AN ABSTRACT OF THE THESIS OF

María Mónica Giusti Hundskopf for the degree of Master of Science in Food Science and Technology presented on April 7, 1995. Title: Radish Anthocyanin Extract as a Natural Red Colorant for Maraschino Cherries.

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Ronald E. Wrolstad

Red radish anthocyanin extract (RAE) was investigated for coloring brined cherries as an alternative to FD&C Red No. 40. Red radish (*Raphanus sativus* L.) anthocyanins were extracted from liquid nitrogen powdered epidermal tissue using acetone, partitioned with chloroform, and isolated using C-18 resin. The monomeric anthocyanin content was determined by pH differential to be 154 ± 13 mg/100 g of epidermal tissue (on pelargonidin-glucoside basis). The major pigments were identified as pelargonidin-3-sophoroside-5-glucoside monoacylated with *p*-coumaric or ferulic acids by HPLC and spectral analyses. Primary and secondary bleached cherries were sweetened to 40° Brix (pH of 3.50), and colored using two concentrations of RAE (600 and 1200 mg/L syrup, designated C1 and C2) and FD&C Red No. 40 (200 ppm). Color was measured for both cherries and syrup. Reflectance measurements (CIE L*, a*, b*), chroma and hue angle, showed that RAE imparted red color to the cherries and syrup extremely close to that of FD&C Red No. 40. RAE C2 gave the primary bleached cherries the closest color characteristics (L* = 18.20, a* = 20.00, b* = 8.47) to FD&C Red No. 40 (L* = 18.00,

a* = 24.35, b* = 12.13). RAE C1 gave the secondary bleached cherries the closest color characteristics (L* = 15.27, a* = 16.21, b* = 5.21) to FD&C Red No. 40 (L* = 16.38, a* = 19.91, b* = 8.99). Color and pigment stability of secondary bleached cherries were evaluated during a year of storage in the dark at 25°C. Monomeric anthocyanin degradation followed first-order kinetics and the half-lives were 29 and 33 weeks for syrups colored with RAE C1 and RAE C2, respectively. However, cherry color showed no significant changes in hue, color intensity nor lightness during storage. Color changes of syrup samples over time were dependant on anthocyanin concentration, higher anthocyanin concentration exerted a protective effect on color stability. Haze formation was observed in syrup samples colored with RAE, possibly due to pigment polymerization.

Syrup samples colored with RAE and FD&C Red No. 40 were also exposed to light for a year at 25°C. Light had a small but significant effect on L*, a*, and monomeric anthocyanin content.

From color and pigment stability data and visual observations we concluded that RAE was effective in coloring secondary bleached cherries with results very similar to those of FD&C Red No. 40 for 6 months of storage.

Radish Anthocyanin Extract as a Natural Red Colorant for Maraschino Cherries

by

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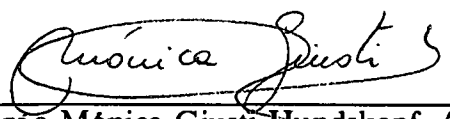

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Para tí...

Lucho

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF TABLES

RADISH ANTHOCYANIN EXTRACT AS A NATURAL RED COLORANT FOR MARASCHINO CHERRIES

INTRODUCTION

Interest in anthocyanins as coloring agents has increased as a result of the continuing official delisting of artificial food dyes, not only in the U.S. but also in many other countries. Anthocyanins are being considered as replacements of banned dyes. Some advantages of the use of anthocyanins are that they have been consumed for countless generations without apparent adverse effects, have bright attractive colors, and are water soluble, making easy their incorporation into aqueous food systems. Their disadvantages are low tinctorial power and stability, usually far below those of artificial dyes (Markakis, 1982).

Many different anthocyanins are currently being used as natural colorants: grape-skin extract, grape colorant and red cabbage, with purplish hue at pH levels above 3.5. Other fruit and vegetable juices are also accepted as anthocyanin natural colorants and exhibit a red hue at pH values below 3.8. However, there is still a demand for a natural red colorant that can effectively substitute for synthetic colorants FD&C Red No. 40 and FD&C Red No. 3.

