

AN ABSTRACT OF THE THESIS OF

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Title: THE INFLUENCE OF ENZYMES ON COLOR DETERIORATION OF STRAWBERRY  
JUICE AND CONCENTRATE

Abstract Approved: \_\_\_\_\_  
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The objective of this research was to gain a better understanding of the relationship between enzymic reactions and color degradation in strawberry products, and the suggestion of possible methods which might be utilized to prevent enzymic breakdown of the red color.

Efforts were made to characterize strawberry polyphenol oxidase (PPO). Several methods were utilized to isolate PPO, including the use of polyvinylpyrrolidone (PPVP), polyethylene glycol (PEG) plus acetone and polyclar-AT plus XAD-4. The enzyme preparations derived from fresh and frozen strawberries showed low levels of PPO activity and erratic results." Catechol solution was applied to the fresh and microwave blanched strawberries in order to demonstrate the presence of PPO. This qualitative test confirmed the PPO activity in strawberries. More

browning occurred in Tioga variety than in Hood variety. Green fruit showed greater browning capacity than ripe fruit.

Strawberry juice and concentrate were made from microwave blanched fruit and from thawed fruit to determine the effect of blanching on the composition and color of the sample. Juice and concentrate were stored at room temperature with one set of juice samples having been sparged with nitrogen. The levels of anthocyanin pigments, total phenolics, flavanols and leucoanthocyanins were determined periodically throughout an eight week storage period. Colorimetric measurements of browning, color density, polymeric color, percent polymeric color and Hunter "a" and "L" value were made over the same interval. Anthocyanin pigments, total phenolics, flavanols and leucoanthocyanins showed a less rapid decline with time in the blanched samples than in the control samples, although there was more browning and percent polymeric color in the blanched samples. The Hunter "a" value demonstrated that the blanched samples had a redder visual appearance. Nitrogen treated samples had a higher level of anthocyanin, total phenolics, flavanols, leucoanthocyanins, browning, percent polymeric color and Hunter "a" value, with a greater retention of red color than the non-nitrogen treated samples. The effects of blanching and nitrogen treatments on composition and color were statistically significant.

The Influence of Enzymes on Color  
Deterioration of Strawberry Juice  
and Concentrate

by

Dai-Lih Doris Lee

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# THE INFLUENCE OF ENZYMES ON COLOR DETERIORATION OF STRAWBERRY JUICE AND CONCENTRATE

## INTRODUCTION

Color has a direct influence on consumer acceptance of strawberry products, and any deviation from the characteristically rich red color may cause consumer rejection because of perceived inferiority. Strawberry products discoloration is a very common occurrence during both processing and storage, and marked reduction in salability due to browning is a major industry concern. Research in this area is needed if improvements are to be made in the retention of the attractive red color that is so closely associated with strawberries.

The loss of this pleasant red color in strawberry products has been attributed to the breakdown of anthocyanin pigments. Factors such as temperature, oxygen, ascorbic acid content, pH, sugars and enzymes have been found to affect anthocyanin degradation (Clydesdale and Francis, 1976). Low temperature ( $0^{\circ}\text{C}$  -  $5^{\circ}\text{C}$ ), lack of oxygen and conditions which decrease the rate of ascorbic acid oxidation are believed to have a decelerating influence on the rate of anthocyanin destruction, thereby improving red color stability (Markakis, 1974).

\* However, only a few reports dealing with the "role of enzymes" in color deterioration in strawberries have been published. This general lack of knowledge in the area of enzymic color degradation needs to be alleviated if further progress is to be made in developing processes that will help stabilize red color during strawberry product manufacture and storage.

This study was undertaken to determine the role of enzymes in color deterioration in order to establish a more effective way of controlling and preventing adverse color change. Enzyme assays were conducted to demonstrate the presence and characteristics of strawberry polyphenol oxidase. Major phenolic compounds and color differences were also studied in an effort to ascertain the effect enzymes have on color degradation in strawberry juice and concentrate.

## REVIEW OF LITERATURE

Many fruits and vegetables undergo color changes during manufacturing and storage. The natural color of fruit and fruit products may be destroyed or masked by the formation of unattractive dark brown or reddish brown pigments. This color deterioration is usually referred to as enzyme-catalyzed oxidative "browning", and nearly all of the oxidative browning of fruits is catalyzed by polyphenol oxidase (PPO) (Joslyn and Ponting, 1951).

Basically, enzymic browning of fruit is the oxidation of phenolic compounds into o-quinones. Early workers had proposed several modes of action of PPO. A major breakthrough was made by Onslow (1931), who concluded that an enzyme which, in the presence of air, catalyzed the oxidation of dihydroxyphenols yields a peroxide or hydrogen peroxide. Nelson and Dawson (1944) subsequently reported that the conversion of a monohydroxyphenol into a dihydroxyphenol is an enzymic function of PPO, with the resulting dihydroxyphenol being dehydrogenated to produce quinone and water. According to recent studies, it is believed that PPO, a complex enzyme, is able to catalyze two different reactions: (1) the hydroxylation of monohydroxyphenols to a o-dihydroxyphenols-- known as cresolase activity, and (2) the oxidation of o-dihydroxyphenols into o-quinones--known as catecholase activity (Corse, 1964; Mathew and Parpia, 1971 and Walker, 1977). Mathew and Parpia (1971) felt that the o-quinones formed from phenolic substrates are themselves

colored from red to reddish-brown, but that the reaction rarely stops there. These phenolic substrates take part in a secondary reaction, and bring about the formation of more intensely colored secondary products. The important secondary reactions, as far as polyphenol substrates are concerned, are coupled oxidation (of substrate with lower oxidized potential, such as anthocyanin), condensation and polymerization.

Because of the widespread distribution of PPO in the plant community, a number of studies have been made to characterize this enzyme. PPO extracted from different sources shows varying degrees of utilization of phenolic substrate. Phenolic acids, catechol, chlorogenic acid, caffeic acid and catechin have been found to be a substrate for PPO oxidation (Joslyn and Ponting, 1967; Mathew and Parpia, 1971; Pifferi and Cultrera, 1974; and Cash et al., 1976). However, most plant PPO requires a basic o-dihydroxyphenol structure for activity (Walker, 1977). Benjamin and Montgomery (1973) identified a cherry PPO which utilized only the dihydroxyphenol substrate. Grape PPO was also reported only as oxidized dihydroxyphenols (Cash et al., 1976). PPO has been found to exhibit a pH optimum between 5.0 and 7.0, depending upon the source from which it was extracted (Reed, 1975). Cash et al., (1976) reported that the optimum activity of grape PPO was in the pH range 5.9 - 6.3. The optimum activity for Royal Ann Cherry PPO occurred over a broad band from pH 6.0 to 8.0 (Benjamin and Montgomery, 1973).

Considerable work has been reported on thermal inactivation of PPO. Dimick et al., (1951) investigated the effect of temperature, time of heating and pH on heat inactivation of PPO in fruit puree.

The authors observed that the rate of inactivation at a constant temperature was different for each type of fruit and that the enzymes in each fruit had a characteristic pH of maximum thermal stability. The optimum blanching conditions for strawberry, black currant, sour cherry, and prune were 2.5 minutes at 85°C, and 2.5 minutes at 85°C, 3 minutes at 80-85°C, and 2.5 minutes at 70-75°C, respectively (Maczynska and Rembowski, 1965). Recently, Chan and Yang (1971) found that 90% destruction of cranberry PPO activity was made by heating at 50, 60, and 70°C for 15.85, 7.05, and 1.37 minutes, respectively. In a study of PPO in grape juice, enzyme inactivation was reached within 6 seconds when temperature varied between 80°C - 89.5°C (Jankov and Kirov, 1972).

The general approach regarding isolation of PPO is to remove phenolic compounds as quickly as possible (Loomis and Battaile, 1966). Mathew and Parpia (1971) pointed out that the presence of PPO in very low concentrations and the occurrence of phenolic substrate side by side were the main problems in the isolation of PPO from tissue.

x Various levels of PVPP and PEG were successfully applied to extract PPO from different sources (Jones et al., 1965; Badran and Jones, 1965; Chan and Yang, 1971; Benjamin and Montgomery, 1973; and Halim, 1977). In the experiment conducted by Badran and Jones (1965), PEG was found to be more effective for isolation of PPO. Benjamin and Montgomery (1973) reported a similar observation and found that PEG could be effectively removed from the enzyme preparation by acetone precipitation. Recently, Cash and Sistrunk (personal communication) developed a new strawberry enzyme extraction technique by using Tris aminoethane buffer (pH 9.5), acetone, sodium acetate buffer (pH 6.2) and calcium

chloride. The PPO activity could be assayed about 20-30 minutes after mixing the substrate solution and enzyme preparation.

\* Although an enzyme in strawberry which could catalyze the oxidation of o-dihydric phenols and cause browning was long ago reported by Onslow (1931), strawberry PPO was not investigated until recently. Pallavicini (1969) studied the action of PPO in frozen strawberry. The author found that PPO activity, determined in acetone-decolorized powders, was reduced to 16% after 4.5 months of storage at  $-20^{\circ}\text{C}$ . The relationship of PPO to pigment degradation in strawberry juice was first recognized by Cash and Sistrunk (1971). PPO was precipitated, with ammonium sulfate, from a water extract of acetone-dried strawberry tissue and used without further purification. The results indicated that a more rapid loss of color occurred in samples containing PPO than in the control, and that the enzymes react preferentially with the pigment to the exclusion of added substrate (catechol).

The red color of the strawberry comes from an anthocyanin (ACN) pigment, pelargonidin-3-monoglucoside (Sondheimer and Kertesz, (1948). \* As early as 1951, Huang found that several crude fungal enzyme preparations exerted a significant decreasing effect on ACN pigment derived from berry fruit. In the study of destruction of tart cherry ACN, Peng and Markakis (1963) concluded that ACN are poor phenolase substrates; but that they are readily decolorized by this enzyme in the presence of better substrates, such as catechol, according to a consecutive-type mechanism. Pifferi and Cultrera (1974) also demonstrated that the degradation of ACN would be attributed to its reaction with the enzymic oxidation products of an appropriate phenol substrate (chlorogenic acid).

