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Title: CAUSATIVE FACTORS OF COLOR DETERIORATION IN STRAWBERRY PRE-  
SERVES DURING PROCESSING AND STORAGE

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An effort was made to determine what factors are responsible for differences in color quality between preserves commercially manufactured from Hood and Tioga strawberry varieties.

Color analyses made on Hood and Tioga preserves, during a 26 week storage period, included spectral measurements of aqueous extracts from the preserve samples. In addition, Hunter color coordinates were determined for both the insoluble residues (remaining after extraction) and the intact preserve samples. Color analyses revealed that color deterioration occurred at a much faster rate in Tioga preserves than in Hood preserves, and that this deterioration was due to a faster rate of browning in Tioga preserves.

Complete chemical analyses of fruit revealed striking compositional differences between Hood and Tioga varieties. The concentrations of free amino acids and metal ions were found to be similar in both varieties. Ascorbic acid, which is believed by many to contribute significantly to color deterioration, was actually present in lower concentration in the Tioga variety. Anthocyanins were present in greater

amounts in the Hood variety, while leucoanthocyanins, flavanols and total phenolics were higher in Tiogas. Recent work, primarily with wine and model wine systems, has shown that leucoanthocyanins, catechins, and possibly other reactive phenolics, will react with anthocyanins to form polymeric pigments. The results of this study are supportive of the hypothesis that a similar reaction in preserves (between anthocyanins and other phenolics) is responsible for color deterioration during storage.

Causative Factors of Color Deterioration in Strawberry  
Preserves During Processing and Storage

by

Julie Ellen Abers

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Typed by researcher for Julie Ellen Abers

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TABLE OF CONTENTS

|  | <u>Page</u> |
|--|-------------|
| INTRODUCTION.....                                | 1           |
| LITERATURE REVIEW.....                           | 3           |
| EXPERIMENTAL.....                                | 8           |
| Color Changes in Preserves.....                  | 8           |
| Preserves.....                                   | 8           |
| Aqueous Extracts.....                            | 8           |
| Color Analyses.....                              | 9           |
| Compositional Analyses of Fruit.....             | 10          |
| Strawberries.....                                | 10          |
| Aqueous Extracts.....                            | 10          |
| Ascorbic Acid.....                               | 11          |
| Metal Ions.....                                  | 11          |
| Free Amino Acids.....                            | 12          |
| Leucoanthocyanins.....                           | 12          |
| Flavanols.....                                   | 13          |
| Anthocyanin Content.....                         | 13          |
| Total Phenolics.....                             | 14          |
| RESULTS AND DISCUSSION.....                      | 15          |
| Color Changes in Preserves.....                  | 15          |
| Compositional Analyses of Fruit.....             | 25          |
| Ascorbic Acid and Metal Ions.....                | 25          |
| Free Amino Acids.....                            | 27          |
| Leucoanthocyanins.....                           | 27          |
| Anthocyanins, Flavanols and Total Phenolics..... | 30          |
| Summary and Conclusions.....                     | 33          |
| BIBLIOGRAPHY.....                                | 34          |
| APPENDIX.....                                    | 38          |

LIST OF TABLES

| <u>Table</u> |  | <u>Page</u> |
|--------------|--|-------------|
| 1            | Comparison of color density and polymeric color in Hood and Tioga preserves stored at 21°C and 37°C. | 18          |
| 2            | Hunter reflectance values for insoluble residues from preserve samples.                              | 24          |
| 3            | Comparison of ascorbic acid content and pH of Hood and Tioga strawberries.                           | 25          |
| 4            | Comparison of metal ion concentrations in Hood and Tioga strawberries.                               | 26          |
| 5            | Comparison of free amino acids in Hood and Tioga strawberries.                                       | 28          |
| 6            | Comparison of relative amounts of leucoanthocyanins in Hood and Tioga strawberries.                  | 29          |
| 7            | Comparison of anthocyanins, flavanols and total phenolics in Hood and Tioga strawberries.            | 30          |

## LIST OF FIGURES

| <u>Figure</u> |  | <u>Page</u> |
|---------------|--|-------------|
| 1             | Effect of storage time and temperature on the (Hunter reflectance) $\tan^{-1}$ a/b function of Hood and Tioga preserves.     | 16          |
| 2             | Effect of storage time and temperature on the (Hunter transmission) $\tan^{-1}$ a/b function of Hood and Tioga strawberries. | 17          |
| 3             | Visible spectrum from an aqueous extract of fresh Tioga preserves.   | 19          |
| 4             | Visible spectrum from an aqueous extract of Tioga preserves at 19 weeks of storage.  | 20          |
| 5             | Effect of storage time and temperature on the formation of brown pigments in Hood and Tioga strawberry preserves.            | 22          |
| 6             | Effect of storage time and temperature on the loss of red anthocyanin pigment in Hood and Tioga strawberry preserves.        | 23          |



# CAUSATIVE FACTORS OF COLOR DETERIORATION IN STRAWBERRY PRESERVES DURING PROCESSING AND STORAGE

## INTRODUCTION

Color deterioration in strawberry preserves has been a persistent problem, concerning the food processor for many years. The bright red color of freshly made preserves is known to deteriorate rapidly when the product is stored at room temperature. This adversely affects consumer acceptance, imparting a relatively short shelf life to the product.

Color deterioration of strawberry preserves is due, at least in part, to degradation of the red anthocyanin (ACN) pigments present in the fresh fruit. Thus, degradation of pelargonidin-3-glucoside (the major ACN pigment in strawberries) in model systems and in strawberry products has been studied extensively. Investigators have identified storage temperature and oxygen as the elements exhibiting the most profound effect on color deterioration. Other factors which have been found to affect ACN degradation include sugar and sugar breakdown products, pH and ascorbic acid.

This investigation was based on the fact that certain strawberry varieties yield preserves with a more acceptable color than do other varieties. The Hood variety is known to produce strawberry preserves with desirable color qualities, while preserves made from the Tioga variety exhibit lower color acceptability upon storage. Differences in chemical composition between these two varieties were determined in an effort to ascertain what factors might contribute to the more rapid color deterioration known to occur in preserves manufactured from the

Tioga fruit. In addition, samples of preserves were measured for various color parameters periodically, over a 26 week period, in order to determine the difference in relative rates of color deterioration between the Hood and Tioga varieties.

## LITERATURE REVIEW

In the first of a series of publications, Kertesz and Sondheimer (1947) reported that, "products from small fruits, especially strawberries and raspberries, show more rapid deterioration in storage than do most other fruit preserves." They noted that a secondary brown discoloration accompanied a loss of red color in these products. Further investigations (Kertesz and Sondheimer, 1948) revealed that a direct relationship existed between the loss of red ACN color in commercially prepared strawberry preserves and the length and temperature of storage. The investigators (Sondheimer and Kertesz, 1948) determined a critical temperature around 65°F, above which considerable acceleration in the loss of red color and subsequent formation of brown was observed. They (Kertesz and Sondheimer, 1948) concluded that, since a major loss of red ACN pigment always preceded a secondary brown discoloration, the latter was not a vital criterion in determining the quality of high-grade strawberry products. Case (1952) observed a similar relationship between pigment loss in strawberry preserves and storage temperature.

Mackinney and Chichester (1952) reported color deterioration in strawberry preserves to be due to at least three causes: loss of natural red ACN pigment, formation of brown pigments and discoloration resulting from such factors as heavy metal contamination. The authors, however, maintained that a heavy loss (50% or more in some cases) of ACN pigment could occur without marked deterioration in color, but that development of a brown discoloration would result in an imme-

diate loss in attractiveness.

