# RECOVERY OF ANTHOCYANIN PIGMENT FROM FERMENTED RED GRAPE SKINS AND ITS STABILITY IN A CARBONATED BEVERAGE

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# ABSTRACT

## RECOVERY OF ANTHOCYANIN PIGMENT FROM FERMENTED RED GRAPE SKINS AND ITS STABILITY IN A CARBONATED BEVERAGE

By

Nickolas Palamidis

Anthocyanin pigment was extracted from fermented Napa-Gamay grape skins and its stability was studied in a carbonated beverage to which it was added as colorant agent.

The following storage conditions of the beverage were used: (a) darkness at 3.5 C, (b) darkness at 10 C, (c) darkness at 20 C, (d) darkness at 38 C, (e) diffuse daylight and 20 C, and (f) continuous fluorescent light and 22 C.

Two solvents, boiling water and 500 ppm $SO_2$ aqueous solution, were compared for their extraction power of the pigment. The extraction was accomplished during grinding of the skins in a mill capable of breaking cells.

Practically the same amount of anthocyanin was extracted with boiling water and $SO_2$ solution: 32.9 and 31.9 g enocyanin equivalent per 100g moisture free skins respectively. Soaking after grinding as well as 1,000

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Nickolas Palamidis

A THESIS

Submitted to
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# INTRODUCTION

Pleasing color makes a food more attractive and desirable. The proper color of many foods is a matter of importance since consumer's acceptance depends on it. Over the years synthetic coloring matters were used in foods to a higher extend than the natural colors because of their higher stability and stronger coloring power.

Because a number of coal tar dyes have been removed from the list of approved colorants for foods (the safety of FD and C red No. 2 is currently under review), considerable interest has recently been generated in the potential use of plant anthocyanins as natural food colorants.

From the various sources of anthocyanins, grapes are considered to be a good potential source of these pigments. Grape pigments can be recovered from grape skins, which are a by-product of the wine industry or the grape juice industry.

For higher color release from grapes it is a common practice in the wine industry to heat the juice and then pump it over the pomace or to heat the crashed grapes in heat exchangers. The heat makes permeable to the pigment the semipermeable membranes of the epidermal cells of the skin which contains the pigment. (In some varieties the pulp of the grape is also pigmented.)

A patent was issued to Peterson and Jaffe (1969) for extracting the color and flavor from pomace of grapes and other fruits with aqueous or alcoholic solutions of sulfur dioxide.

Anthocyanins are unstable compounds and readily undergo undesirable changes under a variety of conditions employed in the processing and storage of fruit products. Several investigations have been made to determine the factors that affect the rate of pigment breakdown in many fruits and fruit products (Lukton *et al.*, 1956; Markakis *et al.*, 1957; Starr and Francis, 1968; Van Buren *et al.*, 1968; Daravingas and Cain, 1968). The most important factors affecting the stability of anthocyanins are enzymes, temperature of processing and storage, oxygen, other cellular constituents such as arcorbic acid, sugars and products of their degradation, hydrogen ion concentration, metals and light.

The objectives of this study were the following:

1. Quantitative recovery of the anthocyanin pigment from the skins of fermented grapes, a by-product of the wine industry, and
2. Study of the stability of this extract, under certain storage conditions of temperature and light, in a non-alcoholic carbonated beverage where it was used as colorant agent.

# LITERATURE REVIEW

## Occurrence, Structure and Properties of Anthocyanins

Anthocyanins are the group of plant pigments responsible for the red, purple, blue, and violet colors that appear in many flowers and fruits (Swain 1965; Harborne, 1967). Two notable exceptions are the tomatoes and red beets.

The term "anthocyanin" composed from the Greek words "antho" (flower) and "cyanin" (blue) used first by Marquart in 1835 to designate the blue pigments of flowers (Markakis, 1974).

Anthocyanins are one class of the larger group of flavonoids which also includes compounds responsible for the yellow color of certain flowers (although more usually this is due to the presence of carotenoid pigments). Yet other flavonoids account for the actual whitness in most white flowers, which without them would appear almost translucent (Swain, 1965).

The flavonoid compounds have been the subject of investigations since the beginning of modern chemistry.

From the chemical point of view the anthocyanin provided a considerable challenge, partly because of their difficulties in their isolation and partly because of their relative lability (Harborne, 1967).

Chemically anthocyanins are glycosides of anthocyanidins. The anthocyanidins are polyhydroxy and methoxy derivatives of the basic structure, 2-phenyl-benzopyrylium or flavylium (Markakis, 1974).

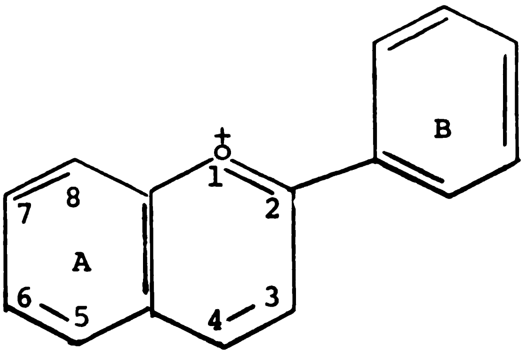

Figure 1. 2-phenyl-benzopyrylium (flavylium) structure.

This is the oxonium (tetravalent oxygen) form of flavylium; actually the positive charge is delocalized over the molecule so that phenolic flavylium salts are resonance hybrids of oxonium ions, carbonium ions, and other charged species:

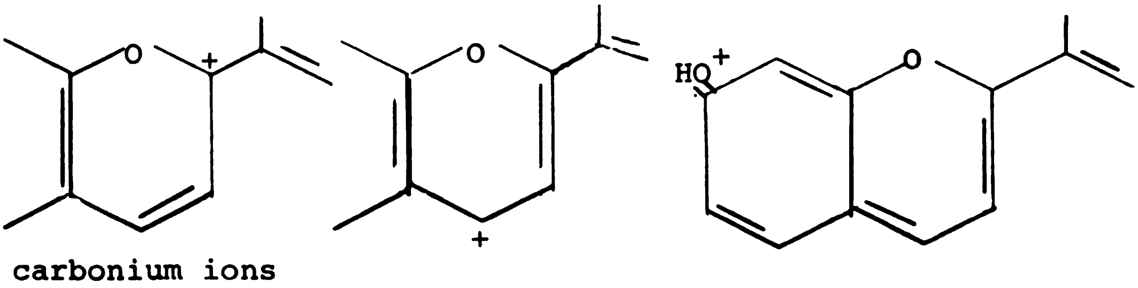

Figure 2. Other species of flavylium structure.

The degree of hydroxylation and methoxylation and the positions of these groups on the flavylium result in different anthocyanidins. In nature 16 anthocyanidins have been found. Of these six (pelargonidin, cyanidin, delphinidin, petunidin, peonidin, malvidin), are the most common in flowers, and fruits. Anthocyanidins are unstable compounds and in nature they occur as glycosides, the anthocyanins (Harborne, 1967).

The sugar moiety of anthocyanins is usually attached to the hydroxyl at position 3. The 5th and 7th hydroxyls may also be glycosylated. The sugars attached may be monosaccharides (chiefly glucose, galactose, rhamnose and arabinose), disaccharides and trisaccharides.

These combinations of the different sugars attached to different positions result in five general classes of anthocyanins: 3-monosides, 3-biosides, 3-triosides, 3,5-diglycosides, 3,7-diglycosides. The 5- (or 7) sugar is always glucose (never rhamnose even when the 3-sugar is rhamnose). All the di- and trisaccharides have at least one glucose unit and the linkages are β1-2, β1-6, or a 1-6. Acylated anthocyanins of all the six common anthocyanidins have also been found in different parts of many plants. All acylated glycosides that have been examined in detail have their acyl group attached to the sugar in the 3-position, but the position of the attachment of the acyl group to the sugar hydroxyl is difficult to determine because of the lability of the ester linkage and because of the propensity of acyl groups on sugars for changing their positions during chemical treatment (Harborne, 1967). The acyl group of these pigments is most often p-coumaric acid and rarely ferulic acid or caffeic acid.

Anthocyanins are soluble in water but are insoluble in benzene, chloroform and ether. With mild acid treatment they are hydrolyzed into the sugar and the aglycone (anthocyanidin) which is insoluble in water, unstable to light and destroyed rapidly by alkali (Harborne, 1967).

The color of anthocyanins is due to the presence of the extensive conjugated double bond system (chromophore) in the molecule. The chromophore creates a condition in the molecule which allows specific wavelengths in the visible region of the spectrum to be absorbed; the remaining wavelengths result in the perception of the color.

The color of anthocyanins is affected by many factors. As the number of hydroxyl groups in the molecule increases the color changes from pink to blue (Harborne, 1965). Methoxyl groups replacing hydroxyl groups have the opposite effect. The hydroxyl group at position 3 appears to be of particular importance, since it shifts the absorption maximum from the yellow-orange into the red region.

Glycosylation at the 5-position has a small effect on color. The 3,5 diglucosides of pelargonidin, peonidin and malvidin appear fluorescent in solution (Harborne, 1965).

The hydrogen ion concentration is another factor affecting the color of anthocyanins. Solutions of anthocyanins are red in acid, blue in alkaline solutions and they fade at intermediate pH values. The cation predominates at low pH, the anion at high pH and the colorless pseudobase with its conjugated double bond system interrupted at neutral pH.

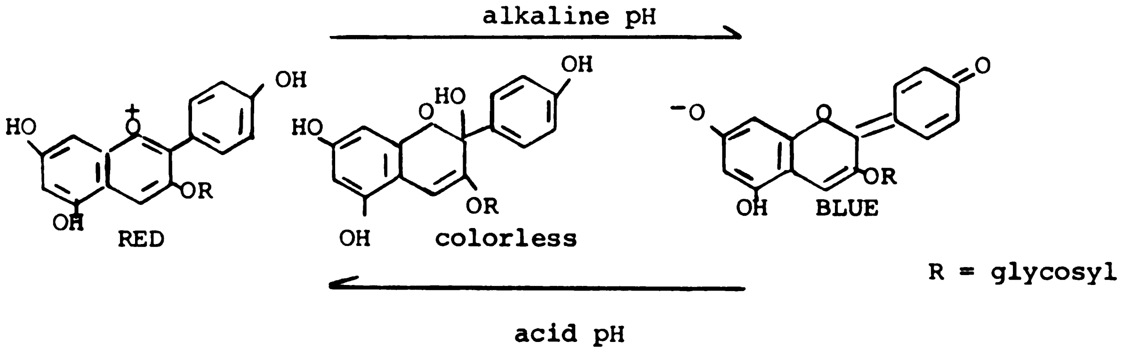

Figure 3. Effect of pH on structure and color of anthocyanins.

Changes of the color of anthocyanins towards the blue may also be due to the formation of complexes with other naturally occurring compounds and metals. The early suggestion by Willstätter that in blue varieties the cell sap is alkaline and thus results in the conversion of the red flavylium salt into blue anion is not valid, since Hayashi and Isaka (1946) and others reported that the pH of flowers, leaves and fruits, irrespective of their color, is always acidic (Jurd, 1966). G.M. and R. Robinson (1932) proposed that great changes in color may result due to the formation of anthocyanin complexes with organic substances (co-pigments) and with metals. Later a blue pigment isolated by Mitsui (1958, 1959) which composed of an atom of Magnesium four molecules of awabanin (delphinidin - 3:5 - dimonoglucoside and p-coumaric acid) and a flavonoid-like compound. Metal complexing is rather responsible for most of the blue colors in berries (Harborne, 1963). Copigmentation is usually associated with the presence of flavones, flavonones and flavans.

The depth of pigmentation in a tissue increases with the concentration of the anthocyanin; when the concentration is sufficiently high as in the skins of fruits, and especially when chlorophyll is also present, the tissue may appear almost black (Swain, 1965).

The synthesis of anthocyanins is controlled genetically. Based on a thorough study of the anthocyanins of _Vitis vinifera_ (by Ribéreau-Gayon) a method of detecting the adulteration of vinifera wines with wines made from hybrid varieties has been developed (Robinson _et al._, 1962). The _Vitis vinifera_ varieties posses on a quantitative basis, almost exclusively monoglucosidic anthocyanins, the American and French hybrids contain considerable diglucosidic and acylated anthocyanins (Van Buren _et al._, 1968).

Anthocyanidins present in _Vitis vinifera_ are: Malvidin, peonidin, delphinidin, cyanidin, petunidin and pelargonidin; those of _Vitis labrusca_ (Concord) are: Cyanidin, delphinidin, peonidin, malvidin and petunidin (Markakis, 1974).

The anthocyanin pigments along with other phenolic substances present in grapes and wines are discussed by Singleton and Esau (1969).

## Extraction of Anthocyanins

The most common extraction method used today is the use of low boiling point alcohols (methanol or ethanol) containing small amounts of hydrochloric acid (0.1-1%), followed by filtration and concentration under vacuum. The acid stabilizes the pigment, but it may cause breaking of the copigments and the association with metals (Markakis, 1974). To obtain the anthocyanins as nearly as possible in the forms in which they exist in plant tissues, some workers preferred to use neutral solvents for the initial extraction; such as, 60% methanol, acetone/ethanol/water-mixture, cold acetone, boiling water and sulfur dioxide, while other workers have preferred methanol containing 1% glacial acetic acid.

Fuleki and Francis (1968), using as extracting solvent 95% ethanol plus 1.5N HCl (85:15), obtained almost 100% of the total anthocyanins from frozen cranberries with one extraction and one washing of the residue. The cranberries, 100g, were macerated in a waring blender with an equal volume of solvent and the slurry stored overnight at 4 C before filtration and washing. Fuller and Francis (1970) compared the yield of pigment (during the cranberry juice production) from fresh berries and frozen thawed berries and found that the frozen berries yielded four times more pigment than the fresh controls. They attributed this difference to the freezing and thawing process which breaks the cells to some extent and allows the pigment to diffuse from its primary site. Chiriboga and Francis (1970) reported that grinding of the cranberry pomace reduces the time of extraction of the pomace pigment from several hours (by stirring), to less than one-half hour.