Subsequently, Cash et al. (1976) investigated the color losses in grape juice and reported that the destruction of ACN pigment could be accelerated by grape PPO.

X Several other enzymes have been implicated in the discolorization of ACN. Huang (1955) showed that fungal glycosidases (anthocyanases) hydrolyzed the ACN to anthocyanidin and sugar, with subsequent spontaneous decolorization of the aglycone. Peroxidase, which catalyzes the oxidation of certain phenolic or aromatic amine compounds by hydrogen peroxide to form dark polymers, has also been shown to decolorize ACN (Grommeck and Markakis, 1964).

It has been observed that condensation and polymerization of polyphenols, after the initial enzymic oxidation, occurred frequently. Markakis et al. (1957) postulated that brown polymerized pigment occurs as a result of the condensation of chalcones formed from the hydrolysis of the pyrylium ring. Similar brown pigments arising from ACN breakdown were reported in cranberry juice (Starr and Francis, 1968). Erlandson and Wrolstad (1972) also isolated red brown pigments from strawberry puree which had chromatography mobility like pelargonidin-3-glucoside. More recently, Timberlake and Bridle (1977) suggested that great increases in the color of ACN solution could be attributed to the formation of new compounds in the presence of catechin.

X In addition to the enzyme effect, ample evidence has accumulated which indicates that the destruction rate of ACN pigment is accelerated by high pH, oxygen, high temperature, ascorbic acid, metals and sugars (Clydesdale and Francis, 1976).

ACNs behave like pH indicators, being reddish at a low pH, bluish at high pH, and almost colorless at intermediate hydrogen for concentration (Markakis, 1974). Low pH was found to stabilize the color of ACN (Sondheimer, 1953). Meschter (1953) showed that increasing acidity has a protective effect on the stability of the pigment in strawberry products. A similar result was also observed by Sistrunk and Cash (1970). It was found that the rate of degradation of pelargonidin-3-glucoside in a model system was influenced to a great extent by pH (Tinsley and Bockian, 1960). Wrolstad et al. (1970) indicated that the pH was the only objective measurement having a high correlation with color quality and suggested that strawberries should have a pH of 3.51 or lower to have acceptable color after freezing.

\* Temperature is another important factor which causes the degradation of color. Early investigations by Kertesz and Sondheimer (1948) led to observations that the rate of acceleration of color loss in strawberry preserves increased when the temperature of storage exceeded 18.3<sup>0</sup>C, thereby resulting in the formation of brown color. Meschter (1953) showed that the rate of color deterioration increases proportionally to the log of temperature increase, while browning increased four times faster than pigment loss. In strawberry jelly, Decarcau et al. (1956) found that temperature exerted a significant effect on the pigment stability. Similar observations in strawberry juice were also reported (Mosorinski, 1975).

x The presence of oxygen was found to be a specific accelerating agent in the degradation of pigments in strawberry juice (Nebesky et al., 1949). Lukton et al. (1956) demonstrated that the rate of ACN breakdown

in strawberry juice occurred much faster in the presence of oxygen than in the presence of nitrogen. Other independent studies also confirmed that pigment losses are greater in the presence of oxygen (Tinsley and Bockian, 1960 and Daravingas and Cain, 1965). In studying the interaction of oxygen, ascorbic acid and pigments, the presence of both oxygen and ascorbic acid was found to cause more destruction of ACN pigment than their single effects (Sondheimer and Kertesz, 1953; Markakis et al., 1957 and Starr and Francis, 1968). Additionally, \* oxidation of ascorbic acid is a possible non-enzymic pathway which results in the browning of fruit juice and concentrate (Eskin et al., 1971).

A number of experiments demonstrated that sugar could also accelerate the degradation of ACN pigment in strawberry products. Meschter (1953) and Markakis et al. (1957) showed that furfural and hydroxymethylfurfural, which are known sugar degradation products, increase the rate of pigment loss. Fructose and glucuronic acid were found by Tinsley and Bockian (1963) to exert a profound deleterious effect on the destruction of pelargonidin-3-glucoside. The authors also indicated that, in the presence of sugar, the rate of disappearance of pelargonidin-3-glucoside followed first order kinetics. Conversely, Andreotti et al. (1969) found that glucose could protect the color of strawberry preserves. In addition, reducing sugars can participate \* in a non-enzymic browning reaction--Maillard reaction (Eskin et al., 1971). This reaction seems to be the major cause of brown color development during heating or prolonged storage of foods. This is

postulated, since the interaction of sugar and amino acid results in the formation of brown pigments or melanoidins (Clydesdale and Francis, 1975).

Based on the above information, the experiment was conducted to determine the role of strawberry PPO in the color deterioration of strawberry products.

## EXPERIMENTAL

### Source and Treatment of Fruit

#### IQF Fruit

Hood strawberries obtained from J. M. Smucker Company, Woodburn, Oregon on June 29, 1977, were individually quick frozen (IQF) at  $-40^{\circ}\text{C}$  and stored in a polyethylene bag at the same temperature.

#### Freeze-dried Fruit

A portion of IQF fruit was freeze-dried using a Hull corporation freeze-drier. The starting temperature was  $-28^{\circ}\text{C}$  and the temperature at shut-down time (123 hours) was  $28^{\circ}\text{C}$ .

#### Nitrogen

IQF and freeze-dried strawberries were immersed in liquid nitrogen and powdered in a stainless steel Waring Blender containing liquid nitrogen. The nitrogen powder was placed in a glass jar lined with a plastic bag and stored at  $-23^{\circ}\text{C}$ .

#### Fresh Fruit

Fresh Hood and Tioga strawberries received from Francis J. Lawrence (Oregon State University, Horticulture Department) on July 7, 1978, were selected and separated into green and ripe fruits. The extraction of enzymes and the qualitative enzyme study were conducted immediately after selection.

Fresh Hood and Benton mixed strawberries were obtained from Clermont West Company, Forest Grove, Oregon on June 27, 1978, for the preparation of juice and concentrate. Fresh fruit were individually quick frozen and stored in the dark at  $-23^{\circ}\text{C}$  before processing.

### Extraction of Polyphenol Oxidase (PPO)

#### Insoluble Polyvinylpyrrolidone (PVPP) Method

The extraction method used for pears by Halim (1977) was modified for strawberry PPO. Fifteen g of IQF strawberries (Hood) were thawed at room temperature and mixed with 1.5 g PVPP (Sigma Chemical Co., pharmaceutical grade). The mixture was blended with 30 ml of 0.05M acetate buffer (pH 5.6) for 15 seconds. The slurry was then centrifuged at  $12,000 \times G$  at  $0^{\circ}\text{C}$  for ten minutes. Enzymic activity of the supernatant was determined as soon as possible.

#### Polyethylene Glycol (PEG) Acetone Method

The method of Benjamin and Montgomery (1973) which has been used for cherries was modified as follows:

Twenty percent of aqueous PEG (Fisher Scientific Company, MW=20,000) solution was freshly prepared with 1.0 M phosphate buffer (pH 8.0). Six ml of aqueous PEG and 4 g of strawberry nitrogen powder were mixed in centrifuge tube to make a thick paste. The mixture was allowed to stand for two minutes before 9 ml of cold acetone ( $0^{\circ}\text{C}$ ) was added. After being stirred for one minute, the sample was centrifuged at  $31,000 \times G$  for five minutes. The supernatant was discarded and the pellet was rinsed three successive times with 9 ml of cold acetone.

The precipitate was stirred with 100 ml of 0.05M acetate buffer (pH 5.6) for twelve minutes and centrifuged at 27,000 X G for ten minutes to obtain an enzyme containing supernatant. The active enzyme was precipitated from the supernatant by the addition of three volume of cold acetone to two volume of supernatant. This mixture was stirred for two minutes and allowed to stand for sixty minutes. The cloudy mixture was centrifuged at 20,000 X G for ten minutes. After centrifugation the precipitate was dissolved in a minimumly small quantity of distilled water and assayed immediately. All solutions were cooled before use and all steps were carried out in an ice bath.

#### Polyclar-AT (PVPP) XAD-4 Method

Two g each of PVPP, XAD-4 (Rohm and Haas Company) and strawberry freeze-dried nitrogen powder were quickly mixed with 15 ml of 0.05M acetate buffer (pH 5.6) and then allowed to stand for two minutes before being centrifuged at 27,000 X G (0°C) for ten minutes. The supernatant obtained from centrifugation was analyzed for PPO activity.

#### PPO Assay Procedure

PPO activity was determined by measuring the initial rate of brown color formation on a Beckman DB recording spectrophotometer at 410 nm. The reference cuvette contained 1.0 ml of 10 mM catechol (freshly prepared in 0.2 M potassium phosphate buffer (pH 7.0)). The sample cuvette contained 1.0 ml of 0.2 M phosphate buffer, 1.01 ml of 10 mM catechol and 1.0 ml enzyme preparation. This sample cuvette was then shaken to promote mixing and the subsequent reaction. The results were measured and recorded at room temperature.

Initial velocity was estimated from the linear slopes of the curve obtained on the recorder. The amount of enzyme that causes a change in absorbance of 0.001 per minute was defined as one unit of enzyme activity.

### Qualitative Study of Enzymes

Fresh Hood and Tioga strawberries were used in this study. One lot of each variety was heated in a microwave oven (Litton Co., system 70/50) to an interval temperature of 82°C - 88°C. Fresh and blanched fruit were halved and placed on white porcelain spot plates. Catechol solution (10 mM) was added to the surface of the strawberries. Distilled water was added to another sample of fresh fruit. Photographs were taken at thirty minutes and four hours after treatment.