In a study of the stability of strawberry juice, Nebesky et al. (1949) found storage temperature and oxygen to be the most specific agents for color deterioration. Mackinney et al. (1955) reported that oxidative conditions also exhibited an accelerating effect on color deterioration in strawberry preserves. These authors observed a linear relationship between increased browning and decreased pigment content; they speculated that the brown product was formed either concomitantly or as a direct result of pigment degradation.

In a study of ACN breakdown in model systems of pelargonidin-3-glucoside, Lukton et al. (195<sup>6</sup>~~9~~) found that an insoluble red-brown precipitate and a soluble brown material were formed to a much greater extent in oxygen than in nitrogen. They postulated that there were at least two possible pathways for the formation of this precipitate. The first possibility was conversion or polymerization of the pseudobase of the pigments to the red-brown precipitate. The second pathway required hydrolysis of the pseudobase to the aglycone with subsequent conversion, directly or through an intermediate, to the red-brown precipitate. (The former was regarded as the most likely.) In both cases, the brown color was thought to arise from glucose, the red-brown precipitate and/or colorless breakdown products.

Markakis et al. (1957) also proposed a tentative scheme for pigment degradation. Their proposed mechanism involved hydrolytic opening of the pyrylium ring (at position 1,2) with the formation of a substituted chalcone, and further degradation to a brownish insoluble

polyphenolic compound. These investigators had also detected a red-brown precipitate, which settled out whenever a pure pigment solution was allowed to stand for a long enough period of time. The precipitate was found to be insoluble in ether, concentrated HCl, concentrated  $H_2SO_4$ , partially soluble in methanol, and completely soluble in 5% NaOH solution, yielding a yellow rather than brown solution (with an absorption maximum at about 420nm). In a previous experiment, 85% of the original activity of  $^{14}C$  labeled pelargonidin-3-glucoside was recovered in a brown precipitate after the radioactive pigment had been stored in citrate buffer solution until virtual discoloration (Livingston and Markakis, 1956). The authors concluded that the brown precipitate they had isolated from strawberry ACN degradation must contribute significantly to the darkening of strawberry preserves in storage.

A brown polyphenolic polymer was also isolated from degraded pelargonidin-3-glucoside by Hamdy et al. (1961). The polymer was found to possess the same solubility properties as that isolated by Markakis et al. (1957). The occurrence of such a precipitate as a product of ACN degradation was further substantiated by Erlandson and Wrolstad (1972) who observed a reddish-brown material in the residue of acidic methanol extracted strawberry puree. (This reddish-brown material was observed to increase with storage time.)

Jurd (1972) has recently demonstrated that synthetic flavylum salts in aqueous acetic acid solutions will dimerize to form benzopyrylium salts. This work supports the feasibility of such a condensa-

tion reaction occurring with natural flavylum salts (aglycones) or ACN's. The mechanism proposed by Jurd for this reaction involves initial condensation of the flavylum salt and its carbinol base.

Other factors, in addition to storage temperature and oxidative conditions, have been shown to increase color deterioration in strawberry products. Sondheimer and Kertesz (1953) studied the participation of ascorbic acid in ACN destruction in strawberry juice and model systems. The authors determined that the rate of ACN loss was influenced by the rate of ascorbic acid oxidation. Cupric ion-catalyzed oxidation of ascorbic acid was believed to be of particular significance, since this reaction results in the production of hydrogen peroxide. (Hydrogen peroxide is known to decolorize ACN's.) The detrimental effect of ascorbic acid oxidation on strawberry ACN pigment has been confirmed by other investigators (Pratt et al., 1954; Markakis et al., 1957; Haginuma, 1962).

Meschter (1953) conducted a study of the effects of carbohydrates on strawberry products. Furfural and hydroxymethylfurfural were found to increase the rate of pigment loss in strawberry juice. Meschter suggested that, since both of these compounds are typical sugar degradation products, similar compounds may react with the ACN in strawberry preserves. Decareau et al. (1956) and Tinsley and Bockian (1960) found that sugar breakdown products produced a similar effect in strawberry jellies and model systems, respectively. The latter study showed that sugars such as fructose, which degrade rapidly at low pH, had a pronounced effect on the rate of pigment degradation. More recently,

Andreotti et al. (1969) have found that using glucose in strawberry preserves improved pigment retention 7 to 8%, but that this improvement decreased after eight months storage at 18°C. Thieme (1970) observed no difference in color between strawberry preserves made using glucose syrups and those made with sucrose syrup following storage at 15°C for 18 months.

The use of antioxidants and metal ions as additives in strawberry products has also been studied by several investigators. Kyzline et al. (1961) found that the addition of cysteine or sulfite improved the color of strawberries in syrup. Adams and Ongley (1973) reported that cysteine enhanced the stability of ACN's in canned strawberries, but its effect was found to be much less pronounced than that of sodium sulfite. Andreotti et al. (1973) have found that the addition of aluminum, in concentrations of 500 ppm, improves the color of strawberry jam. The authors have claimed that this improved color is maintained even when browning sets in.

## EXPERIMENTAL

Color Changes in PreservesPreserves

Hood strawberries from Woodburn, Oregon were received at a commercial plant (June 24, 1976) and frozen in a concentration of five parts fruit to one part sugar. Tioga strawberries from Watsonville, California were also received at a commercial plant (August 5, 1976) and frozen in the same manner. The fruit was shipped in 55 gallon drums to a processing plant (The J. M. Smucker Company, Salinas, California) and, following a ten week storage period, manufactured into preserves. (Processing dates were as follows: Hood fruit, September 2, 1976; Tioga fruit, October 20, 1976.) Samples of preserves were shipped to Oregon State University and, after two weeks (from the date of processing), stored in the dark at 21°C and 37°C for subsequent analyses.

Aqueous extracts

Preserve samples of 240 g (125 g fruit) were extracted with 250 ml of boiling acetone (reagent grade) and 50 ml of distilled water in a Waring Blendor. The macerated mixtures were allowed to stand in a refrigerator for a period of four hours, then filtered through Whatman No. 1 filter paper in a Buchner funnel. The residues were re-extracted with 150 ml of boiling acetone and again placed in a refrigerator for four hours prior to filtration. Following a second re-ex-



traction (with 150 ml of boiling acetone) of the residues, the mixtures were kept in the refrigerator overnight (12 to 16 hours) and filtered the following morning. The (three) filtrates were combined and shaken in a separatory funnel with a volume of chloroform (reagent grade) equal to 1.5 times the total volume of filtrate. After allowing the mixture to separate at room temperature for 1.5 hours, the bulky chloroform-acetone phase was discarded and the aqueous phase retained and filtered gravimetrically through Whatman No. 2 filter paper.

### Color Analyses

Hunter coordinates of preserve samples were determined periodically on a Hunter color difference meter (CDM), Model D25P-2. The samples were measured in a ten ml sample cell against a red tile standard CSR 0093 ( $L = 26.8$ ,  $a = +45.4$ ,  $b = +15.1$ ). Transmission values of Hunter coordinates were determined for preserve samples in addition to the standard reflectance values. Reflectance values for the insoluble residues, remaining after acetone extraction, were measured in a ten ml sample cell against a dull red tile standard CDR 0081 ( $L = 24.1$ ,  $a = +14.5$ ,  $b = +3.4$ ).

