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Title Changes in Raspberry Pigments During Processing and Storage

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The retention of pigments in processed raspberries is a function of the process method and conditions of subsequent storage. The presence of sucrose and the nature of the headspace gas also influences the retention of the pigment. The effect of some of the above variables on the pigments was investigated. Variables imposed were: (1) concentration of sirup, (2) headspace gas atmosphere, (3) temperature, and (4) time of storage. Separation of the pigment of Willamette red raspberries and black raspberries was made by column chromatography and the pigments were analyzed.

spectrophotometrically.

The results showed the following:

1. The pigments of Willamette red raspberries consisted of four separate cyanins as based on the R_f values and characteristic wavelengths of maximum absorption. The pigments of black raspberries consisted of three fractions.

2. On the basis of R_f values and wavelengths of maximum absorption only two of the constituent fractions were the same.

3. The wavelength of maximum absorption of all the anthocyanins shifted towards shorter wavelengths after the first month of storage.

4. Time and temperature of storage and their interaction each contributed significantly to the destruction of the cyanin pigments. An increase in either variable resulted in a greater destruction of the pigments.

5. As the concentration of the ingoing sirup was increased, the amount of individual cyanins decreased.

6. Oxygen in the headspace gas was shown to be detrimental to the retention of the cyanins I, II, and IV of the Willamette raspberries and to cyanins II and III of the black raspberries.

7. Changes in the total pigment concentration were greatly influenced by the cyanin in greatest concentration in the species.

8. Heat processing destroyed approximately 20 percent of the pigments of the raspberries.

CHANGES IN RASPBERRY PIGMENTS DURING PROCESSING AND STORAGE

by

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CHANGES IN RASPBERRY PIGMENTS DURING PROCESSING AND STORAGE

INTRODUCTION

Color is an important attribute of the term "quality" in fresh and processed fruits and vegetables. Fresh and processed products must have a pleasing color before they are accepted by the consumer. It follows that prevention of undesirable changes in the color of these products during processing, storage and distribution becomes of importance to the food technologist. The chemistry of the natural or synthetic pigments present in or added to foods assumes paramount importance in solving some of the problems associated with loss of color.

The main classes of natural pigments in fruits and vegetables are: (1) carotenoids, (2) chlorophylls, (3) anthoxanthins and (4) anthocyanins. The latter group, the subject of this research, is the principle coloring material in raspberries, strawberries and other red or pink colored fruits. Maintenance of color in processed pigmented fruit is of importance because of the pronounced instability of the anthocyanins due to such factors as:

(1) pH, radiant energy (light), bacteria and

enzymes;

(2) presence of other food ingredients such as ascorbic acid, sugars, and metallic ions.

(3) temperature and time of processing and storage; and

(4) variations in methods of preservation, i.e. canning, freezing, dehydration and other methods of preservation.

Degradation of the pigments cannot be said to be entirely due to one single factor. In fact, the degree of anthocyanin degradation is a function of the variables mentioned above and of their interaction.

In an attempt to clarify some of the reasons for loss or change in the anthocyanins, two types of raspberries, i.e. red and black were canned, their pigments subsequently separated by column chromatography and analyzed to determine the effect of:

(1) level of added sucrose,

(2) type of headspace gas, and

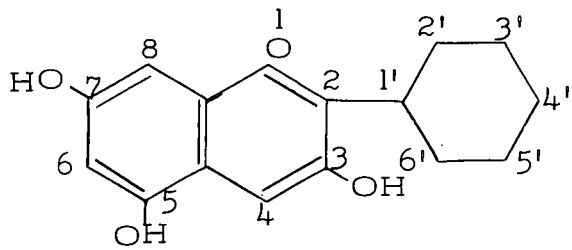
(3) time and temperature of storage

on the retention or loss of the individual anthocyanin and of the collective natural pigmentation of the fruit.

LITERATURE REVIEW

Nature of the pigment

The flavonoid pigments present in raspberries and other berries are anthocyanins, which are glucosides of anthocyanidins in the three and/or five position:



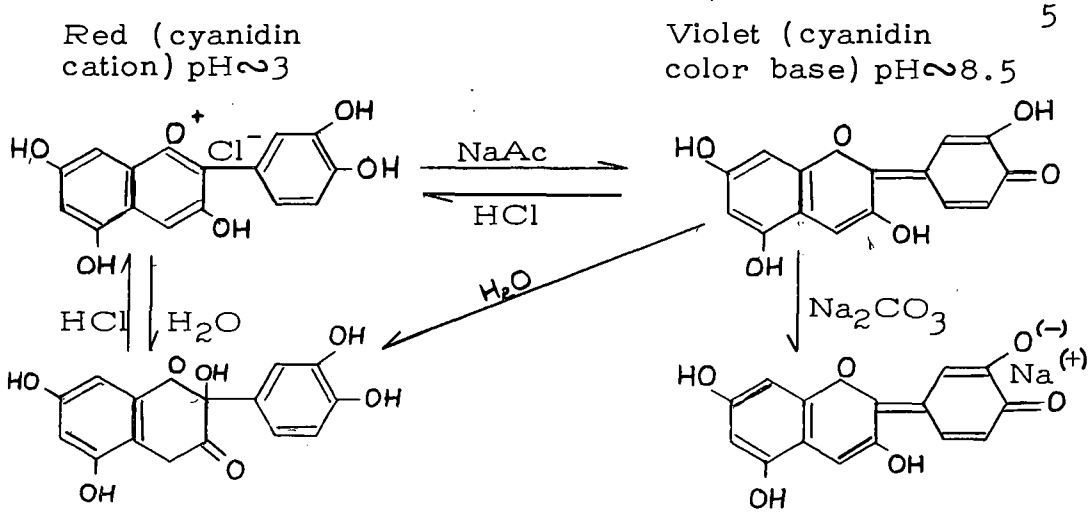
(71, p. 648; 73, p. 2476; 64, p. 1650-1664; 46, p. 790; 40, p. 153-157; 64, p. 1687-1705). Willstäter and Bolton (82, p. 113-136) first reported the existence of one glucoside of cyanin in raspberries which they termed a cyanidin bioside. Harib, et al. (25, p. 482) showed that the red pigment of raspberries was composed of four components. They used talc-silicon earth columns (3:1 v/v) and two percent acetone solution as solvents for chromatographic separation, and reported the corresponding Rf values of the four components. Lamort (40, p. 153) reported that the anthocyanin pigments in raspberries (Newberg variety) were cyanins, separated them into four fractions by paper chromatography, and

measured the Rf values and wavelength of maximum absorbancy.

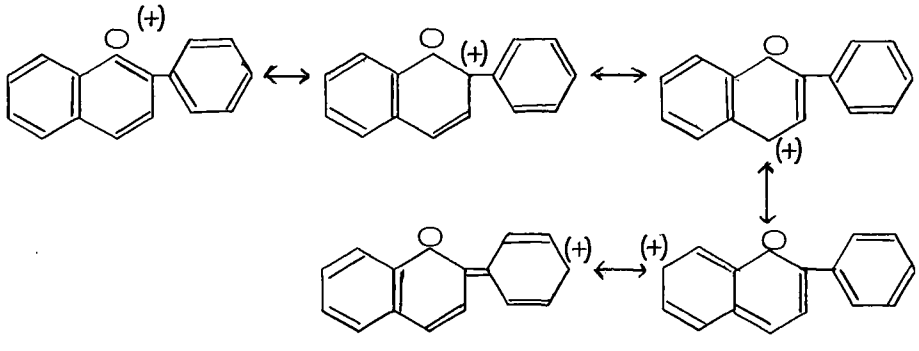
The above authors did not completely identify the pigments on a physical and chemical basis.

Suomalainen and Erriksson (77) found for fruits grown in northern latitudes that the anthocyanins of the berries of the bramble were localized in the peel. The number of the anthocyanins varied with the type of fruit but in the same type of fruit, the number also varied with the ripeness.

Generally, anthocyanins are amphoteric substances and form oxonium salts with either mineral or organic acids (33, p. 562; 20, p. 1325). They are crystalline substances soluble in water but insoluble in non-hydrophilic solvents such as benzene, ether, etc. The form in which an anthocyanin or a anthocyanidin exists, because of its amphoteric character, is a function of the pH. For cyanin the following equilibrium exists at different levels of hydrogen ion concentration (20, p. 1325; 67, p. 861).



The colorless pseudobase can not exist in the case of cyanin because the hydroxyl group, in the 3-position, is blocked. The vivid reds and blues of anthocyanin are associated with the distribution of positive charges throughout an aryl substituted chroman ring system (11, p. 519).



In the case of strawberry juice, pH plays an important role in determining the form of the anthocyanin. In neutral

solutions or solutions close to neutrality, it is believed that the pigment exists in the free state: ROH. Acidification can shift the equilibrium toward the colored benzopyrylium salt R^+ (7, p. 358; 70, p. 1507). This molecule has high resonance and therefore exhibits greater light absorption and bathychromy. The equilibrium may be represented as:

$$R^+ + 2H_2O \rightleftharpoons ROH + H_3^+O$$

The pK of this reaction may be calculated by the Henderson-Hasselbalch equation: $pK = pH + \log \frac{(salt)}{(base)}$, (81, p. 116; 80, p. 101).

Determination of the anthocyanin pigment

Sondheimer and Kertesz (72, p. 245) developed a method for the determination of anthocyanins in strawberries. The amount of the red anthocyanin pigment was determined by measuring the optical density at 500 mμ of extracts at pH 2.0 and 3.4. The O.D. at pH 3.4 was subtracted from the O.D. at pH 2.0 and the difference was proportional to the concentration of the anthocyanin in the solution (65, p. 246). A solution of Congo-red was used as the standard of color intensity. As previously discussed, the pH of the solution

affects the absorbance of the solution (66, p. 7). A decrease in pH in the acid region causes an increase in the intensity of absorption in the visible wavelengths, because of the change in equilibrium. Therefore by measuring the difference in O.D. at two standard pH levels, the difficulty is overcome.

Alcohols affect the wavelength of maximum absorbancy of anthocyanin solutions by shifting it to higher wavelengths. Alcohol solutions are to be avoided in such spectrophotometric measurements (72, p. 248).

Absorption of the pigment

Lamort (41, p. 70-72) showed that there were four main anthocyanin pigments in Newberg raspberries which are cyanins. The absorption peaks were found at 504, 510, and 514 mμ with Rf values of 0.43, 0.32, 0.26, and 0.22 respectively.

The absorption peaks for the corresponding cyanidins were shifted to longer wavelengths as compared to the cyanins.

Gizis (21) reported that the absorption peak of the raspberry pigment was observed at 510 mμ.

Guardani and Nimmo (22) also reported that the

absorption peak for pigments extracted from frozen raspberries occurred at 513 mμ.

Extraction of the pigment

In the chromatographic analysis of the anthocyanins of plant pigment tissues, one is concerned with selecting the proper solvent to extract all of the anthocyanins from the tissue without interfering substances or modification of the structural characteristics of the molecule.

Willstäter and his co-workers early in the century in a series of reports on anthocyanins used a dilute solution of hydrochloric acid (one percent) aqueous or methanolic, as the solvent for the extraction of anthocyanin and ten percent HCl or higher for hydrolysis of the pigment into anthocyanidin and sugar (82, 83).

Karrer, et al. also in their extended research on the anthocyanins used dilute HCl-MeOH solutions (34, p. 729-759; 35, 36).

Later workers (63, 24, 68, 45, 15, 41) have standardized the extraction solvent as one percent HCl:MeOH solution. Harbonne (24) emphasized that this solvent system was the best because of the extraction properties and ease of

concentration.

Chromatographic analysis

Column and paper chromatography have been used for the separation and identification of anthocyanin pigments separately or in combination. There are certain advantages and disadvantages according to the method used which will be discussed later (24). First, the two main methods of chromatographic analysis for anthocyanins will be reviewed.

Column chromatography. Paul Karrer first successfully applied column chromatography for the separation and identification of the anthocyanins of plant origin (35, p. 1025-1027). He used columns packed with specially prepared gypsum of specific particle size and separated althaein into enin and the monoglucoside of delphinidin 3'-Me-ether. In another work, he used a column of activated Al_2O_3 for analysis of peonin-chloride with water as the solvent and dilute HCl acid solutions for elution. The cyanin-chloride bands were purple (35, p. 25-28).

In 1950 Spaeth, et al. (76, p. 1321-1326) reported a separation of synthetic anthocyanidin mixtures. They used

columns of silicic acid with ten percent phosphoric acid as the stationary phase and a mixture of phenol-toluene as the non-aqueous phase. This method was suggested for the quantitative separation and analysis of small amounts of synthetic anthocyanidins. Ice, et al. (28, p. 1616) and Pearl and Dickey (59, p. 863-869) also reported on the use of "Magnesol" columns for separation of flavonol pigments.

In 1954, Parkinson used column chromatography to separate the anthocyanins of Victoria plums with cellulose powder (58, p. 239). He found that one of the pigments was chrysanthemin (cyanidin-3-glucoside) from the observed R_f values of the isolated and synthetic pigments, which were between 0.25 to 0.30 in a Bu-OH: Ac-OH: H_2O (40:10:50 v/v) solvent.

Lukton, et al. (46, p. 790) reported on separation of strawberry pigments by column chromatography. A cellulose column was used with Bu-OH: Ac-OH: H_2O-(40: 10:50 v/v) as the solvent. It was found that the secondary pigment was cyanidin-3-monoglucoside with an R_f value of 0.32 for both the isolated and synthesized cyanidin-3-monoglucoside. A modification of the Spaeth Method was applied by Li, et al. (45, p. 978) using as a solvent n-Bu-OH:

Ac-OH: H_2O (4:1:5 v/v) and 3.5 x 54 cm columns packed with 250 g. of silicic acid. A repetition of the chromatography was recommended. Endo (14, p. 378-379) used column chromatography with different kinds of cellulose powder in 4 x 35 cm tubes to separate anthocyanins from Viola tricolor maxima. He used solvents that were a mixture of n-Bu-OH: 36% HCl: H_2O (5:1:4 v/v).