### Preparation of Strawberry Juice and Concentrate

IQF strawberries (Hood and Benton mixed varieties) were thawed at room temperature. A large sample of unthawed IQF fruit was heated in a microwave oven for 3-4 minutes to an interval temperature of 82°C - 88°C. Juice was prepared from both thawed and blanched fruit using an Oster's Automatic Juice Extractor. Pectinol L (Rohm and Haas Company), in the amount of 0.2% of the total juice volume, was added to the juice and this mixture was incubated in a warm water bath at 37°C for 3.5 hours. In order to complete clarification, the juice was strained through a nylon cloth and then filtered under vacuum through Whatman No. 1 filter paper coated with a 1 cm layer of celite.

A portion of the juice was concentrated on a rotary evaporator to 58° Brix, (water bath = 37°C, pressure 29"Hg).

The juice and concentrate preparations were transferred to 500 ml glass stopper erlenmeyer flasks. Potassium sorbate (0.1% of volume) was added to the juice to prevent microbial growth. All samples were stored in the dark at 20°C. One set of blanched and unblanched juice was allowed to stand in the presence of air, while another set had nitrogen bubbled through it to eliminate the presence of oxygen. Chemical and color analyses were conducted periodically during a total of eight weeks of storage.

### Analyses of Chemical Parameters

#### Anthocyanin (ACN) Pigment Content

The pH differential method (Wrolstad, 1976) was utilized to determine ACN pigment content of both juice and concentrate. This pigment content expressed as mg of pelargonidin-3-glucoside per g fruit, was calculated by using a molecular weight of 433.2 g and molar absorbance of 22,400. Samples were diluted with buffer before measuring the absorbance at 496 nm and 700 nm on a Beckman DB-G spectrophotometer. Determinations were performed in duplicate.

#### Total Phenolics

The improved method of Singleton and Rossi (1965) utilizing Folin-Ciocateau reagent and 20% Na<sub>2</sub>CO<sub>3</sub> solution was followed for the measurement of total phenolics in strawberry juice and concentrate. The total

phenolic content was calculated as gallic acid equivalents and reported as the mean of two determinations.

### Flavanols and Leucoanthocyanins

Flavanol and leucoanthocyanin contents were determined according to the procedure of Swain and Hillis (1959). These determinations were performed in duplicate and results are reported as mean values.

### Color Analyses

#### Browning, Color Density, Polymeric Color, and Percent Polymeric Color

Color parameters were measured by the potassium meta bisulfite method which was developed by Somers (1971) for wines and then adapted for other ACN-containing products by Wrolstad (1976). This method is based on the resistance of polymeric tannin pigments to bisulfite bleaching. Color density, defined as the sum of the absorbance of the untreated sample at 420 nm and 496 nm, is an indication of total color. Polymeric color is expressed as the sum of the absorbance of the bisulfite-treated samples at 420 nm and 496 nm. The ratio of polymeric color to color density then measures the percent of polymeric color. The degree of browning is determined by recording the absorbance at 420 nm of the bisulfite treated samples. All of those samples were diluted in order to obtain an accurate absorbance reading.

### Colormetric Measurement

Juice and concentrate samples were stored at 20°C in a plastic parafilm covered cell, measuring 5 cm x 5 cm x 1 cm. Color specifications were obtained by standardizing the Hunter Color Difference

Meter (CDM), model D25 P-2 with a white tile standard (standard no. DC122, L = +94.02, a = -0.9, b = +1.2).

Transmittance values of "L" (lightness), "a" (redness), and "b" (yellowness) were obtained with the light source being pivoted and the specular component being excluded (Arrangement III). The "Y" values were recorded in both arrangement I (light source and exit port are in alignment, and specular component is included) and arrangement III. The percent of Haze was then expressed as the ratio of "Y" value in arrangement I to "Y" value in arrangement III. The square root of  $a^2 + b^2$  and  $\tan^{-1} \frac{a}{b}$  were also calculated to demonstrate the color changes in the sample.

#### Statistical Procedures

Three-way analysis of variance (blanched, control and/or nitrogen treatment vs time) were run for the following sets of data: anthocyanin, total phenolics, flavanols, leucoanthocyanins, browning, color density, polymeric color and percent polymeric color. Levels of significance were determined at the 95% and 99% level. The change of anthocyanin content vs time was analyzed by the regression test in order to determine if the log of the pigment concentration showed a linear relationship for each treatment type.

## RESULTS AND DISCUSSION

### Isolation of PPO from Strawberry

Characterization of strawberry PPO is dependent upon the successful extraction of this enzyme. Several methods were tried in order to find the most effective procedure for obtaining a PPO preparation with the highest activity.

Table 1 shows the results of the PVPP method wherein buffer pH and concentration were the variables for determining the level of PPO activity. Five buffer pH values and three buffer concentrations were compared for both IQF (Hood) and Fresh (Driscoll) strawberries. The greater activity was recorded at pH 5.6 with 0.05M buffer concentration, but the results proved inconsistent with a range varying between 0 unit/ml and 562 units/ml in ten runs. A higher buffer concentration (0.1M and 0.5M) was used in the next set of tests, with resultant inconsistency and low activity. Next, the buffer pH was raised to 7.0 with poor results, and then to 8.0 with zero activity.

An effort to eliminate the possible detrimental effects of freezing, fresh fruit was tested. Freezing may disturb the cell structure and cause inactivation of enzymes. Results, however, showed low activity with fresh fruit when tested over a pH range of 5.0-7.0 with buffer concentration at 0.05M.

Due to the inconsistency and low level of activity resulting from the PVPP method, two alternate processes were investigated. The

Table 1. Isolation of strawberry PPO by PVPP method.

Buffer pH	Buffer Concentration	Source of Fruit	Variety	Activity (units/ml)
5.6	0.05M	IQF	Hood	0, 402, 562, 16, 10, 0, 22, 14, 10, 10
5.6	0.1M	IQF	Hood	6, 8, 14
5.6	0.5M	IQF	Hood	0, 14, 0
7.0	0.05M	IQF	Hood	16, 12
7.0	0.1M	IQF	Hood	8,10
8.0	0.1M	IQF	Hood	0, 0
5.6	0.05M	Fresh	Driscoll	0, 8
5.0	0.05M	Fresh	Driscoll	8, 0
6.0	0.05M	Fresh	Driscoll	0, 0
7.0	0.05M	Fresh	Driscoll	12, 0

first was the PEG and acetone procedure, the results of which are presented in Table 2. These data show that IQF (Hood) fruit had the highest activity in one of the eight runs, but inconsistency was still a problem. While fresh fruit were in season, several different types of tests were conducted using Driscoll, Hood, Tioga and Benton varieties. Most of the tests on the fresh fruit produced consistent, but low activity runs. The same was true when using green fruit (Tioga), although green fruit and the core of the green fruit did have higher activity. In order to achieve uniformity and for the sake of convenience, a nitrogen powder preparation was made and tested. The consistently low level of enzymic activity made this process unacceptable for further use.

The second alternate and final process used to determine PPO activity was the PVPP and XAD-4 method. After a number of trials, it was found that a 1:1:1 ratio of PVPP, XAD-4 and freeze dried nitrogen powder (Hood) was the best combination for testing enzymic activity. On tests run at pH 5.6 and 7.0 with a buffer concentration of 0.05M and 0.1M, the results (Table 3) showed consistently low activity. With fresh fruit (Hood, Tioga, Benton) being substituted for the nitrogen powder, similar results were obtained. Green fruit did show a higher level of activity, however.

After an exhaustive review of the three aforementioned procedures for testing PPO activity, it was determined that these methods were inadequate for use in this study. Due to the generally low level of enzymic activity, it would be very difficult to conduct experiments

Table 2. Isolation of strawberry PPO by PEG and acetone method.

Source of Fruit	Variety	Activity (units/ml)
IQF	Hood	0, 478, 0, 0, 18, 14, 14, 10
N <sub>2</sub> powder	Hood	10, 10
Freeze-dried N <sub>2</sub> powder	Hood	30, 62, 84, 50, 52
Fresh	Driscoll	0, 0
Fresh	Hood	0, 0
Fresh	Tioga	0, 0
Fresh (green)	Tioga	2, 10
Fresh (green and core)	Tioga	18, 16
Fresh (green)	Hood and Benton	20, 14

Table 3. Isolation of strawberry PPO by PVPP and XAD-4 method.

Buffer pH	Buffer Concentration	Source of Fruit	Variety	Activity (units/ml)
5.6	0.05M	Freeze-dried N <sub>2</sub> powder	Hood	0, 14, 6, 8
7.0	0.05M	Freeze-dried N <sub>2</sub> powder	Hood	10, 6
7.0	0.1M	Freeze-dried N <sub>2</sub> powder	Hood	18, 10, 10, 12
7.0	0.1M	Fresh	Hood and Benton	18. 8
7.0	0.1M	Fresh	Hood	0, 0
7.0	0.1M	Fresh	Tioga	0, 0
7.0	0.1M	Fresh (green)	Tioga	20, 8

on pH stability, heat tolerance of PPO, and the effect of PPO in the browning of strawberry products. The problem with low activity could be due to the biochemical composition of the strawberry, the interference by high levels of phenolic compounds, and/or the special characteristics of strawberry PPO. Further research needs to be conducted in this area.

#### Qualitative Study of Enzymes

Due in part to the results of the quantitative study and in part to the need for further analysis of PPO activity, a qualitative study was conducted to evaluate enzyme action in strawberries. Although more subjective in nature, the qualitative study produced some very obvious and profound results, which are presented in Table 4. As can be seen in this table, the blanched strawberries with catechol showed little, if any, browning; whereas the unblanched with catechol exhibited slight to extensive browning, with green Tioga and Hood varieties being more susceptible to enzymic action than mature strawberries. The unblanched fruit and distilled water exhibited virtually no browning, except for the green Tioga fruit which stood for four hours. Tioga variety exhibited generally more PPO activity than Hood when receiving the same treatment. It should be noted that most of the browning occurred in the central ring of fibro-vascular bundles and fibro-vascular bundles leading to the seeds.