The pigments of red radish have been identified by previous workers, the major pigments being pelargonidin-3-sophoroside-5-glucoside with p-coumaric, ferulic and caffeic acid esterified to the sugar substituents. Pelargonidin has an orange-red hue; acylation would be expected to shift the hue to a longer wavelength (red hue) and impart improved stability.

The objectives of this study were 1) the extraction, isolation, identification, and quantification of the anthocyanin pigments in the red radish (*Raphanus sativus* L.), cultivar Fuego, epidermal tissue, 2) the examination of the feasibility of the pigment isolate for coloring brined cherries for maraschino cherries, as an alternative to FD&C Red No. 40, 3) the evaluation of the color and pigment stability of cherries and syrup colored with radish anthocyanins, compared to FD&C Red No. 40, during a year of storage in the dark at 25°C and 4) the evaluation of the effect of light in color and pigment stability during the storage time.

LITERATURE REVIEW

COLOR IN FOODS

Food is eaten not only for nutrition, but also for pleasure. Therefore, taste, aroma, texture and appearance are of crucial importance for food (Food Advisory Committee, Great Britain, 1987; Robert-Sargeant, 1988). Consumers first judge the quality of a food product by its color: color helps us judge its wholesomeness, relish its flavor and appreciate its texture (Parkinson and Brown, 1981; Newsome, 1986). Indeed, the color of foods governs its palatability. Food without color or without the right color is either unacceptable or assumed to be so (Gordon and Bauernfeind, 1982; Robert-Sargeant, 1988; Hallagan, 1991). In 1958, Hall studied how people reacted to sherbets which had flavor and color mismatched. This study showed that color far outweighs flavor in the impression it makes on the consumer, even when the flavors are pleasant and the food is a popular one, and that color influences not only the consumer's ability to identify the flavor, but also his or her estimation of its strength and quality (Newsome, 1986). Color of food is more than a matter of taste (Robert-Sargeant, 1988). Foster, studying the psychological aspects of food color, listed 5 functions to be considered in understanding human reactions to color in foods: perception, for food selection or judgement of quality; motivation, since color of foods influences our desire or appetite for it; emotion, since attractive foods are considered as pleasure giving while unattractive foods are avoided as painful; learning what colors to expect, we usually predict the properties a food will have by

its color and our memory of similar appearance; and thinking, since our reaction to unusual properties may change if they have an explanation (Meggos, 1994).

Food colorants: color additives

Coloring foods to make them attractive was not an invention of contemporary society. Spices and condiments were probably used as color as long as 3,000 years ago (Newsome, 1986; Anonymous, 1988). From the earliest times, people added color to their foods: the Egyptians to their confectionery, the Romans to their wine, the Indians to their curries (Robert-Sargeant, 1988). The changing aspect in more recent times has been the choice of colorants (Gordon and Bauernfeind, 1982).

Color additives are defined by USA Food and Drug Administration (FDA) as follows:

> any dye, pigment or other substance made by a process of synthesis or similar artifice, or extracted, isolated, or otherwise derived, with or without intermediate or final change of identity, from a vegetable, animal, mineral, or other source and that, when added or applied to a food, drug, cosmetic or to the human body or any part thereof, is capable (alone or through reaction with another substance) of imparting a color thereto (FDA, 1986).

Food colors may be added to foods for the following reasons (Newsome, 1986; Dziezak, 1987):

- To restore the original appearance of the food where the natural colors have been destroyed by heat processing or storage.
- To ensure uniformity of color from batch to batch, due to natural variations in color intensity, ensuring equal acceptability.

- To enhance colors naturally occurring in foods but in less intensity than the consumer would expect.
- To give an attractive appearance to certain, otherwise virtually colorless foods (such as jelly, sweets and others).
- To help protect flavor and light sensitive vitamins during shelf-storage by a sun screen effect.
- To help preserve the identity or character by which foods are recognized.
- To serve as visual indication of quality.

However, colors should not be added to food products to hide poor quality, spoilage or adulteration (Newsome, 1986). Coloring should neither be merely used as a matter of fancy or fashion (Robert-Sargeant, 1988; Hallagan, 1991).