ACN content, color density, polymeric color and percent contribution by tannin were measured from aqueous extracts of preserve samples taken at 19 and 26 weeks of storage. All values, except ACN content, were determined by the potassium metabisulfite method developed by Somers (1972) for measuring color parameters of wine. This method has

been applied to other ACN-containing products by Wrolstad (1976), who has outlined a procedure for the method in a recent agricultural experiment station bulletin (624). The values obtained from this procedure were recalculated to represent a total volume of 125 ml (ie. a concentration of one ml per g of fruit) for each of the aqueous extract samples.

ACN content was determined by the pH differential method also reported by Wrolstad (1976). The ACN concentrations, expressed as mg of pelargonidin-3-glucoside per g fruit, were calculated using a molar absorbance of 22,400 (Wrolstad, 1976). (It should be noted that all measurements from aqueous extracts were determined immediately following filtration of the extracts, since degradation of the pigments was found to proceed rapidly.)

### Compositional Analyses of Fruit

#### Strawberries

Hood strawberries from Woodburn, Oregon and Tioga strawberries from Watsonville, California were received at commercial plants on the dates previously mentioned (Hood fruit, June 24, 1976; Tioga fruit, August 5, 1976). Samples of fruit were individually quick frozen (IQF), packed in polyethylene bags and stored in the dark at  $-10^{\circ}\text{C}$ .

#### Aqueous Extracts

IQF fruit samples of 250 g were thawed at room temperature for

approximately four hours, then extracted with 500 ml of boiling acetone (reagent grade) in a Waring Blendor. The macerated mixtures were allowed to stand in a refrigerator for a period of four hours, then filtered through Whatman No. 1 filter paper in a Buchner funnel. The residues were re-extracted with 300 ml of boiling acetone, kept in the refrigerator overnight (12 to 16 hours) and filtered the following morning. The (two) filtrates were combined and shaken in a separatory funnel with a volume of chloroform (reagent grade) equal to 1.5 times the total volume of filtrate. After allowing the mixture to separate at room temperature for 1.5 hours, the chloroform-acetone phase was discarded and the aqueous phase retained and filtered gravimetrically through Whatman No. 2 filter paper. Half of the total volume of aqueous extract was extracted six times with equal volumes of ethyl acetate (reagent grade) to remove flavanols for subsequent analysis.

#### Ascorbic Acid

Ascorbic acid determinations were made on 150 g samples of IQF fruit (thawed at room temperature for four hours), using the procedure of Loeffler and Ponting (1942). Determinations were performed in duplicate and results expressed as mean values.

#### Metal Ions

IQF strawberry samples (of approximately 100 g) were thawed as described for the ascorbic acid determinations. Thawed samples were pureed in a Waring Blendor, then freeze dried to approximately 10% of

the original weight of the berries. The samples were analyzed on a Jarrel-Ash model 750 Spectrophotometer by the direct reading spark emission spectroscopy procedure of Chaplin and Dixon (1974). Reported values represent the means of results obtained from samples run in duplicate.

### Free Amino Acids

The free amino acid contents of the aqueous extracts were determined using updated single column procedures on a Beckman 120B amino acid analyzer. The method of Spackman et al. (1958) was followed (using pH 2.2 sodium citrate buffer, 0.2N in sodium) with the exception that no hydrolysis of the sample was performed prior to analysis. Results were determined from single runs of aqueous extracts representing 250 g of Hood and Tioga fruit.

### Leucoanthocyanins

The relative concentrations of leucoanthocyanins in the whole fruit, aqueous extract and filter cake residue of the strawberries were measured by the method reported by Swain and Hillis (1959). Prior to analysis, the filter cake residues (remaining after acetone extraction) were covered loosely with plastic film and allowed to dry slowly in a refrigerator. (If left uncovered at room temperature, the residues were observed to change from a whitish color to a pink, then brown color.) Quantitative estimations were based on the transformation of leucoanthocyanins to anthocyanidins by heating in acid solu-

tion. This transformation, however, is not quantitative (with the leucoanthocyanin reagent of Swain and Hillis, yield is on the order of 25%), and careful attention must be paid to uniform heating conditions. For this reason, and to eliminate discrepancies occurring as a result of different storage times (or conditions) of the residues and extracts, samples of Hood and Tioga strawberries were prepared on the same day and analyzed at the same time. Reported values represent means of results obtained from samples run in duplicate.

#### Flavanols

Ethyl acetate extracts were brought up to a standard volume of one liter. Suitable aliquots of the extracts (0.5 to 2.0 ml) were taken to dryness on a Buchler rotary evapo-mix, and then brought back into solution with two ml of distilled water. These aqueous samples were analyzed for flavanols using the procedure of Swain and Hillis (1959). Determinations were performed in triplicate and results reported as mean values.

#### ACN Content

ACN content was determined from the aqueous extracts using the pH differential method described for the preserve samples. (Results represent single determinations performed immediately following filtration of Hood and Tioga aqueous extracts.)

#### Total Phenolics

Aqueous extracts were analyzed for total phenolics by the Folin-Denis test described in the AOAC Official Methods of Analysis (1960). Results were obtained from a standard curve of tannic acid and expressed as means of samples run in triplicate.

## RESULTS AND DISCUSSION

Color Changes in Preserves

Relative rates of color deterioration in Hood and Tioga preserves were established from periodic measurements of Hunter coordinates. All of the preserve samples exhibited a decrease in the Hunter +a value (a measure of redness) with increasing storage time. This measurement alone, however, is not an accurate index for determining color deterioration.

Livingston et al. (1959) have studied the colorimetry of strawberry preserves extensively. These authors have determined that hue, expressed as the  $\tan^{-1} a/b$  function in the Hunter system, is the most significant instrumental (reflectance) value for correlating the color acceptability of strawberry preserves with objective color measurement. The changes in  $\tan^{-1} a/b$  (reflectance) for the various preserve samples, with respect to storage time, are shown in Figure 1 (page 16). The results confirm that color deterioration proceeds at a faster rate in preserves manufactured from Tioga strawberries. A similar plot of the transmission data (Figure 2, page 17) reveals that the  $\tan^{-1} a/b$  function does not have the same relation with color acceptability when transmission, rather than reflectance, data are used. The significance of the transmission data is not known, since, to date, no work has been published regarding the colorimetry of strawberry preserves using instrumental transmission values.