The aforementioned data reveals very striking differences between blanched and unblanched samples, since browning did not occur in any of the blanched fruit. These results substantiate the fact that

Table 4. Effect of enzymes on the browning of Hood and Tioga strawberries.

Variety	Maturity	Treatment*	Time** (hour)	
			0.5	4
Tioga	Green	A	-	-
Tioga	Green	B	++	+++
Tioga	Green	C	-	+
Tioga	Ripe	A	-	-
Tioga	Ripe	B	+	++
Tioga	Ripe	C	-	-
Hood	Green	A	-	-
Hood	Green	B	+	++
Hood	Green	C	-	-
Hood	Ripe	A	-	-
Hood	Ripe	B	-	+
Hood	Ripe	C	-	-

\*A. Blanched fruit + catechol  
 B. Unblanched fruit + catechol  
 C. Unblanched fruit + distilled H<sub>2</sub>O

\*\* - No browning  
 + Slight browning  
 ++ Moderate browning  
 +++ Extensive browning

✕  
PPO does exist in strawberries. Greater PPO activity occurs in green fruit, however, the anthocyanin pigment in ripe strawberries may partially mask the browning so that it is not as perceptible in ripe fruit as in green strawberries. It has been reported that there is a decrease in PPO activity during the ripening of grapes, apples and stone fruits (Traverse-Reuda and Singleton, 1973; Dang, 1971; and Zocca and Ryugo, 1975.) Hence, there may not be as much enzymic browning in ripe fruit as there is in immature fruit. Abers and Wrolstad (1978) reported that strawberry preserves derived from Tioga variety showed greater color degradation than preserves derived from Hood variety. The investigators suggested that greater color deterioration could be due to the higher level of flavanols, leucoanthocyanins and total phenolics in Tioga fruit. This higher level of phenolics could serve as a substrate for PPO, thereby enhancing its activity and subsequent browning of the fruit. The results of this study show that Tioga strawberries have a high level of PPO activity, which might account for, or at least contribute to, the greater color degradation of Tioga fruit than in Hood fruit.

### Analyses of Chemical Parameters

#### Anthocyanin (ACN) Pigment Content

In an effort to study the effects of ACN pigment degradation on the red color of strawberry juice and concentrate, research was conducted to determine the effects of blanching and a nitrogen environment on the rate of ACN breakdown. The values used for

plotting were the mean of the two readings from two determinations. The variation from the mean for these two readings is much less than  $\pm 0.01\%$ .

As can be seen in Figure 1, ACN pigments showed rapid change during storage. The initial ACN content of the blanched samples was substantially higher than that of the unblanched. This difference has been attributed to enzymic action which may have occurred in the control sample during the depectinizing stage of juice production. All samples tested showed similarly rapid deterioration during the first two weeks, wherein the majority of ACN present broke down. After this initial two week period, the rate of degradation was markedly reduced, and relatively little degradation occurred between the fifth and eighth week. The ACN pigment levels in all of the samples were very low at the termination of eight weeks storage at 20°C. The concentration of ACN dropped to less than 6 percent of the original concentration in all samples.

Based on the results of statistical analyses (Table 5), there is a significant difference between treatments. The differences are as follows: 1) the blanched samples had a higher level of total ACN pigment than the control throughout the experiments; 2) samples in a nitrogen environment demonstrated less degradation than those in the presence of air; 3) the combination treatment of blanching and nitrogen retained a greater amount of ACN pigment. This is generally consistent with the reports of Lukton *et al.* (1958) and Tinsley and Bockian (1960) which stated that destruction of strawberry ACN pigment was materially lower in the presence of nitrogen rather than oxygen.

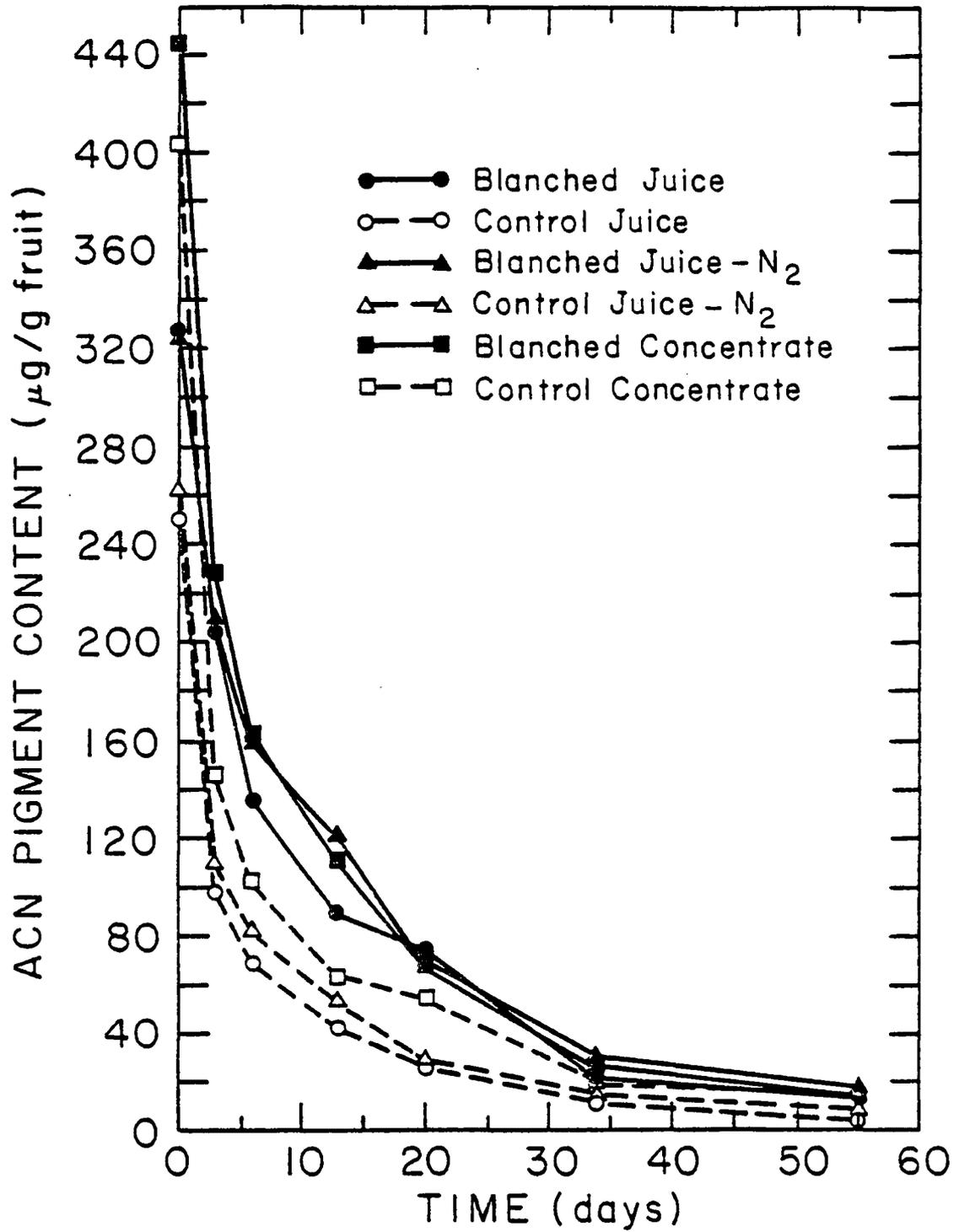


Figure 1. Degradation of ACN pigment during storage.

Table 5. Results of statistical analysis\*--three-way analysis of variance.

Variable	Blanched vs Control	Air treated vs N <sub>2</sub> treated	Interaction
ACN	P=.001	P=.001	P=.002
Total phenolics	P=.001	P=.432	P=.746
Flavanols	P=.001	P=.01	P=.138
Leucoanthocyanins	P=.001	P=.002	P=.164
Browning	P=.001	P=.001	P=.001
Color density	P=.242	P=.001	P=.205
Polymeric color	P=.004	P=.001	P=.001
% Polymeric color	P=.001	P=.001	P=.105

- \*1) If  $P < 0.05$ , there is a significant difference between treatments (based on 95% level).
- 2) If  $P < 0.01$ , there is a significant difference between treatments (based on 99% level).

In addition, the log of the ACN content was plotted against time (Figure 2) in order to determine the kinetics of the pigment degradation. The destruction rate of ACN did not plot in a straight line.

\* The results of the regression test (Table 6) also suggest that there is no significant linear relationship between ACN content and time in most of the samples. The time square values of blanched juice and control juice are insignificant, which shows that the linear relationship is probably correct. However, these results are questionable because the sample size was small and few data points were used. The above observation does not correlate with other studies which reported a logarithmic nature in pigment loss in strawberry products (Meschter, 1953; Decareau et al., 1956; and Markakis et al., 1957).