Phillip (1974) suggested the extraction of anthocyanin from grape wine pomace and grape juice residues with methanol containing 1% or 0.1% tartaric acid, respectively. He neutralized part of the acidity with KOH solution and filtered off the precipitated potassium hydrogen tartate (Phillip, 1974).

## Purification

Chromatographic techniques have been used for the purification of individual anthocyanins. Although valuable as methods for the examination of anthocyanins they are not practical for the purification of anthocyanins in large quantities. Ion exchange resins could also be used for the purification of anthocyanins, but certain disadvantages limit their application for commercial purposes. Weak cationic resins have low pigment capacity while strong cationic resins require large volumes of solvent for complete elution of anthocyanins. According to Kazmierczak (1966) the anthocyanins from various berry juices can be completely absorbed on Amberlite IRC-50, IR-120 and Dowex 50 columns, but easily eluted only from Amberlite IRC-50; elution from the other resins is difficult.

Fuleki and Francis (1968) evaluating the performance of some ion exchange resins (Amberlite CG-50 and IR-4B from Rohm and Haas Co., Rexyn 102 from Fisher Sci. Co., AG-50 from Bio-Rad Lab., Zeo-Karb. Permutit Q and H-70 from Permutit Co.) on the recovery and concentrating power of cranberry anthocyanins found CG-50 to be the best. Based on the ionic structure of the molecule of anthocyanins Markakis (1960) used paper electrophoresis for separation and purification of anthocyanins of cherries.

## Quantitative Determination

Since anthocyanins absorb light in the visible region of the spectrum they can be measured by spectrophotometry. The amount of an individual anthocyanin present in a solution can be determined by measuring the absorbance of the solution at the $\lambda$max of this anthocyanin and knowing the molar absorptivity of that anthocyanin. Values of $\lambda$max and molar absorptivity for many anthocyanins are given in the literature. If more than one anthocyanins are present in the solution an average $\lambda$max and an average molar absorptivity must be used for the determination of total anthocyanin content. Fuleki and Francis (1968) used this method for determination of the total anthocyanin in cranberries. They used the 535 nm wavelength since the cranberry anthocyanins have absorption maxima in the 510-550 nm region, (Harborne, 1958) in which region other common phenolic compounds extracted with the anthocyanins do not absorb. Since the absorption maximum and the molar absorptivity affected by pH and the solvent (Harborne, 1958) in these determinations the same pH and solvent should be used as for determining the molar absorptivity.

If in the solution of the anthocyanins there are substances which absorb at the same wavelength as anthocyanins and interfere in the determination, the substractive or differencial methods used for measuring anthocyanin concentration. The substractive method consists in destroying (bleaching) the anthocyanins and measuring the absorption of the solution before and after bleaching. By substraction, the absorption of anthocyanin is found and converted to anthocyanin concentration by means of a calibration curve. Dickinson and Gawler (1956) used sodium sulphite for bleaching the anthocyanins working with syrups from bottled Victoria plums. Swain and Hillis (1959) used hydrogen peroxide as a bleaching agent. Hydrogen peroxide destroyes irreversible the anthocyanins and they cannot be recovered as they can after treatment with sodium sulphite.

The differential method used first by Sondheimer and Kertesz (1948) based on the observation that pH changes cause changes in the absorbance of the anthocyanins but they do not change the absorbance of other substances (products of browning reaction or of degradation of anthocyanins) present in the solution. By substracting the values obtained at two different pH levels the absorbance due to the anthocyanins is found. The differential absorbance is converted to anthocyanin concentration by means of a caliblation curve. For highest sensitivity and accuracy the absorbance difference should be the greatest possible between the two pH values, the anthocyanin should be stable at both levels during determination and slight deviation of pH around these values should not cause large changes in absorbance.

Fuleki and Francis (1968) proposed the use of the degradation index as indicative of the proportion of degraded anthocyanin in a sample, particularly in cases where the original anthocyanin content is not known, and a measure of the state of anthocyanin degradation is needed. Degradation index is defined the ratio of absorbances of a sample at two different pH levels, one at which the pigment absorbs strongly and another at which the degradation products of the pigment absorb. Absorption ratios such as 515/415 for cranberry juice, 440/500 strawberry juice, and 520/420 for grape juice and 420/520 for wine are used widely (Fuleki and Francis, 1968).

## Degradation of Anthocyanins

Because of the electron deficiency of the flavylium nucleus, anthocyanins are highly reactive compounds and very easily undergo structural and color changes under the conditions used in processing and storage. This electron deficiency makes anthocyanins succeptible to attack by nucleophilic reactants such as, water, peroxides, sulfur dioxide, etc. (Jurd, 1972).

Kinetic investigations have been done to study the factors that affect the rate of pigment breakdown in strawberry, raspberry, cranberry (Sondheimer and Kertesz, 1951, 1953; Markakis _et_ _al_., 1957; Daravingas and Gain, 1968; Starr and Francis, 1968), and other fruits.

That ascorbic acid, naturally occurring in plant tissues, play a significant role in the red color deterioration of fruit juices was first pointed out by Beattie _et_ _al_. (1943) who observed parallel losses of ascorbic acid and anthocyanins during storage. Sondheimer and Kertesz (1953) demonstrated that the breakdown of anthocyanin related to the rate of ascorbic acid oxidation. Under conditions which decrease the rate of oxidation of ascorbic acid (lack of oxygen and addition of thiourea) the rate of destruction of the main strawberry anthocyanin, pelargonidin-3-monoglucoside, was decreased indicating that it was the oxidation products of ascorbic acid, hydrogen peroxide, that interacts with the pigment than the ascorbic acid itself. Markakis _et_ _al_. (1957) concluded from work with model systems that in the simultaneous presence of oxygen and ascorbic acid the destruction of the strawberry main anthocyanin was greater than the addition of the single effects of oxygen and ascorbic acid, this indicating a positive interaction of these two factors in relation to the pigment. Starr and Francis (1968) reached the same conclusion studying the effect of different concentrations of ascorbic acid and different levels of headspace oxygen on the anthocyanin pigments of cranberry juice.

As ascorbic acid oxidation results in hydrogen peroxide formation, practical interest was generated on the effect that the $H_2O_2$ would have on the anthocyanins. Sondheimer and Kertesz (1952) have studied the kinetics of this oxidation and suggested two possible mechanisms for the reaction (Sondheimer and Kertesz, 1951).

Sugars, and other natural constituents, and their degradation products upon heating with acids (furfural and 5-hydroxymethyl furfural) accelerate also the degradation of anthocyanins (Meschter, 1953; Tinsley and Bockian, 1960; Markakis _et_ _al_., 1957; Daravingas and Cain, 1968).

Low pH within certain limits, has a stabilizing effect on the color of anthocyanins. Meschter (1953) showed that decreasing the pH in the range 5.0 to 1.0 increased the stability of pigment extracted from strawberries, in buffered strawberry juice concentrate. But for strawberry syrup the optimum pH was 1.8. Below that deterioration of the pigment was faster as pH decreased. He correlated that with the high rate of browning of sugars at low pH values and suggested that reactivity of these sugar degradation products with the pigment is more important than the stability contributed to the pigment by the low pH value. Daravingas and Cain (1968) studying the effect of hydrogen ion concentration on the thermal degradation of black raspberry anthocyanin in model systems, found that a decrease in pH from 4.25 to 0.95 enhanced the pigment retention.

Lucton et al. (1956) showed that pigment destruction is strongly pH-dependent in the presence of oxygen but it had little effect when the air replaced by nitrogen.

Treatment of fruits and juices with $SO_2$ results in bleaching of anthocyanins. This bleaching may be reversible or irreversible. The decoloration is a reaction between the anthocyanin carbonium ion and the bisulfite ion to form a colorless chromen-2 (or 4) -sulfonic acid (Jurd, 1962) which is similar in structure and properties to the anthocyanin pseudobase. In alkaline solution these sulfonic acids destroyed.

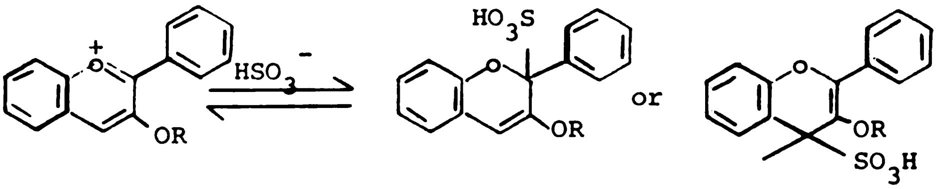

Figure 4. Decoloration of anthocyanins by bisulfite.

Decoloration of anthocyanins can also result of anzymatic activity. It may be caused by glycosidases (Huang, 1956) which hydrolyze the glycosidic bond and form unstable anthocyanidins or by phenolases. Peng and

Markakis (1963) showed that catechol is required for activation of phenolases and proposed the following mechanism for their action.

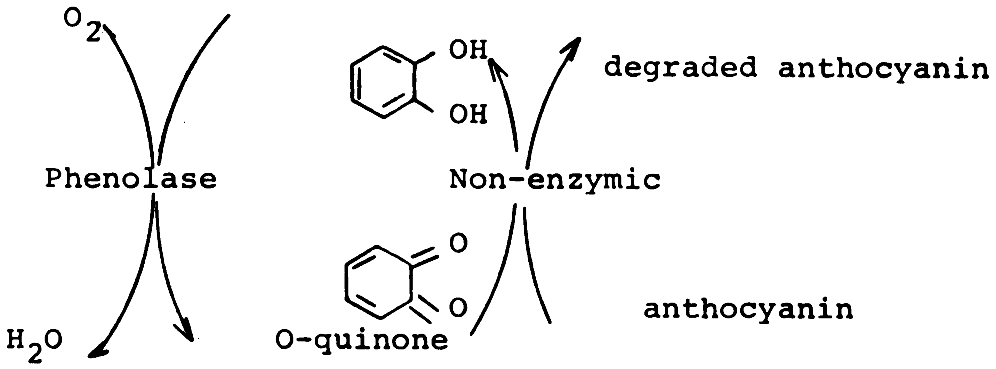

Figure 5. Degradation of anthocyanins by phenolase.

Horseradish peroxidase also was found to have decolorizing effect on anthocyanin pigments (Grommeck and Markakis, 1963).

The temperature during processing and the following storage affects the destruction of anthocyanins. Mackinney, Lukton, and Chichester (1955) showed on strawberry preserves that by low temperature vacuum evaporation 90% of the original anthocyanin may be retained in contrast to the 10-50% by conventional techniques. Meschter (1953) showed decrease of the half-life of strawberry preserves from 1300 hours to 240 hours as the storage temperature increased from 20 C to 38 C and he proposed storage temperature of 4 C to extend the half life to 6,000-8,000 hours. Markakis _et_ _al_. (1957), suggested a short time-high temperature treatment for higher retention of the strawberry anthocyanin and the use of the lowest possible temperature on storage.

Robinson _et al._ (1966) keeping under storage at 120 F wines containing a single isolated pigment, observed that diglucosides were more resistant in wines to browning than monoglucosides.

Van Buren _et al._ (1968) studying the color stability of wines during storage observed that light has a decolorizing effect on the pigments and that acylated pigments were more stable to light than the non-acylated. They also reported that diglucosidic pigments were more stable than the monoglucosidic to the combined effect of light (250 ft. candles) and temperature (50 C). Their results confirm the observation of Robinson and _et al._ (1962) on single purified pigments dissolved in white wines and stored at 120 F.

Based on informations available in literature Markakis (1974) summarized the degradation of anthocyanins into two possible pathways as shown in Figure 6.

According to the first pathway anthocyanins before hydrolysis of the glycosidic bond takes place, partially converted into their corresponding chalcones and partially decomposed with loss of the B ring to give coumarin diglycoside.