Federal regulations classify the approved color additives into two broad categories: certified colors and colors exempt from certification (Meggos, 1994). Certified colors are compounds that are chemically synthesized to a high degree of purity and are required to be tested every time they are produced. Color additives exempt from certification include colors obtained from natural sources: vegetables, animals or minerals, and "nature-identical" compounds, which are synthetic counterparts of natural derivatives (Dziezak, 1987). These color additives are also referred as "natural colors", even though FDA does not recognize the description "natural" for these colors (Lauro, 1991; Meggos, 1994). The process of colorant selection usually is accomplished by answering the following issues: the target shade, the physical/chemical attributes of the food, the processing (both by processor and

consumer), the type of packaging to be used, the storage conditions of the final product, the country where the product will be marketed, and regulation (Meggos, 1994).

Certified colors: synthetic colorants

The discovery of the first synthetic dye, mauve, in 1856 by Sir William Henry Perkins in England prompted search for other dyes (Newsome, 1986). Synthetic dyes were superior to many of the natural extracts in tinctorial value, stability and uniformity. Synthetic colors usually have stronger color; therefore, very small amounts are required. Furthermore, many are stable to light, and to sterilizing temperatures (Robert-Sargeant, 1988).

Seven synthetic colorants are currently permitted by the FDA for general use in foods and in orally ingested drugs and cosmetics: FD&C Red No. 40 (Allura Red), FD&C Red No. 3 (Erythrosine), FD&C Yellow No. 5 (Tartrazine), FD&C Yellow No. 6 (Sunset yellow), FD&C Blue No. 1 (Brilliant blue FCF), FD&C Blue No. 2 (Indigotine), and FD&C Green No. 3 (Fast green FCF). Citrus red No. 2 is permitted only for coloring skins of oranges and Orange B is permitted only for coloring the casings or surfaces of frankfurters and sausages (Parkinson and Brown, 1981; Hallagan, 1991, USA FDA, 1991). The seven FD&C colors have all undergone a complete battery of toxicity testing in a variety of species, including lifetime carcinogenicity studies, metabolism studies, reproduction and teratology studies, and other toxicological studies (Hallagan, 1991). Each batch of color made is tested by FDA for compliance with chemical specifications established by the agency. After

testing, the batch is issued a certified lot number and a certificate of acceptance or "certification". This process was made mandatory by the Federal Food, Drug and Cosmetic Act of 1938 (Dziezak, 1987). There are two forms of the FD&C color additives: FD&C Dyes and FD&C Lakes.

FD&C Dyes

The dye form is the color additive itself, and is water soluble (Hallagan, 1991). These compounds represent four distinct chemical classes (azo, trimethyl-methane, xanthene, and indigoid dyes) and the solubility is conferred by one or more sulfonic or carboxylic acids groups. Sulfonation decreases fat solubility and also appears to decrease toxicity of related compounds by enhancing urinary excretion of the dye and its metabolites (Parkinson and Brown, 1981). Dyes are manufactured in the form of powders, granules, liquids, blends, pastes and dispersions. The physical properties of each form confer advantages and disadvantages to the dye, making some forms more suitable for certain application over others (Dziezak, 1987). Their commercial use includes a variety of food products, carbonated beverages, still beverages, dry mixes, baked goods, confections, dairy products, sausage casings and pet foods. There are no specific concentration limits for dyes; however, good manufacturing practice suggests that they be used at less than 300 ppm (Newsome, 1986).

FD&C Lakes

The lake form of the color additive is the dye attached to an aluminum or calcium substrate in order to make it insoluble (Hallagan, 1991). Lakes are formed

by chemically extending the corresponding dye onto an alumina hydrate (aluminum hydroxide) substratum. The dye content generally varies from 10 to 40 percent. The color of lakes is manifested through dispersion, rather than solubilization as for dyes. Important commercial application of lakes include oil-based products and other types of products which do not contain sufficient moisture for dye solubilization. The certified FD&C lakes may also be used in foods or any materials that may come in contact with foods and be ingested (printing inks, paper or plastic). Typical applications include direct compression tablets and coated tablets, icings and fondant coatings, oil based coatings, cake and donut mixes, hard candy, gum products and many others. Lakes are generally more stable than dyes. It is believed that alumina substrate dissolves in the stomach, liberating the water soluble dye. Consequently, FDA considers lakes toxicologically equivalent to their corresponding dyes; however, the agency has not yet established regulations for the use of lakes in foods. Except for the lake of FD&C Red No. 40, all lakes are provisionally listed by FDA (Parkinson and Brown, 1981; Newsome, 1986).