### Total Phenolics

To facilitate further analysis of color degradation in strawberry juice and concentrate, experiments were conducted to determine the relationship between the breakdown of phenolics and the effects of enzyme presence. The results of these tests are shown in Table 5 and Table 7. The phenolic content of the juice samples was higher for the blanched samples than for the control. The blanched juice also had a smaller percentage loss of phenolics than the control. The nitrogen treated samples did not show a significantly higher level of phenolics than those samples not treated with nitrogen. The strawberry concentrate samples, both blanched and control, had phenolics losses during storage, but to a lesser extent than the juice. Statistical analyses (Table 5) indicate that only blanching has a significant effect on

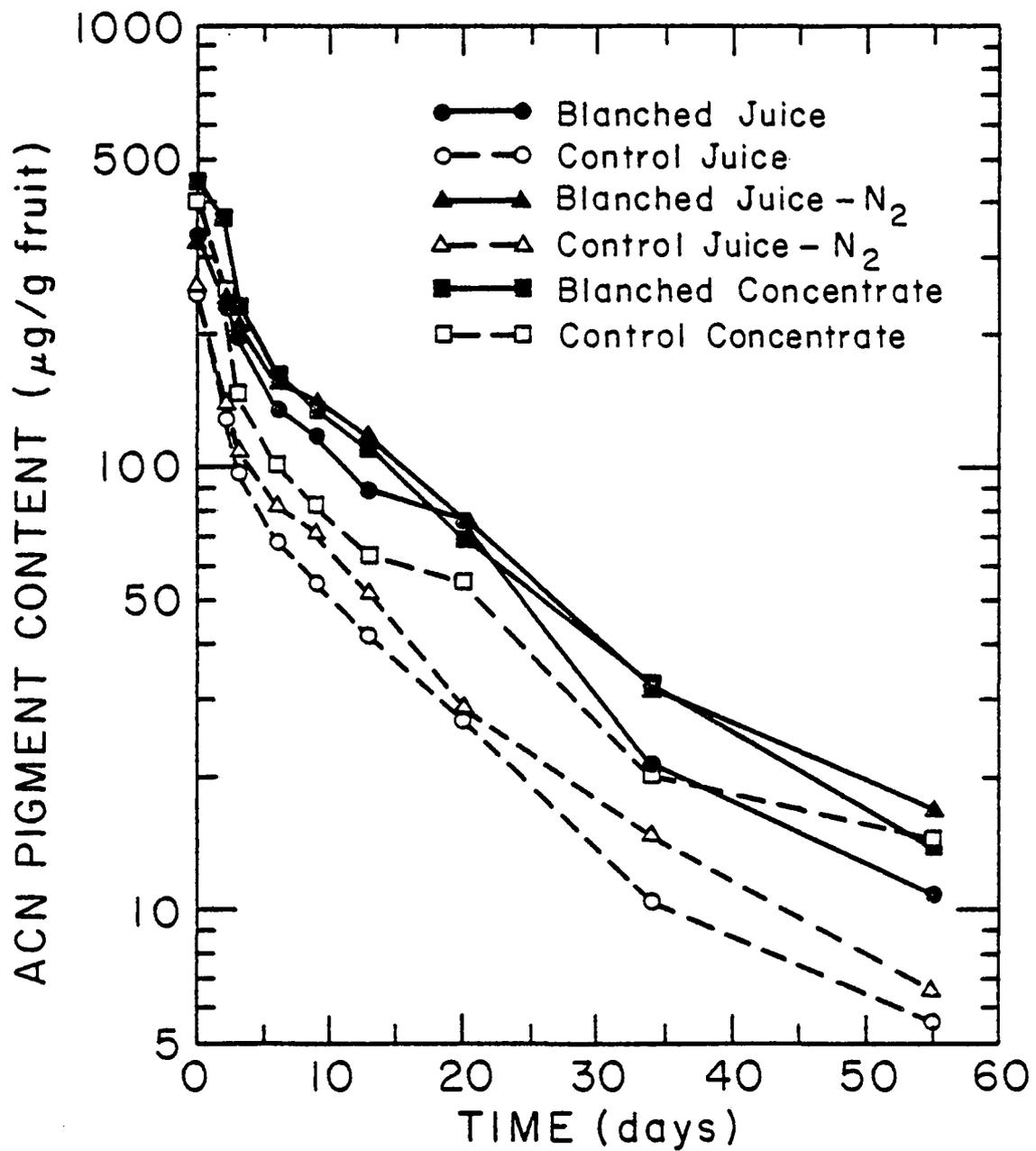


Figure 2. Degradation of ACN pigment during storage - plot in log scale.

Table 6. Regression test\* of ACN pigment content during storage.

	Time	(Time) <sup>2</sup>	Overall R square	Increase in R square from second term
Blanched juice	P=.000	P=.074	.98	.02
Blanched juice-N <sub>2</sub>	P=.000	P=.011	.99	.02
Blanched concentrate	P=.000	P=.001	1.00	.02
Control juice	P=.001	P=.115	.92	.06
Control juice-N <sub>2</sub>	P=.000	P=.022	.98	.05
Control concentrate	P=.000	P=.015	.97	.08

\*If  $P < 0.05$ , there is a significant importance of the term (based on the 95% level).

Table 7. Effect of storage on Total Phenolics Content\* in strawberry juice and concentrate.

Sample Day	0	13	55	% Loss
Blanched juice	1.35 $\pm$ 2.2%	1.34 $\pm$ 0.8%	0.97 $\pm$ 0.2%	28.2
Blanched juice-N <sub>2</sub>	1.49 $\pm$ 1.2%	1.16 $\pm$ 0.2%	1.04 $\pm$ 0.2%	30.2
Blanched concentrate	1.30 $\pm$ 1.7%	1.18 $\pm$ 0.5%	1.00 $\pm$ 0.6%	23.1
Control juice	1.05 $\pm$ 0.5%	0.78 $\pm$ 0.7%	0.67 $\pm$ 0.4%	36.2
Control juice-N <sub>2</sub>	1.09 $\pm$ 0.5%	0.82 $\pm$ 0.3%	0.73 $\pm$ 0.2%	33.0
Control concentrate	0.90 $\pm$ 1.2%	0.85 $\pm$ 0.2%	0.74 $\pm$ 0.2%	17.8

\*1) Expressed as mg gallic acid per g fruit.

2) Reported values represent the mean of two determinations.

the loss of total phenolics.

### Flavanols and Leucoanthocyanins

As with total phenolics, experiments were conducted to determine the effects of blanching and nitrogen treatment on the level of flavanols and leucoanthocyanins in stored strawberry juice and concentrate.

Tables 8 and 9 show the results of tests which point to a higher level of both flavanols and leucoanthocyanins in the blanched rather than the control samples. The data reflects a pattern established earlier with ACN pigment and total phenolics. The higher level of flavanols and leucoanthocyanins in the nitrogen treated samples is also consistent with the pattern established with ACN. Flavanol and leucoanthocyanin degradation occurred during the eight week storage period, with the control juice losing a larger percentage than the blanched sample. The non-nitrogen treated samples had a greater loss of these compounds than the nitrogen treated samples. Both the control and blanched concentrates showed a reduction in flavanols and leucoanthocyanins. There was a greater loss of leucoanthocyanins in the control than in the blanched concentrate, however, a lesser loss of flavanols in the control than in the blanched concentrate. Statistical analyses (Table 5) reveal that both blanching and nitrogen treatments have a significant effect on the retention of flavanols and leucoanthocyanins, while the interaction effect of blanching and nitrogen treatments is not significant.

The above chemical analysis tests have shown a general trend for

Table 8. Effect of storage of flavanols content\* in strawberry juice and concentrate.

Sample Day	0	13	55	% Loss
Blanched juice	6.78 $\pm$ 1.5%	5.72 $\pm$ 4.2%	3.27 $\pm$ 0.7%	51.8
Blanched juice-N <sub>2</sub>	7.05 $\pm$ 0.2%	6.13 $\pm$ 1.3%	4.45 $\pm$ 0.9%	36.9
Blanched concentrate	4.73 $\pm$ 6.2%	3.85 $\pm$ 0.1%	3.82 $\pm$ 1.0%	19.2
Control juice	5.38 $\pm$ 5.7%	4.17 $\pm$ 4.0%	2.34 $\pm$ 0.1%	56.5
Control juice-N <sub>2</sub>	5.64 $\pm$ 0.8%	4.34 $\pm$ 0.2%	2.96 $\pm$ 1.1%	47.1
Control concentrate	3.25 $\pm$ 0.4%	3.19 $\pm$ 0.2%	2.72 $\pm$ 0.1%	16.1

\*1) Expressed as absorbance units per g fruit.

2) Reported values represent the mean of two determinations.

Table 9. Effect of storage on leucoanthocyanins content\* in strawberry juice and concentrate.

Sample Day	0	13	55	% Loss
Blanched juice	1.88 $\pm$ 0.2%	1.26 $\pm$ 2.5%	0.78 $\pm$ 0.1%	58.5
Blanched juice-N <sub>2</sub>	1.92 $\pm$ 0.2%	1.87 $\pm$ 3.4%	0.91 $\pm$ 0.1%	52.6
Blanched concentrate	1.47 $\pm$ 0.3%	1.12 $\pm$ 2.4%	1.03 $\pm$ 0.1%	30.0
Control juice	1.42 $\pm$ 0.2%	0.69 $\pm$ 3.5%	0.50 $\pm$ 0.1%	64.8
Control juice-N <sub>2</sub>	1.53 $\pm$ 0.2%	1.27 $\pm$ 4.1%	0.59 $\pm$ 0.2%	61.4
Control concentrate	1.12 $\pm$ 0.2%	9.82 $\pm$ 0.2%	0.64 $\pm$ 0.2%	42.7

\*1) Expressed as absorbance units per g fruit.

2) Reported values represent the mean of two determinations.

greater degradation of ACN pigment, total phenolics, flavanols and leucoanthocyanins in the control (unblanched) samples and in those samples that did not receive the nitrogen treatment.

### Color Analyses

#### Browning, Color Density, Polymeric Color, and Percent Polymeric Color

In order to better understand the relationship between the color and composition changes in strawberry juice and concentrate, a number of color analyses were made.

#### Browning

Figure 3 shows the results of the browning measurements of juice samples during storage. These results are expressed as an increase in absorbance at 420 nm of the bisulfite treatment sample. The plotted points represent the mean of two determinations. The blanched samples showed more browning than the control, but both blanched and control samples showed similar rates of increases during storage. The same pattern was obtained with blanched nitrogen juice as compared with control nitrogen juice. However, the non-nitrogen treated samples had less browning than the nitrogen treated samples. This phenomenon could possibly be explained by the oxidative reactions which may have occurred in the air-exposed samples. That is, chromophoric groups of polymeric compounds could have been oxidized with a resulting bleaching effect.