Lamort (40) also reported the use of column chromatography to quantitatively separate raspberry pigments. Cellulose powder, Whatman standard grade, and n-Bu-OH: Ac-OH: H_2O (4:1:2.5 v/v) were used in 5 x 65 cm columns. Another use of the absorption medium, Al_2O_3, with one percent HCl:MeOH as the solvent for separation of the anthocyanins of Rubus arcticus (bramble) was reported by Kerainen. A further purification of the anthocyanin was obtained with the solvent system H_2O: Ac-OH: HCl (82:15:3) in columns packed with cellulose powder (37, p. 46).

A suggestion for a new type of absorption medium was made by Chandler and Swain (10, p. 989) for separation of glycosides from aglycons in mixtures. The medium used was nylon powder in combination with a mixture of Bu-OH: 2N HCl (1:6 v/v) as the solvent. According to the authors

the separation was due to the greater solubility of the glycosides in H_2O as compared to the aglycon and to the strong absorptive capacity of nylon for the H-bonding phenolic groups. According to the authors this technique was thought to be a superior method for the separation of anthocyanins from the leuco-anthocyanins. Sakamura and Francis (68, p. 318-321) reported on the separation of anthocyanins of American cranberry. The columns (5 x 40 cm) were packed with 350 g. silicic acid and 193 ml H_2O. The solvent was the upper phase of n-Bu-OH: Ac-OH: H_2O (4:1:2 v/v). The disadvantage of this method was the long time (7 days) required for development of the column.

Garber, Redding and Chorney (18, p. 801-802) published a modification of the cellulose powder procedure using as the solvent the upper phase of Bu-OH: Ac-OH: H_2O (5:1:4 v/v). They considered this method superior to the others in both separating capacity and time required. According to these authors, other procedures which have been previously discussed did not give sharp separation.

Paper chromatography. All of the methods used for the separation of anthocyanins by paper chromatography are

basically modifications of the method introduced by Bate-Smith and Westall (4, p. 421). The primary differences in the methods are due to the solvent system used. Their method can be used for either anthocyanins or anthocyanidins. The solvent systems used are: (1) the classical n-Bu-OH: Ac-OH: H_2O (40:10:50 v/v) herein after referred to as (BAW) and (2) m-cresol: Ac-OH: H_2O (50:2:48 v/v). Both solvents gave good results. Natural and synthesized anthocyanins have been chromatographed and identified by the R_f values using BAW.

Fuassin, et al. (15, p. 173-192), Forsyth, et al. (15, p. 511-515), Garber, et al. (17, p. 240-243), Abe, et al. (1, p. 577), Jiraček, et al. (30, p. 659) have used this method or slight modifications there-of for separation and identification of the natural or synthetic anthocyanins.

Lamort (40, p. 153) reported on the separation of raspberry pigment using Whatman No. 1 paper and BAW as the solvent. He used citrate buffer for elution.

Anthocyanins have been separated by the use of circular paper chromatography (6, p. 844).

Geissman (19) reviewed the chemistry, physiological importance, purification, identification, chromatography and

elucidation of structure of anthocyanins and flavonoids. Useful tables with R_f values, for different solvents, were cited for reference purposes.

Harbonne (24) reviewed the chromatographic methods used up to 1959 in his excellent paper. He concluded that paper chromatography was generally superior to column chromatography when concerned with (a) accuracy of determination, (b) identification of small amounts of pigments and (c) ease of handling and economy of materials. Column chromatography was recommended for separation of large amounts of pigments. He summarized the principal solvent systems used and gave R_f values for a number of known anthocyanins with different solvent systems. He proceeded further and made generalizations correlating the R_f values measured and the structure of anthocyanins. The correlations given may be applied for either column or paper chromatography and are:

(a) Hydroxylation: The greater the number of hydroxyl groups in the anthocyanidin molecule the lower the R_f value in both alcoholic and aqueous solvents.

(b) Methylation: Reverses the effect of hydroxylation.

(c) Glycosidation: There is a direct relationship between R_f value and number of sugar residues which is quite independent from the nature of the anthocyanin. In aqueous solvents an increase of R_f values is observed and in alcoholic solvents, the reverse.

(d) Acylation: Increases the R_f values in solvents based on n-Bu-OH but lowers the R_f values in aqueous solvents.

Electrophoresis. Markakis (52, p. 1092) approached the separation of anthocyanins by using zone electrophoresis. Anthocyanins from sour cherries, strawberries, and other fruits when placed on paper strips migrated towards the cathode at pH values less than 6 and towards the anode at pH values greater than 7 by application of 7-8 Volts per cm for 5-10 hours.

Degradation of the pigment

Influence of storage condition. Pederson, et al. (60, p. 82) in a study on the color of raspberry, strawberry and currant juices reported that the storage temperature was an important factor in the stability of the pigment of these juices. The

juices were pasteurized and contained approximately ten percent natural sugars. The retention of the color after four months in storage at 86°F was less than half of that at 33°F for the variety "Newberg" and less than two-thirds for the variety "Latham". Almost the same results were reported by Tessler, et al. (79, p. 98) for the color of raspberry juice. They showed that the color was greatly affected by the temperature and time of storage. Similar results were observed for strawberry juice.

Beattie, et al. (5, p. 395-404) showed that the rate of destruction of ascorbic acid and color in pasteurized strawberry, raspberry and currant juices, containing about ten percent natural sugars, was higher at higher pasteurization temperatures. The color change was extensive after three months of storage. The red and yellow color (Lovibond colorimeter) of juice stored at 32°C (89.8°F) was less than half as intense as the color of juice stored at 1°C (34°F) for two months.

Lee, et al. (42, p. 16) reported that the storage temperature was of extreme importance in color retention; the lower the temperature of storage the better the color retention in systems where anthocyanins are the primary

pigments. They showed that the color of the juice of black raspberries was more stable than that of red raspberries and the latter was more stable than the color of strawberry juice.

Pederson, et al. (61, p. 14,15) indicated a parallel lowering in intensity of the red color of apple-raspberry juice combinations and the ascorbic acid concentration. The higher the storage temperature and the longer the storage time, the greater the color reduction. In another experiment with red raspberry juice (61, p. 17) prepared from frozen fruit, the color of the juice was found to decrease in both red and yellow color (in Lovibond units) in three months of storage at room temperature.

Color analysis by the spectrophotometer showed that as the color intensity at 400 mμ increased, the principal juice color intensity decreased in the range 500-520 mμ, but not to the same extent. In the case of black raspberry juice stored at 45°C (113°F), the color intensity was decreased to one half that of the original after 22 weeks storage and of purple raspberry juice to 1/4 of the original in nine weeks when measured at 520 mμ (61, p. 24). Red raspberry juice, in less than 12 months storage under the same conditions,

showed a reduction in the color intensity to one-fourth that of the original juice.

Nebesky (57, p. 261-274) and his co-workers studied the stability of the color of fruit juices during storage. The juices contained 15 percent natural sugars and were pasteurized (190°F) and stored for 6 months at 100°, 80°, 70°, 35° and 0°F in darkness and at 70° and 80°F in the daylight. Severe changes were observed as time and temperature increased.

In 1952 a study on the effect of time and temperature of storage on canned strawberry jam was reported by Case (9, p. 16). The jams were stored at -20°, 70° and 100°F for 1,2,3,4,5 and 12 months. The color was measured by the method of Sondheimer and Kertesz (72, p. 245-248). The results showed that storage at -20°F resulted in only a slight decrease in the pigment during the first nine months of storage. Samples examined after 12 months of storage showed a noticeable reduction in the intensity of the color. Storage at 70°F for one month resulted in a loss of one-half of the initial pigment content; this loss was of the same level as storage at -20°F for 12 months. Storage at 100°F caused a loss of approximately two-thirds of the original pigment content

in one month, and three-fourths in six months. Storage at 70°F and 100°F for 12 months caused almost complete destruction of the pigment. The results generally followed the pattern for both strawberry and raspberry jams.

Decereau, et al. (13, p. 125) working on the effect of storage at 100°F upon strawberry jellies found 26 percent retention of the color after 19 days of storage 100 percent destruction after 6 months of storage at 100°F. The onset of browning resulted in a masking of the color differences, due to anthocyanin system.

Kertesz and Sondheimer (38, p. 106) also pointed out that strawberry preserves must be stored at temperatures lower than 60°F because the rate of destruction of the color increased as the storage temperature approached 65°F. They also concluded that the onset of browning due to non anthocyanin constituents for storage from two to eight months occurred at 80°F when the red color already had been partially or completely destroyed.

Kitson (39, p. 16) showed that the color of strawberry pie fillings, changed from bright red to dull brown, when stored at high temperatures. Upon storage at 40°F, a slight browning occurred in six months, while at

70°F the same change occurred in one month and at 100°F a completely unacceptable dark brown color was produced in one month. A shift of the color maxima to shorter wavelengths (hypsochromic effect) was also observed.

In 1957 Guadagni and Nimmo (22, p. 653) reported that the absorption peak for pigments extracted from frozen raspberries occurred at 513 mμ. The effect of storage also was studied. Storage at 30°F for two weeks did not shift the wavelength of maximum absorption in fresh frozen raspberries. A pigment transfer occurred from the berry flesh to the sirup. The magnitude of the transfer varied with the original pigment concentration in the berries, the temperature, and time of storage. After one year of storage at 0°F only a slight increase in the color of the sirup was observed.

The authors stated that the color change was the direct result of the sum of all the time-temperature experiences which the product encountered due to mishandling during storage. The same authors in later work showed (23, p. 306) that the color changes in frozen raspberries were proportional to the storage temperatures.

In 1962, Gizis, (21, p. 51-54) reported that the wavelength of maximum absorption in liquid taken from canned

raspberries was 510 mμ. An increase in the intensity of the color of the sirup (60° Brix) was found during the first 14 days storage at 100°F. After the 14th day a definite decrease in the color intensity was obtained. No significant differences were observed in the color intensity of the sirup after 28 days storage at 100°F as compared to the intensity of the sirup after processing.

Lamort (40, p. 153-157) studied the kinetics of the degradation of anthocyanin pigments of raspberry (Newberg) and reported that it followed the rate of a first order reaction. He wrote that the reported K values were not absolutely correct because of the difficulty in the separation of the pigment. In a later (41, p. 70) work the same author showed that the degradation of the corresponding cyanidins of raspberries at 22°C and pH 1 did not follow the first order reaction. In the case of the pelargonidin, he found a very close approximation to a first order reaction.

Markakis, et al. (51, p. 118) reported on the kinetics of the main anthocyanin of raspberries, pelargonidin-3-monoglucoside, in buffer solution at pH 3.4 and pH 2.0. First order reaction rates were observed for a range of temperatures from 45°C (113°F) to 110°C (230°F) and a

straight line relationship for the thermal destruction curve was obtained.

Sugar . Meschter (55, p. 575) investigated the effect of different sugars on anthocyanin pigments of strawberry products stored at 38°C. Sugars used in this work were: (a) fructose, arabinose, and sorbose and (b) maltose and sorbitol. The first group which included the more heat-labile sugars showed a higher rate of pigment degradation than the second group which included the more stable sugars. Typical degradation products of sugars such a 5-hydroxy-methyl-furfural and furfural tended to increase the rate of degradation of the anthocyanin pigment. Later work on the effect of sugars on the anthocyanin pigments in model systems pointed out that fructose is more active than sucrose (49, p. 396). An interaction was also shown between amino acids and sugars.

Decereau, Livingston and Fellers (13, p. 126) determined the breakdown of the pigment in strawberry jellies, in the presence of different sugars. A replacement of sucrose by fructose resulted in an increase of the half-life of the pigment in the jelly.

Aref (2, p. 293-297), et al. found a significant change in the color of frozen strawberries due to types or levels of sugars in terms of the "L" value of the Hunter color and color difference meter. Minor changes in the dominant wavelength were also observed.

Later Tinsley (78, p. 2) showed that gluconic acid and sorbitol did not have a noticeable effect on the rate of pigment degradation of the anthocyanin pigment of strawberries in model systems at pH 3.4 and a temperature of 90°C. However, fructose and glucuronic acid had a marked effect in increasing the rate of degradation. The rate of pigment degradation was paralleled, by an increase in the concentration of fructose and glucuronic acid. In the presence of furfural and 5-hydroxymethylfurfural, a rapid rate of pigment degradation was observed. The degradation products of 5-hydroxymethylfurfural, levulinic and formic acids, produced only a slight increase in the rate of degradation of the pigment (78, p. 54). Amino acids, such as arginine and cystine at concentrations comparable to that found in strawberry juice, also resulted in increased rate of degradation in strawberry juice when in glucose and fructose model systems (78, p. 25-27).

Meschter reported (54, p. 574) that pH is an

important factor governing the stability of strawberry pigments and that the maximum retention occurred at pH 1.8. Below this value the stability was lower because a reaction took place between sugar degradation products and pigment.

Tinsley (78, p. 30) reached the same conclusion and reported that the pseudobase form of the anthocyanin is more active in the degradation reaction.

Effect of headspace gases. Pederson (60, p. 75) observed that deaeration was essential for the retention of pigments in raspberry juice. Changes in color during storage at room temperature were mostly caused by small quantities of air. Air also effected ascorbic acid destruction and color degradation of raspberry and strawberry juices (5, p. 495-503).

Nebesky, et al. (57, p. 261-274) reached the same conclusion working with raspberry juice. Headspace played an important role in the color retention; deaerated samples had a higher color retention as compared with five samples having air in the headspace. The kind of gas in the headspace influenced the pigment stability of canned raspberries (25, p. 482). When oxygen was included in the headspace, an increase in the rate of pigment degradation was observed.

Strawberries and raspberries showed similar patterns of degradation insofar as the effect of oxygen in the headspace was concerned. Markakis, et al. (51, p. 121) in a study of the interaction between oxygen and ascorbic acid with the pigment, reported that when air was replaced by nitrogen less pigment degradation occurred. Strawberry juice packed under air retained only 3% of the original pigment after storage of 50°C for 160 hours, while juice under nitrogen retained 30 percent of the pigment under the same conditions.

Lukton, et al. (47, p. 427) suggested, from their experimental data on strawberry juice packed with air in the headspace, that the insoluble red-brown precipitate and the soluble brownish substance which formed resulted from the oxidation of the strawberry pigment. First a conversion of the pseudobase occurred followed by its hydrolysis and the development of the red-brown precipitate. Strawberry juice packed under nitrogen had only a slight precipitate formed. Much earlier, in 1953, another approach to the pathway of pigment degradation in strawberry juice was reported (75, p. 478). The detrimental effect of oxygen was said to be due mostly to the oxidation of ascorbic acid rather than the direct

oxidation of the pigment giving rise to the insoluble precipitate.