The vast majority of certified colors in the United States are manufactured domestically. In 1990, 6.36 million pounds of FD&C dyes and 1.99 million pounds of FD&C lakes were produced in the USA and certified by the FDA, FD&C Red No. 40 being the dye that is produced and certified in the largest amount, 2.6 million pounds in 1990 (Hallagan, 1991).

FD&C Red No. 40

FD&C Red No. 40 (6-Hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonic acid, disodium salt), also known as allura red, was developed in the mid-1960's (Newsome, 1986). It has been approved in the USA for use in foods, drugs and cosmetics since 1971. Its use expanded dramatically in 1976, when FD&C Red No. 2 was banned by regulatory action. By the end of 1978, FD&C Red No. 40 was the most widely used of the certified colors, and it still is (Collins and Black, 1980; Borzelleca *et al.*, 1991). FD&C Red No. 40 is less expensive than all natural red colorants (Wiesenborn *et al.*, 1991). Approximately, 2,595,720 pounds were certified by FDA in 1990 (Hallagan, 1991). The maximum average anticipated human consumption in the USA is 12.45 mg/person/day, corresponding to a daily intake of 0.18 mg/Kg body weight/day for a 70-Kg person (Borzelleca *et al.*, 1991).

Canada and many countries of the European Economic Community initially refused to permit the use of the color in 1974 after scientists of the Canadian Health and Protection Branch (HPB) of Health and Welfare concluded that data submitted by the manufacturer to support its safety were inadequate. However, it was given approval for use in the USA. The major test on this food color was terminated after 21 months, instead of the scheduled 24 months, when pneumonia swept the rat colony. FDA accepted the test results to that date as being adequate proof of safety, while the Canadian HPB did not (Newsome, 1986). Since that year, many other studies have been completed (Collins and Black, 1980; Brown and Dietrich, 1983; Borzelleca *et al.*, 1989; Collins *et al.*, 1989; Borzelleca *et al.*, 1991), and no significant,

consistent, compound-related adverse effects have been reported. The dye is non-genotoxic, non-teratogenic, and long term toxicity/carcinogenicity studies have failed to demonstrate any compound-related adverse effects (Borzelleca *et al.*, 1991). After subsequent studies, the use of this colorant has been approved in Canada since 1986 (Newsome, 1986), and since 1991 in Japan (Hallagan, 1991). However, there is concern about the presence of impurities, which originate from the use of technical grade intermediates and from side reactions that occur during the manufacture of the color additive (Richfieldt-Fratz and Bailey, 1987). Of concern is the presence of *p*-cresidine, which is a contaminant of cresidine sulfonic acid and has been proven to be carcinogenic in laboratory animals.

Colors exempt from certification

Colorants from natural sources have been widely used in both animal feed and human food applications for quite some time. Particularly during the last decade there has been an increased tendency for the use of "natural" colorants as a consequence of both legislative action and consumer awareness to the use of synthetic additives in their food (Shi *et al.*, 1992b; Garcia and Cruz-Remes, 1993).

The colorants exempt from certification are pigments that are obtained from animal, vegetable, or mineral sources, and include (Freund, 1985):

- Colors obtained by direct use of approved pigmented vegetable and fruit products. Included in this group are dehydrated beets, dried paprika powder, dried turmeric powder, dried saffron powder, vegetable juices and fruit juices.