\* The concentrate samples, shown in Figure 4, also demonstrated an

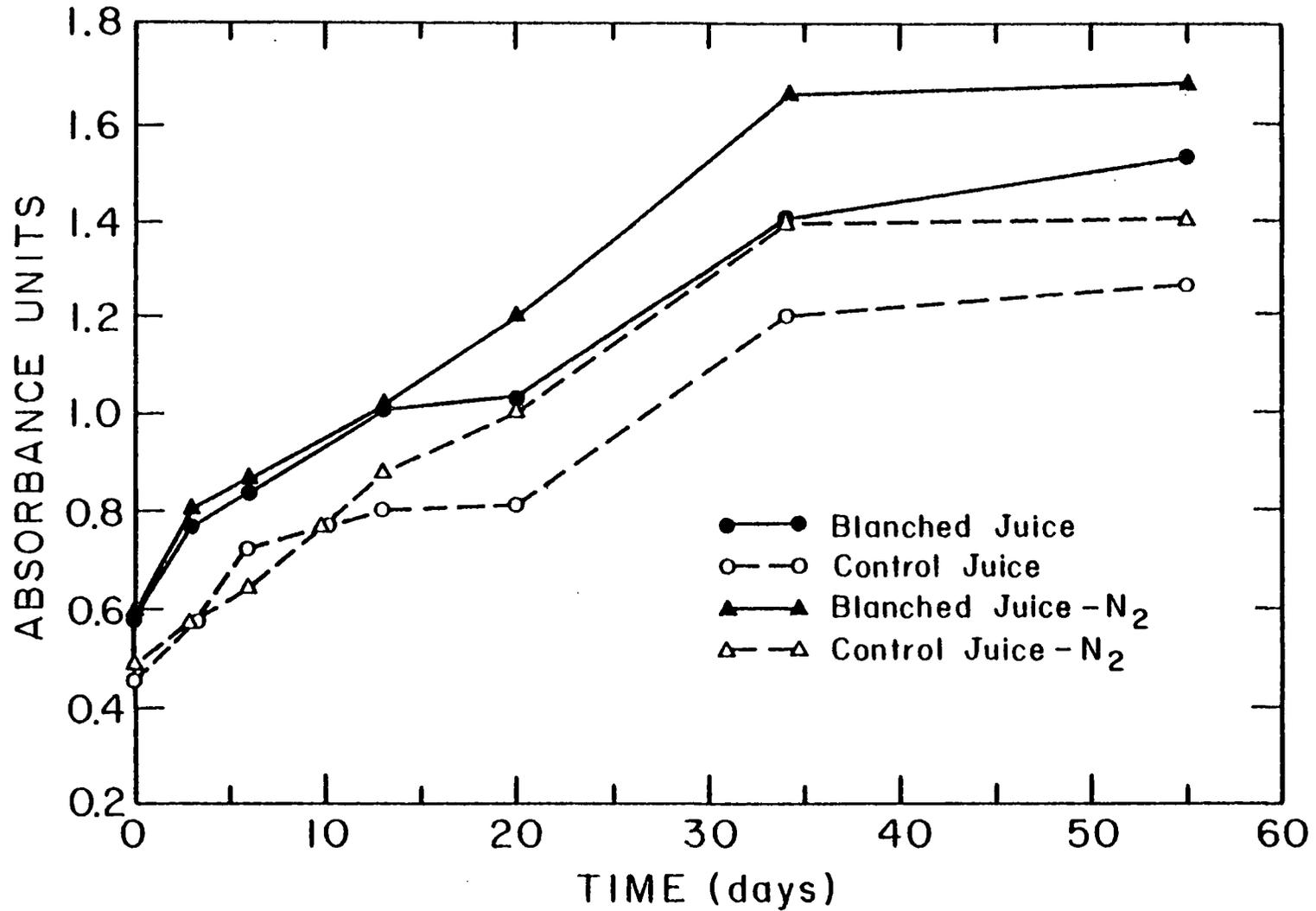


Figure 3. Browning in strawberry juice during storage--absorbance at 420 nm of bisulfite treated sample.

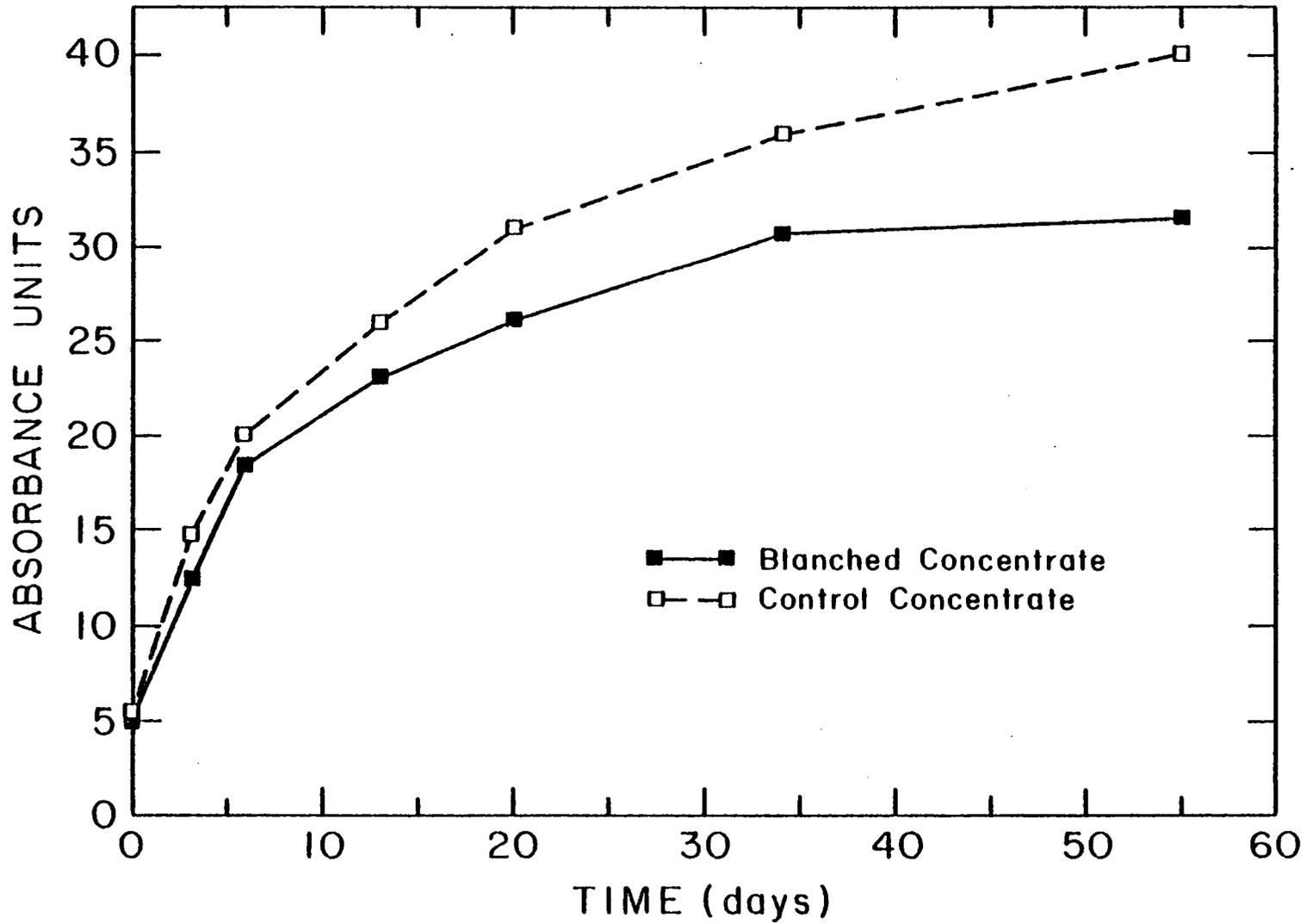


Figure 4. Browning in strawberry concentrate during storage--absorbance at 420nm of bisulfite treated sample.

increase in browning, with more browning in the control than in the blanched sample. This result suggests the possibility of an enzymic effect. Once enzymes oxidize the phenolics, the resulting oxidized compounds could be further oxidized during storage. The additional browning occurs readily when initial oxidation has begun.

The statistical analyses (Table 5) support the above observation that blanching and nitrogen treatments have a significant influence on the increased formation of brown color.

### Color Density

Color density is an indication of the total amount of color, and the changes in color density for the samples during storage are shown in Table 10. The blanched juice and control juice had similar trends with decreasing color density through the 34th day, followed by a slight increase. The differences between these two samples are not significant (Table 5). In addition, both blanched nitrogen and control nitrogen treated juice showed decreasing color density throughout the storage period. According to the statistical data (Table 5), nitrogen treated samples maintained a significantly higher color density than non-nitrogen treated samples. The blanched and control concentrates had decreased color by the third day of storage with density increasing by the sixth day. Further, the control concentrate showed greater color density by the end of the storage period than at the beginning of the experiment. This increase in color density can be explained by the increase in browning during storage.

Table 10. Effect of storage on the change of color density\* in strawberry juice and concentrate.

Sample Day	0	3	6	9	13	20	34	55
Blanched juice	7.73	6.10	4.90	5.09	3.93	3.47	3.07	3.27
Blanched juice-N <sub>2</sub>	8.17	6.20	5.41	5.04	4.60	3.99	3.68	3.32
Blanched concentrate	62.0	50.1	51.6	55	53.5	53.6	54.6	56.4
Control juice	6.04	3.56	3.50	2.71	2.52	2.43	2.43	2.48
Control Juice-N <sub>2</sub>	5.68	3.70	3.51	2.97	2.87	2.53	2.68	2.57
Control concentrate	54.9	47.3	50.8	51.4	52.3	59.1	62.0	66.5

\*1) Expressed as absorbance units.