According to the second pathway, hydrolysis of the glycosidic bond first takes place and then anthocyanidins

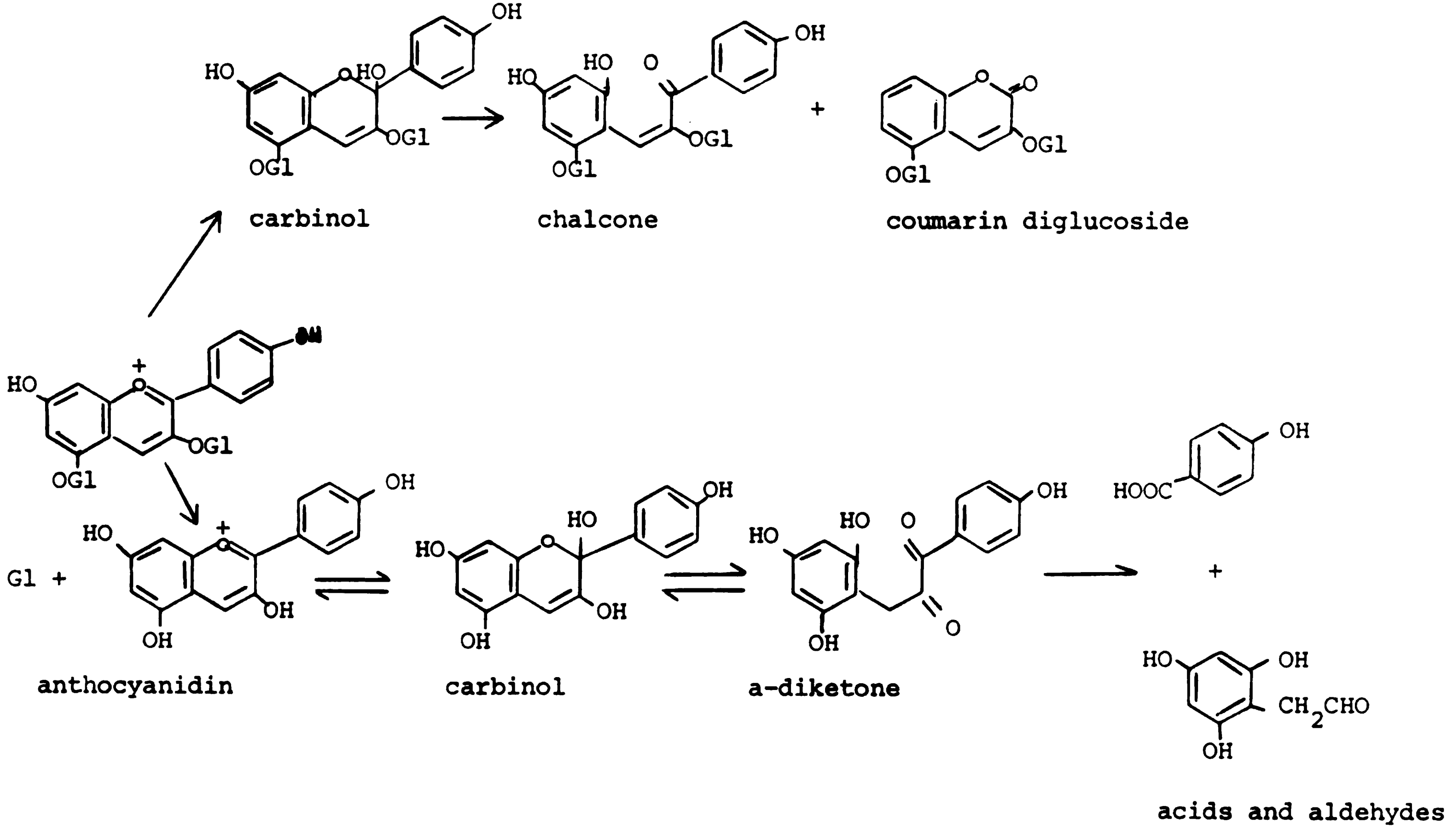

Figure 6. Degradation pathways of anthocyanins.

converted to a-diketones which are converted to acids and aldehydes.

# METHODS AND MATERIALS

The fermented undistilled grape pomace used for the extraction of anthocyanins was obtained from the Christian Brothers Winery in California, and it was of the Napa-Gamay grape variety. It was received in the laboratory by air mail in a plastic bag and stored in the freezer (-14 C). Storage at low temperature was necessary to prevent further fermentation of the pomace, growth of molds on it and possible degradation of the antocyanins.

Before extraction, the grape skins were separated by hand from the seeds and pieces of stems. Stems and seed extracted with the skins would impart to the anthocyanin extract considerable amounts of tannins.

An attempt to dry the pomace in air current for easier separation of the skins from the seeds resulted in very low anthocyanin recovery probably because of the oxidation of anthocyanins during drying.

The composition of the pomace was 45% seeds and stems and 55% skins. The moisture content of the skins was 60% as it was determined with an infrared moisture balance.

## Extraction

Two extracting liquids were used (a) boiling water and (b) aqueous solution of sulfur dioxide.

The $SO_2$ generated in a Kipp-type flask from $NaHSO_3$ and concentrated HCl and trapped in cold water. This concentrated $SO_2$ solution (solution A) was stored in the refrigerator and used for the preparation of the working sulfur dioxide solution (solution B) when it was required. The concentration of $SO_2$ in solution A, 9,000 ppm, was determined iodometrically. By successive dilutions, the solutions B were prepared containing 500, 1,000, 2,000, ppm $SO_2$.

Five grams of ground skins free from seeds and stems, and containing 60% moisture were mixed with five times as much extracting medium and ground with a mill capable of breaking cells (Polytron marketed by Brinkman). The extraction lasted 3 minutes and, after the Polytron was washed with the extraction liquid, the macerate, approximately 80 ml, was filtered under suction on a Whatman No. 1 filter paper through a Buchner funnel. The sediment was ground again three more times with fresh solvent. Filtration followed after each extraction and the four filtrates were combined, concentrated and analyzed for anthocyanin content. In the case of determining the proportions of anthocyanin extracted in each extraction, the extracts were concentrated, purified and measured for anthocyanin content each one separately.

For the purpose of preparing enough anthocyanin powder to be used with the beverages, each sample was extracted twice only, since it was found that the two first extractions give more than the 90% of the total extracted anthocyanin.

## Concentration

The filtrates concentrated in a flush evaporator at a 50-55 C temperature until a thick solution was obtained and the $SO_2$ (in the case it was used for extraction) escaped almost completely.

The concentrates transferred to a volumetric flask of 50 ml or 25 ml in the case of separate extracts and the flask of the evaporator was rinsed twice with small amounts of distilled water which was added to the concentrate. The concentrates were made to 50 ml or 25 ml with distilled water.

## Purification

The concentrates were separated from other particulate skin constituents (gums, proteins, pectin) by centrifugation in a SORVALL-superspeed RC 2-B automatic refrigerated centrifuge (30,000 xg).

## Anthocyacin Determination

Enocyanin A, a commercial coloring which contains less than 50% anthocyanin, and has the same maximum

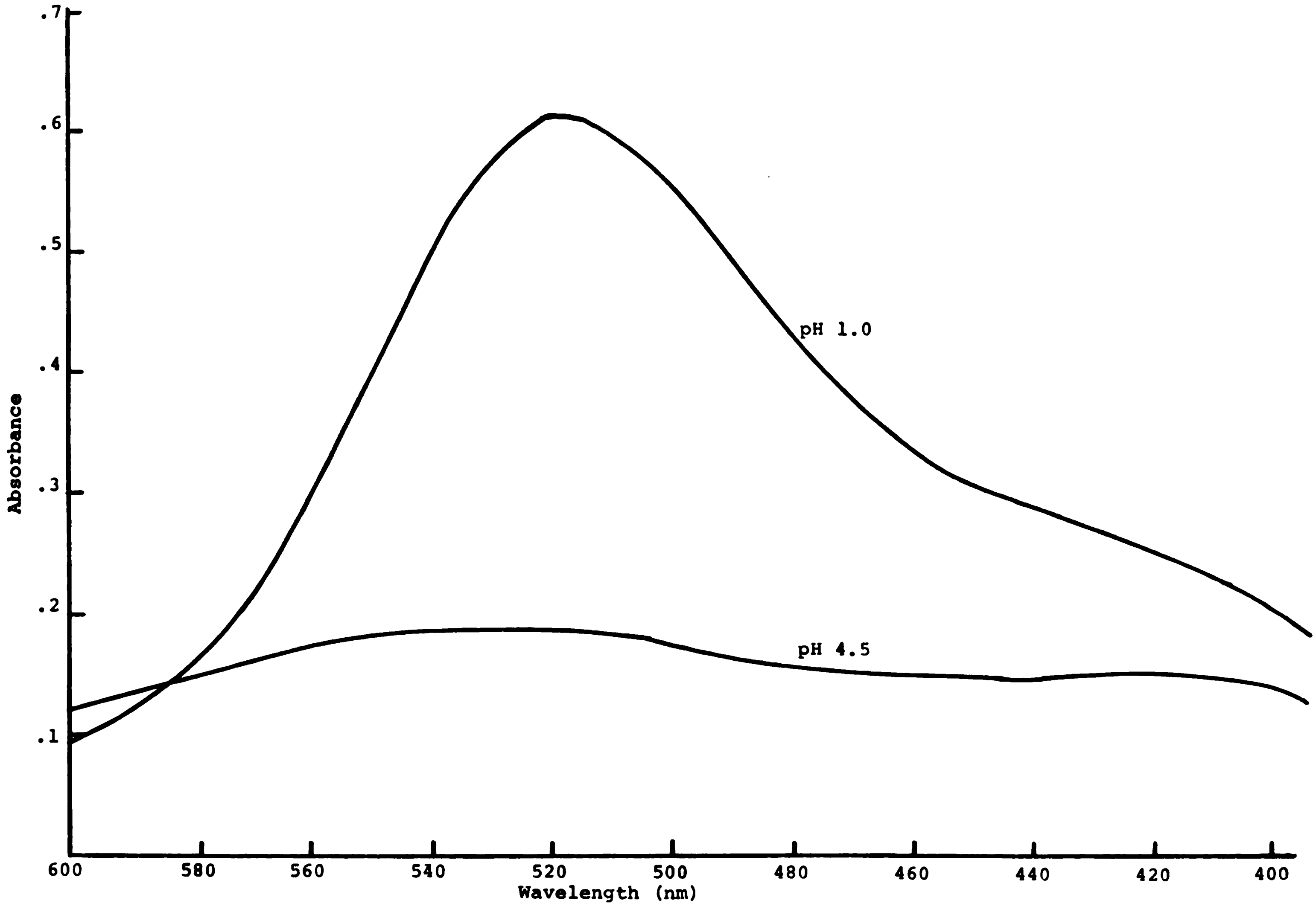

Figure 7. Absorption spectra of enocyanin A at pH 1.0 and pH 4.5

absorbance wavelength as the extract (Figure 7), 520 nm, was used as reference for color measurement.

Since in the crude extract of anthocyanins and in the beverages later anthocyanin degradation products might be present that could interfere with the determination of total anthocyanin, the differential method (pH 1.0 and 4.5) was used for the total anthocyanin determination. The wavelength used for comparing the absorbance of the extract at the two pH levels was 520 nm at which the extract showed the maximum absorbance (Figure 8).

The following buffers were used:

pH 1.0 : 0.12 M HCl - 0.05 M KCl

pH 4.5 : 0.05 M HCl - 0.5 M Sodium acetate

Two of 0.2 ml aliquots of the concentrate were diluted each with 9.8 ml of buffer pH 1.0. Similarly another two 0.2 ml aliquots were diluted with 9.8 ml of buffer pH 4.5. The diluted samples were allowed to equilibrate in the dark, at room temperature, for one hour. The absorbance of the samples at the wavelength at 520 nm was measured on a Beckman DU Spectrophotometer using the corresponding buffers as blanks. The absorbance difference was obtained by subtracting the absorbance at pH 4.5 from the absorbance at pH 1.0 and from the reference curve (Figure 10) the content of anthocyanin in equivalent mg enocyanin/ml was obtained. By using the proper dilution factors the anthocyanin content was calculated in g of standard enocyanin per 100 g moisture-free skins.

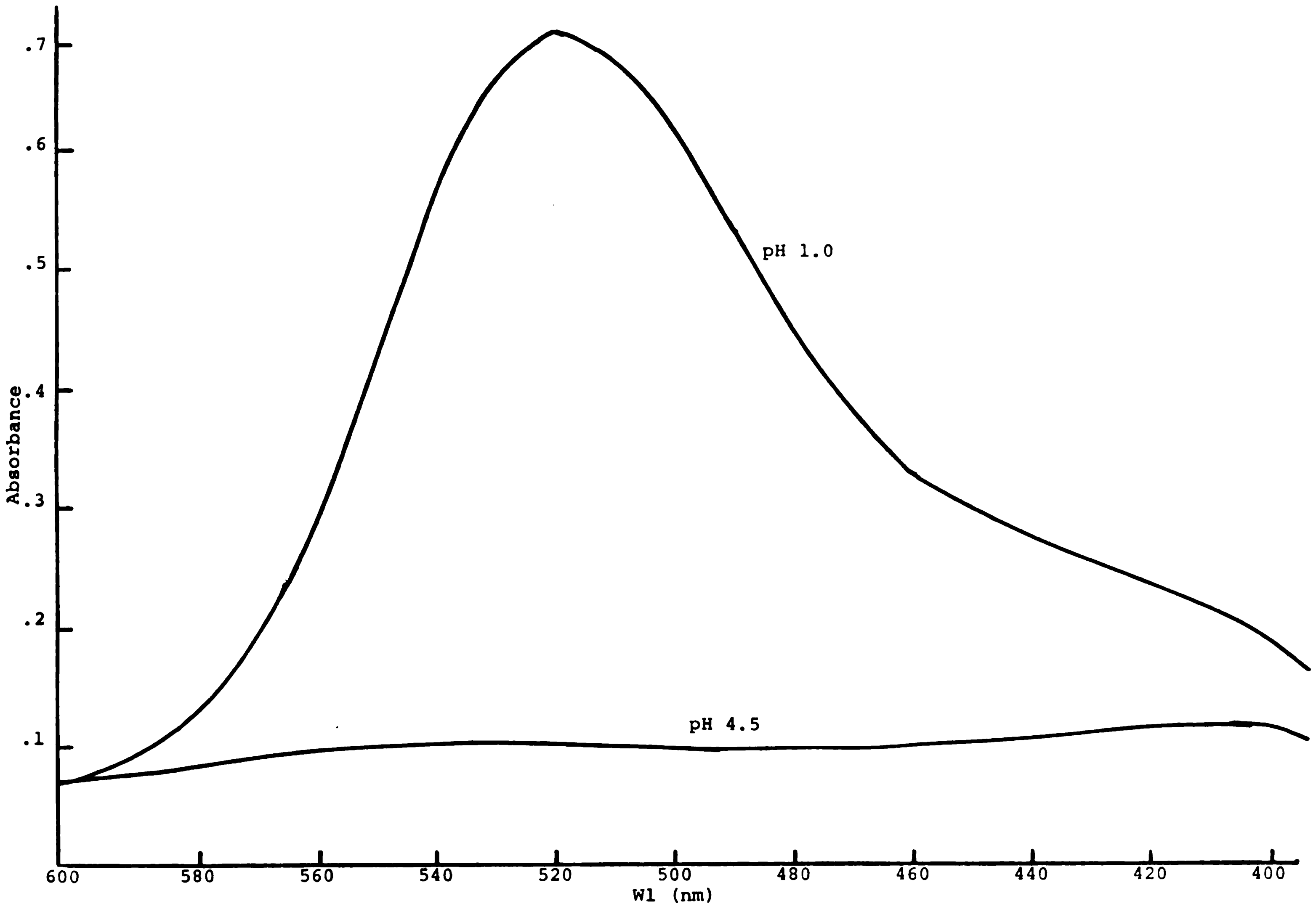

Figure 8. Absorption spectra of an anthocyanin extract from grape skins at pH 1.0 and pH 4.5.