Spectral measurements, determined from aqueous extracts of pre-

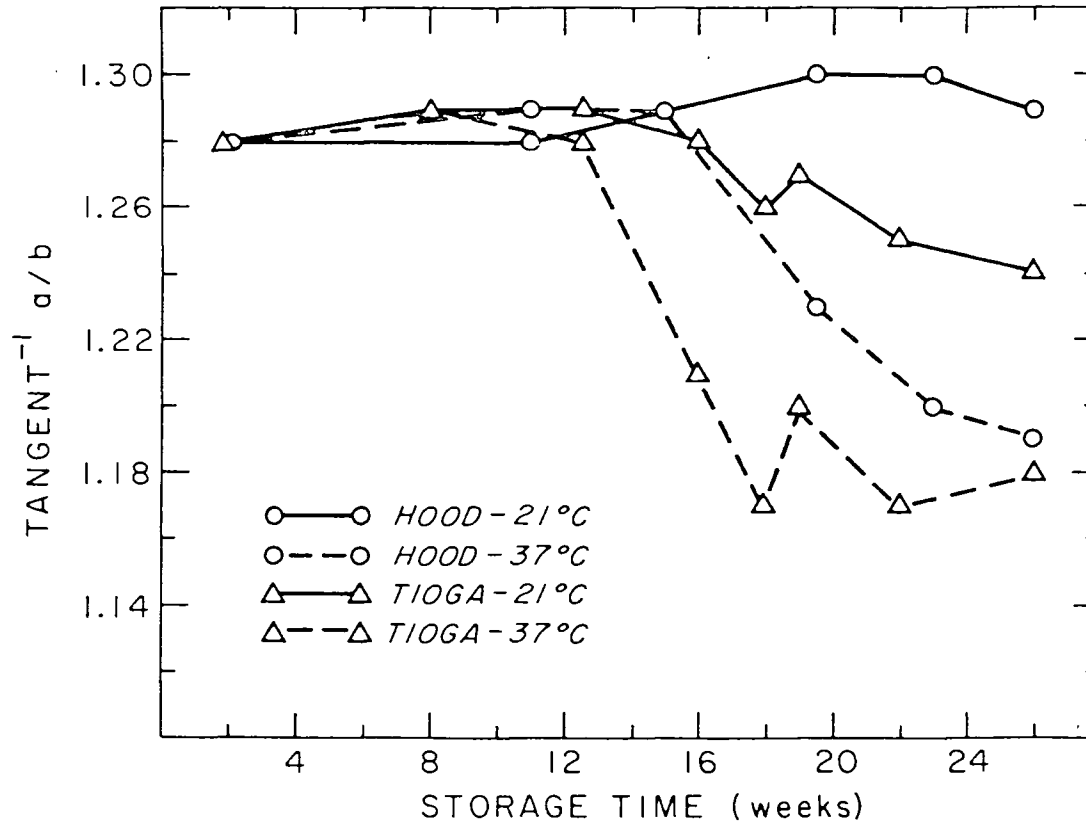


Figure 1. Effect of storage time and temperature on the (Hunter reflectance)  $\tan^{-1} a/b$  function of Hood and Tioga preserves.



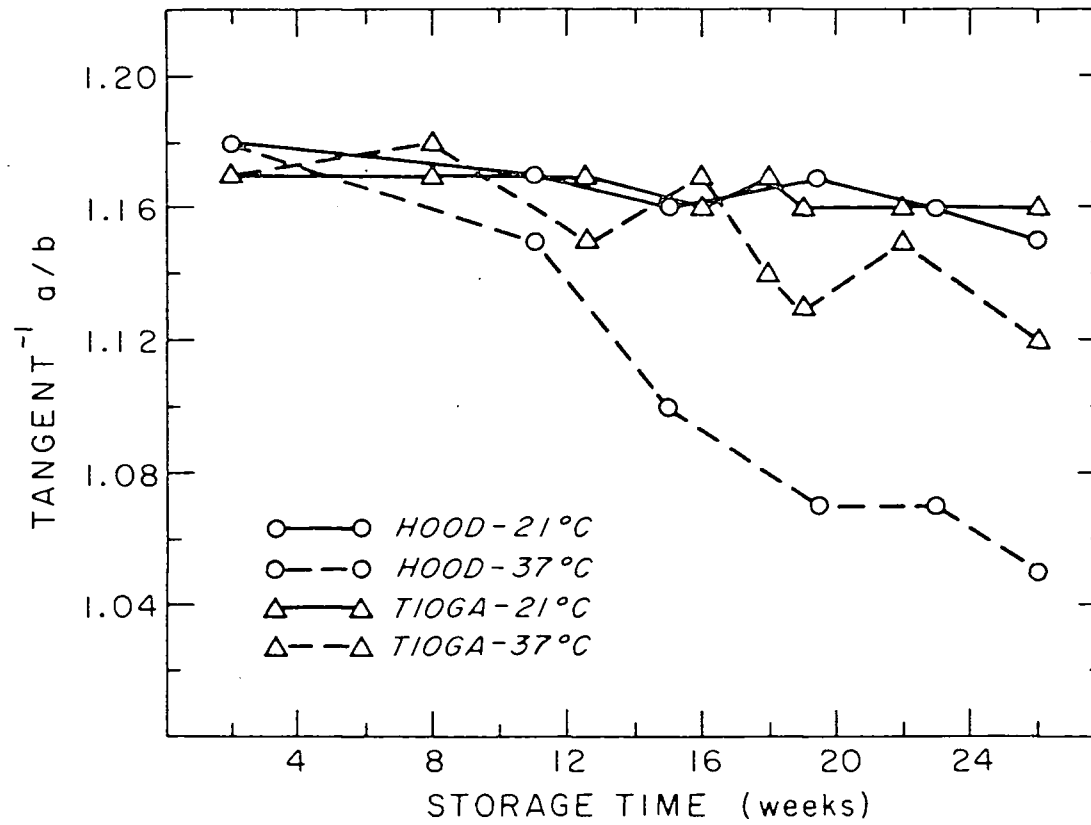


Figure 2. Effect of storage time and temperature on the (Hunter transmission)  $\tan^{-1} a/b$  function of Hood and Tioga preserves.

serve samples, serve to characterize the color changes taking place during storage. The pelargonidin-3-glucoside pigment of strawberries has a maximum absorbance in the orange-red region and an inflection in the brown (420 to 450nm) region of the visible spectrum. These characteristics are displayed in the spectrum obtained from an extract of fresh Tioga preserves (Figure 3, page 19). Extracts from older samples, however, exhibited fading in the orange-red region (495nm in this case), with a corresponding increase in the brown region (Figure 4, page 20). The increase in brown color is related to the amount of polymeric pigment present in the extract. This polymeric "tannin" pigment, unlike ACN pigment, is resistant to bisulfite bleaching, enabling the following measurements to be made:

Color density = the sum of the absorbance of the untreated extract at 420 and 495nm.

Polymeric color = the sum of the absorbance of the bisulfite treated extract at 420 and 495nm.

Table 1. Comparison of color density and polymeric color in Hood and Tioga preserves stored at 21°C and 37°C.

| Variety | Storage Time (weeks) | Temperature (°C) | Color Density | Polymeric Color |
|---------|----------------------|------------------|---------------|-----------------|
| Hood    | 2                    | 21               | 6.35          | 0.48            |
| Hood    | 19                   | 21               | 2.95          | 0.58            |
| Hood    | 26                   | 21               | 3.73          | 0.96            |
| Hood    | 19                   | 37               | 1.87          | 0.99            |
| Hood    | 26                   | 37               | 1.94          | 1.40            |
| Tioga   | 2                    | 21               | 6.74          | 0.74            |
| Tioga   | 19                   | 21               | 3.71          | 1.47            |
| Tioga   | 26                   | 21               | 3.66          | 1.48            |
| Tioga   | 19                   | 37               | 3.02          | 2.19            |
| Tioga   | 26                   | 37               | 3.03          | 2.21            |