- Colors obtained by extraction and concentration of colors derived from approved plant materials. Included in this group are annatto extract, turmeric extract, tumeric oleoresin, paprika oleoresin, beet color extract, grape skin extract, grape color extract and carrot oil.
- Colors obtained by extraction and concentration of colors derived from approved animal materials. The only approved colors in this group are cochineal extract and carmine derived from the extraction of the red pigment from the insect *Coccus cacti.*
- Colors derived by heat processing of food-grade carbohydrates. The primary color in this group is caramel color.
- Pigments found in nature but chemically synthesized. Included in this group are the carotenoids, beta-carotene, beta-apo-8'-carotenal, and canthaxanthin
- Other coloring materials not requiring certification but limited in amounts and types of foods in which they may be used. Included in this group are ferrous gluconate, titanium dioxide, and riboflavin.

All these colorants are subjected to the same scrutiny as synthetic colorants prior to approval for use in foods, but they are not required to undergo certification testing by FDA (Newsome, 1986).

Although the use of "natural" colors is increasing, some of the properties of these substances make a wider-spread use by the food processing industry difficult. Some of their limitations are their performance in food processing, the lack of suitable physical presentations for adequate handling, reduced shelf life, stability to

light, etc. (Garcia and Cruz-Remes, 1993). Generally speaking, naturally derived colors are more expensive and more heat and pH sensitive, and these characteristics vary from product to product. Colors obtained from natural sources may also impart undesirable flavor and odor characteristics (Anchorage, 1992).

Red colorants exempt from certification

While food processors rely heavily on synthetic red colorants, there is increased consumer demand for natural colorants (Francis, 1989; Hong and Wrolstad, 1990; Wiesenborn *et al.*, 1991; Chandra *et al.*, 1993). FD&C Red No. 2 and FD&C Red No. 4 were delisted by the FDA in 1976. FD&C Red No. 3 was found to cause benign thyroid tumors in rats and banned for use in certain products in 1990, including all uses of FD&C Red No. 3 lake and cosmetic uses of the dye (Hallagan, 1991; Rumore *et al.*, 1992). The foods and ingested drug uses of this dye remain approved until some future regulatory action. The only remaining synthetic red colorant available for widespread use is FD&C Red No. 40. However, even if the use of FD&C Red No. 40 is permitted in the future, consumers increasingly perceive a natural food ingredient to be a more healthful ingredient (Wiesenborn *et al.*, 1991).

Cochineal and carmine

Carminic acid, also known as Natural Red 4, is normally contained in cochineal, a natural red dyeing agent obtained from an aqueous-alcoholic extract of the air-dried bodies of the pregnant female insect *Dactylopius coccus* Costa. This insect is indigenous to Central America, and a parasite of the plant *Opuntia ficus*

indica (Ford *et al.*, 1987; Mori *et al.*, 1991; Loprieno *et al.*, 1992). The dye obtained is widely used to impart various shades of red to food, cosmetics and drug products (Freund, 1985; Loprieno *et al.*, 1992). The term cochineal refers to red coloring material consisting of dried crushed bodies of female *D. coccus*, whereas carmine refers to the aluminum lake of the active principle, carminic acid, that has been extracted from the insect. The pigment carmine can be solubilized by alkali for various applications (Parkinson and Brown, 1981). However, the use of these colorants is limited by stability characteristics of the pigment (Freund, 1985). Cochineal, which has been called "the best of all natural colors" (Murai and Wilkins, 1990; Riboh, 1977), is extremely stable to heat and light but is susceptible to color change at low pH (Murai and Wilkins, 1990).

Carotenoids

Carotenoids are a group of aliphatic or aliphatic alicyclic acid, fat soluble compounds widely distributed in nature. These yellow, red and orange pigments are the most widespread class of natural colors in both plant and animal kingdom (Parkinson and Brown, 1981; Newsome, 1986; Dziezak, 1987). At least 300 naturally occurring carotenoids are related to the parent compound, lycopene, the red pigment of tomatoes, only a dozen of which are approved for food use. The most important commercial carotenoids are ß-carotene, ß-apo-8'-carotenal and canthaxanthin. Carotenoid food colorants may be natural or synthetic (Parkinson and Brown, 1981).