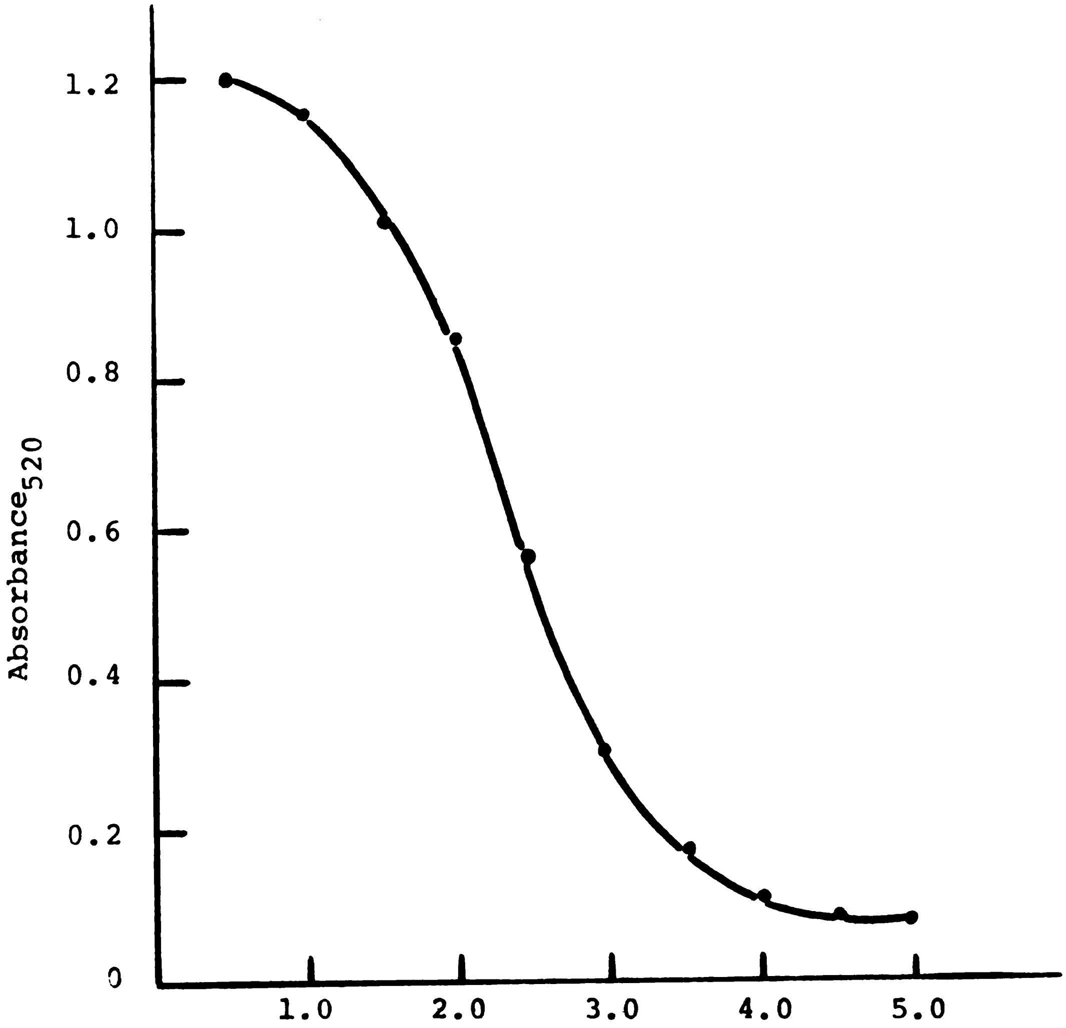

Figure 9. **Effect of pH on the absorbance of an anthocyanin extract from grape skins.**

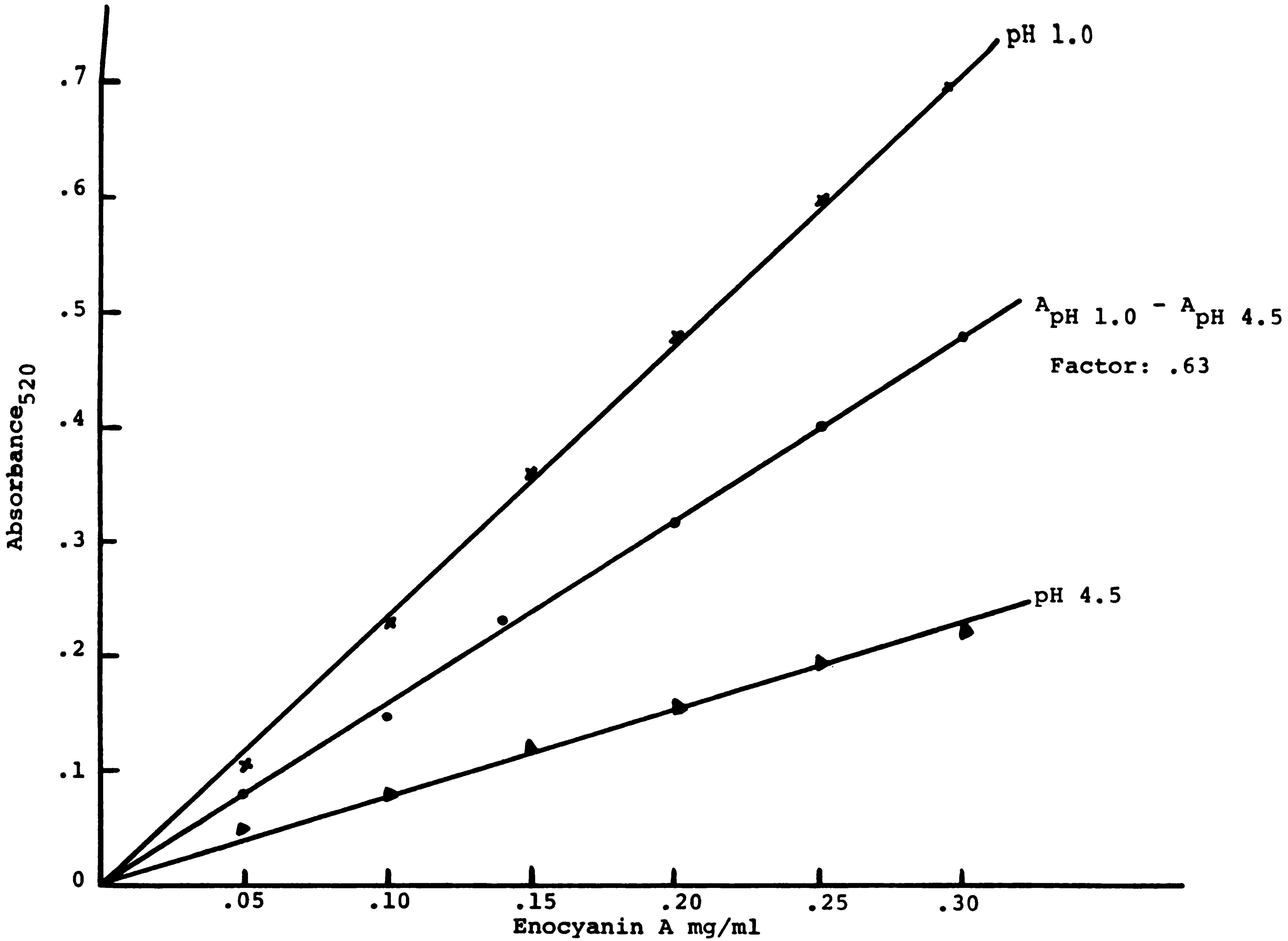

Figure 10. Standard curve of enocyanin A.

## Drying

The concentrated and purified extract was lyophilized. It was frozen solid in a flask using acetone and dry ice (solid $CO_2$). The flask was attached to a vacuum pump and the water removed by sublimation. After 24 to 30 hours the sample was dried completely.

## Preparation of the Beverages

Carbonated nonalcoholic beverages are defined as beverages that are generally sweetened, and flavored, sometimes acidified and containing salts and which are artificially impregnated with carbon dioxide (Jacobs, 1959).

Two types of sweeteners are used: natural and artificial. The most common natural sweeteners are sucrose, dextrose and fructose. The only two artificial sweeteners allowed to be used in the United States are saccharin and aspartame. Their principal functions are (a) to provide sweetness, to balance the acid and other tastes contributed by other ingredients, (b) in the case of sugars to give sufficient body to raise the beverage out of the watery class, and (c) to carry the flavor and deposit it uniformily when consumed. The major food value of carbonated beverages comes from the sugar ingredients.

The acids give tang to the flavor, act as preservatives by lowering the pH and contribute to the sour taste of the beverages. The most common used acids are citric, tartaric, malic, lactic, acetic and phosphoric acid or mixtures of them (Hall _et al_., 1972).

The flavors for soft drinks are made from essential oils, fruit juice concentrates, aromatic chemicals, extracts of various roots, herbs, beans and barks, and used mainly as blended flavors of two or more single ones.

Coloring agents used in beverages are almost exclusively the eight certified coal-tar dyes and caramel. In addition to these, there are also fruit and vegetable colors (Hall _et al_., 1972).

Carbon dioxide contributes a unique taste to carbonated beverages, giving them "life" and sparkle.

In solution it reacts with water to form carbonic acid, which dissociates to form a hydrogen ion and bicarbonate ion. Its solubility depends mainly on the pressure and temperature of the liquid into which it is dissolved and is affected slightly by the dissolved solids. The $CO_2$ dissolved is measured in "gas volumes," which are defined as the amount of gas in milliliters that a one ml volume of water will absorb at atmospheric pressure (760 mm Hg) and at 60 F.

The filling of beverages is made most often with the three-stage process, by which a measured amount of the flavored and acidified syrup is first placed in each bottle. Then the carbonated water is added, the bottles are closed and inverted for the syrup and carbonated water to be mixed.

An average composition of grape-flavored carbonated beverage is the following (Jacobs, 1959).

| Sugar % °Brix | $CO_2$ Gas Volume | Acid % As Citric |
|---|---|---|
| 12.2-13.2 | 2.2-2.4 | .10% |

The beverage prepared for these experiments had the following composition:

| Anthocyanin Extract, % w/v | Citric Acid % w/v | Na Benzoate % w/v | Flavor w/v | Sugar % w/v | $CO_2$ Gas Volume |
|---|---|---|---|---|---|
| 0.7 | 0.10 | 0.05 | 0.205 | 13.0 | 1.7 |

Na benzoate was used as preservative at the concentration commonly used for carbonated beverages (Furia, 1973). The grape flavoring used was provided by the Coca Cola Export Corporation.

Two batches of beverages, 1600 ml each, were prepared differing only in the way the anthocyanin extract used was extracted from the grape skins; namely either boiling water or 500 ppm $SO_2$ solution.

In a 2000 ml Erlenmeyer flask containing approximately 400 ml distilled water the proper amounts of ingredients were added and dissolved with the following order: (a) Na benzoate, (b) anthocyanin extract, (c) flavor, and (d) acid. After they were mixed well, the syrup was added and the volume made to 1600 ml. The syrup was prepared by dissolving the proper amount of sugar in distilled water and boiling for 5 minutes for the purpose of pasteurization. After mixing well the beverage was cooled to OC by keeping it in an ice bath in the refrigerator. The beverage was then transferred into a bottle equipped with a side tube near its bottom and $CO_2$ was bubbled into the beverage from a $CO_2$ storage tank while the bottle was kept under ice. Carbonation at atmospheric pressure and OC would result in 1.7 gas volume $CO_2$ in the beverage (Jacobs, 1953). Under continuous carbonation and at OC the beverage was transferred through the side tube into glass ampules 10 ml in each of them. The ampules were maintained deeped in ice until they were sealed in gas oxygen flame. Before sealing $CO_2$ was flushed repeatedly into the head space of the ampule so that the air was excluded completely. The resulting beverages had a pH 3.7 (measured with a pH meter, Model LS, SARGENT), soluble solids content 13.4° Brix and color intensity equivalent to 580.5 mg enocyanin per 100 ml beverage for the hot water anthocyanin extract (beverage A) and 640.0 mg per 100 ml beverage for the $SO_2$ anthocyanin extract (beverage B).

## Storage Conditions

Beverages A and B were stored under the following conditions:

a. In darkness at 3.5 ± 2C

b. In darkness at 10 ± 2C

c. In darkness at 20 ± 2C

d. In darkness at 38 ± 1C (a common U.S. army food storage specification)

e. In diffused day light and 20 ± 2C

f. In continuous fluorescent light (80 ft. candles) and 22 ± 2C

Determination of the color intensity was made every 15 days, and the loss of anthocyanin was followed. In each analysis two ampules were analyzed and dublicate determination of the color of each ampule was made using the differential method, at pH levels 1.0 and 4.5. Two 0.5 ml aliquots of each ampule were made to 10.0 ml with buffer pH 1.0. Similarly another two 0.5 ml aliquots were made to 10.0 ml with buffer pH 4.5. The pH of the buffers did not change after mixing with the samples. The diluted samples were allowed to equilibrate in dark at room temperature for one hour. The absorbance of the samples were measured at 520 nm on the Beckman DU spectrophotometer using the buffers pH 1.0 and pH 4.5 as blank. The absorbance difference was obtained by subtracting the absorbance at pH 4.5 from the absorbance at pH 1.0. From the reference curve (Figure 10) the content of anthocyanin in equivalent of mg enocyanin/ml was obtained. By using the proper dilution factors the anthocyanin in the beverages was calculated as equivalent of mg of enocyanin per 100 ml beverage.