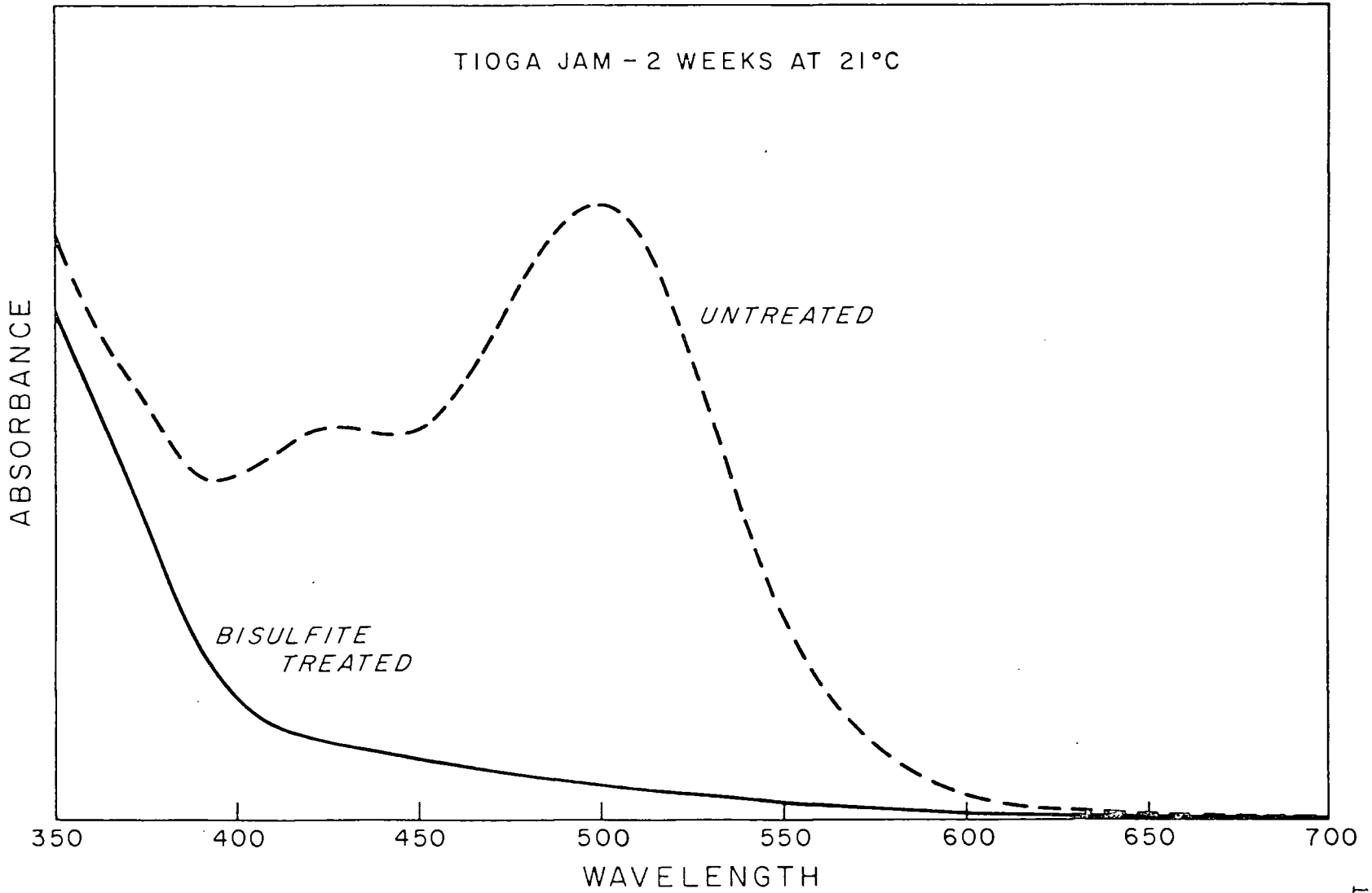


Figure 3. Visible spectrum from an aqueous extract of fresh Tioga preserves.

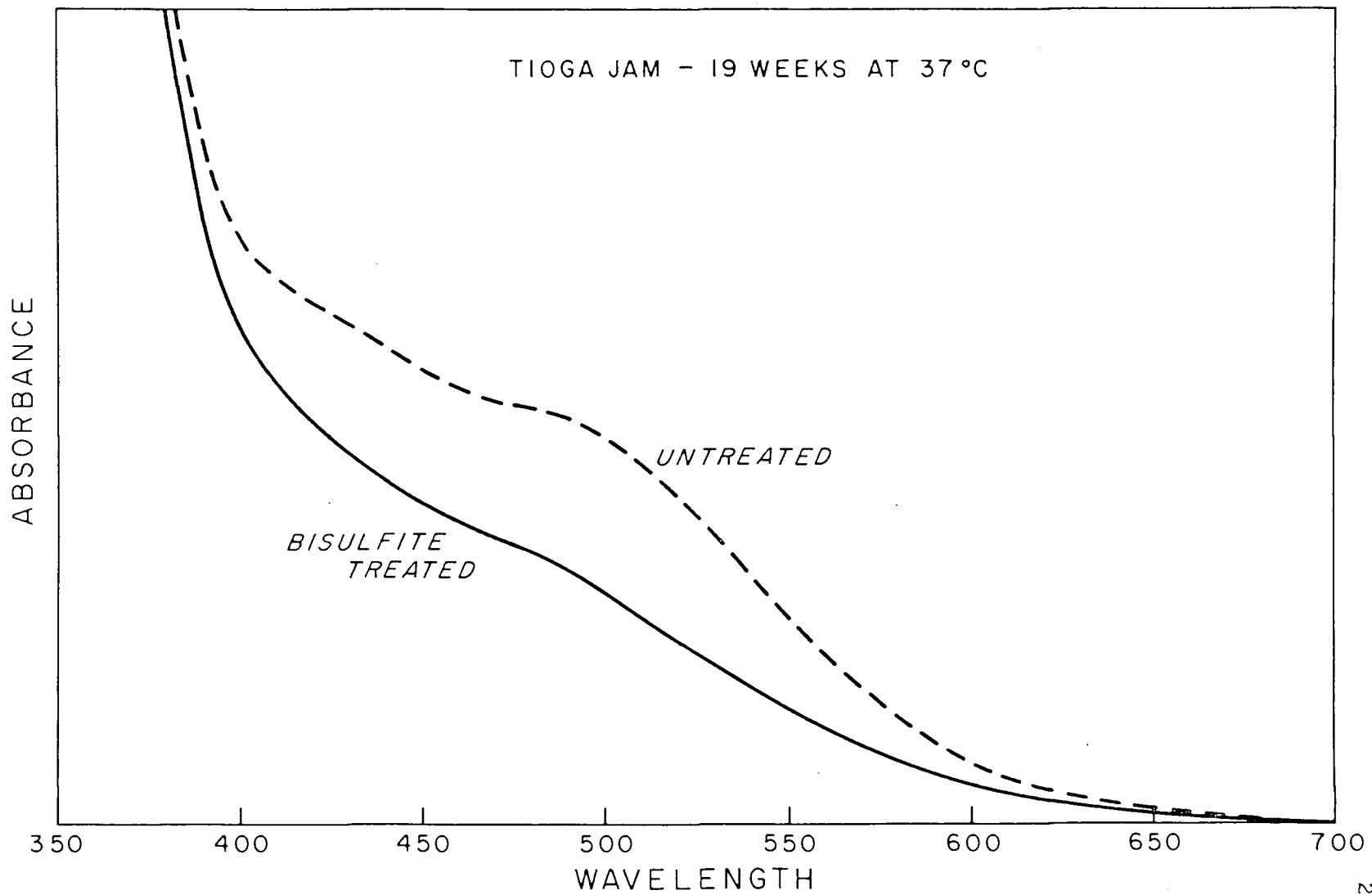


Figure 4. Visible spectrum from an aqueous extract of Tioga preserves at 19 weeks of storage.