Carotenoids are sensitive to heat, light, or radiation, with the formation of 5,6-epoxide, which results in color loss. During food processing, thermal isomerization

is also a cause of color loss. Indirect oxidation can result from co-oxidation with unsaturated lipids. Despite these limitations and high cost, the carotenoids enjoy many advantages, including synthetic availability, highly desirable colors and high tinctorial strength, vitamin A activity, stability in the presence of reducing agents (ascorbic acid), non corrosivity, and antioxidant activity (Parkinson and Brown, 1981). They are also absorbers of light energy, oxygen transporters and powerful quenchers of singlet oxygen (Gordon and Bauernfeind, 1982).

Betalaines: betacyanins and betaxanthines

These are a small group of red and yellow plant pigments found only in the family *Centrospermae*, which includes red beets, pokeberries, cactus fruit, red chard and bougainvillea flowers (Newsome, 1986). Red-violet betacyanins and yellow betaxanthines are water soluble quaternary ammonium derivatives of 4-vinyl-5,6-dihydropyridine-2,6-dicarboxylic acid (Parkinson and Brown, 1981). The best known betacyanin in the red pigment of the table beet, whose purified form is betanine. Beets are the most common commercial source for these pigments, and they are approved for use in foods as beet powder or beet juice (Newsome, 1986). Betanines are stable between pH 4 and 7. Below pH 4 and above pH 7, the color shifts to red-violet to violet, respectively, due to changes in the ionization of acidic groups. The thermal stability of betanine is pH dependent and is greatest in the range of pH 4.0 to 5.0, and is enhanced when betanine is inside juice or other foods. Other factors that influence betanine stability include air, light, radiation, water activity, and metal ion contamination (Parkinson and Brown, 1981; Von Elbe *et al.*, 1974; Simon *et al.*,

1993). Natural sulfhydryl groups, as those present in the egg yolk, will react with the beet color extract causing degradation of the pigment, resulting in a yellow-brown color instead of the desired red shade (Freund, 1985).

Anthocyanins

Anthocyanins comprise a diverse group of intensely colored water-soluble orange, red, purple and blue pigments responsible for the appealing and often spectacular colors of many fruits, vegetables, flowers, leaves, roots and other storage organs (Francis, 1989; Newsome, 1986; Parkinson and Brown, 1981). Anthocyanins were probably the first flavonoids to be studied because of their visible colors and their high concentrations (Stafford, 1990). These ubiquitous compounds are fascinating in that they can exist in many structural forms, both simple or complex, with profound effects on their colors and stabilities. Anthocyanins in flowers are believed to ensure fertilization and seed dispersal by animals and in leaves, they act as light screens against UV radiation and have been associated with resistance to pathogens. Anthocyanins have also been implicated as enhancers of photosynthesis in leaves of tropical rain forest plants (Mazza and Miniati, 1993). Interest in anthocyanins has increased in recent years because of their potential as natural colorants and their beneficial therapeutic properties. Anthocyanins are used to enhance sight acuteness in ophthalmology, for treatment of capillary permeability and fragility in illnesses involving tissue inflammation, and for treatment of ulcers. Extracts of blueberries *Vaccinium myrtillus*, containing a rich mixture of simple

glycosides of common anthocyanidins, have been shown to inhibit blood platelet aggregation and to inhibit porcine elastase *in vitro* (Mazza and Miniati, 1993).

As many as 260 different naturally occurring anthocyanin pigments have been reported (Francis, 1989). Anthocyanins consist of the aglycon, or flavylium nucleus (chromophore), with a C6-C3-C6 structure, one or more sugar groups and often acylating acids (Figure 1). Of the 17 naturally occurring aglycons only six commonly occur in foods: cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin. The sugars substituted on the aglycon are in order of occurrence in nature: glucose, rhamnose, xylose, galactose, arabinose. They occur as monoglycosides, diglycosides or triglycosides (Francis, 1989).