# RESULTS AND DISCUSSION

## Extraction

Two solvents were used in extraction of anthocyanins from the grape skins, (a) boiling water and (b) aqueous solution of sulfur dioxide. Sulfur dioxide is permitted as food additive. (In preparation of this extract the $SO_2$ was removed during concentration.) Both processes would stabilize the anthocyanin extract against enzymatic action since boiling water would destroy enzymes, while the $SO_2$ would inactivate them.

The quantitative recovery of the anthocyanins is shown in Table 1. The amount of anthocyanin recovered expressed as of enocyanin equivalent (EE)/100 g dry skins was almost the same with both solvents; 32.9 g with boiling water and 31.9 g with $SO_2$.

Concentrations of $SO_2$ higher than 500 ppm did not give considerable higher yields in anthocyanin. The yield with 1000 ppm $SO_2$ was 33.8 g of EE per 100 g moisture-free skin, while extraction with 2,000 ppm $SO_2$ gave an EE yield of 34.8 g/100 g moisture-free skins.

Evaluating the effect that soaking might have on the efficiency of extraction, samples after their first

Table 1.--Anthocyanin extracted from grape skins, in g of enocyanin equivalent/100 g of moisture free skins.

| Sample | With Boiling Water | With 500 ppm $SO_2$ in Water |
|---|---|---|
| 1 | 32.70 | 30.40 |
| 2 | 33.10 | 32.80 |
| 3 | 33.10 | 30.30 |
| 4 | 32.10 | 29.40 |
| 5 | 32.60 | 33.20 |
| 6 | 36.30 | 32.60 |
| 7 | 31.00 | 35.10 |
| Average | 32.90 | 31.90 |

maceration with the solvent in the Polytron, were left to soak for 0, 4, 8, and 16 hours. They were filtered and the extraction was repeated three more times without previous soaking. The results (Table 2), showed a small increase in the recovered pigment with increase in the soaking period for the $SO_2$ solution, which showed no considerable change in the case of boiling water.

Table 2.--Effect of soaking on the extraction of anthocyanin. (Pigment equivalent to g of enocyanin/ 100 g moisture-free skins.)

| Soaking Time, Hours | Boiling Water | 500 ppm $SO_2$ Solution |
|---|---|---|
| 0 | 31.30 | 32.00 |
| 4 | 31.00 | 34.10 |
| 8 | 29.50 | 36.30 |
| 16 | 28.50 | 35.20 |

The slight decrease in the amount of recovered anthocyanin with increasing soaking time in water might be due to the destruction of anthocyanin during the soaking period in water while the pigment was stabilized in the presence of $SO_2$. This almost complete recovery of the pigment without soaking probably is due to the very fine maceration of the skins by the mill (Polytron) used for extraction and the breaking down of the cells that allowed the pigment to diffuse very fast from its site to the solvent. Chiriboga and Francis (1970) reported increase of the extracting power of the solvent (acidified methanol) from 68% to 81% with increase of extraction time from 0.5 to 4.5 hours. Extraction was made by stirring the mixture of pomace and solvent and holding it at 23°F. Maceration of the sample for 2 minutes in Waring Blender preceded the extraction.

By concentrating, purifying and measuring the anthocyanin content of each extract separately the following profile of the extraction procedure was obtained (Table 3). The results show that 80% of the total pigment was recovered with the first extraction while with the two additional extractions almost all the pigment was obtained. The fourth extraction contributed very little to the recovery of the pigment. Extraction with either of the two solvents followed the same profile.

The anthocyanin content of the lypohilized pigments, determined with the differential method, was equal to 0.788 g enocyanin per 1 g for the pigment extracted with

Table 3.--Recovery of total anthocyanin from grape skins.

| | *Recovered total anthocyanin | | | |
|---|---|---|---|---|
| | With boiling water | | With 500 ppm $SO_2$ aqueous solution | |
| | as g enocyanin A per 100 g moisture free skins | % of total | as g enocyanin A per 100 g moisture free skins | % of total |
| First Extraction | 26.66 | 82.11 | 26.17 | 80.00 |
| Second Extraction | 3.93 | 12.17 | 4.39 | 13.33 |
| Third Extraction | 1.36 | 1.36 | 1.43 | 4.34 |
| Fourth Extraction | .47 | 1.46 | .75 | 3.14 |

*Numbers represent average of three samples.

boiling water and 0.905 g enocyanin per 1 g for the pigment extracted with $SO_2$ solution. This difference might be due to the fact that with boiling water other cellular constituents (tannins) extracted in higher amount than with the $SO_2$ solution and thus lowered the amount of anthocyanin per g of dry pigment.

## Stability

Both factors investigated in the stability experiment, i.e., temperature and light, were found to accelerate the rate of the anthocyanin degradation in the beverages. The stability of the pigment was studied by determining the color loss during the storage period, the rate constants for the reaction, the half life of the pigment under the storage conditions and their activation energy.

During the storage period the red color gradually diminished and a light brown color appeared eventually. This change in color was studied by determining the decrease in anthocyanin content and calculating the percent anthocyanin remained in the beverage.

Figures 11 and 12 show the effect that various storage conditions had on the anthocyanin content of the beverages. As the storage temperature increased from 3.5C to 38C, in the dark, the loss of anthocyanin increased reaching 10% after 135 days at 3.5°C and 77% in the same period of storage at 38°C, for the pigment extracted with boiling water. Ten degrees increase in temperature (comparison between 10C and 20C in the dark) resulted in an increase in loss from 16% to almost 30%, a $Q_{10}$ approximately 2. Exposure to daylight at 20C, resulted in a 20% increase in pigment loss (from 30% to almost 50%) at the end of 135 days, while storage under 22C and continuous fluorescent lighting resulted in 70% loss in anthocyanin.

Tables 4 and 5 show the concentration of anthocyanin in the beverages at the different intervals of the storage period, for the pigment which was extracted with boiling water and that extracted with 500 ppm $SO_2$ aqueous sln.

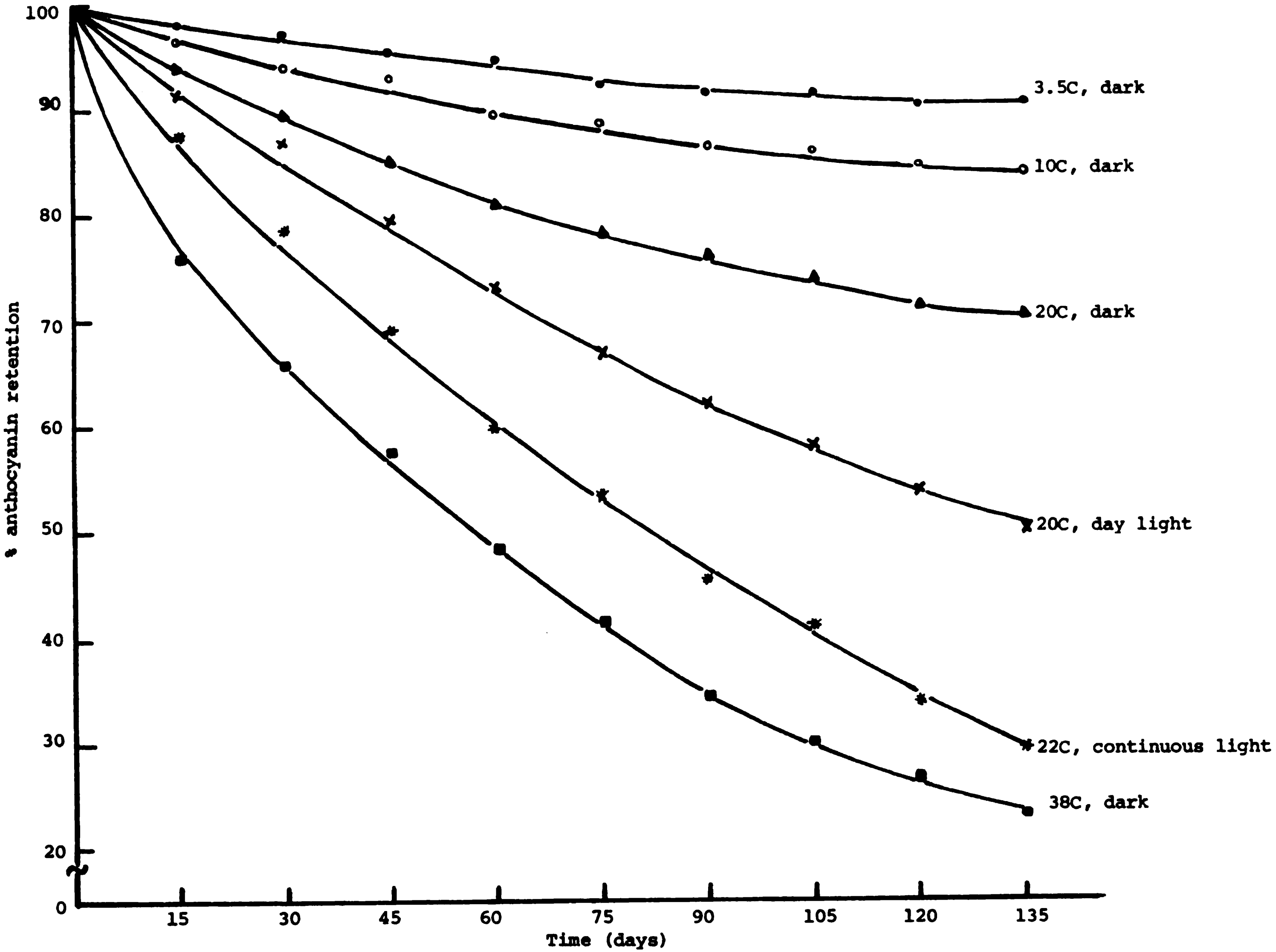

Figure 11. Effect of various storage conditions on anthocyanin retention in a beverage colored with anthocyanin extracted with boiling water from red grape skins.

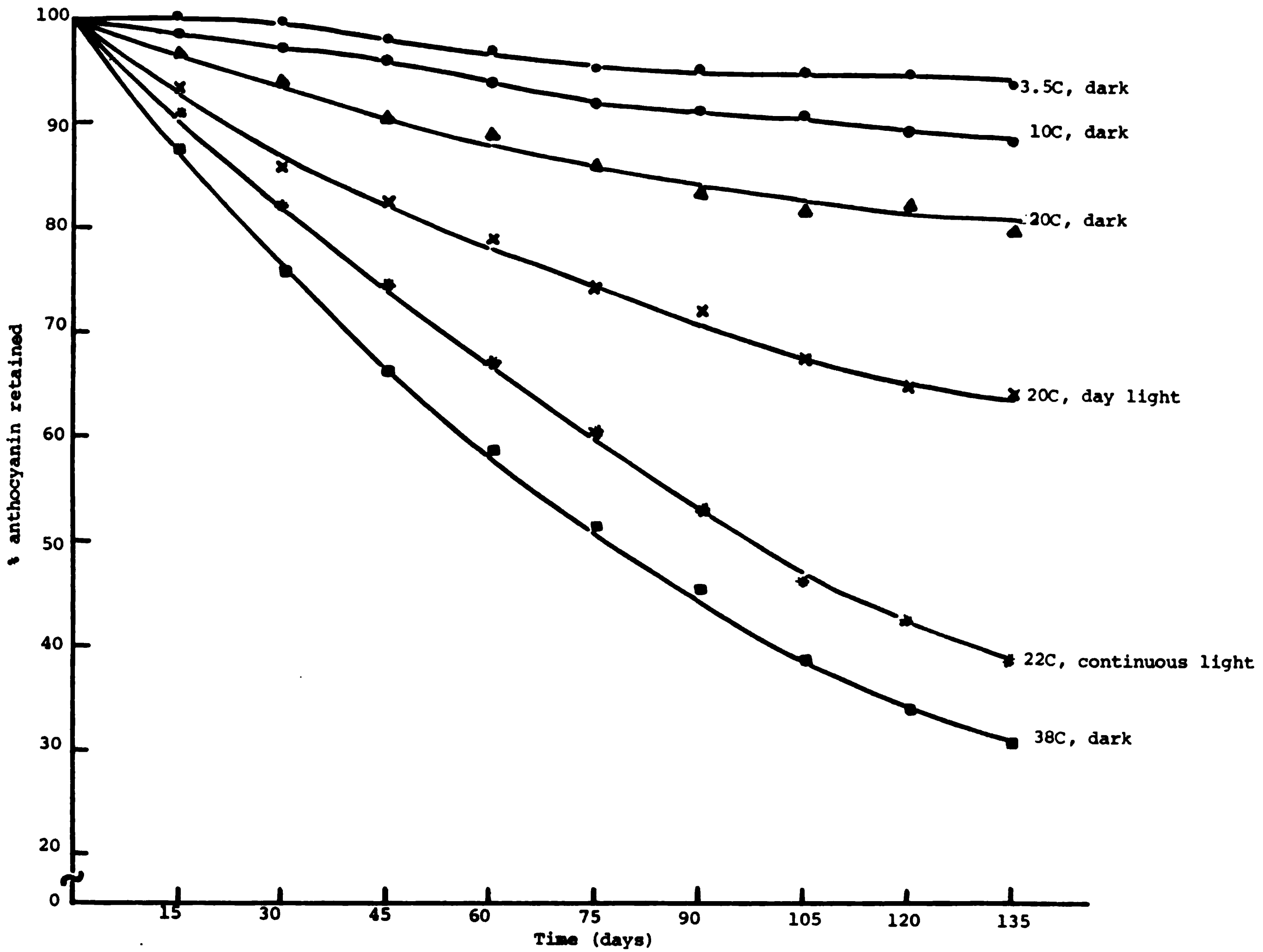

Figure 12. Effect of various storage conditions on anthocyanin retention in a beverage colored with anthocyanin extracted with 500 ppm $SO_2$ sln from red grape skins.