In the presence of nitrogen rather than of oxygen, a marked decrease of the rate of the pigment degradation was observed in model system for pelargonidin-3-monoglucoside by Tinsley (78, p. 25). Comparison between nitrogen and oxygen as headspace gases showed that nitrogen gave good protection (41, p. 70-72).

pH . Nebesky, et al. (57, p. 271) reported that pH played a minor role in the stability of the pigment in raspberry juices. The pH of the medium in strawberries affected the rate of degradation as reported by Lukton, et al. (47, p. 428). The lower the pH the higher the degree of pigment stability. In the high-acid region, the pigment exists in the cationic R^+ form and is dependent upon the pH value of the medium. The rate constant of pigment degradation is a function of the R^+ form concentration. The higher the concentration of the R^+ form the greater the degree of pigment stability.

Harib, et al. (25, p. 482) observed that the pH of the medium affected the rate of pigment degradation in processed canned raspberries. Decreasing the pH value of the medium resulted in an increase in the retention of the pigment.

A significant difference in color retention was shown between canned berries at pH 3.5 and those canned at pH 7.5 and 8.5.

Meschter (54, p. 576), on the other hand, correlated the effect of pH on pigment stability in strawberries with the sugar content of the sirup. Maximum stability of the pigment occurred at pH 1.8.

Ascorbic acid. Ascorbic acid is an important factor influencing the rate of degradation of anthocyanins. Nebesky (57, p. 261-274) reported that ascorbic acid accelerated the degradation of the color in raspberry, strawberry and currant juices. Pederson, et al. (61, p. 1) reported that the loss of ascorbic acid and loss of red color were related and occurred simultaneously in strawberry juice.

Sondheimer and Kertesz (75, p. 476) showed that ascorbic acid possessed an accelerating property on the rate of pigment breakdown. An assumption was made for the mechanism of this breakdown. They suggested that the oxidative degradation of ascorbic acid resulted in the formation of hydrogen peroxide which was responsible for the degradation of the pigment of strawberry products. The proposed scheme

for that oxidation was (74, p. 288):

$$H_2O_2 + Fe^{++} \longrightarrow HO^{\cdot} + HO^{-} + Fe^{+++}$$

$$OH^{\cdot} + Fe^{++} \longrightarrow HO^{-} + Fe^{+++}$$

$$Fe^{+++} + \text{ascorbic acid} \longrightarrow Fe^{++} + \text{dehydro-ascorbic acid}$$

$$HO^{\cdot} + \text{anthocyanin} \longrightarrow ? \longrightarrow \text{oxidative product}$$

Markakis, et al. (51, p. 121) reached the same conclusions in respect to the effect of ascorbic acid. With a nitrogen headspace, 50 moles of ascorbic acid were involved. in the oxidation of 1 mole of pigment.

Processing methods. Joslyn (31, p. 308) reported that changes in the pigment of fruits occurred during processing due to decomposition reactions or other chemical interreaction among the different constituents in the can. Pederson, et al. (60, p. 78-83) pasteurized strawberry and raspberry juices at 180°F and compared the degree of color and flavor of the juice with those pasteurized at 190° and 200°F. They reported that the degradation of the pigment was more pronounced than the change in the flavor of the juice as the pasteurization temperature was increased.

Mackinney also (49, p. 324) reported that when

oxygen was introduced during the processing of strawberry preserves, a breakdown of the pigment occurred.

Markakis, et al. (51, p. 117) concluded in the study of the breakdown of strawberry pigments that care must be taken during processing to use a combination of time and temperature which results in the least loss of the pigments. A short-time-high-temperature treatment was found to be superior to heating at relatively lower temperatures but for a longer time. Excessive heating resulted in a fading of the color and an increase of its absorption in the region of 400 - 460 mμ in strawberry and boysenberry juices (62, p. 271-478).

Other factors. Colloids have been used to improve the quality of frozen raspberries in two respects: (1) the increase of drained weight and (2) the retention of color (43, p. 31). Pectins and gums of plant origin have been used to the greatest extent.

Barton (3, p. 30) reported an improvement in the retention of color in both red and purple canned raspberries by dissolving colloidal agents such as gum tragacanth, 150 grade high-methoxyl pectin, 320 grade low-methoxyl pectin in

50 percent sirup which was added to the can.

Heavy metal cations are also known to form complexes. Stannous salts, for instance, have been shown to change the tint of the anthocyanins (31, p. 308; 12, p. 107).

Browning reaction. Lukton (47, p. 429) reported that the degradation products in canned strawberries were a red-brown insoluble precipitate and a soluble brown pigment. The latter absorbed at 440 mμ in comparison to the absorbance of anthocyanin pigment which absorbed at 500 mμ (47, p. 427; 81, p. 626). In the same work (47, p. 429) it was observed that the ratio of absorption at 440 mμ to that of 500 mμ tended to increase with the time of storage and approached the limit of 2.3 when the anthocyanin pigment was completely broken down. The mechanism of the pigment degradation was not reported.

In 1953, Hodge (27, p. 928) reviewed and classified the browning reactions:

(a) Amino-carbonyl reactions: In this category first, amines, amino acids, peptides and proteins, and second, aldehydes, ketones and reducing sugars are mainly involved in the reaction. This reaction (known as the Maillard type)

involves a low order of energy for initiation and proceeds autocatalytically.

(b) Caramelization: Polyhydroxycarbonyl compounds such as sugars and their derivative acids are involved. Caramelization requires a high order of activation energy expressed as heat. Neither one of the above types of browning necessarily involves oxygen.

(c) Oxidative browning: Involved is the conversion of ascorbic acid and polyphenols into di- or poly-carbonyl compounds.

The two first mechanisms of browning do not involve enzymatic action (49, p. 302). In canned strawberries two possible mechanisms exist for the rise of the red-brown insoluble substances. The first (8, p. 1573) is characterized by the conversion or polymerization of the pseudobase form of the pigment to the red-brown substance. The second is characterized by a hydrolysis of the pseudobase form of the aglycon and simultaneously to the red-brown substances.

There is evidence that first a colorless degradation product is formed which in turn gives rise to a brown soluble pigment which then changes to the red-brown precipitate

(69, p. 517; 26, p. 667; 47, p. 592). In model systems with anthocyanidin, discoloration was not followed by formation of the red-brown precipitate. Therefore, it was concluded that the formation of the precipitate was due to either the pseudobase of the anthocyanin or to the sugar part, or to both.

MATERIALS AND METHODS

The purpose of this experiment was to study the effect of time and temperature of storage on the anthocyanin pigments of Willamette red raspberries and black raspberries. The effect of added levels of sucrose and of the nature of the headspace were also investigated.

Raw Materials

The raspberries were obtained from the Lewis-Brown farm of the Department of Horticulture. After harvest they were held in storage at 34°F until processed.

Processing

The berries were washed in a small McLaughlan washer, sorted and inspected (to remove immature fruit and extraneous material) on a vibrating table and then caught and drained on 8 x 8 mesh stainless steel wire trays.

The drained berries were weighed (275 g) into 303 x 406 fruit enameled cans and 170 g of the packing media were added.

The headspace gas adjustment was accomplished by:

(1) Atmospheric headspace: The cans were filled with berries and sirup (50° Brix) at 170°F in the proper proportion and closed.

(2) Vacuum headspace: The cans were filled with the proper amount of berries and evacuated for two minutes at 25 inches of vacuum. Then the cans were filled with 50° Brix sirup under vacuum and weighed. The vacuum was re-applied for one minute and the can was closed under 25" vacuum.

(3) Nitrogen headspace: The closing machine was connected with a nitrogen tank and a sirup container. A vacuum of 25 inches was applied to the filled can for two minutes and the vacuum broken by nitrogen. This was repeated twice in order to replace the air from the can with nitrogen. Then the can was filled with 50° Brix sirup under vacuum, the vacuum broken, and the can weighed. The siruped, weighed can was then gradually evacuated to 25 inches, the vacuum broken with nitrogen and the can closed. Care was taken in all operations because the berries were raised in the can and the sirup bubbled when the vacuum was applied. Regulation of the operations were made by observing the contents of the can through a glass window in the chamber.

Heat Processing

The sealed cans were cooked in a water bath for ten minutes at 212°F (29, p. 537-539) then they were water cooled and stored at three different temperatures: 34°F, 70°F and 100°F for 30, 60, and 90 days.

After cooling, cans were selected for the initial (0 time) analyses.

Extraction of the Pigment

The cans were opened and the contents were ground in a blender for three minutes. One portion of 12.5 g from the homogeneous mixture was immediately taken and mixed thoroughly with 15 ml one percent HCl: MeOH solvent. The mixture was left at room temperature for 12 hours and then filtered under slight vacuum. The slightly red-colored residue was mixed with a second portion of 15 ml of the solvent and left to extract for two additional hours and then filtered. The filtrates and the wash-solutions were combined and made to 50 ml volume. The second filter-cake was free of pigment, indicating that the pigment had been completely extracted as the hydrochloric acid salt. Care was taken in

stirring the extraction mixture to avoid the gelation of the mixture due to the pectin content of the raspberry. The gel was extremely difficult to extract and filter. In the case of black raspberries 4.167 g were taken as a sample because of the higher concentration of the pigment in this fruit.

Column Chromatography

Column chromatography was applied in this experimental work to quantitatively separate the pigments of the raspberry. The columns used were 3 x 60 cm and were carefully packed, so as to insure uniform density with the dry cellulose powder (Whatman standard grade). The columns were exhaustively washed first with H_2O and then with the upper phase of BAW (5:1:4 v/v) and then the columns were irrigated with methanol and dried by drawing warm air through them. Seven grams of cellulose powder, so treated, were obtained from the upper part of the column. Five mls of the Me-OH: HCl extract of the anthocyanins of the raspberries were absorbed on three grams of the above cellulose powder and dried under vacuum at room temperature (18, p. 801). The dried cellulose was carefully placed on the top of the column to form a uniform layer and then the evaporation

dish was washed with two, one gram portions with the above cellulose powder. Finally two grams of cellulose were placed on the top of the column and the column was packed with washed and dried glass wool so as to retain the cellulose column when the tube was inverted.

The column was then inverted into a beaker containing Bu-OH: Ac-OH: H_2O (5:1:4 v/v) solvent and slight suction was applied to the orifice in order to permit the solvent to rise gradually in the column through the glass-wool without causing air bubbles. After the contact of the solvent with the cellulose powder, the suction was disconnected and the solvent was allowed to ascend by adsorption forces. The development was complete after 18-20 hours and the column was eluted through the orifice. The same solvent was used for elution as for development.

By this method four well defined bands of anthocyanins were obtained with the Willamette raspberries. The column could be used repeatedly after an appropriate washing with all solvents used and drying of the column. It was impossible to apply this method to Black raspberries because the separation was incomplete. Several of the normally used solvents were applied but without success. The best separation

on the columns was obtained by a H_2O: Ac-OH (85:15 v/v) solvent which had been applied by Luh in paper chromatography (p.c.). The solvent gave three very well defined bands in the column and was used for all analyses of the black raspberries. The fractions were held at -18°F until needed for further analysis. R_f values were measured on the column.

Concentration of the Pigment

The fractions of red raspberries in the BAW mixtures were freed from n-Butanol first by a selective extraction with petroleum ether in a separatory funnel. In this separation, Butanol was extracted from the aqueous lower phase which contained the pigment. Then the mixture was divided in two equal portions and carefully evaporated under vacuum until dry. The temperature was kept almost constant at 12°C (53°F) with an infra-red lamp (150 watts). The heating was fixed by the distance of lamp to the samples.

Absorption Spectra

The dried pigment was dissolved in an appropriate amount of buffer at pH 3.4 and pH 2.0. For the first (I),

second (II), and fourth (IV) bands of the Willamette raspberries and the first (I) band of the black raspberry, five ml of citrate buffer were used. For the rest of the bands, 15 ml of citrate buffer were used in order to obtain an appropriate dilution.

Sörensen buffer, pH 3.4, was prepared by mixing 0.1 N HCl and sodium citrate solution in the ratio 5.5:4.5 (v/v). Buffer pH 2.0 was prepared by mixing 0.1 N HCl and sodium citrate solution in a ratio 7:3 (v/v). The pH was adjusted with Beckman pH meter to the proper pH value.

Sodium citrate solution was made by dissolving 21.008 g of citric acid in 200 ml N NaOH and diluting to one liter.

The two buffered solutions of pigment were filtered through No. 4 Whatman filter paper and held for one hour at room temperature. Then the absorption spectra from 700 mμ to 400 mμ were obtained with a Beckman Model DK-1 Spectrophotometer using the buffer solution in the reference cell. The cells used were plastic Beckman cells of one cm width. The Beckman DK spectrophotometer was used to obtain the absorption spectra of samples immediately after processing (0 time) and to periodically compare the results as measured

by a Model B Beckman spectrophotometer. The latter instrument was used in most of this work. The results of both instruments were in agreement.

A reference curve also was prepared with Congo red according to the Sondheimer and Kertesz method (72, p. 245-248) in which 20 mg percent congo red in 0.01 N Na_2CO_3 were systematically diluted. The calibration curve was obtained by dilution of the above solution and plotting O. D. at 500 m against concentration. The O.D. at pH 3.4 was subtracted from the O.D. pH 2.0 and this net reading was converted to mg percent of congo red. These values multiplied by 1.2 gave the equivalent of the anthocyanin at that absorption.

Anthocyanin Tests

Color reactions were made for the fractions obtained in order to attempt a gross identification of the kind of anthocyanin (19, p. 464-466). The sodium acetate test and the color test with Na_2CO_3 and NaOH was used.

The corresponding anthocyanidins were formed by hydrolysis of the fractions with equal amounts of 12 N HCl and boiling for 30 seconds. After cooling of the solution, an

extraction was performed with amyl alcohol. Benzene was then added and the pigment was extracted with a small amount of one percent HCl solution. For cyanidin, a reddish violet color was observed with Ac-ONa which was converted to a blue color by $FeCl_3$.