2) Reported values represent the mean of two measurements.

### Polymeric Color

Polymeric color is the measurement of those polymeric pigments which are not bleached by bisulfite. As can be seen in Table 11, the blanched juice had more polymeric color than the control juice. The same was true of the blanched nitrogen sample, when compared with the control nitrogen sample. The concentrates had substantially higher polymeric color than any of the juice samples. The blanched concentrate had more color than the control and samples increased in polymeric color over the storage period. The aforementioned effect of blanching and/or nitrogen treatment is significant on the basis of statistical analyses (Table 5).

### Percent Polymeric Color

A comparison of the percent polymeric color, as is seen in Figure 5, shows that all samples had similar initial percent polymeric color, followed by pronounced increases during the storage period. In the juice samples, the blanched, nitrogen blanched and control samples seemed to have a similar rate of increase in percent polymeric color level. The nitrogen control samples had a greater increase than the other three juice samples. Further, the blanched samples showed less percent polymeric color when compared with the control samples. The nitrogen treated samples, however, had greater percent polymeric color than the non-nitrogen treated samples. This observation could be again attributed to the possible oxidative reaction with a resulting bleaching effect.

Table 11. Effect of storage on the change of polymeric color\* in strawberry juice and concentrate.

Sample	Day	0	3	6	9	13	20	34	55
Blanched juice		0.74	1.09	1.19	1.57	1.63	1.74	2.17	2.28
Blanched juice-N <sub>2</sub>		0.76	1.18	1.20	1.45	1.61	1.91	2.49	2.51
Blanched concentrate		6.35	19.2	26.0	29.9	33.0	38.1	46.4	48.9
Control juice		0.54	0.76	0.98	1.09	1.15	1.18	1.64	1.86
Control juice-N <sub>2</sub>		0.58	0.78	0.85	1.04	1.30	1.60	2.24	2.16
Control concentrate		6.25	23.0	28.0	34.6	38.0	45.3	54.5	61.2

\*1) Expressed as absorbance units.

2) Reported values represent the mean of two measurements.

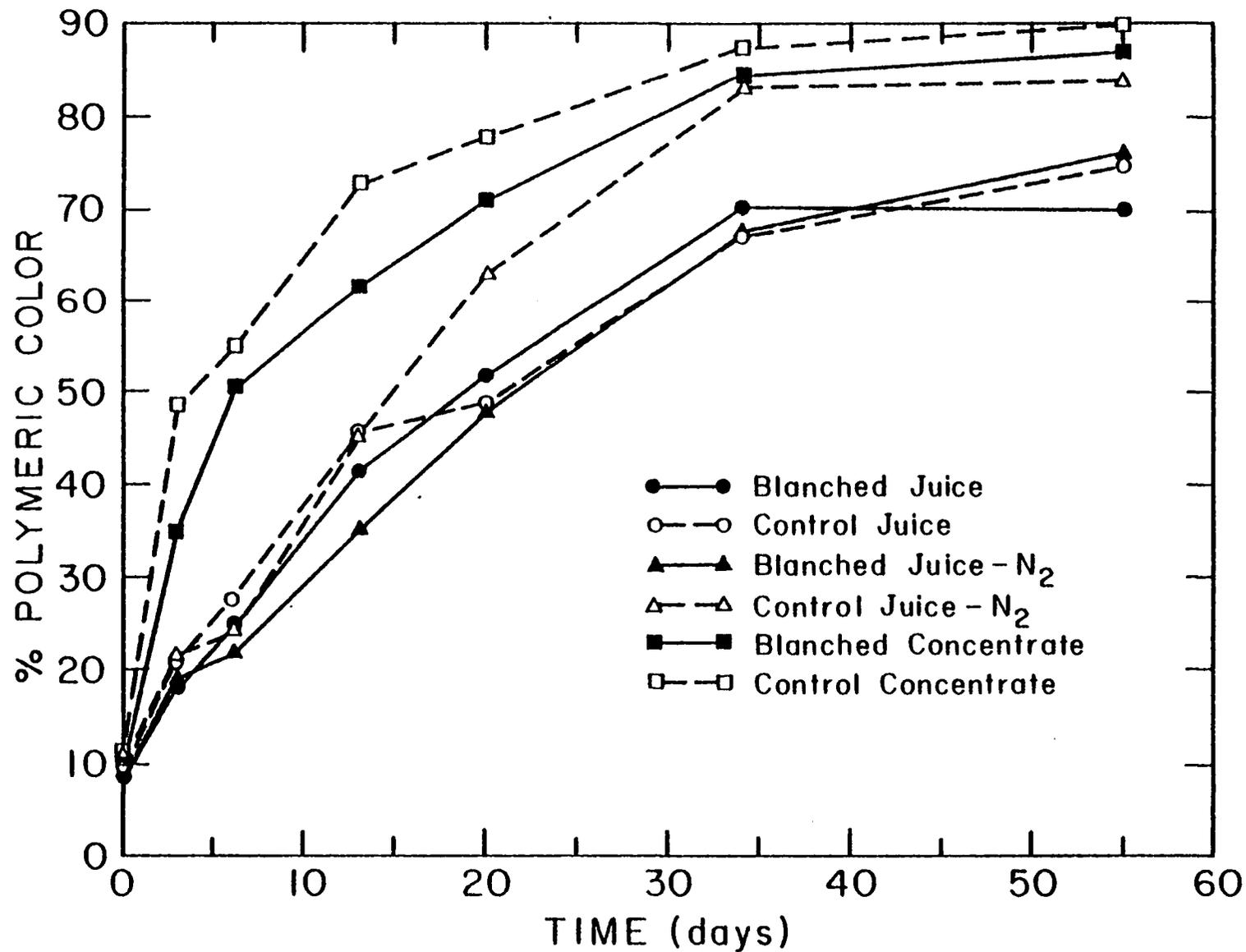


Figure 5. Formation of percent polymeric color in strawberry juice and concentrate during storage.

The increase in color level occurred faster in the concentrate samples than in the juice. The control sample had a slightly greater overall percent polymeric color than the blanched. It was anticipated that the concentrate would have a more rapid rise in color levels because of the concentration effect which accounts for the rapid formation of polymeric compounds.

It was also noted that, in all of the paired samples, the control had a higher percent polymeric color level than the blanched. This result could be due in part to the action of PPO. The more PPO in a sample, the greater the quantity of oxidative products and resulting increase in percent polymeric color. Statistical analyses substantiate that blanching and/or nitrogen treatment have a significant effect (Table 5).

The experimental results on browning, color density, polymeric color, and percent polymeric color seem to indicate that enzymic reaction, which is an oxidation of phenolics by PPO in the presence of oxygen, might not be the only browning reaction. The Maillard reaction, which is the reaction between sugar and amino groups under anaerobic conditions, also produces a browning effect. Another possibility for the increase in brown color is the oxidation of ascorbic acid. The aforementioned reactions could occur simultaneously with all of them contributing to the increase of brown color in the sample.

#### Colorimetric Measurement

The tests conducted to determine pigment degradation indicated a trend toward greater amounts of ACN, flavanols and leucoanthocyanins

in the blanched and nitrogen treated samples rather than in the control and non-nitrogen treated samples, throughout the storage period. Although the levels of ACN pigments were low in all samples at the end of storage, browning tests indicated that the blanched and nitrogen treated samples had a greater degree of browning than the control. Alternately, the control concentrate had more browning than the blanched concentrate. However, visual appearance indicated that the blanched samples did have a redder appearance.

Hunter measurements were then applied to corroborate the above observation. The "a" value is a measure of the amount of redness. As shown in Figure 6, all of the juice samples had similar initial values, with a generally decreasing trend during storage. The nitrogen blanched and blanched samples maintained a higher "a" value than the nitrogen control and control juice samples. The blanched and control concentrates had much lower initial "a" value, but both had similar losses during the storage period. The blanched concentrate retained a slightly higher "a" value than the control concentrate.

The "a" value in the strawberry juice samples did decline over the storage period, but still maintained relatively high levels during storage. On the other hand, ACN pigments virtually disappeared by the end of the storage period. This data supports the idea that the red color which exists in the juice samples at the end of storage may not be an ACN pigment, but some other red color compound(s) formed during storage. In experiments conducted by Timberlake and Bridle (1977), it was suggested that greatly increased color could be attributed to the formation of new red compounds in the presence of anthocyanins and