The ratio of polymeric color to color density yields a value, defined as "percent contribution by tannin", which can be used as an index of browning. Changes in this value, with respect to storage time, are shown for the preserve samples in this study (Figure 5, page 22). The data indicate that browning occurred at a much faster rate in the Tioga preserves.

Changes in ACN content, with respect to storage time, are shown in Figure 6 (page 23). Hood preserves are shown to have initially contained almost 50% more ACN pigment than Tioga preserves. Both varieties, however, exhibited essentially the same color acceptability in fresh preserves (Figure 1). This indicates that ACN content does not always exert a marked influence on the color quality of strawberry preserves. Hood preserves are also shown to have experienced a more rapid loss of ACN pigment, further substantiating the theory that ACN loss is not the paramount problem affecting the color of the product during storage.

The results obtained from these color analyses point to the formation of brown pigments, rather than ACN pigment loss, as the primary cause of the more rapid color deterioration observed in Tioga preserves. This conclusion is corroborated by the earlier findings of Mackinney and Chichester (1952). These authors stressed the importance of the role of browning in the loss of color acceptability of strawberry preserves.

The data presented thus far have been concerned with measurements from aqueous extracts as well as intact preserve samples. There was,

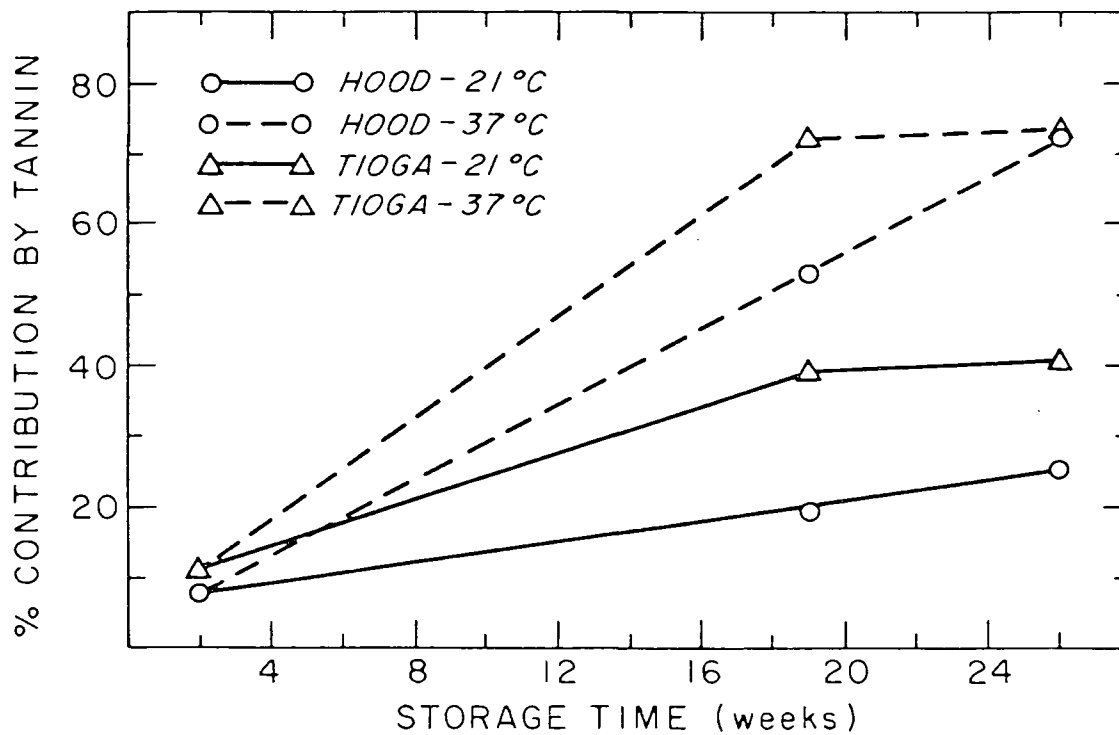


Figure 5. Effect of storage time and temperature on the formation of brown pigments in Hood and Tioga strawberry preserves.

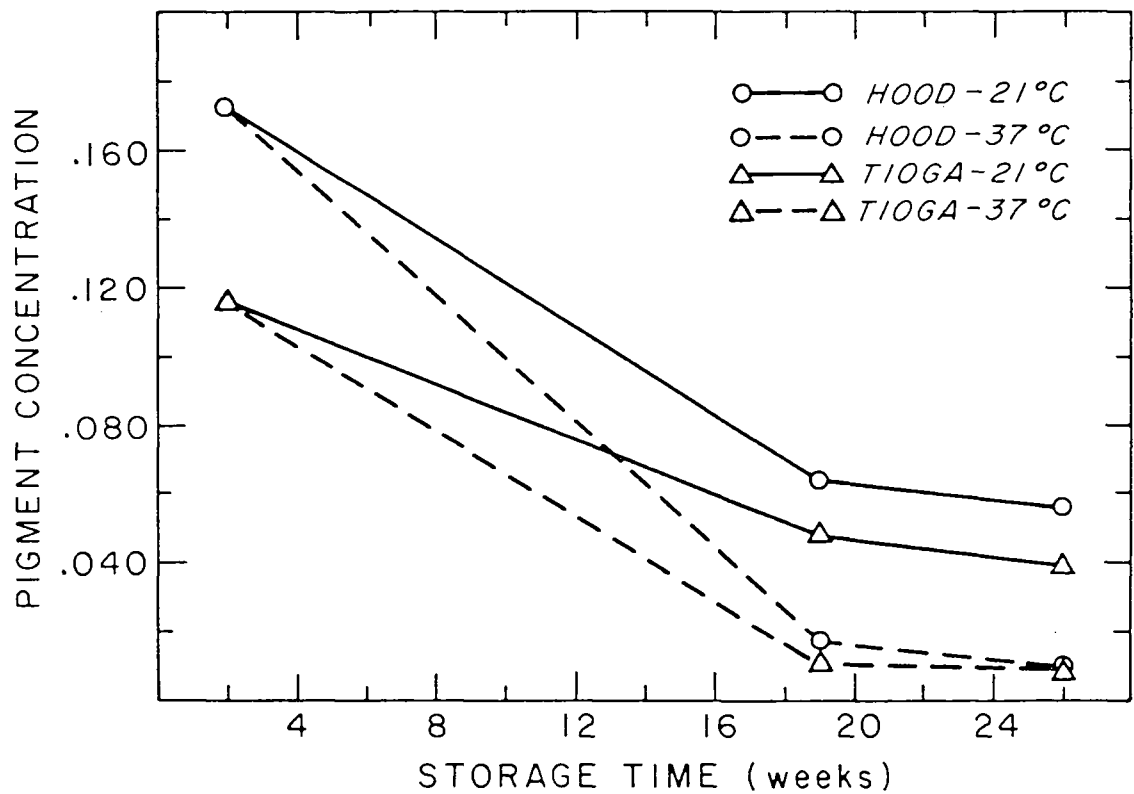


Figure 6. Effect of storage time and temperature on the loss of red ACN pigment in Hood and Tioga strawberry preserves. (Pigment concentration is expressed as mg ACN/g fruit.)

however, a certain amount of unextractable pigment which remained in the residues of preserve samples following extraction. Measurements from the aqueous extracts cannot serve to characterize the color contributed by these pigments. Therefore, Hunter color coordinates were determined separately for the insoluble residues. The results are shown below in Table 2.