Table 4.--Anthocyanin concentration (EE mg/100ml beverage) in the beverages during storage. The pigment was extracted from grape skins with boiling water.

| Storage Conditions | Days | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 | 135 |
| 3.5C, dark | 580.5 | 568.8 | 563.0 | 554.3 | 548.5 | 536.9 | 531.1 | 531.0 | 524.8 | 525.3 |
| 10C, dark | 580.5 | 560.2 | 542.7 | 536.3 | 519.5 | 514.2 | 501.5 | 497.7 | 491.4 | 487.3 |
| 20C, dark | 580.5 | 545.7 | 519.8 | 493.4 | 527.5 | 452.8 | 441.2 | 429.5 | 415.0 | 410.4 |
| 20C, day light | 580.5 | 531.1 | 505.0 | 460.9 | 425.7 | 388.9 | 359.9 | 336.7 | 313.5 | 293.1 |
| 22C, continuous light | 580.5 | 507.9 | 455.7 | 400.5 | 348.3 | 309.8 | 265.3 | 238.6 | 195.0 | 172.1 |
| 38C, dark | 580.5 | 446.9 | 383.1 | 333.8 | 281.5 | 240.9 | 200.8 | 174.1 | 154.9 | 136.7 |

Table 5.--Anthocyanin concentration (EE mg/100ml beverage) in the beverage during storage. The pigment was extracted from grape skins with 500 ppm $SO_2$ aqueous sln.

| Storage Conditions | Days | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 | 135 |
| 3.5C, dark | 640.0 | 640.0 | 636.8 | 627.2 | 620.8 | 611.2 | 608.0 | 607.3 | 604.8 | 601.6 |
| 10C, dark | 640.0 | 632.3 | 620.8 | 614.4 | 601.6 | 588.8 | 584.4 | 582.4 | 572.5 | 566.4 |
| 20C, dark | 640.0 | 619.5 | 602.2 | 579.2 | 570.9 | 552.4 | 535.7 | 526.0 | 526.6 | 512.0 |
| 20C, day light | 640.0 | 599.0 | 550.4 | 528.0 | 505.6 | 476.8 | 462.1 | 428.8 | 416.3 | 409.6 |
| 22C, continuous light | 640.0 | 582.4 | 524.8 | 476.8 | 429.1 | 387.2 | 339.2 | 297.2 | 272.0 | 249.6 |
| 38C, dark | 640.0 | 560.0 | 486.4 | 425.6 | 374.4 | 330.7 | 291.2 | 255.2 | 217.6 | 197.5 |

## Order of the Reaction

For both extracts and under all the storage conditions studied the loss of anthocyanin was found to follow a first order reaction. By plotting the logarithm of concentration vs time straight lines were obtained for all cases (Figure 13 and 14).

The rate constants of the reaction for all cases calculated from the formula

$$K = \frac{1}{(\text{anth. conc.})\ \text{av.}} \times \frac{\Delta\ (\text{anth. conc.})}{\Delta t}\ \text{av.}$$

and the curves on Figure 11 and 12, where:

(anth. conc.) av. = is the average concentration of anthocyanin during the period of storage.

$\frac{\Delta\ (\text{anth. conc.})}{\Delta t}$ av. = the average change of the anthocyanin concentration divided by the time.

The values of K are given in Table 6.

## Half-Life

The half-life ($T_{1/2}$) of the pigment was calculated by the formula:

$$T_{1/2} = 0.693/K$$

where K: is the rate constant.

The values for both pigments under all storage conditions are given in Table 6.

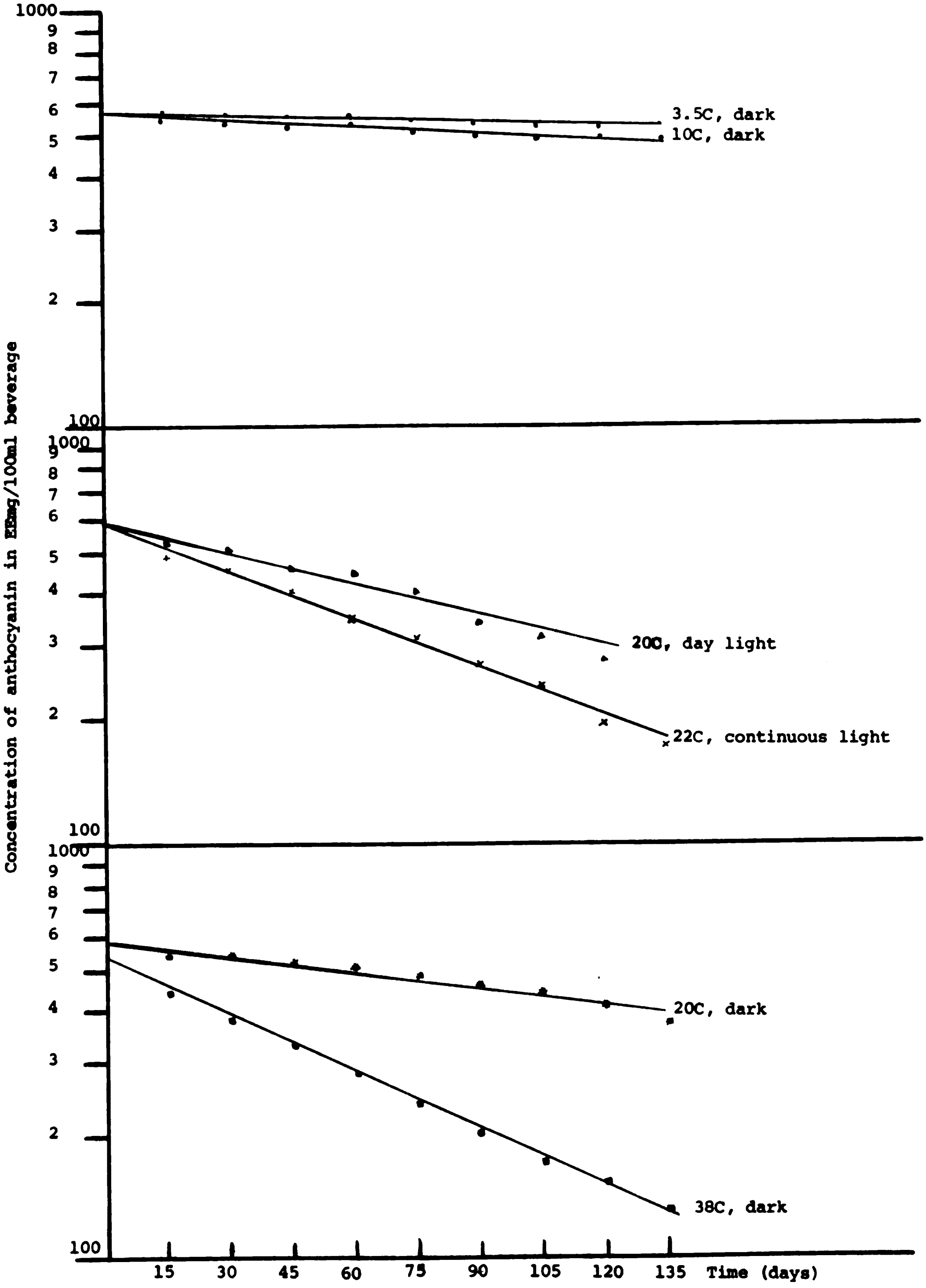

Figure 13. Relation between log concentration of anthocyanin and storage period showing first order reaction. (Pigment extracted with boiling water.)

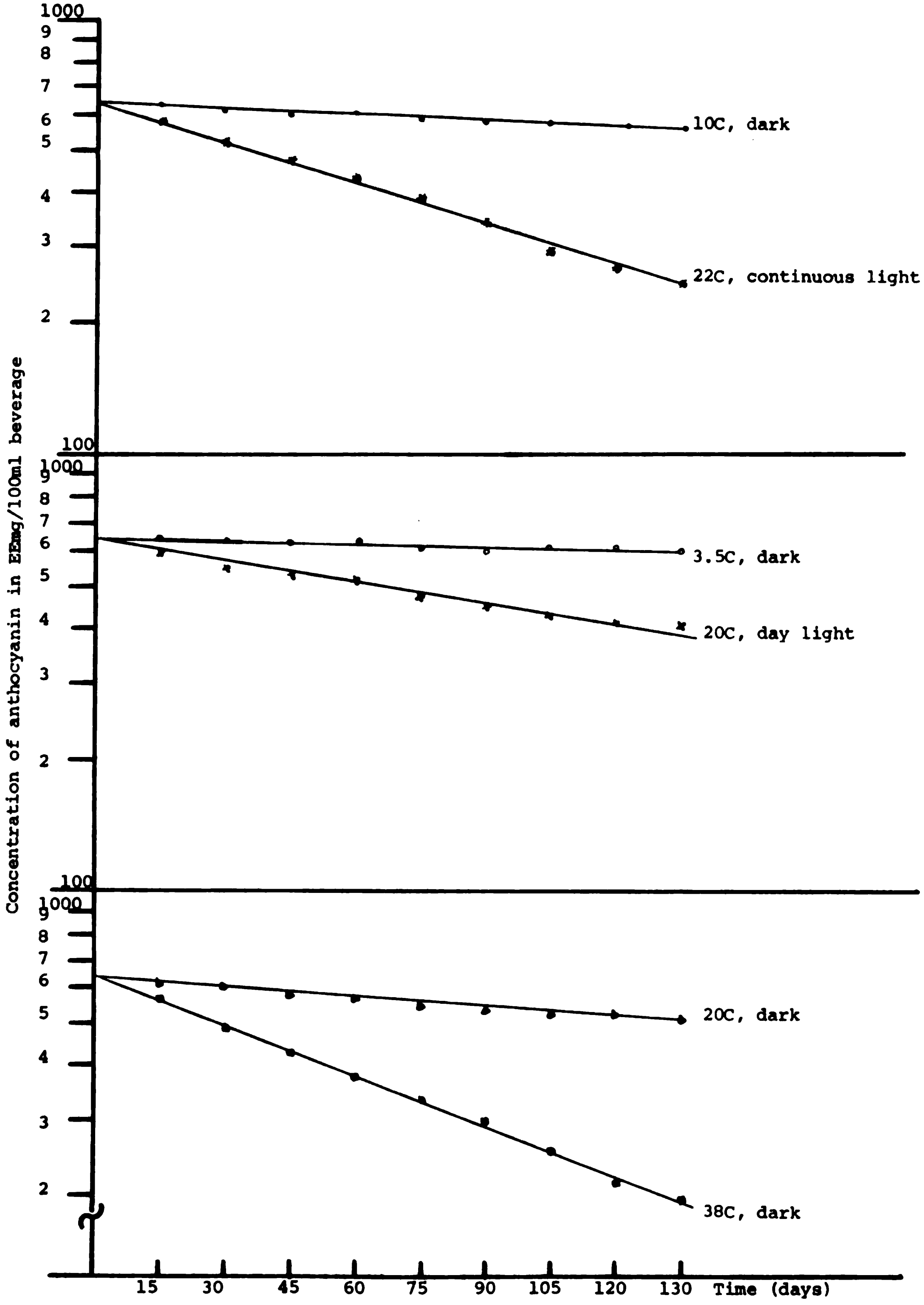

Figure 14. Relation between log concentration of anthocyanin and storage period showing first order reaction. (Pigment extracted with 500 ppm $SO_2$ sln.)

Table 6.--First order rate constants (K) (days$^{-1}$) and half-life ($T_{1/2}$) x days of the pigments.

| Storage Conditions | Anthocyanin extracted with | | | |
|---|---|---|---|---|
| | Boiling Water | | $SO_2$ aqueous sln | |
| | K | $T_{1/2}$ | K | $T_{1/2}$ |
| 3.5C, dark | 0.000736 | 941 | 0.000451 | 1536 |
| 10C, dark | 0.001320 | 525 | 0.000901 | 769 |
| 20C, dark | 0.002684 | 258 | 0.001665 | 416 |
| 20C, day light | 0.005068 | 136 | 0.003518 | 197 |
| 22C, continuous light | 0.008715 | 79 | 0.006836 | 101 |
| 38C, dark | 0.011123 | 62 | 0.008684 | 80 |

## Activation Energy

The activation energy was calculated by using data from the storage conditions at 3.5C and dark, 10C and dark, 20C and dark, and 38C and dark and the Arrhenius equation:

$$K = A\ e^{-E^*/RT}$$

which expresses the relation between the rate constant K and the temperature.

The Arrhenius equation converted to the logarithmic form:

$$\log_{10}K = \log_{10}A - \frac{E^*}{2.303RT}$$

in which $-\frac{E^*}{2.303RT}$ expresses the slop (λ) of the straight line obtained by plotting $\log_{10}K$ vs $\frac{1}{T}$ (where T = temperature in degrees Kelvin = 273 + C). The plotting $\log_{10} K$ vs $\frac{1}{T}$ is shown in Figures 15 and 16.

The activation energy was calculated from the final equation:

$$E^{*} = -2.303 \times \lambda \times R$$

where R = 1.987 cal/mole (gas constant); and was found 13,743.69 cal/mole for the pigment extracted with boiling water and 14,729.37 cal/mole for the pigment extracted with $SO_2$ solution.

Both pigments were affected similarly by the storage conditions employed (comparison of Figures 11 and 12), although the loss of anthocyanin in the beverage colored with the boiling water extract of grape skins was higher than that observed in the beverage colored with the $SO_2$ extract of grape skins under all storage conditions. This is also evident from the higher rate constants calculated for the extract with boiling water, under all storage conditions (Table 6) and its lower activation energy. The faster loss of anthocyanin in this case might be due to the fact that with boiling water more cellular constituents, that accelerate the loss of anthocyanin, were extracted than with the sulfur dioxide solution.