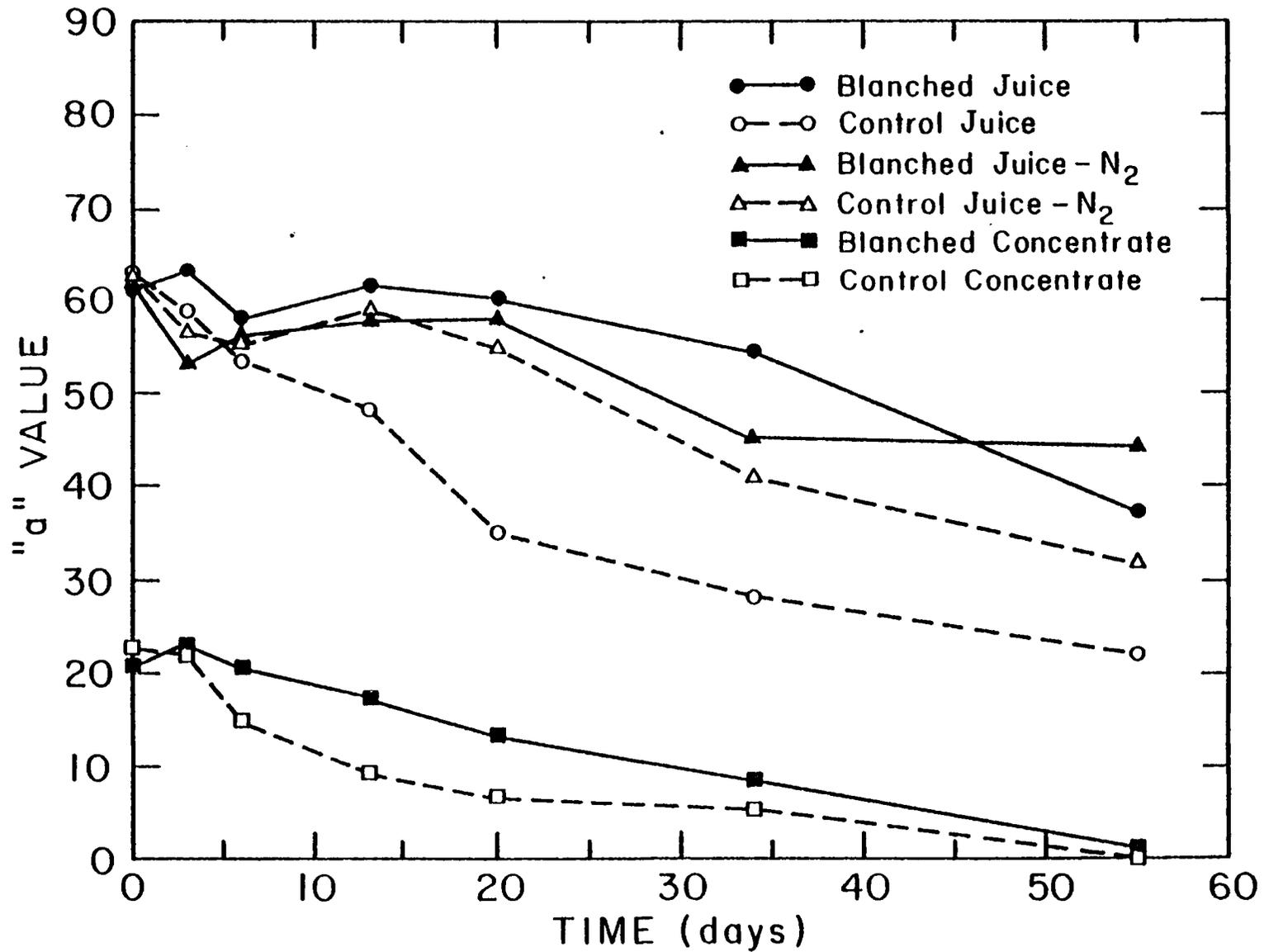


Figure 6. Change of "a" value (transmittance) in strawberry juice and concentrate during storage.

catechins.

Figure 7 shows the "L" value of the juice samples during storage. "L" value, a measure of lightness, generally increased throughout the storage period with the greatest "L" value occurring in the control juice and lowest in the blanched juice sample. The pronounced increase in "L" value in the control sample indicates bleaching and lightening as a result of oxidation, polymerization and precipitation. The precipitation occurred most rapidly during the third week of storage. The blanched sample, which had the lowest "L" value, had virtually no precipitate. The nitrogen treated samples had very similar "L" values after three weeks of storage. The above results seem to suggest the possibility of a direct relationship between the "L" values, polymeric color and color density, that is, a greater color level in the blanched samples.

\* Figure 7 also shows the "L" value for the concentrate samples, which darken with storage. This decrease in "L" value can be explained by the fact that precipitation did not easily take place due to the high viscosity of the concentrate. Therefore, polymeric (darkening) compounds formed from oxidation or polymerization remained in solution. \* Unlike the juice samples, the results derived from concentrate tests do not show the same trend in polymeric color and color density measurements.

### Yield

An incidental finding during the juice preparation was the fact that blanched strawberries yielded 73.7 percent juice, while the

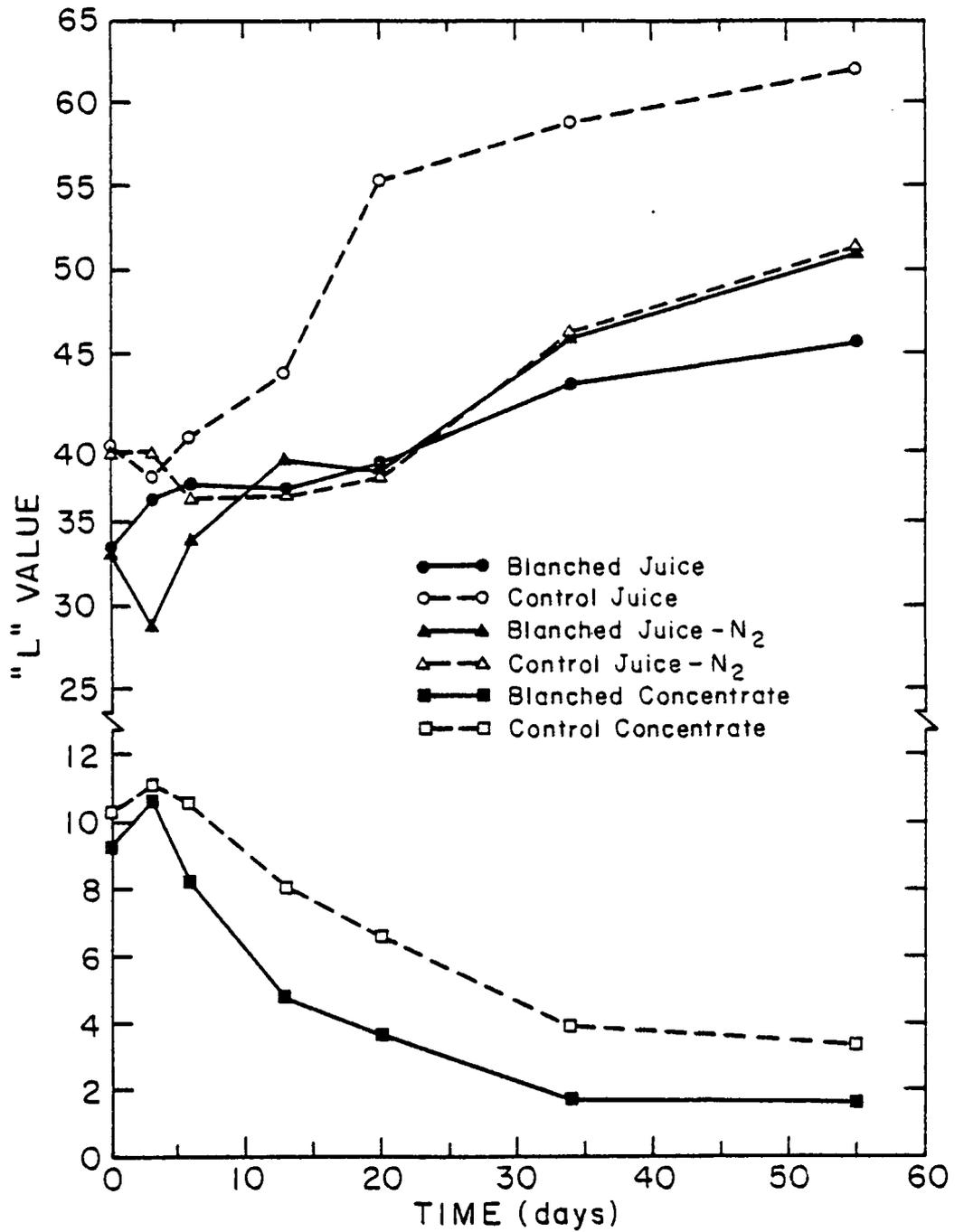


Figure 7. Change of "L" value (transmittance) in strawberry juice and concentrate during storage.

unblanched fruit produced juice amounting to only 66.4 percent of raw weight. It was also noted that the production of juice was achieved more readily with the microwave blanched fruit. These observations may be of interest to the food industry when considering the economic factors involved with juice production.

### Summary and Conclusion

The purpose of this study was to determine the influence of enzymes on color deterioration in strawberry juice and concentrate during manufacture and storage.

Attempts were made to isolate PPO from strawberries by utilizing the PVPP, PEG-Acetone, and PVPP-XAD-4 procedures. The enzyme preparations showed low levels of activity and inconsistent results. Therefore, no further PPO characterization was conducted.

The presence of PPO, however, was confirmed through qualitative tests. Unblanched strawberries (Hood and Tioga varieties) treated with catechol experienced a greater degree of browning than blanched fruit. Tioga variety generally had more browning than Hood, thereby indicating greater enzymic activity. Green fruit also appeared to sustain more browning than ripe fruit.

Blanching had a significant effect on the pigment level and on color changes in strawberry juice and concentrate which were made from frozen strawberries. Chemical analyses showed a progressive decrease of anthocyanin, total phenolics, flavanols and leucoanthocyanins content during storage. This decrease was greater in the control samples than in the blanched samples. Monomeric anthocyanin pigments degraded

rapidly during the first two weeks of storage and had virtually disappeared by the end of the storage period. The low level of flavanols and leucoanthocyanins in the unblanched samples suggested that they could have been utilized as a PPO substrate. Color analyses revealed that there was a great increase in browning and percent polymeric color in all of the samples during storage. However, colorimetric measurements of "a" value showed that blanched samples retained a better red color appearance. The significant influence of blanching treatment was confirmed by the statistical analyses. Nitrogen treated samples also had a higher level of ACN pigment, flavanols and leucoanthocyanins during storage than the non-nitrogen treated samples. A greater increase in browning and percent polymeric color was found in the nitrogen treated samples, but the Hunter "a" value indicated that these samples exhibited a better red color than the air-exposed samples. Statistical analyses showed that there was a significant effect because of the nitrogen treatment, which implied that nitrogen sparging could be beneficial for color retention.

The summation of the experimental results of this research suggests that polyphenol oxidase does exist in the strawberry. Blanched and nitrogen treated strawberry juice and concentrate had a higher level of anthocyanin pigments, total phenolics, flavanol, leucoanthocyanin and showed a redder visual appearance than the nontreated samples.

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