Table 2. Hunter reflectance values for insoluble residues from preserve samples.

| Variety | Storage Time (weeks) | Temperature (°C) | L    | +a  | +b  |
|---------|----------------------|------------------|------|-----|-----|
| Hood    | 2                    | 21               | 26.8 | 5.8 | 5.5 |
| Hood    | 19                   | 21               | 25.7 | 6.9 | 5.9 |
| Hood    | 26                   | 21               | 24.4 | 6.7 | 5.9 |
| Hood    | 19                   | 37               | 23.2 | 7.7 | 7.9 |
| Hood    | 26                   | 37               | 19.2 | 6.9 | 7.4 |
| Tioga   | 2                    | 21               | 27.0 | 5.7 | 7.8 |
| Tioga   | 19                   | 21               | 26.7 | 6.8 | 8.3 |
| Tioga   | 26                   | 21               | 26.4 | 7.2 | 8.6 |
| Tioga   | 19                   | 37               | 21.9 | 9.0 | 9.6 |
| Tioga   | 26                   | 37               | 22.4 | 8.5 | 9.6 |

Insoluble residues from all of the preserve samples displayed an increase in both +a (redness) and +b (yellowness) values upon storage. The increases in +a and +b were accompanied by corresponding increases in darkness (represented by a decline in the L values during storage), and appeared to be greater in samples stored at 37°C. It seems likely that the observed increases in color were caused by the formation of the insoluble red-brown precipitate that has been reported as an end



product of ACN degradation by several investigators.

### Compositional Analyses of Fruit

#### Ascorbic Acid and Metal Ions

The rate of ascorbic acid oxidation has been shown to influence the rate of ACN loss in strawberry products and model systems (Sondheimer and Kertesz, 1953; Pratt *et al.*, 1954; Markakis *et al.*, 1957, Haginuma, 1962). For this reason, ascorbic acid determinations were performed on Hood and Tioga IQF fruit samples. The results are displayed in Table 3. (Analysis of a sample of fresh Hood strawberries revealed the loss of ascorbic acid, resulting from the thawing of IQF samples, to be negligible.)

Table 3. Comparison of the ascorbic acid content and pH of Hood and Tioga strawberries.

| Variety | Ascorbic Acid<br>(mg/100 g fruit) | pH   |
|---------|-----------------------------------|------|
| Hood    | 48.2                              | 3.69 |
| Tioga   | 18.8                              | 3.50 |

Ascorbic acid was found to be present in higher concentration in the Hood strawberries. It is apparent, therefore, that ascorbic acid content was not one of the factors contributing to the more rapid color deterioration seen in Tioga preserves. The higher concentration of this compound in the Hood fruit, however, may have contributed to the more rapid loss of ACN pigment observed in the preserves manufactured from this variety.

Cupric ion-catalyzed (aerobic) oxidation of ascorbic acid has been thought to play a significant role in the ascorbic acid-induced destruction of pelargonidin-3-glucoside (Sondheimer and Kertesz, 1953). Although these (copper) ions were detected in higher concentration in the Tioga strawberries (Table 4), the importance of the cupric ion-catalyzed reaction in the preserve samples is questionable since, as previously mentioned, ACN loss occurred at a faster rate in Hood preserves.

Table 4. Comparison of metal ion concentrations in Hood and Tioga strawberries.

| Variety | ← % dry weight → |      |      |      | ← ppm dry weight → |    |    |    |    |    |
|---------|------------------|------|------|------|--------------------|----|----|----|----|----|
|         | K                | P    | Ca   | Mg   | Mn                 | Fe | Cu | B  | Zn | Al |
| Hood    | 1.45             | 0.26 | 0.10 | 0.06 | 9                  | 44 | 2  | 12 | 17 | 14 |
| Tioga   | 1.86             | 0.31 | 0.16 | 0.11 | 71                 | 13 | 8  | 12 | 17 | *  |

\*below detection levels of instrument

Erlandson (1972) reported that stannic, stannous and aluminum ions improved the color stability of strawberry puree, while the presence of either ferrous or ferric ions resulted in the appearance of a dark purplish-black discoloration. Addition of aluminum, in concentrations of 500 ppm, has also been shown to improve the color stability of strawberry jam (Andreotti *et al.*, 1973). However, the 500 ppm added to strawberry jam by these investigators is considerably higher than the 14 ppm of aluminum naturally present in Hood strawberries. This indicates that any color stability contributed by the higher concentration of aluminum in Hood fruit would probably be negligible. The lower concentration of ferrous and ferric ions in the Tioga variety precludes

the likelihood of these ions playing a major role in the discoloration of Tioga preserves.

### Free Amino Acids

Maillard browning often occurs in food products containing both reducing sugars and amines and was, accordingly, considered as a possible cause of browning in the preserve samples. However, since the concentration of reducing sugars was assumed to be identical in both Hood and Tioga preserves (both varieties were processed with the same amount of added sugar and adjusted to the same pH) any differences in the rate of Maillard browning would have, of necessity, had to arise from differences in the concentrations of free amino acids.

The results of free amino acid analysis are shown in Table 5 (page 28). The values in this table are in general agreement with those reported by other investigators (Tinsley and Bockian, 1959; Drawert et al., 1970). The higher concentrations of both amino sugars and total amino acids in Hood fruit indicate that preserves from this variety would be more prone to undergo Maillard browning, and thus, this type of a reaction cannot be responsible for the more rapid browning of Tioga preserves.

### Leucoanthocyanins

Leucoanthocyanins were detected in the relative amounts displayed in Table 6 (page 29).

Table 5. Comparison of free amino acids in Hood and Tioga strawberries.

| Amino Acid             | (μmoles amino acid/100 g fruit) |       |
|------------------------|---------------------------------|-------|
|                        | Hood                            | Tioga |
| Glutamine & Asparagine | 269                             | 242   |
| Alanine                | 60.2                            | 63.7  |
| Glutamic Acid          | 65.7                            | 49.7  |
| Amino Sugars           | 68.6                            | 23.2  |
| Aspartic Acid          | 33.5                            | 32.6  |
| Threonine              | 18.7                            | 15.3  |
| Arginine               | 6.17                            | 3.59  |
| Valine                 | 4.50                            | 3.47  |
| Histidine              | 4.24                            | 1.24  |
| Isoleucine             | 2.71                            | 1.25  |
| Leucine                | 2.76                            | 0.84  |
| Glycine                | 2.43                            | 3.54  |
| Tyrosine               | 1.36                            | 2.95  |
| Methionine             | trace                           | trace |
| Proline                | trace                           | trace |
| Total                  | 540                             | 442   |

Table 6. Comparison of relative amounts of leucoanthocyanins in Hood and Tioga strawberries.

| (absorbance units/g fruit) |             |                 |         |
|----------------------------|-------------|-----------------|---------|
| Variety                    | Whole Fruit | Aqueous Extract | Residue |
| Hood                       | 4.69        | 3.89            | 0.166   |
| Tioga                      | 5.65        | 6.11            | 0.234   |

The presence of these compounds in strawberries has also been reported by Co and Markakis (1968). When leucoanthocyanins were fractionated into water-methanol insoluble, ethyl acetate soluble and water soluble fractions, these authors found the latter fraction to be the largest of the three. In the analysis of Hood and Tioga strawberries also, the vast majority of leucoanthocyanins were detected in the water soluble fraction. Earlier analysis of the ethyl acetate extract, and corresponding ethyl acetate-extracted aqueous extract, revealed that only a small amount of leucoanthocyanins (approximately 6%) were removed from the aqueous phase. Hence, leucoanthocyanin determinations were performed only on the aqueous extracts, residues and whole fruit samples. (All determinations involving the aqueous extracts, including analyses of free amino acids, ACN's and total phenolics, were performed on the portion of the extracts not subjected to ethyl acetate extraction.)