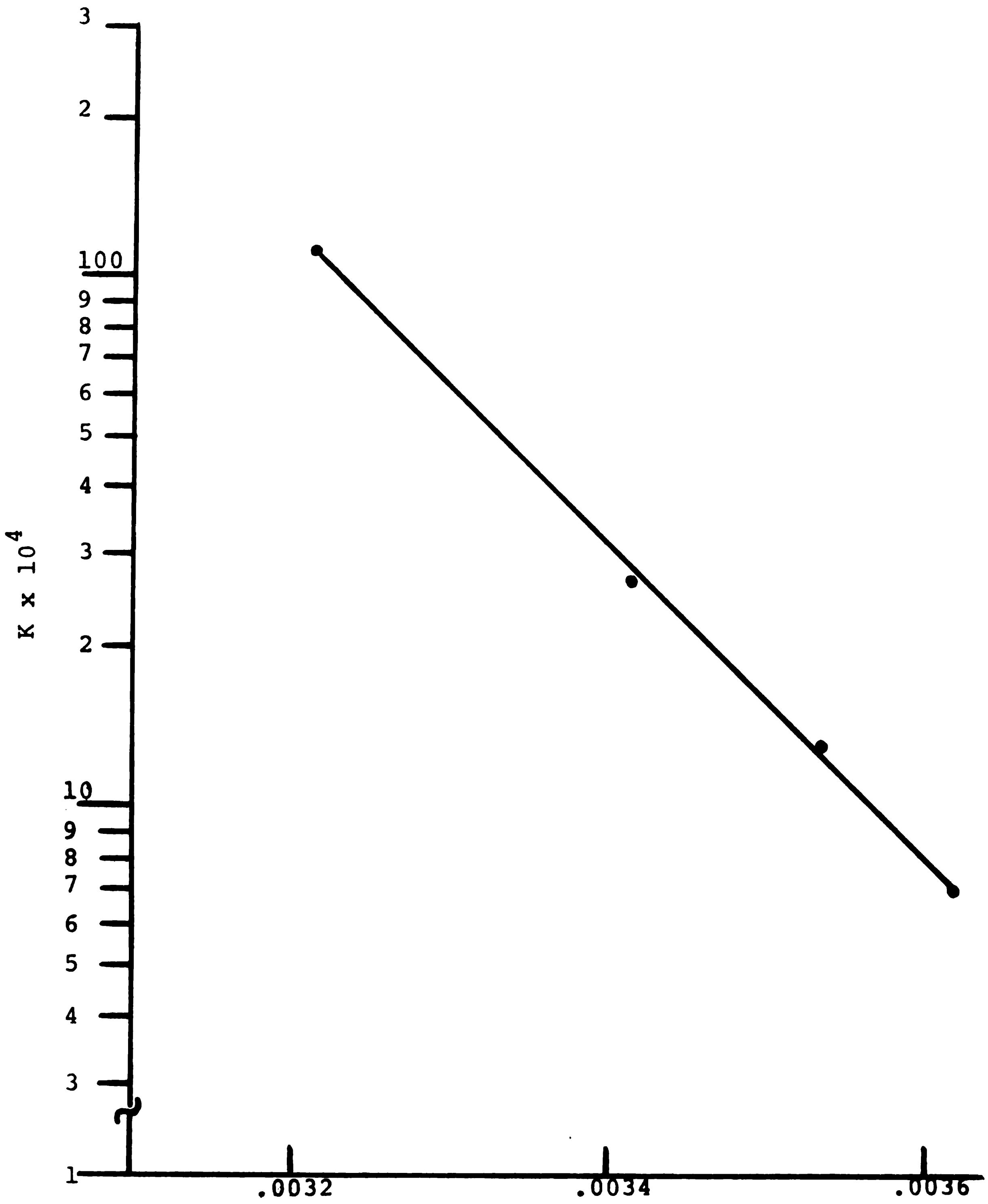

Figure 15. Plot of log K vs $\frac{1}{T}$ for the pigment extracted with boiling water.

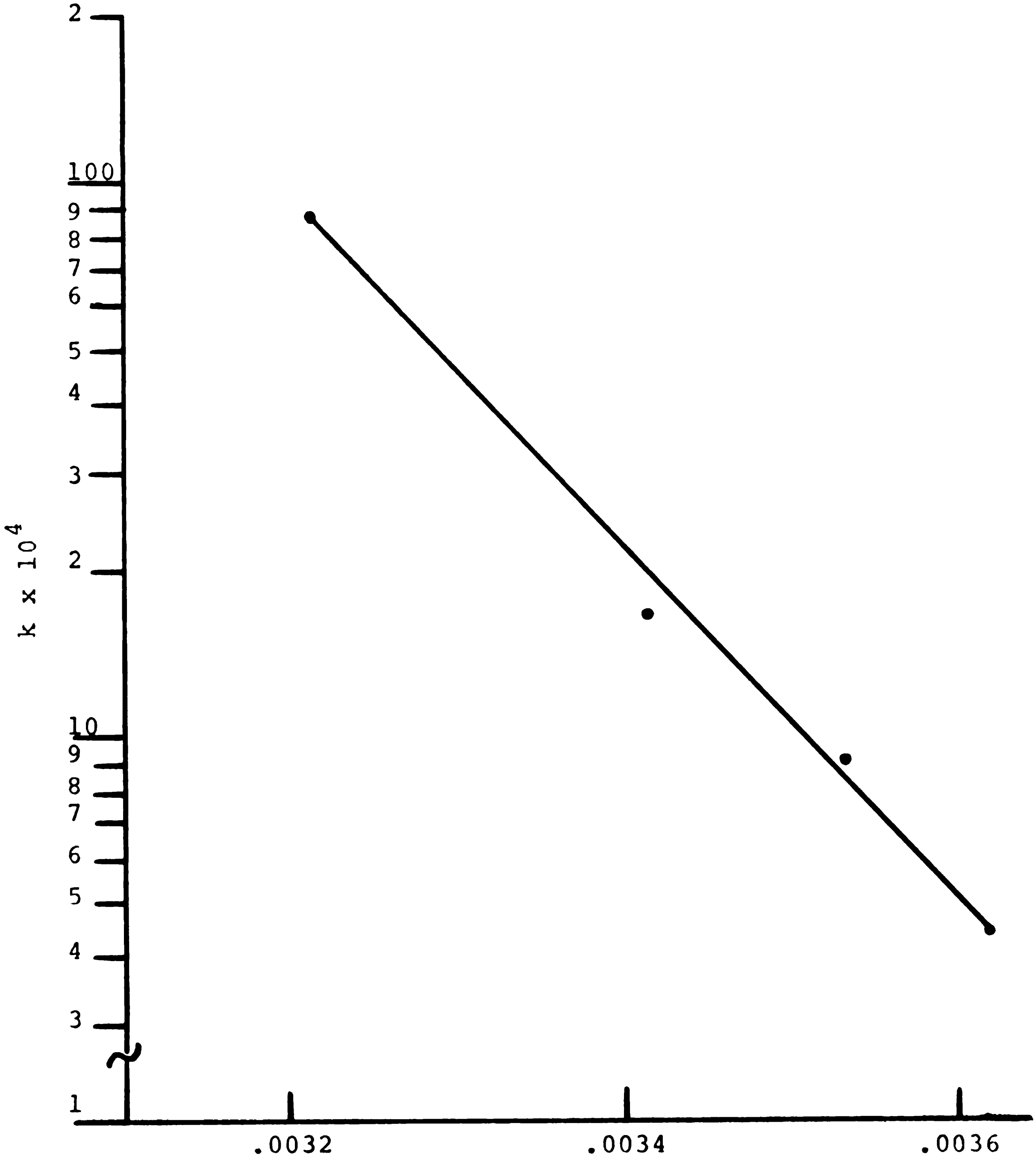

Figure 16. Plot of log K vs $\frac{1}{T}$ for the pigment extracted with 500 ppm $SO_2$ sln.

# SUMMARY AND CONCLUSIONS

The anthocyanins of the fermented Napa-Gamay grape skins were extracted and the extract was used as a coloring agent for a non-alcoholic carbonated beverage. The stability of the anthocyanin extract was studied under six different storage conditions.

Extraction was carried with boiling water and $SO_2$ solution in water in a mill capable of breaking cells (Polytron). Practically the same amount of anthocyanin was extracted by both solutions; i.e., 32.9 g enocyanin equivalent with boiling water and 31.9 g enocyanin equivalent with 500 ppm $SO_2$ solution per 100 g moisture-free skins. With 1,000 ppm $SO_2$ sln and 2,000 ppm $SO_2$ solution the enocyanin equivalents 33.8 g and 34.8 g per 100 g moisture-free skins were obtained, respectively.

With increasing soaking period, after the first maceration in a Polytron disintegrator, some decrease in the amount of anthocyanin recovered was noticed for the extraction with boiling water, while it was increased slightly in the case of 500 ppm $SO_2$ solution.

The pigment obtained by lyophilization of the extract with $SO_2$ solution contained more anthocyanin per g dry powder than the powder obtained from boiling water extract.

The storage conditions employed were:

a. Darkness at 3.5 ± 2C

b. Darkness at 10 ± 2C

c. Darkness at 20 ± 2C

d. Darkness at 38 ± 1C

e. Diffused daily light and 20 ± 2C

f. Continuous fluorescent light (80 ft. candles) and 22 ± 2C.

As the storage temperature increased the loss of pigment in the beverages increased. Light accelerated the anthocyanin destruction at the two temperatures tried.

The half-life of the anthocyanin in darkness varied from 62 days at 38C to 941 days at 3.5C for the anthocyanin extracted with boiling water. The half-life of the anthocyanin at 20C was 258 days in darkness and 136 days in diffused (northern window) day light for the extract with boiling water.

The anthocyanin extracted with $SO_2$ solution displayed greater stability than that anthocyanin extracted with boiling water; e.g., at 20C in darkness the $SO_2$ extract of anthocyanin had a half-life of 416 days, while that extracted with boiling water had a half-life of 258 days.

In both extracts the loss of pigment followed first order reactions.

The first-order rate constants for the pigment extracted with boiling water were higher than those for the pigment extracted with $SO_2$ solution.

The activation energy for the later was higher than the former extract thus showing higher stability of the pigment extracted with $SO_2$ solution.

Results of this experiment indicate that the anthocyanins extracted from grape skins can be used as colorants for carbonated beverages since.

1. Grape skins are available in large quantities as by-products of wine and grape juice industries and thus can be a cheap source of anthocyanin extracts.
2. With the usual chemical composition of carbonated beverages, a representative of which used in this experiment (pH 3.7, $CO_2$ 1.7, 13.4 °Brix), and for their marketing under "room temperature" (refrigeration is not necessary for their preservation) the half life of the pigments is long enough to permit their market. Lower pH or, refrigerated temperature if employed, will further enhance the retention of the color. The cans would be more suitable than the glass bottles for the protection of color, since the effect of diffuse day light decreases the half-life of the pigment almost to one half.

Further research should be directed towards the study of behavior of the pigment in commercial size containers and the possible use of the extract as coloring matter for other foods.

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# APPENDIX

# APPENDIX

I. Calculation of activation energy for the pigment extracted with boiling water.

From Figure 15 we take:

$$\text{slope } (\lambda) = \frac{\log 0.006250 - \log 0.001000}{0.003300 - 0.003565}$$

$$= \frac{-2.204100 + 3.000000}{-0.00265} = -\frac{0.795900}{0.000265}$$

$$= -3{,}003.39$$

$$E^{*} = -2.303 \times R \times \lambda = 2.303 \times 1.987 \times 3{,}003.39 =$$

$$= 13{,}743.69 \text{ cal/mole}$$

II. Calculation of activation energy for the pigment extracted with sulfur dioxide solution.

From Figure 16 we take:

$$\text{slope } (\lambda) = \frac{\log 0.008684 - \log 0.0004516}{0.003215 - 0.003616}$$

$$= \frac{-2.0615 + 3.3458}{-0.000399} = - \frac{1.2843}{0.000399}$$

$$= -3{,}218.79$$

$$E^* = - 2.303 \times R \times \lambda = 3{,}218.79 \times 1.987 \times 3{,}218.79 =$$

$$= 14{,}729.37 \text{ cal/mole}$$

III. Calculation of the rate constant K (days $^{-1}$) for the pigment extracted with boiling water under storage conditions. (Data obtained from Figure 11.)