Statistical Treatment

The experiments as previously designed each had three variables: (1) sucrose concentration, (2) time and (3) temperature of storage or (1) headspace atmosphere, (2) time and (3) temperature of storage.

The design was a factorial experiment with 3 x 4 x 3 treatments studied simultaneously. Analysis of variance was applied and the significance of the results was decided by comparison of the obtained F values with the table F at the 5 percent level.

The different treatments were compared to each other by using the L S D (.05) as suggested by Li (44).

RESULTS AND DISCUSSION

Identification of the pigments

Willamette raspberry. The anthocyanins are the main red pigments of the fruit of the genus Rubus. Four separate cyanin pigments are responsible for the total pigment complex of this genus (40, 41, 16).

Special tests for cyanins were made which gave positive evidence that all the fractions of both varieties were cyanins. The R_f values of the four cyanin bands of the Willamette variety were measured as well as the wavelength of maximum absorption and are reported in Table 1.

Table 1

Pigment fractions of fresh Willamette raspberries

Fraction	R_f	Wave length mμ	Pigment per 100 g fruit (mg)	Percent of the total Pigment
I	0.69	497.5	1.12	2.8
II	0.59	504.5	2.24	5.6
III	0.52	510 .0	35.10	88.6
IV	0.39	511 .0	2.00	4.9
Total	--	--	40.46	100.0

The R_f values could not be used for the identification of the four pigments since reference R_f values for column chromatography, using the BAW solvent and the cellulose medium, are not available.

The R_f value 0.32 (46, p.790) was reported for cyanidin-3-monoglucoside in a similar system which is close to the R_f of band IV. The wavelength of maximum absorption of band II corresponded to that reported by Lamort (40, p. 156) for the Newberg variety as cyanin "I", and band III corresponded to that reported as cyanin "II". The wavelengths for maximum absorption reported by Lamort for his bands "III" and "IV" were not observed in this work. This may be due to the technique since the methods used were not exactly similar. On the basis of Harborne's generalization and the observed R_f values one would expect that, since the anthocyanins are in alcoholic solution, an increase of the level of glycosidation of bands I to IV or a decrease in the degree of methylation or both occurs in the separate pigments.

The R_f values, reported in Table 1, are the mean values for the pigments as developed on the cellulose columns. The wavelengths refer to the pigment of fresh raspberry and to that of the pigment immediately after processing. A shift

of the wavelength of maximum absorption which occurred during storage will be discussed subsequently.

Black raspberries. The R_f values and wavelengths of maximum absorption of the pigment of black raspberries are shown in Table 2.

Table 2

Pigment fractions of fresh black raspberries

Fraction	R_f	Wave length mμ	Pigment per 100 g fruit (mg)	Percent of the total Pigment
I	0.65	515	103.5	23.2
II	0.52	511	315.1	71.0
III	0.40	510	26.6	5.8
Total	--	--	445.2	100.0

Since the solvent for the separation of the pigment was aqueous, the application of the Harborne generalizations would suggest that the pigment of band I had more sugar moieties than that of band III. On the basis of R_f values and the wavelengths, the two types of berries appeared to possess identical pigments in bands III and IV of the

Willamette raspberry and bands III and II respectively for the black raspberry. Band I in black raspberries had a wavelength of maximum absorption similar to that reported by Lamort (40, 41) for bands "III" and "IV" in the Newberg variety.

The R_f values obtained for the pigments of the black raspberry were in agreement with Harborne's generalizations. In this case, however, the level of glycosidation appears to decrease from band I to band III while the level of methylation appears to increase.

Only three pigments were obtained with black raspberries compared to four pigments with the Willamette red raspberries. This could be due to the presence of only three pigments or to incomplete separation of the bands.

Shift in wavelength of the pigment

A slight tendency for a shift of the absorption peak to shorter wavelengths irrespective of the source of the fractions was observed as the storage time increased. The slight shift which occurred after processing (hypsochromic effect) was pronounced after one month storage and increased with time. The maximum wavelengths were on the order of

504 to 514 mμ in the fresh berries and shifted to 494 to 498 mμ in the processed and stored fruit.

The effect of degree of sirup concentration, and of time and temperature of storage on the quantity of the anthocyanin pigments in canned Willamette raspberries.

Band I. The effect of the three main variables on the quantity of the pigment of band I of Willamette raspberries is shown in Table 3. Statistical analysis of the results is presented in Table 4. The mean values for variations in the main effects are presented in Table 5. It is readily apparent that an increase in concentration of the ingoing sirup, and an increase in the storage temperature and time lowers the retention of the first fraction (Band I).

The statistically significant interaction effect shown between time and temperature in band I, can possibly be explained by the fact that the rate of the destruction at 34° and 100°F appeared to be less than the rate at 70°F between 30 and 60 days of storage. Whether or not the significance is of any real consequence needs to be tested by more refined techniques.

This substantiates the research by Tinsley (78) who

showed that, in model systems, an increase in the concentration of the sugar lowered the amount of extractable anthocyanins in strawberries. The loss of the pigment was noted to be partially dependent upon the formation of degradation products. An increase in the concentration of the ingoing sirup in the presence of a low pH would be expected to result in more degradation products and thus high losses of the pigment.

Band II. The results showing the quantity of the pigment in band II of Willamette raspberries are shown in Table 6. When analyzed statistically the importance of sugar, storage temperature and storage time and the interaction of the latter two effects were shown to be significant at the five percent level.

The analysis revealed that there was a significant lowering of the amount of the band II pigment with an increase from 0° to 25° Brix. An increase from 25° to 50° Brix in the ingoing sirup did not significantly lower the amount of extractable pigment in band II (Table 7). It is to be noted that a significant interaction occurred between time and temperature of storage.

Table 3

Effects of sirup concentration, temperature, and time of storage on the quantity of band I pigment of Willamette raspberries.

Temperature of Storage (°F)	Sirup Concentration (°Brix)	Fresh Fruit	Storage Time (Days) 0	30	60	90
			(mg per 100 g fruit)			
34	0	1.12	1.04	1.04	1.04	0.96
	25	1.12	1.04	1.04	0.96	0.72
	50	1.12	1.04	0.88	0.80	0.50
70	0	1.12	1.04	0.88	0.80	0.72
	25	1.12	1.04	0.88	0.72	0.76
	50	1.12	1.04	0.80	0.64	0.40
100	0	1.12	1.04	0.80	0.48	0.23
	25	1.12	1.04	0.64	0.32	0.19
	50	1.12	1.04	0.32	0.30	0.18

Table 4

Statistical analysis of the main effects on the pigment of band I of Willamette raspberries.

Variation Due to	D. F.	F	Critical Region	Significance
Sugar	2 and 12	15.670	3.885	Yes
Temperature	2 and 12	74.430	3.885	Yes
Time	3 and 12	81.770	3.490	Yes
Sugar x Temperature	4 and 12	1.290	3.259	No
Sugar x Time	6 and 12	2.430	2.996	No
Time x Temperature	6 and 12	8.940	2.996	Yes

Table 5

Means of main effects on the band I pigment of Willamette raspberries.

Main Effect	Treatment	Mean (mg/100g)	Difference	Significance
Sugar (°Brix)	0	0.84		
	25	0.76	0.08	Yes
	50	0.66	0.10	Yes
		(LSD 0.05 = 0.066)		
Temperature (°F)	34	0.92		
	70	0.79	0.13	Yes
	100	0.54	0.25	Yes
		(LSD 0.05 = 0.066)		
Time (Days)	0	1.04	0.24	
	30	0.80	0.24	Yes
	60	0.67	0.13	Yes
	90	0.49	0.18	Yes
		(LSD 0.05 = 0.076)		

Table 6

Effect of sirup concentration, temperature, and time of storage on the quantity of band II pigment of Willamette raspberries.

Temperature (°F)	Sirup Concentration (°Brix)	Fresh Fruit	Storage Time (Days) 0	30	60	90
			(mg per 100 g fruit)			
34	0	2.24	1.92	1.84	1.68	1.60
	25	2.24	1.92	1.68	1.52	1.44
	50	2.24	1.92	1.68	1.60	1.36
70	0	2.24	1.92	1.60	1.60	1.20
	25	2.24	1.92	1.44	1.36	1.28
	50	2.24	1.92	1.44	1.20	0.96
100	0	2.24	1.92	1.60	1.44	0.72
	25	2.24	1.92	1.50	0.40	0.24
	50	2.24	1.92	1.20	0.34	0.24

Table 7

Means of main effects on the band II pigment of Willamette raspberries.

Main Effect	Treatment	Mean (mg/100g)	Difference	Significance
Sugar (°Brix)	0	1.58		
	25	1.38	0.20	Yes
	50	1.31	0.07	No
		(LSD 0.05 = 0.122)		
Temperature (°F)	34	1.68		
	70	1.49	0.19	Yes
	100	1.12	0.37	Yes
		(LSD 0.05 = 0.122)		
Time (Days)	0	1.92		
	30	1.56	0.36	Yes
	60	1.24	0.32	Yes
	90	1.00	0.24	Yes
		(LSD 0.05 = 0.140)		

Band III. The results showing the effect of the variables on the quantity of the band III pigment of Willamette raspberries, are shown in Table 8.

Table 8

Effect of sirup concentration, temperature, and time of storage on the pigment of band III of Willamette raspberries.

Temperature (°F)	Sirup Concentration (°Brix)	Fresh Fruit	Storage Time (Days) 0	30	60	90
			(mg per 100 g fruit)			
34	0	35.10	25.90	24.00	13.04	20.88
	25	35.10	25.90	23.50	23.40	21.60
	50	35.10	25.90	23.50	21.60	18.00
70	0	35.10	25.90	22.56	22.32	14.40
	25	35.10	25.90	16.80	12.48	12.00
	50	35.10	25.90	15.12	10.80	9.60
100	0	35.10	25.90	10.10	4.32	1.30
	25	35.10	25.90	9.36	3.12	1.20
	50	35.10	25.90	9.12	3.12	1.00

Table 9

Means of main effects on the band III pigment of Willamette raspberries.

Main Effect	Treatment	Mean (mg/100g)	Difference	Significance
Sugar (° Brix)	0	18.38		
	25	16.76	(No Significant Difference)	
	50	15.80		
Temperature (°F)	34	23.10		
	70	17.15	5.95	Yes
	100	10.03	7.12	Yes
		(LSD 0.05 = 3.903)		
Time (Days)	0	25.90		
	30	17.12	7.78	Yes
	60	13.80	3.32	No
	90	11.10	2.70	No
		(LSD 0.05 = 4.507)		

From the statistical analysis of band III data, the following conclusions were drawn. The significant effects of temperature, time and the time-temperature interaction were the same as with bands I and II. A variation in sirup concentration from 0° to 50° Brix did not significantly affect the degradation of the pigment. The non-significant effect of sirup in this case may be explained by the fact that the quantity of pigment of band III was approximately 20 times more concentrated than the pigment in bands I or II. Therefore, with the same degree of sugar degradation and formation of active products but with a pigment concentration of 20-fold, the effect of "X" amount of active products was not sufficient to result in a statistically significant lowering of the pigment concentration.

As shown in Table 9, a significant loss of pigment occurred during the first 30 days of storage. The degree of loss due to storage was not significant for each succeeding 30 day interval, but only for the period from 30 to 90 days.

Band IV. The results for band IV (Tables 10 and 11) were more in agreement with the results of bands I and II than with band III.

The statistical analysis of the results of this band showed that the influence due to sugars on the pigment degradation was significant. This supports the previous assumption for the action of the sugar since this band did not have a high concentration. Also the effect of temperature, time and the interaction of time and temperature with this band was the same as with bands I and II.

In respect to the effect of different concentration of sucrose the change was significant between 0° and 25° Brix and between 0° and 50° Brix but not between 25° and 50° Brix. This last result is not in agreement with the results obtained with bands I and II.

Change of total pigment. The total pigment in the Willamette red raspberry was found to be 40.46 mgs per 100 grams of fresh fruit. The pigment in the four bands was not equally distributed. The third band contained 88.6% of the total pigment. The concentration of each of the other bands was shown in Table 1. The effect of sugar upon the percent loss of pigment in each band and upon the total pigment is shown in Figure 1.

Table 10

Effect of sirup concentration, temperature, and time of storage on the pigment of band IV of Willamette raspberries.

Temperature (°F)	Sirup Concentration (°Brix)	Fresh Fruit	Storage Time (Days) 0	30	60	90
			(mg per 100 g of fruit)			
34	0	2.00	1.80	1.76	1.52	1.44
	25	2.00	1.80	1.68	1.52	1.28
	50	2.00	1.80	1.68	1.44	1.20
70	0	2.00	1.80	1.68	1.60	1.52
	25	2.00	1.80	1.44	1.12	1.12
	50	2.00	1.80	1.44	1.12	1.12
100	0	2.00	1.80	1.36	0.36	0.24
	25	2.00	1.80	0.96	0.64	0.18
	50	2.00	1.80	0.80	0.32	0.18

Table 11

Mean of main effects on the band IV pigment of Willamette raspberries.