Leucoanthocyanins are very reactive compounds and are undoubtedly responsible for the color changes observed in the fresh filter cake residues. (When left uncovered at room temperature, the residues were observed to change from a whitish color to a pink, then brown color.)

Their higher concentration in Tioga strawberries may be of some significance, since recent work with wine tannins has shown that leucoanthocyanins may polymerize with ACN's. The ACN pigments of a new wine are known to disappear rapidly during maturation and ageing. Isolation (by gel filtration) of condensed flavonoid pigments from a mature wine (Somers, 1966) revealed the presence of ACN's in the wine tannin structure. Somers (1966, 1970) postulated that the ACN's of a new wine are actually incorporated into a polymeric complex of leucoanthocyanin-ACN. The possibility of such a reaction occurring in strawberry preserves seems feasible, especially since the variety of strawberry producing the more rapidly browning preserve is also higher in leucoanthocyanins.

#### ACN's, Flavanols and Total Phenolics

The results from analyses of ACN content, flavanols and total phenolics are displayed in the composite table below (Table 7).

Table 7. Comparison of ACN's, flavanols and total phenolics in Hood and Tioga strawberries.

|                           | Hood<br>Fruit | Tioga<br>Fruit |                            |
|---------------------------|---------------|----------------|----------------------------|
| ACN's                     | 0.377         | 0.276          | (mg/g fruit)               |
| Flavanols                 | 0.404         | 0.876          | {absorbance units/g fruit) |
| Total Phenolics           | 2.05          | 3.12           | (mg/g fruit*)              |
| *expressed as tannic acid |               |                |                            |

ACN was detected in Hood and Tioga fruit in essentially the same ratio as it was found to exist in the preserves. It has previously

been established, however, that the higher amount of ACN did not improve the color acceptability of Hood preserves (see page 21). ACN was also shown to degrade at a slower rate in Tioga preserves and, thus, cannot be the influencing factor on color deterioration.

The concentration of flavanols in Tioga fruit was found to be more than twice as high as that in Hood fruit. The extreme difference in relative amounts of the flavanol compounds suggests that they may play an important part in the browning of Tioga preserves.

The flavanol reagent of Swain and Hillis (1959) reacts only with compounds containing an undeactivated phloroglucinol (or resorcinol) nucleus. Due to this fact, the flavonols of strawberries (quercetin and kaempferol), reported in concentrations of 37 to 79 mg/kg of fruit (Ryan, 1971), cannot yield a positive test with the reagent. Other phenolics which have been reported in strawberries include the aforementioned leucoanthocyanins, and catechin (Co and Markakis, 1968; Stöhr and Herrmann, 1975). (Stöhr and Herrmann reported (+)-catechin in strawberries in concentrations of 10 to 70 mg/kg of fruit.) Both leucoanthocyanins and catechin are capable of producing a positive test with the flavanol reagent. Nevertheless, since the concentration of leucoanthocyanins in the ethyl acetate extract was found to be only very slight, it is reasonable to assume that catechin was the principal component detected by the flavanol reagent.

Jurd (1967) has shown that synthetic flavylum salts and catechin, in dilute acetic acid solutions, will form dimeric flavylum-flavan pigments. More recently, Timberlake and Bridle (1976) have studied the

interactions of ACN's, phenolic compounds and acetaldehyde in model wine (10% ethanol) systems. These investigators found that, on storage, mixtures of ACN's and catechin gradually lost color in the red region of the spectrum while increasing in the brown region. When the spectra of mixtures of ACN's and catechin were compared with the sum of the spectra of each component held separately, it was revealed that the mixtures had experienced a small net loss of ACN and a net increase in browning. This is indicative of interaction between the components, and supports the hypothesis of interaction between catechin and ACN during storage of preserves.

The Folin-Denis test for total phenolics measures all phenolic compounds, including ACN's, catechin, leucoanthocyanins and flavonols. However, the more reactive phenolics (ie. leucoanthocyanins and catechin) will react with the reagent to a greater extent than will the other phenolics. Therefore, the higher value of total phenolics determined in Tioga strawberries confirms the results of the leucoanthocyanin and flavanol analyses (indicating a greater quantity of the reactive phenolics in the Tioga variety).

A further significance of the higher value of total phenolics in Tioga fruit lies in the possible function of phenolics as substrates for polyphenoloxidase (PPO). This enzyme has been identified in strawberries (Pallavicini, 1969) and may be active during the thawing of frozen lots of strawberries prior to manufacture into preserves. Quinones formed by PPO action are very prone to polymerization, and these components may be partially responsible for the browning of the



strawberry preserves.

#### Summary and Conclusions

It was determined from storage studies of the preserve samples that the more rapid color deterioration in Tioga preserves was due to a faster rate of browning in this variety. The formation of brown pigments was not attributable to metal ion complexing or to a Maillard type of reaction.

Chemical analyses did reveal a considerably higher concentration of reactive phenolics (leucoanthocyanins and catechins) in the Tioga strawberry variety. Studies have shown that these compounds can form dark colored polymers with ACN's in wine and model systems. Nonetheless, it cannot be unequivocally stated that browning in preserves is analogous to the reactions hypothesized from these studies. Even though it is impossible, at this point, to determine the mechanism of the reaction, the results of this investigation point to polymerization (of phenolics) as a major cause of color deterioration in strawberry preserves.

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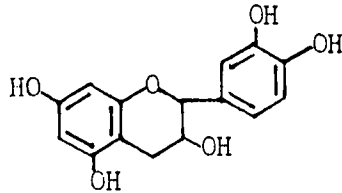
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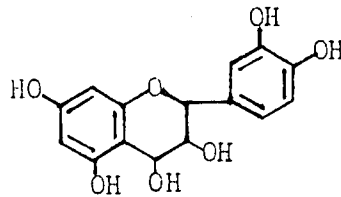
## APPENDIX

## CHEMICAL STRUCTURES OF SOME PHENOLIC COMPOUNDS

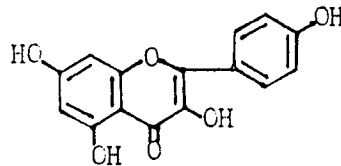
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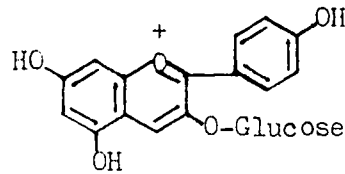
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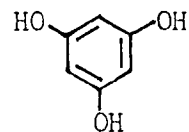
Kaempferol (flavonol):



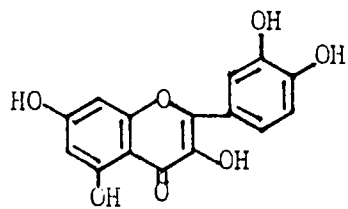
Pelargonidin-3-glucoside (ACN):



Phloroglucinol:



Quercetin (flavonol):



Rescorcinol:

