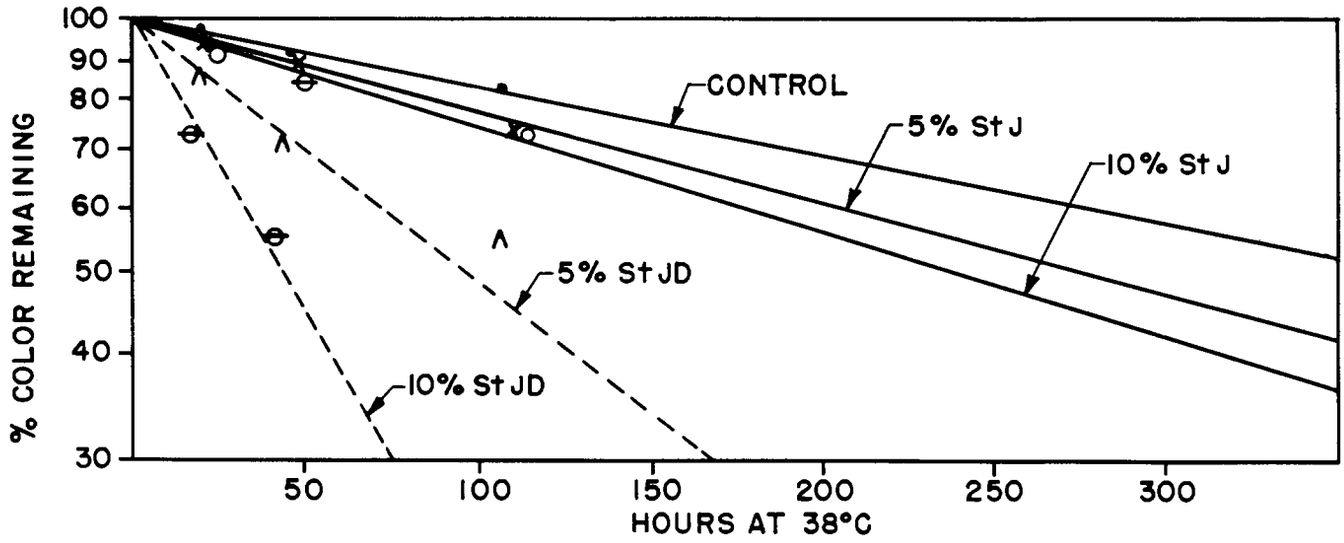


Figure 8. Effect of addition of concentrated strawberry juice and heat-damaged strawberry juice on rate of color loss in buffered solutions

tivity, various carbohydrates with their corresponding half lives. This list is essentially the order of ring stability or carbonyl activity demonstrated by these sugars in other reactions of this type (*1*, *5*).

Table III. Relative Effect of 40% Concentration of Various Carbohydrates on Stability of Water-Soluble Pigment (St B)

Carbohydrate	Hours to Half Life
Corn sirup	650–700
Methyl α-D-glucoside	
Mannose	
Glycerol	
Sucrose	
Maltose	
Sorbitol	
Control, no sugar	
Arabinose	300
Levulose	
Sorbose	240

The source of the carbohydrate that might enter into such a reaction need not necessarily be an added one. Indeed, there is evidence that the source material is already present in the strawberry juice itself. Figure 8 shows the effect of the addition of concentrated strawberry juice and heat-damaged strawberry juice upon pigment loss. The juice must contain an agent or agents, which are increased upon heat treatment, capable of reacting with the pigment. Such a mechanism could also explain the difficulty in making uniform measurement of rate of pigment loss on natural strawberry products, as these unidentified products may be present in varying quantities from time to time.

Further evidence in support of this explanation is given by the data in Table IV. Here, the rate of anthocyanin loss was measured in regular unfermented strawberry juice in the presence of several sugars. Then some of the same juice was fermented by *Saccharomyces cerevisiae* and any pigment lost during the fermentation was replaced by the addition of water-soluble pigment. Carbohydrates were similarly added to this fermented juice after fermentation. The rate of pigment loss in the two juices was then followed, with the results given.

Table IV. Effect of Fermentation upon Rate of Color Loss in Presence of Various Carbohydrates in Strawberry Juice (St J)

Carbohydrate	Hours to Half Life	
	Fermented	Unfermented
Corn sirup	380	250
Sucrose	250	143
Dextrose	250	150
Levulose	168	114
Control, no sugar	205	100

After most of the natural carbohydrates have been removed by fermentation, the stability of the pigment on storage is greatly increased. Differences in the reactivity of the various added carbohydrates are evident. The relative effect of these sugars has always remained in this order, regardless of the environment in which the reactivity of the sugars with the pigment has been studied.

Loss of sugar is not the only change in the juice upon fermentation. But the data strongly suggest that some ingredient normally reactive with the pigment has been removed and it would be plausible to assume that this ingredient is a carbohydrate.

It is to be expected that under the conditions of these experiments, as well as in finished preserves, some sucrose will be inverted and thus the sucrose samples will contain both dextrose and levulose.

Acknowledgment

The writer is indebted to Gordon Mackinney, University of California, for his vital interest and helpful cricitism of the work, and to Sidney M. Cantor, American Sugar Refining Co., for his encouragement and valuable assistance in the evaluation and interpretation of the data.

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Received for review March 17, 1953. Accepted June 19, 1953. Presented before the Division of Carbohydrate Chemistry, Symposium on the Use of Sugars and Other Carbohydrates in the Food Industry, at the 123rd Meeting of the AMERICAN CHEMICAL SOCIETY, *Los Angeles, Calif.*