Main Effect	Treatment	Mean (mg/100g)	Difference	Significance
Sugar (°Brix)	0	1.41		
	25	1.28	0.13	Yes
	50	1.22	0.06	No
	(0 vs 50)		0.19	Yes
		(LSD 0.05 =0..107)		
Temperature (°F)	34	1.58		
	70	1.46	0.12	Yes
	100	0.87	0.59	Yes
		(LSD 0.05 =0..107)		
Time (Days)	0	1.80		
	30	1.42	0.38	Yes
	60	1.07	0.35	Yes
	90	0.92	0.15	Yes
		(LSD 0.05 =0.124)		

Figure 1

Main effects of sugar on percent loss of pigments in Willamette raspberries

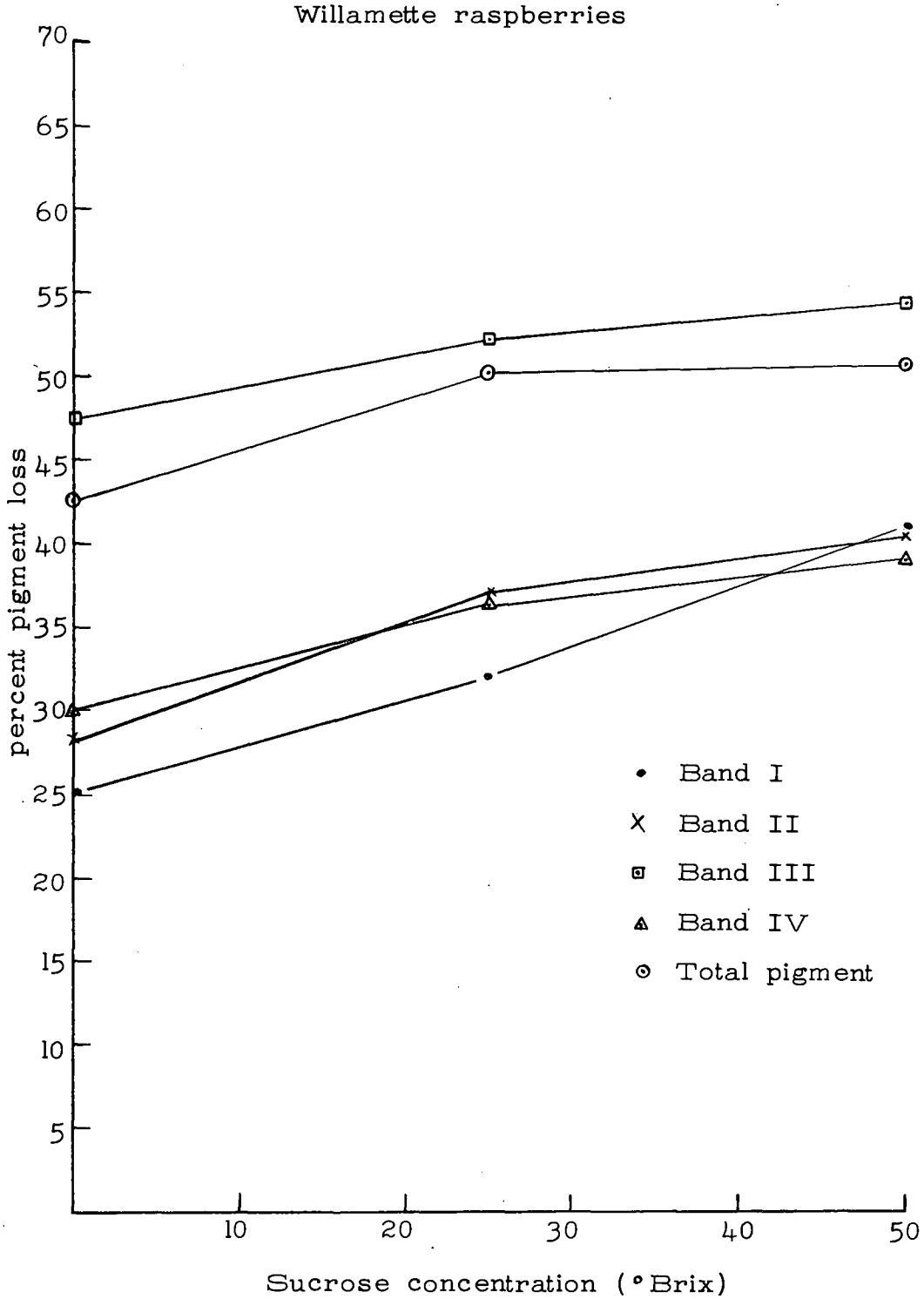


Figure 2

Main effect of temperature of storage on percent loss of pigments in Willamette raspberries

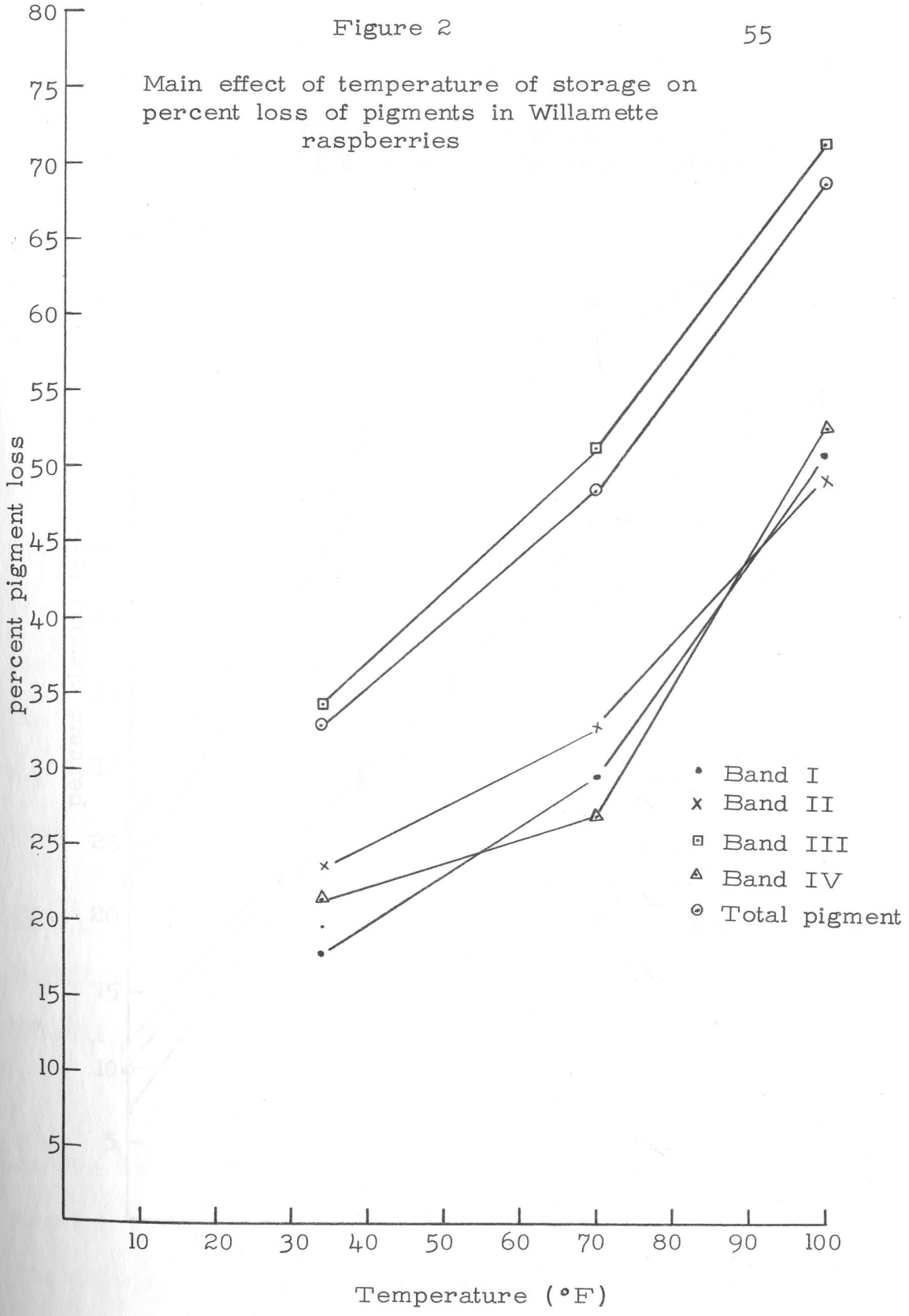
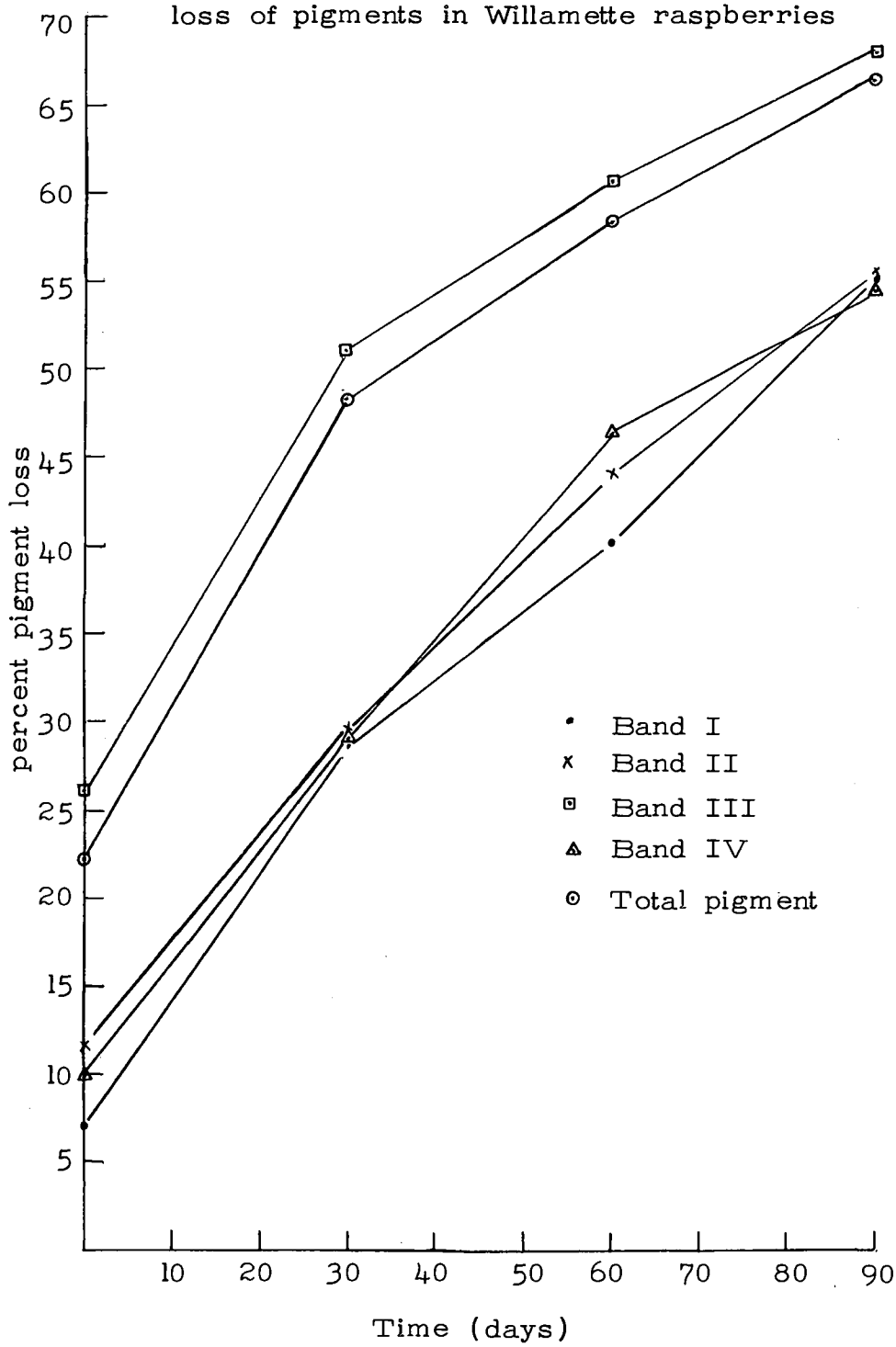


Figure 3

Main effects of time of storage on percent loss of pigments in Willamette raspberries



It is seen that the changes in total pigment due to sugar were primarily influenced by the sugar effect on band III, since the plot for the total change followed the pattern of that of band III. The three other bands each showed a similar percent loss due to the increase of the sirup concentration.

The effects of temperature and time on the percent loss of pigment is shown in Figures 2 and 3 respectively. The same conclusion as stated above may be drawn in respect to the importance of band III to the total pigment change as affected by time and temperature. The percent loss of pigment occurring in bands I, II and IV was almost the same. The percent loss of band III pigment was much higher and this resulted in a parallel change of the total pigment in the Willamette red raspberry.

Effect of headspace atmosphere and the temperature and time of storage on the stability of the pigments of Willamette raspberries.

The second experiment was carried out to investigate the influence of the headspace on the stability of the pigments as influenced by the time and temperature of storage. The different headspace conditions, as previously mentioned,

were air atmosphere (A), nitrogen (N), and vacuum (V).

Band I. The results of this experiment for the pigment of band I (Table 12) were treated statistically , as for all the bands, at the 5% level of significance (Table 13). The statistical analysis indicated that the observed changes due to all factors and interaction between them were significant. Tables 26 and 27 show the interaction between headspace and time and headspace and temperature. It was also observed that the effects of the time and temperature variables within these main factors were each significantly different.

As far as the gaseous headspace was concerned, it was found that the retention of the berry pigment in the vacuum and nitrogen packed cans was significantly higher as compared to that of the cans packed without exclusion of air. This result could be due to the direct or indirect detrimental effect of oxygen on the pigment as has been previously discussed.

The effect of using nitrogen in the headspace on the retention of the pigment was not significantly superior to the retention under vacuum conditions. This was probably due to traces of oxygen remaining in the headspace and in the cavity of the berries.

Table 12

Effect of head space, temperature, and time of storage on the pigment of band I of Willamette raspberries.

Temperature (°F)	Head Space	Fresh Fruit	Storage Time (Days) 0	30	60	90
			(mg per 100 g fruit)			
34	A[1]	1.12	1.04	0.72	0.40	0.20
	V[2]	1.12	1.04	0.88	0.80	0.48
	N[3]	1.12	1.04	0.96	0.80	0.48
70	A	1.12	1.04	0.64	0.40	0.28
	V	1.12	1.04	0.72	0.64	0.40
	N	1.12	1.04	0.70	0.64	0.40
100	A	1.12	1.04	0.20	0.12	0.04
	V	1.12	1.04	0.32	0.30	0.18
	N	1.12	1.04	0.37	0.35	0.16

[1] Air atmosphere [2]Vacuum [3]Nitrogen

Table 13

Means of main effects on the band I pigment of Willamette raspberries.

Main Effect	Treatment	Mean (mg/100g)	Difference	Significance
Head Space	A[1]	0.51		
	V[2]	0.65	0.14	Yes
	N[3]	0.67	0.02	No
	(A vs N)		0.16	Yes
		(LSD 0.05 = 0.022)		
Temperature (°F)	34	0.74		
	70	0.66	0.08	Yes
	100	0.43	0.23	Yes
		(LSD 0.05 = 0.022)		
Time (Days)	0	1.04		
	30	0.61	0.43	Yes
	60	0.49	0.12	Yes
	90	0.23	0.26	Yes
		(LSD 0.05 = 0.026)		

[1] Air atmosphere [2]Vacuum [3]Nitrogen

Band II. The results for the pigment of band II (Table 14 and 15) were similar to those of band I in respect to the effect of headspace, temperature, time and the interaction between temperature and time. The interactions involving headspace with the other two main effects were not significant.

The same results as with band I were obtained for band II with respect to the effect of specific time and temperature treatments, each being significantly different.

In the case of the headspace, only the nitrogen packing treatment showed a significant effect on the retention of the pigment when compared with air. Vacuum did not result in a statistically significant improvement in the retention of the pigment even though in most of the samples the effect was apparent.

Band III. With the exception of the headspace effect, results obtained with band III (Tables 16 and 17) were in agreement with those shown for band II above.

The calculated F value for the effect of headspace was very close to the limit of the critical region but was not such as to prove the significant effect of headspace on the retention of the pigment. Inspection of the data in the Table

Table 14

Effect of head space, temperature, and time of storage on the pigment of band II of Willamette raspberries.

Temperature (°F)	Head Space	Fresh Fruit	Storage Time (Days) 0	30	60	90
			(mg per 100 g fruit)			
34	A[1]	2.24	1.92	1.68	1.56	1.20
	V[2]	2.24	1.92	1.76	1.36	1.36
	N[3]	2.24	1.92	1.68	1.68	1.52
70	A	2.24	1.92	1.60	1.20	0.64
	V	2.24	1.92	1.84	1.44	0.96
	N	2.24	1.92	1.76	1.20	0.80
100	A	2.24	1.92	1.00	0.40	0.08
	V	2.24	1.92	1.40	0.37	0.30
	N	2.24	1.92	1.60	0.34	0.16

[1] Air atmosphere [2] Vaccum [3] Nitrogen

Table 15

Means of main effects on the band II pigment of Willamette raspberries.

Main Effect	Treatment	Mean (mg/100g)	Difference	Significance
Head Space	A[1]	1.26		
	V[2]	1.29	0.03	No
	N[3]	1.39	0.10	Yes
	(A vs N)		0.13	Yes
		(LSD 0.05 = 0.087)		
Temperature (°F)	34	1.64		
	70	1.43	0.21	Yes
	100	0.95	0.48	Yes
		(LSD 0.05 = 0.087)		
Time (Days)	0	1.92		
	30	1.61	0.31	Yes
	60	1.06	0.55	Yes
	90	0.78	0.28	Yes
		(LSD 0.05 = 0.100)		

[1] Air atmosphere [2] Vacuum [3] Nitrogen

16 and the mean values for headspace in Table 17 shows that the nitrogen pack tended to retain more pigment than the other two treatments.

The differences found in retention of the pigment due to the specific time and temperature variables and their interaction were significant.

Band IV. The changes in band IV (Tables 18 and 19) due to the main effects were similar to those reported for bands I and II. The destruction of the pigment in band IV due to the specific time and temperature variables and their interaction was significant. As the temperature and time of storage were increased the retention of the pigment was lowered.

The headspace also significantly affected the retention of the pigment. Nitrogen headspace, as compared to both air and vacuum headspace, was significantly superior. Vacuum packing did not result in better retention of the pigment when compared with air packing.

As an over-all conclusion drawn from this experiment, it may be said that all three factors: time, temperature, and headspace, are of major importance in the stability of the

Table 16

Effect of head space, temperature, and time of storage on the pigment of band III of Willamette rapsberries.

Temperature (°F)	Head Space	Fresh Fruit	Storage Time (Days) 0	30	60	90
			(mg per 100 g fruit)			
34	A[1]	35.10	25.90	21.36	20.64	20.64
	V[2]	35.10	25.90	23.52	21.60	18.00
	N[3]	35.10	25.90	24.00	21.36	18.48
70	A	35.10	25.90	17.28	11.50	9.40
	V	35.10	25.90	17.30	12.20	9.72
	N	35.10	25.90	17.30	12.00	9.60
100	A	35.10	25.90	5.52	1.52	0.85
	V	35.10	25.90	8.88	2.88	1.20
	N	35.10	25.90	8.88	2.68	2.40

[1] Air atmosphere [2] Vacuum [3] Nitrogen

Table 17

Means of main effects on the band III pigment of Willamette raspberries

Main Effect	Treatment	Mean (mg/100g)	Difference	Significance
Head Space	A[1]	15.53	(No significant differences)	
	V[2]	16.12		
	N[3]	16.20		
Temperature	34	22.28		
	70	16.21	6.07	Yes
	100	9.38	6.83	Yes
		(LSD 0.05 = 0.62)		
Time (Days)	0	25.90		
	30	16.00	9.90	Yes
	60	11.87	4.13	Yes
	90	10.03	1.84	Yes
		(LSD 0.05 = 0.714)		

[1] Air atmosphere [2] Vacuum [3] Nitrogen

anthocyanin of canned raspberries. Nitrogen packing was superior, in most cases, to the other two headspace atmospheres.

Since the pigment of band III was the major constituent in the anthocyanin pigment complex of the Willamette raspberry, one might conclude that by using nitrogen in the headspace, one would retain more of the original color than with air packing.

Change in total pigment. The effect of three variables upon the percent loss of pigment in each band and in the total pigment is shown in Figure 4,5, and 6. The main effect of the vacuum headspace (Figure 4) is to slightly lower the percent loss of pigment in bands II and IV and to greatly improve the retention of the pigment of bands I and III. The over-all result is controlled by band III which accounted for 88% of the total pigment of the fruit. The decrease in the loss of pigment from vacuum to nitrogen headspace was very slight.

The main effect of temperature (Figure 5) and time (Figure 6) on the individual bands and on the total pigment was the same as in the previous experiment. The plot of

Table 18

Effect of head space, temperature, and time of storage on the pigment of band IV of Willamette raspberries.

Temperature (°F)	Head Space	Fresh Fruit	Storage Time (Days) 0	30	60	90
			(mg per 100 g fruit)			
34	A[1]	2.0	1.80	1.65	1.60	1.04
	V[2]	2.0	1.80	1.68	1.44	1.20
	N[3]	2.0	1.80	1.76	1.52	1.20
70	A	2.0	1.80	1.44	1.20	0.96
	V	2.0	1.80	1.52	1.12	1.12
	N	2.0	1.80	1.52	1.20	1.04
100	A	2.0	1.80	0.72	6.20	0.08
	V	2.0	1.80	0.80	0.32	0.18
	N	2.0	1.80	0.96	0.64	0.32

[1] Air atmosphere [2] Vacuum [3] Nitrogen

Table 19

Means of main effects on the band IV pigment of Willamette raspberries.

Main Effect	Treatment	Mean (mg/100g)	Difference	Significance
Head Space	A[1]	1.20		
	V[2]	1.23	0.03	No
	N[3]	1.30	0.07	Yes
	(A vs N)		0.10	Yes
		(LSD 0.05 = 0.058)		
Temperature (°F)	34	1.54		
	70	1.38	0.16	Yes
	100	0.80	0.58	Yes
		(LSD 0.05 = 0.058)		
Time (Days)	0	1.80		
	30	1.49	0.31	Yes
	60	1.03	0.46	Yes
	90	0.78	0.25	Yes
		(LSD 0.05 = 0.066)		

[1] Air atmosphere [2] Vacuum [3] Nitrogen

Figure 4

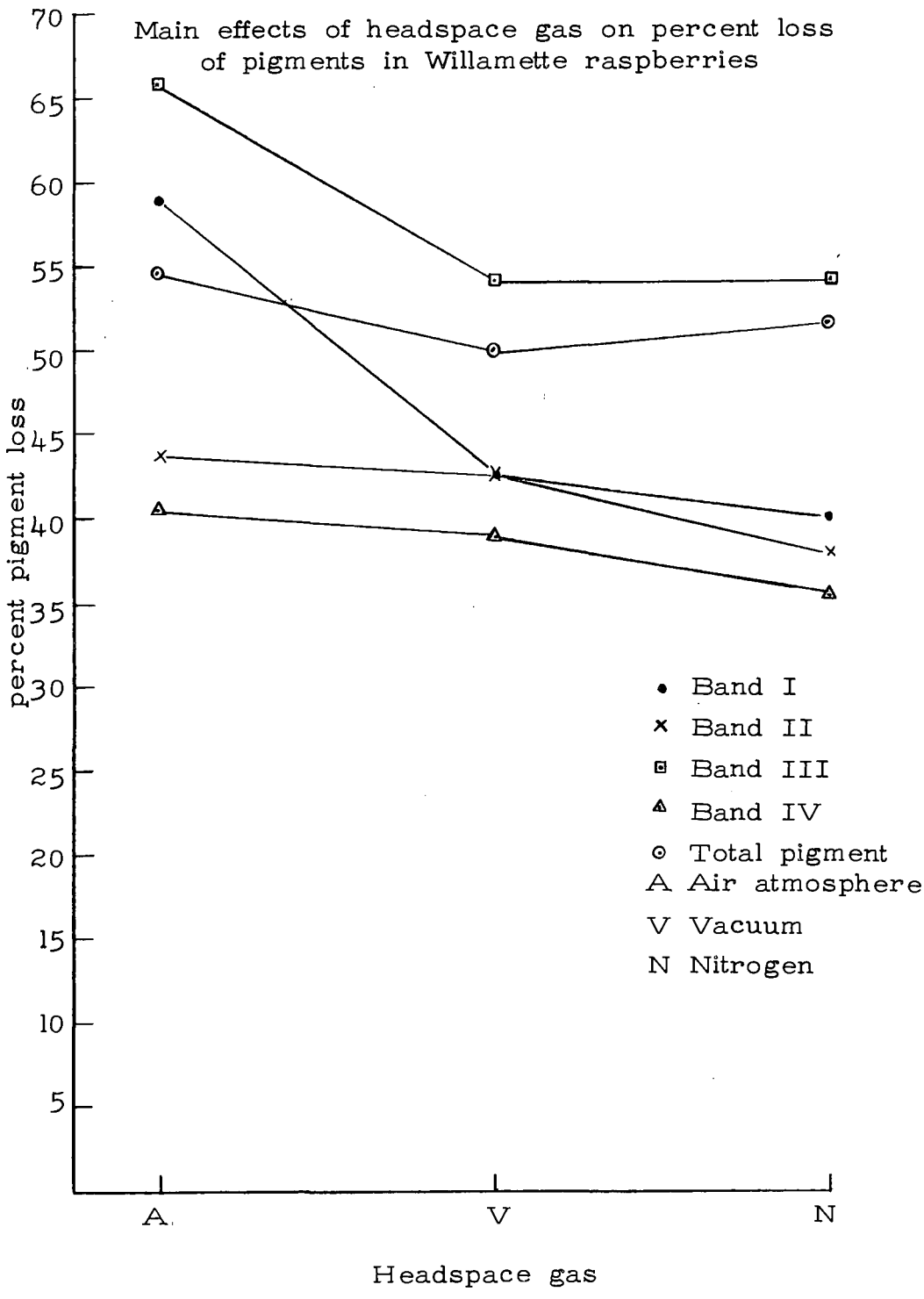

Main effects of headspace gas on percent loss of pigments in Willamette raspberries
70
65
60
55
50
45
40
35
30
25
20
15
10
5
percent pigment loss
A
V
N
Headspace gas
Band I
Band II
Band III
Band IV
Total pigment
A Air atmosphere
V Vacuum
N Nitrogen

Figure 5

Main effects of temperature of storage on percent loss of pigments in Willamette raspberries

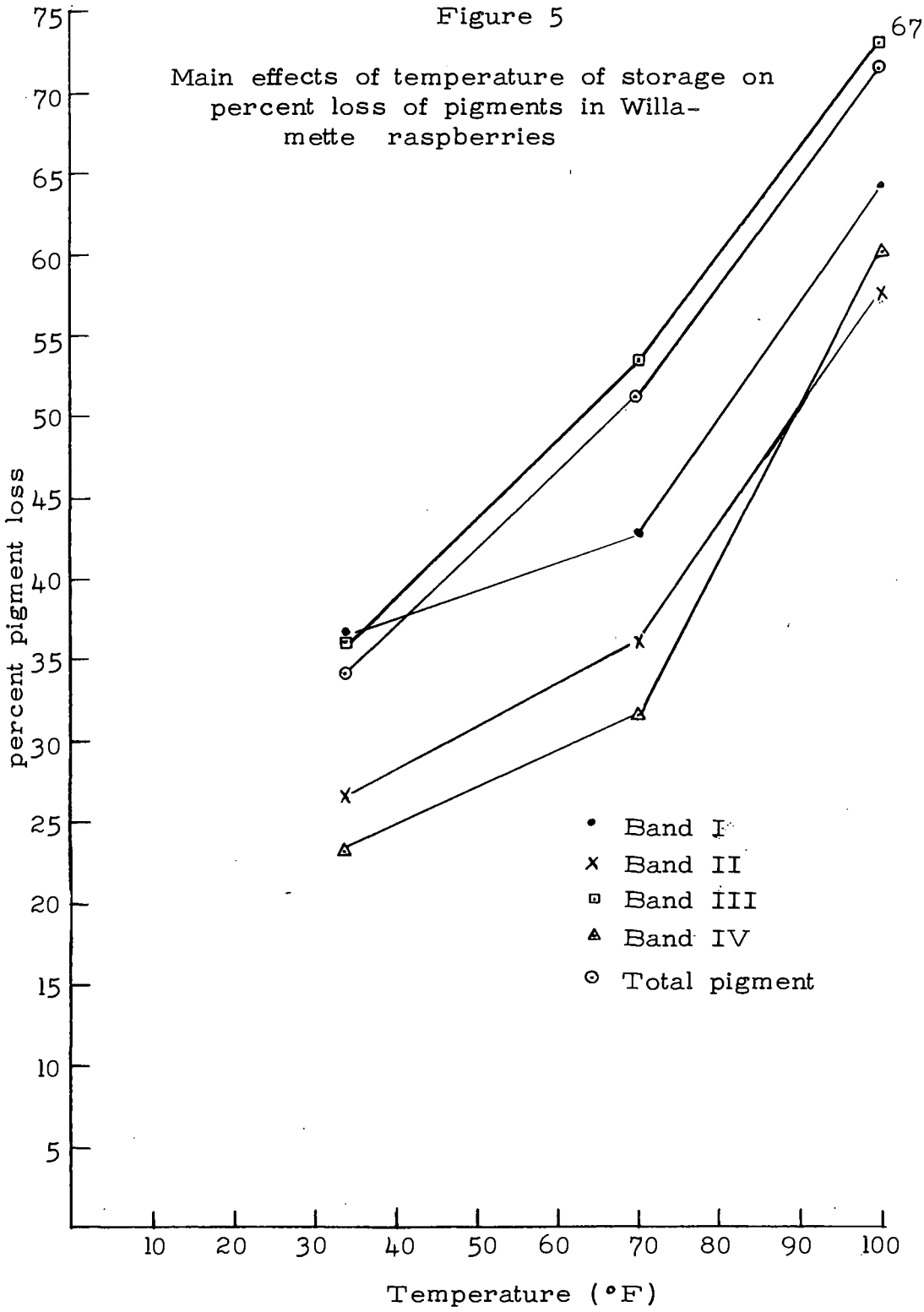
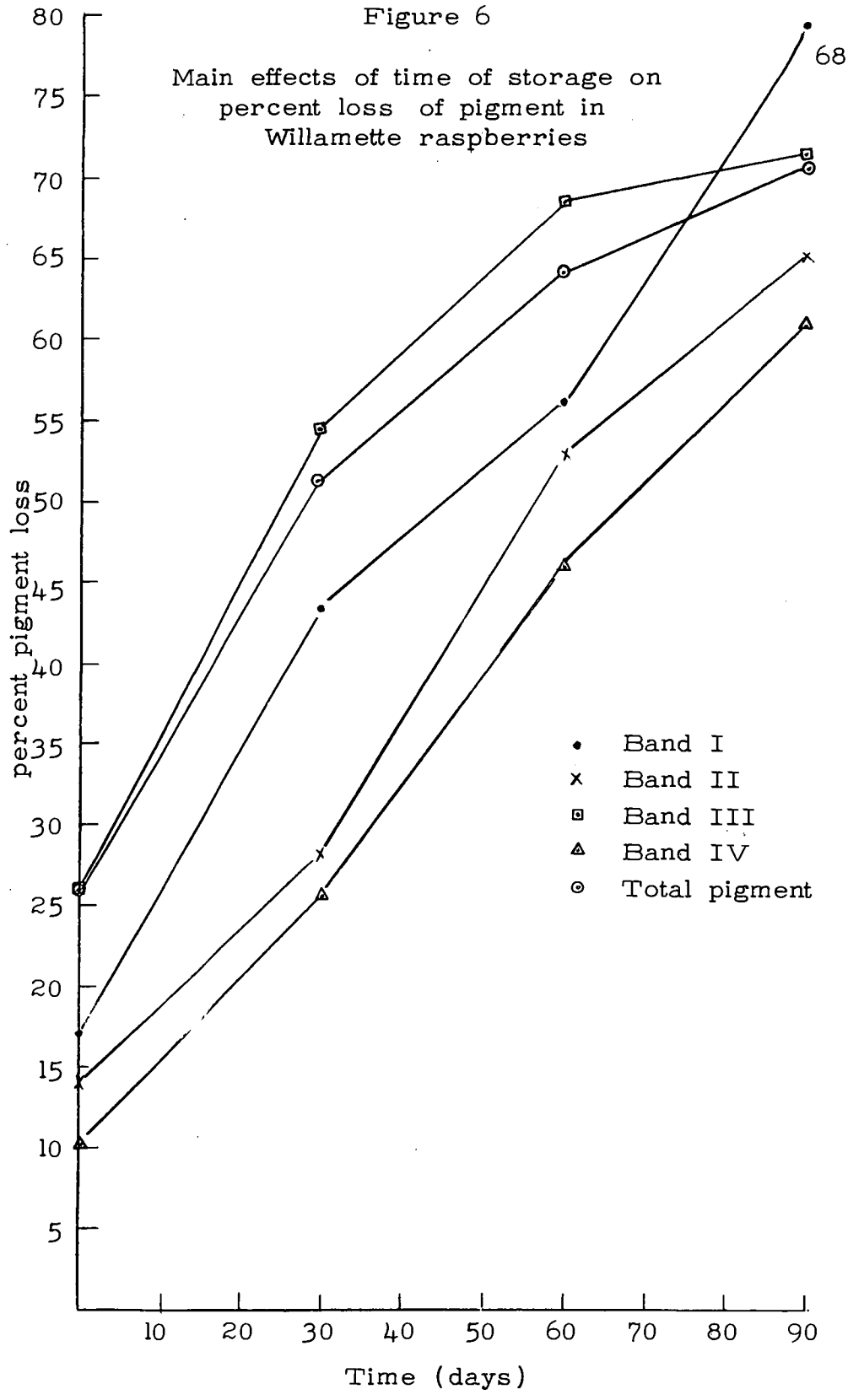


Figure 6

Main effects of time of storage on percent loss of pigment in Willamette raspberries



the total pigment loss was paralleled by that of band III.

Effect of headspace atmosphere and of temperature and time of storage on the stability of the pigment of black raspberries

The purpose of this experiment was to study the effect of the nature of the headspace, temperature and time of storage on the stability of the pigments in canned black raspberries.

Band I. Statistical analysis of the results for band I (Table 21) indicated that the headspace atmospheres did not affect the destruction of the pigment nor did the data show a consistent effect with respect to the influence of oxygen. A significant effect of the headspace variable might possibly be revealed by using more refined techniques since the differences between headspace treatments began to approach that of the critical region for significance.

Changes in the pigment due to specific temperatures, times of storage, and their interaction were significant. An increase of storage temperature and time resulted in a progressive destruction of the pigment.

Table 20

Effects of the head space, temperature and time of storage on the band I pigment of black raspberries.

Temperature (°F)	Head Space	Fresh Fruit	Storage Time (Days) 0	30	60	90
			(mg per 100 g fruit)			
34	A[1]	103.5	89.70	88.55	88.55	51.75
	V[2]	103.5	89.70	87.50	80.50	80.50
	N[3]	103.5	89.70	81.65	80.50	59.50
70	A	103.5	89.70	57.50	57.50	23.00
	V	103.5	89.70	59.50	59.50	29.90
	N	103.5	89.70	47.20	47.20	47.20
100	A	103.5	89.70	18.40	18.40	17.20
	V	103.5	89.70	32.20	32.20	26.40
	N	103.5	89.70	24.20	24.20	21.90

[1] Air atmosphere [2] Vacuum [3] Nitrogen

Table 21

Means of main effects on the band I pigment of black raspberries.

Mean Effect	Treatment	Mean (mg/100g)	Difference	Significance
Head Space	A[1]	58.83		
	V[2]	65.13	(No significant differences)	
	N[3]	60.18		
Temperature (°F)	34	80.67		
	70	64.02	16.65	Yes
	100	44.47	19.55	Yes
		(LSD 0.05 = 6.52)		
Time (Days)	0	89.70		
	30	61.85	27.85	Yes
	60	54.28	7.57	Yes
	90	39.71	14.57	Yes
		(LSD 0.05 = 7.540)		

[1] Air atmosphere [2] Vacuum [3] Nitrogen

Band II. The results obtained for band II of the black raspberries were not similar to those found for band I. This band showed changes in the retention of the pigment which were significant for each of the main effects (Table 22 and 23). Significant interactions were also shown between (1) time and temperature and (2) headspace and time of storage (Table 28 and 29).

Statistical analysis of the data obtained for the headspace variables showed a significantly higher retention of band II pigment for black raspberries packed under nitrogen than for those packed under vacuum or normal atmosphere. It is to be recalled that the interaction between headspace and time of storage was also significant and definite conclusions cannot be made. The interaction may be observed in Table 26.

The retention of band II pigment was found to be significantly different between each of the three storage temperatures and between each of the storage periods in this experiment.

Band III. Results relative to band III revealed that the three main variables studied significantly affected the stability of

Table 22

Effect of the head space, temperature and time of storage on the band II pigment of black raspberries.

Temperature (°F)	Head Space	Fresh Fruit	Storage Time (Days) 0	30	60	90
			(mg per 100 g fruit)			
34	A[1]	315.10	257.60	211.60	201.30	200.10
	V[2]	315.10	257.60	224.30	218.50	171.90
	N[3]	315.10	257.60	253.00	241.00	174.80
70	A	315.10	257.60	126.50	103.50	55.20
	V	315.10	257.60	111.20	104.00	54.10
	N	315.10	257.60	128.80	109.30	43.70
100	A	315.10	257.60	33.40	29.90	23.00
	V	315.10	257.60	46.00	43.70	25.30
	N	315.10	257.60	77.00	54.00	23.00

[1] Air atmosphere [2] Vacuum [3] Nitrogen

Table 23

Mean of main effects on the band II pigment of black raspberries.

Main Effect	Treatment	Mean (mg/100g)	Difference	Significance
Head Space	A[1]	160.89		
	V[2]	160.96	0.07	No
	N[3]	169.10	8.14	Yes
		(LSD 0.05 = 6.204)		
Temperature (°F)	34	236.80		
	70	127.66	109.14	Yes
	100	108.30	19.36	Yes
		(LSD 0.05 = 6.204)		
Time (Days)	0	315.20		
	30	133.64	181.56	Yes
	60	121.93	11.71	Yes
	90	85.64	36.29	Yes
		(LSD 0.05 = 7.144)		

[1] Air atmosphere [2] Vacuum [3] Nitrogen

the pigment. There was a significant interaction between (1) temperature and time of storage and (2) headspace and temperature of storage (Table 27). Thus, the effect of headspace gases on the stability of the pigment was affected by the temperature of storage. In the same way, the effect of time of storage on the stability of the pigment could be expected to vary with the storage temperature. Inspection of the data in Table 24 and 25 show that both vacuum and nitrogen packing, although not different from each other, provided significantly better protection of the pigment than atmosphere packing. The effect of temperature was such as to show that although storage at 34° and 70°F were not significantly different, both were more effective in retaining the pigment than storage at 100°F.

The effect of time of storage was such that each succeeding interval of storage resulted in an increased loss of the pigment of band III.

Change in total pigment. The effect of headspace on the percent loss of pigment of each band and in the total pigment is shown in Figure 7.

Bands I and III showed a lower percent loss when

Table 24

Effect of head space, temperature and time of storage on the band III pigment of black raspberries.

Temperature (°F)	Head Space	Fresh Fruit	Storage Time (Days) 0	30	60	90
			(mg per 100 g fruit)			
34	A[1]	26.60	20.80	14.20	13.60	13.90
	V[2]	26.60	20.80	18.90	17.70	16.60
	N[3]	26.60	20.80	16.20	14.60	12.90
70	A	26.60	20.80	14.40	16.90	7.70
	V	26.60	20.80	17.30	16.20	15.40
	N	26.60	20.80	16.50	16.30	7.80
100	A	26.60	20.80	9.00	6.90	2.00
	V	26.60	20.80	8.90	8.10	6.90
	N	26.60	20.80	15.40	12.70	7.30

[1] Air atmosphere [2] Vacuum [3] Nitrogen

Table 25

Means of main effects on the band III pigment of black raspberries.

Main Effect	Treatment	Mean (mg/100g)	Difference	Significance
Head Space	A[1]	13.28		
	V[2]	15.70	2.42	Yes
	N[3]	15.04	0.66	No
	(A vs N)		1.76	Yes
		(LSD 0.05 = 1.198)		
Temperature (°F)	34	16.66		
	70	15.58	1.08	No
	100	11.80	3.78	Yes
		(LSD 0.05 = 1.198)		
Time (Days)	0	20.80		
	30	14.53	6.27	Yes
	60	13.16	1.37	Yes
	90	10.17	2.99	Yes
		(LSD 0.05 = 1.336)		

[1] Air atmosphere [2] Vacuum [3] Nitrogen

packed under vacuum than when canned with the air atmosphere headspace or nitrogen headspace. Nitrogen was superior in lowering the percent loss of the pigments.

The plot of changes in total pigment shown in Figure 7 parallels that of band II, which represents 71.0% of the total pigment in black raspberries and therefore controls the total pigment effect.

The total change of the pigment shows that nitrogen headspace was superior to vacuum headspace and vacuum headspace was slightly better than air atmosphere headspace.

The percent total losses of pigment due to temperature and time of storage (Figure 8 and 9) were greatly influenced by band II. The loss of Band II pigment almost paralled that of the over-all pigment while the other two bands, although less seriously affected, showed similar results.

Effect of heating on degradation of the pigment of Willamette red raspberries and black raspberries.

The destruction of the pigment in the bands I, II, III, IV of Willamette raspberries due to the processing was 7.2, 14.3, 26.1, and 10.9 percent respectively. The highest destruction due to the processing practices was observed for

the band which had the higher amount of pigment. The overall reduction of the total pigment due to processing was 42.3 percent.

Black raspberries showed 12.5, 20.2, and 21.0 percent in color reduction for bands I, II, and III respectively. The overall color destruction due to processing was 18.4 percent of the original pigment of the fresh fruit.

Since the pigments primarily responsible for visual color were Band III in red raspberries and Band II in black raspberries, comparison between the two varieties confirmed that black raspberry pigment was more stable to the processing treatment (i.e. 180°F for 10 minutes).