A. <u>3.5C, dark</u>

| Time (days) | conc. of anth. (EE mg/ 100 ml beverage) | Δ(conc. of anthocyanin) | $\frac{\Delta(\text{conc. anth.})}{\Delta t}$ |
|---|---|---|---|
| 0 | 580.50 | | |
| 15 | 568.89 | 11.61 | .77 |
| 30 | 563.08 | 5.81 | .38 |
| 45 | 554.37 | 8.31 | .55 |
| 60 | 545.67 | 8.70 | .58 |
| 75 | 539.86 | 5.81 | .38 |
| 90 | 531.15 | 8.71 | .58 |
| 105 | 528.25 | 2.90 | .19 |
| 120 | 525.35 | 2.90 | .19 |
| 135 | 525.35 | 0.00 | .00 |

(conc. anth.) av. = 546.24 $\frac{\Delta(\text{conc. anth.})}{\Delta t}$ av. = .4022

$$\frac{1}{(\text{conc. anth.})\text{ av.}} = .001830$$

$$K = \frac{1}{(\text{conc. anth.})\text{ av.}} \times \frac{\Delta(\text{conc. anth.})}{\Delta t}\text{ av.} =$$

$$= .001830 \times .4022 = .000736$$

B. <u>10C, dark</u>

| Time (days) | conc. of anth. (EE mg/ 100 ml beverage) | Δ(conc. of anthocyanin) | $\frac{\Delta(\text{conc. anth.})}{\Delta t}$ |
|---|---|---|---|
| 0 | 580.50 | | |
| 15 | 560.18 | 20.32 | 1.35 |
| 30 | 545.67 | 14.51 | .96 |
| 45 | 534.06 | 11.61 | .77 |
| 60 | 519.54 | 14.52 | .96 |
| 75 | 507.93 | 11.61 | .77 |
| 90 | 502.13 | 6.80 | .45 |
| 105 | 496.32 | 5.81 | .38 |
| 120 | 490.52 | 5.80 | .38 |
| 135 | 487.62 | 2.90 | .19 |

$(\text{conc. anth.})\ \text{av.} = 522.44 \qquad \frac{\Delta(\text{conc. anth.})}{\Delta t}\ \text{av.} = .69$

$$\frac{1}{(\text{conc. anth.})\ \text{av.}} = .001914$$

$$K = \frac{1}{(\text{conc. anth.})\ \text{av.}} \times \frac{\Delta(\text{conc. anth.})}{\Delta t}\ \text{av.} = .001914 \times .69 =$$

$$= .001320$$

C. 20C, dark

| Time (days) | conc. of anth. (EE mg/ 100 ml beverage) | $\Delta$(conc. of anthocyanin) | $\frac{\Delta(\text{con. anth.})}{\Delta t}$ |
|---|---|---|---|
| 0 | 580.50 | | |
| 15 | 560.18 | 20.32 | 1.35 |
| 30 | 519.54 | 40.64 | 2.71 |
| 45 | 493.42 | 26.12 | 1.74 |
| 60 | 470.20 | 23.22 | 1.54 |
| 75 | 452.79 | 17.41 | 1.16 |
| 90 | 441.18 | 11.61 | .77 |
| 105 | 429.57 | 11.61 | .77 |
| 120 | 415.05 | 14.52 | .96 |
| 135 | 406.35 | 8.70 | .58 |

$$(\text{conc. anth.})\ \text{av.} = 476.87 \qquad \frac{\Delta(\text{conc. anth.})}{\Delta t}\ \text{av.} = 1.28$$

$$\frac{1}{(\text{conc. anth.})\ \text{av.}} = .002097$$

$$K = \frac{1}{(\text{conc. anth.})\ \text{av.}} \times \frac{\Delta(\text{conc. anth.})}{\Delta t}\ \text{av.} = .002097 \times 1.28 =$$

$$= .002684$$

D. 38C, dark

| Time (days) | conc. of anth. (EE mg/ 100 ml beverage) | $\Delta$(conc. of anthocyanin) | $\frac{\Delta(\text{conc. anth.})}{\Delta t}$ |
|---|---|---|---|
| 0 | 580.50 | | |
| 15 | 446.98 | 133.52 | 8.90 |
| 30 | 383.13 | 63.85 | 4.25 |
| 45 | 333.78 | 49.35 | 3.29 |
| 60 | 284.44 | 49.34 | 3.28 |
| 75 | 238.00 | 46.44 | 3.09 |
| 90 | 200.27 | 37.73 | 2.51 |
| 105 | 174.15 | 26.12 | 1.74 |
| 120 | 150.93 | 23.22 | 1.54 |
| 135 | 139.32 | 11.61 | .77 |

$$(\text{conc. anth.})\ \text{av.} = 293.07 \qquad \frac{\Delta(\text{conc. anth.})}{\Delta t}\ \text{av.} = 3.26$$

$$\frac{1}{(\text{conc. anth.})\ \text{av.}} = .003412$$

$$K = \frac{1}{(\text{conc. anth.})\ \text{av.}} \times \frac{\Delta(\text{conc. anth.})}{\Delta t}\ \text{av.} = .003412 \times 3.26 =$$

$$= .011123$$

E. <u>20C, day light</u>

| Time (days) | conc. of anth. (EE mg/ 100 ml beverage) | Δ(conc. of anthocyanin) | $\frac{\Delta(\text{conc. anth.})}{\Delta t}$ |
|---|---|---|---|
| 0 | 580.50 | | |
| 15 | 531.10 | 49.4 | 3.29 |
| 30 | 493.40 | 37.7 | 2.51 |
| 45 | 461.50 | 31.9 | 2.12 |
| 60 | 423.70 | 37.8 | 2.52 |
| 75 | 388.90 | 34.8 | 2.32 |
| 90 | 359.90 | 29.0 | 1.93 |
| 105 | 336.70 | 23.2 | 1.54 |
| 120 | 313.40 | 23.2 | 1.55 |
| 135 | 293.10 | 20.3 | 1.35 |

$(\text{conc. anth.})\ \text{av.} = 418.22 \qquad \frac{\Delta(\text{conc. anth.})}{\Delta t} = 2.12$

$$\frac{1}{(\text{conc. anth.})} = .002391$$

$$K = \frac{1}{(\text{conc. anth.})\ \text{av.}} \times \frac{\Delta(\text{conc. anth.})}{\Delta t}\ \text{av.} = .002391 \times 2.12 =$$

$$= .0050689$$

F. 22C, continuous light

| Time (days) | conc. of anth. (EE mg/ 100 ml beverage) | Δ(conc. of anthocyanin) | $\frac{\Delta(\text{conc. anth.})}{\Delta t}$ |
|---|---|---|---|
| 0 | 580.50 | | |
| 15 | 505.00 | 75.50 | 5.0 |
| 30 | 446.90 | 58.10 | 3.9 |
| 45 | 394.70 | 52.20 | 3.5 |
| 60 | 348.30 | 46.40 | 3.1 |
| 75 | 310.50 | 37.80 | 2.5 |
| 90 | 269.90 | 40.60 | 2.7 |
| 105 | 238.00 | 31.90 | 2.1 |
| 120 | 200.30 | 37.70 | 2.5 |
| 135 | 171.20 | 29.10 | 1.9 |

$$(\text{conc. anth.})\ \text{av.} = 346.5 \qquad \frac{\Delta(\text{anth. conc.})}{\Delta t}\ \text{av.} = 3.02$$

$$\frac{1}{(\text{conc. anth.})\ \text{av.}} = .002886$$

$$K = \frac{1}{(\text{conc. anth.})\ \text{av.}} \times \frac{\Delta(\text{conc. anth.})}{\Delta t}\ \text{av.} =$$

$$= .0028886 \times 3.02 = .008715$$

IV. Calculation of the rate constant K ($days^{-1}$) for the pigment extracted with 500 ppm $SO_2$ sln under storage conditions. (Data obtained from Figure 12.)

A. <u>3.5C, dark</u>

| Time (days) | conc. of anth. (EE mg/ 100 ml beverage | Δ(conc. of anthocyanin) | $\frac{\Delta(\text{conc. anth.})}{\Delta t}$ |
|---|---|---|---|
| 0 | 640.00 | | |
| 15 | 640.00 | 0.00 | 0.0 |
| 30 | 636.80 | 3.20 | .21 |
| 45 | 627.20 | 9.60 | .64 |
| 60 | 620.80 | 6.40 | .42 |
| 75 | 611.20 | 9.60 | .64 |
| 90 | 608.00 | 3.20 | .21 |
| 105 | 608.00 | 0.00 | .00 |
| 120 | 604.80 | 3.20 | .21 |
| 135 | 601.60 | 3.20 | .21 |

(conc. anth.) av. = 619.84 $\frac{\Delta(\text{anth. conc.})}{\Delta t} = .28$

$$\frac{1}{(\text{conc. anth.})\ \text{av.}} = .001613$$

$$K = \frac{1}{(\text{conc. anth.})\ \text{av.}} \times \frac{\Delta(\text{conc. anth.})}{\Delta t} = .001613 \times .28 =$$

$$= .0004516$$

B. <u>10C, dark</u>

| Time (days) | conc. of anth. (EE mg/ 100 ml beverage | $\Delta$(conc. of anthocyanin) | $\frac{\Delta(\text{conc. anth.})}{\Delta t}$ |
|---|---|---|---|
| 0 | 640.00 | | |
| 15 | 630.60 | 9.60 | .64 |
| 30 | 620.80 | 9.60 | .64 |
| 45 | 611.20 | 9.60 | .64 |
| 60 | 601.60 | 9.60 | .64 |
| 75 | 588.80 | 12.80 | .85 |
| 90 | 582.40 | 6.40 | .42 |
| 105 | 576.00 | 6.40 | .42 |
| 120 | 572.80 | 3.20 | .21 |
| 135 | 566.40 | 6.40 | .42 |

(conc. of anth.) av. = 599.04 $\frac{\Delta(\text{conc. anth.})}{\Delta t} = .54$

$$\frac{1}{(\text{conc. anth.})\ \text{av.}} = .001669$$

$$K = \frac{1}{(\text{conc. anth.})\ \text{av.}} \times \frac{\Delta(\text{conc. anth.})}{\Delta t} = .001669 \times .54 =$$

$$= .000901$$

### C. 20C, dark

| Time (days) | conc. of anth. (EE mg/ 100 ml beverage) | Δ(conc. of anthocyanin) | $\frac{\Delta(\text{conc. anth.})}{\Delta t}$ |
|---|---|---|---|
| 0 | 640.00 | | |
| 15 | 617.60 | 22.40 | 1.49 |
| 30 | 595.20 | 22.40 | 1.49 |
| 45 | 579.20 | 16.00 | 1.06 |
| 60 | 563.20 | 16.00 | 1.06 |
| 75 | 550.40 | 12.80 | .85 |
| 90 | 537.60 | 12.80 | .85 |
| 105 | 528.00 | 9.60 | .64 |
| 120 | 518.40 | 9.60 | .64 |
| 135 | 512.00 | 6.40 | .42 |

(conc. of anth.) av. = 564.12 $\frac{\Delta(\text{conc. anth.})}{\Delta t}$ av. = .94

$$\frac{1}{(\text{conc. anth.})\ \text{av.}} = .001772$$

$$K = \frac{1}{(\text{conc. anth.})\ \text{av.}} \times \frac{\Delta(\text{conc. anth.})}{\Delta t}\ \text{av.} = .001772 \times .94 =$$

$$= .001665$$

D. <u>38C, dark</u>

| Time (days) | conc. of anthoc. (EE mg/ 100 ml beverage) | Δ(conc. of anthocyanin) | $\frac{\Delta(\text{conc. anth.})}{\Delta t}$ |
|---|---|---|---|
| 0 | 640.00 | | |
| 15 | 560.00 | 80.00 | 5.33 |
| 30 | 486.40 | 73.60 | 4.90 |
| 45 | 425.60 | 60.80 | 4.05 |
| 60 | 371.20 | 54.40 | 3.62 |
| 75 | 326.40 | 44.80 | 2.98 |
| 90 | 281.60 | 44.80 | 2.98 |
| 105 | 246.40 | 35.20 | 2.34 |
| 120 | 217.60 | 28.80 | 1.92 |
| 135 | 198.40 | 19.20 | 1.28 |

(conc. of anth.) av. = 375.36 $\frac{\Delta(\text{conc. anth.})}{\Delta t} = 3.26$

$$\frac{1}{(\text{conc. anth.})\ \text{av.}} = .002664$$

$$K = \frac{1}{(\text{conc. anth.})\ \text{av.}} \times \frac{\Delta(\text{conc. anth.})}{\Delta t} = .002664 \times 3.26 =$$

$$= .008684$$

E. 20C, day light

| Time (days) | conc. of anth. (EE mg/ 100 ml beverage | $\Delta$(con. of anthocyanin) | $\frac{\Delta(\text{conc. anth.})}{\Delta t}$ |
|---|---|---|---|
| 0 | 640.00 | | |
| 15 | 592.00 | 48.00 | 3.2 |
| 30 | 553.60 | 38.40 | 2.5 |
| 45 | 524.80 | 27.80 | 1.8 |
| 60 | 499.20 | 25.60 | 1.7 |
| 75 | 476.80 | 22.40 | 1.5 |
| 90 | 454.40 | 22.40 | 1.5 |
| 105 | 432.00 | 22.40 | 1.5 |
| 120 | 419.20 | 12.80 | .8 |
| 135 | 409.60 | 9.60 | .6 |

$$(\text{conc. of anth.})\ \text{av.} = 500.16 \qquad \frac{\Delta(\text{conc. anth.})}{\Delta t}\ \text{av.} = 1.76$$

$$\frac{1}{(\text{conc. anth.})\ \text{av.}} = .001999$$

$$K = \frac{1}{(\text{conc. anth.})\ \text{av.}} \times \frac{\Delta(\text{conc. anth.})}{\Delta t}\ \text{av.} =$$

$$= .001999 \times 1.76 = .0035182$$

F. 22C, continuous light

| Time (days) | conc. of anth. (EE mg/ 100 ml beverage | Δ(conc. of anthocyanin) | $\frac{\Delta(\text{conc. anth.})}{\Delta t}$ |
|---|---|---|---|
| 0 | 640.00 | | |
| 15 | 582.40 | 57.60 | 3.84 |
| 30 | 524.80 | 57.60 | 3.84 |
| 45 | 476.80 | 48.00 | 3.20 |
| 60 | 428.80 | 48.00 | 3.20 |
| 75 | 387.20 | 41.60 | 2.70 |
| 90 | 339.20 | 48.00 | 3.20 |
| 105 | 297.60 | 41.60 | 2.70 |
| 120 | 272.00 | 26.60 | 1.70 |
| 135 | 249.60 | 22.40 | 1.50 |

$$(\text{conc. of anth.})\ \text{av.} = 419.8 \qquad \frac{\Delta(\text{conc. anth.})}{\Delta t}\ \text{av.} = 2.87$$

$$\frac{1}{(\text{conc. anth.})} = .002382$$

$$K = \frac{1}{(\text{conc. anth.})\ \text{av.}} \times \frac{\Delta(\text{conc. anth.})}{\Delta t}\ \text{av.} =$$

$$= .002382 \times 2.87 = .0068363$$