Figure 7

Main effects of headspace gas on percent loss of pigment in black raspberries

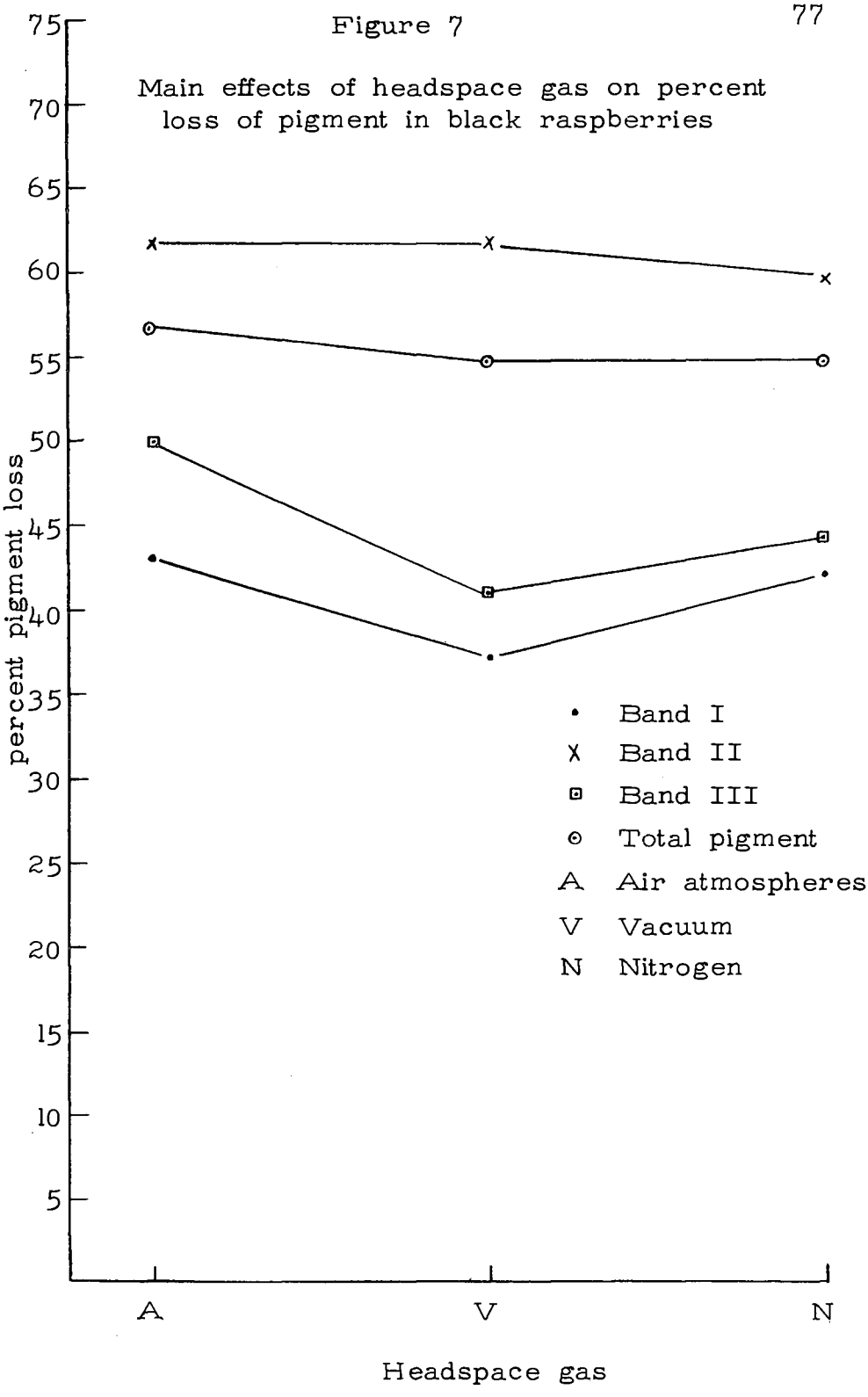


Figure 8

Main effects of temperature of storage on percent loss of pigments in black raspberries

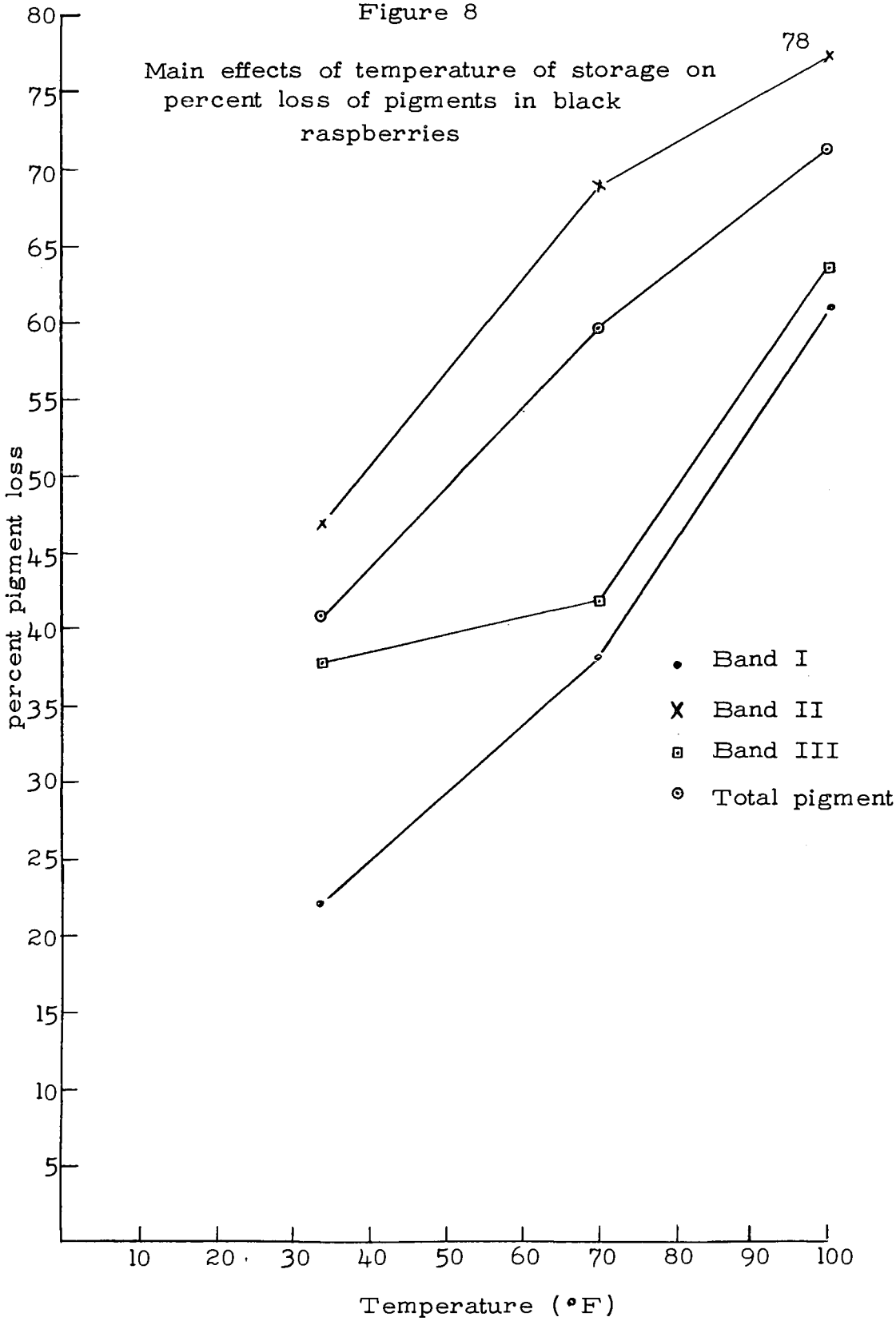


Figure 9

Main effects of time of storage on percent loss of pigment in black raspberries

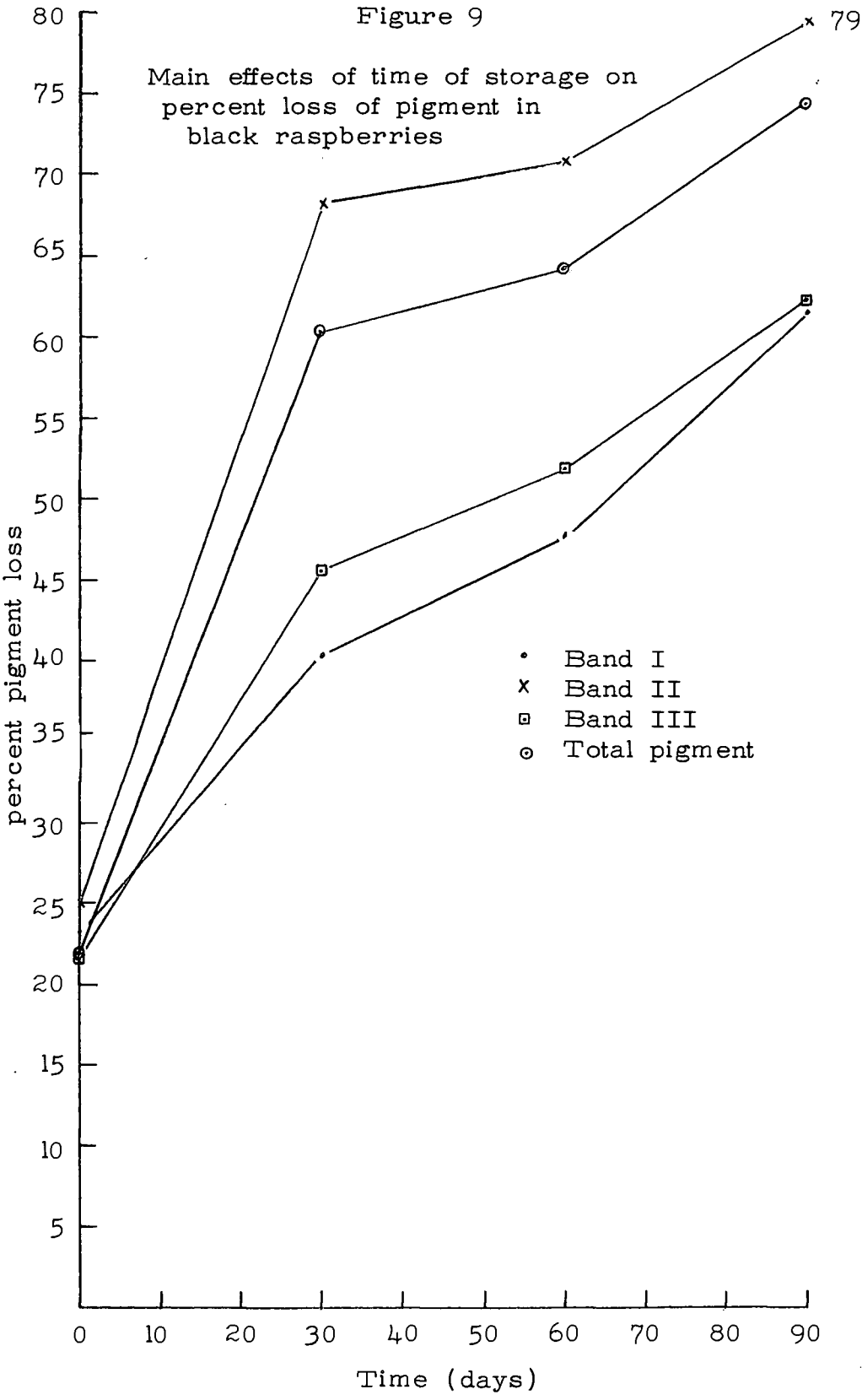


Table 26

Head space x time interaction in band I pigment of Willamette raspberries.

Head Space	Storage Time (Days)			
	0	30	60	90
	(mgs per 100g fruit)			
A[1]	1.04	0.52	0.31	0.17
V[2]	1.04	0.64	0.58	0.35*
N[3]	1.04	0.68	0.60	0.35*

* Points of interaction [1] Air atmosphere [2] Vacuum [3] Nitrogen

Table 27

Head space x temperature interaction in band I pigment of Willamette raspberries.

Head Space	Storage Temperature (°F)		
	34	70	100
	(mgs per 100g fruit)		
A	0.59	0.59	0.35
V	0.80	0.70*	0.46
N	0.82	0.69*	0.48

* Points of interaction

Table 28

Head space x time interaction in band II pigment of black raspberries.

Head Space	Storage Time (Days)			
	0	30	60	90
	(mgs per 100g fruit)			
A[1]	257.60	123.80	111.60	92.20*
V[2]	257.60	127.20	122.10	83.80*
N[3]	257.60	152.90	134.80	80.50*

* Points of interaction [1] Air atmosphere [2] Vacuum [3] Nitrogen

Table 29

Head space x temperature interaction in band III pigment of black raspberries.

Head Space	Storage Temperature (°F)		
	34°	70°	100°
	(mgs per 100g fruit		
A	20.93	18.60	13.59
V	24.66*	22.63*	14.90*
N	21.03*	20.47*	18.73*

* Points of interaction

SUMMARY AND CONCLUSIONS

The purpose of this thesis was to investigate the changes of color in Willamette red raspberries and black raspberries during processing and storage.

Variables imposed in the experimental design were: (1) Concentration of sirup, (2) temperature, and, (3) time of storage; and (1) headspace atmosphere, (2) temperature, and, (3) time of storage. Concentrations of sugars used were 0°, 25°, and 50° Brix. Headspace atmospheres used were air, vacuum, and nitrogen as affecting the anthocyanins of the canned berries stored at 34°, 70° and 100°F for 90 days.

Anthocyanin pigments were separated by column chromatography and subsequently analyzed spectrophotometrically.

On the basis of this experiment the following conclusions were drawn.

1. The components of the anthocyanin pigment of the Willamette raspberry consisted of four separate cyanins as based on the R_f values and characteristic wavelengths of maximum absorption. The anthocyanin pigments of black

raspberries were separated into three cyanin pigments using a new solvent Ac-OH: H_2O (15:85 v/v).

2. On the basis of R_f values and wavelengths of maximum absorption it appeared that only two of the constituent pigments separated from the two kinds of raspberries were common to both species.

3. The wavelength of maximum absorption of all anthocyanins shifted towards shorter wavelengths after the first month of storage. The shift was more pronounced as the time of storage increased. The maximum extent of the shift observed after 90 days of storage was approximately 19 mμ.

4. Time and temperature and their interaction were shown to significantly affect the retention of the pigments of raspberries. Increasing the storage temperature or time resulted in progressively greater destruction of all of the pigments.

5. High levels of the ingoing sirup resulted in a greater destruction of the cyanins in three out of the four pigments of the Willamette raspberry. This affect could not be shown in the case of the most concentrated band. This may have been due to the high concentration of the pigment.

6. Changes in pigment retention due to the nature of the headspace revealed that three of the bands of the Willamette raspberry and two of the bands of the black raspberry were affected. Exclusion of oxygen generally reduced the destruction of the pigment.

7. Changes in total pigment concentration were greatly influenced by the cyanin in greatest concentration. These cyanins were band III in Willamette and band II in black raspberries.

8. The individual pigments separated from both species of raspberries were in every case adversely affected by the heat process. Heat processing resulted in a 24% and 18% loss in total pigments of the Willamette and black raspberries respectively.

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