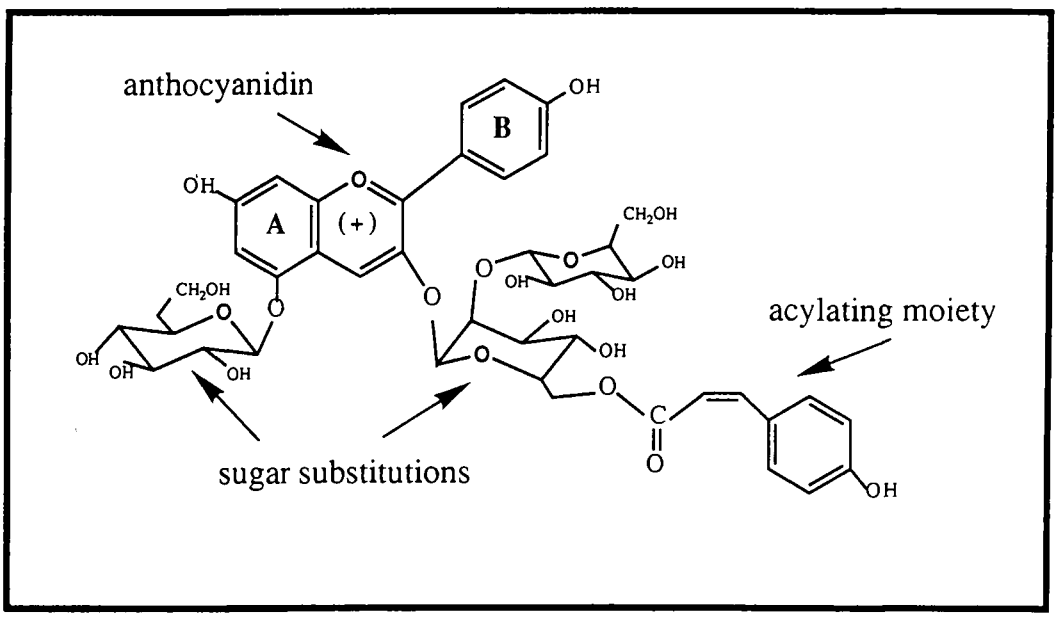


Figure 1. Structure of an anthocyanin, showing the anthocyanidin, sugar substitutions and acylating moiety. Proposed structure for pelargonidin-3-sophoroside-5-glucoside acylated with *p*-coumaric acid.

Anthocyanins have the same biosynthetic origin as other natural flavonoid compounds. Radioactive labeled precursors have established that the carbon skeleton of all flavonoids is derived from acetate and phenylalanine. The A-ring (Figure 1) is derived from three acetate units (malonate), and phenylalanine gives rise to the B-ring and the 3-C chain of the C-ring. The C-15 skeleton is formed by a chalcone synthase (CHS) which catalyzes the condensation of a molecule of 4-coumaroyl CoA with 3 molecules of malonyl CoA. The chalcone is then converted into flavanone by a chalcone isomerase. The various flavonoids formed result from the changes which occur in the different rings, essentially hydroxylations, oxidations, methylations and glycosylations. Methylation is involved only after the formation of the flavonoid ring structure, and glycosylation occurs after all substitutions and modifications of the flavonoid ring structure (Macheix *et al.*, 1990).

Stability of anthocyanins in foods

Anthocyanin-containing products are susceptible to color deterioration during processing and storage, resulting in the combined anthocyanin degradation and brown pigment formation (Abers and Wrolstad, 1979; Skrede *et al.*, 1992).

In aqueous media, most of the natural anthocyanins behave like pH indicators, being red at low pH, and bluish at higher pH. It has been demonstrated that four anthocyanin structures exist in equilibrium: the red flavilium cation (AH^+), the blue quinonoidal (anhydro) base (A), the colorless carbinol pseudobase or hemiketal (B), and the colorless chalcone (C) (Figure 2). The blue quinonoidal base is protonated to give the red flavylium cation, which can then hydrate to form a colorless carbinol

pseudo-base. The carbinol pseudo-base exists in equilibrium with its chalcone. The relative proportions of these pigments vary at different pH values and the color changes according to the prevailing form (Francis, 1985; Jackman and Smith, 1991; Mazza and Miniati, 1993). The equilibrium affects the stability of the product in storage, since the AH^+ form is the most stable. Therefore, anthocyanins show more stability in acidic solutions than in neutral or alkaline solutions. The presence of oxygen increases anthocyanin degradation, and usually stability of juices and dry

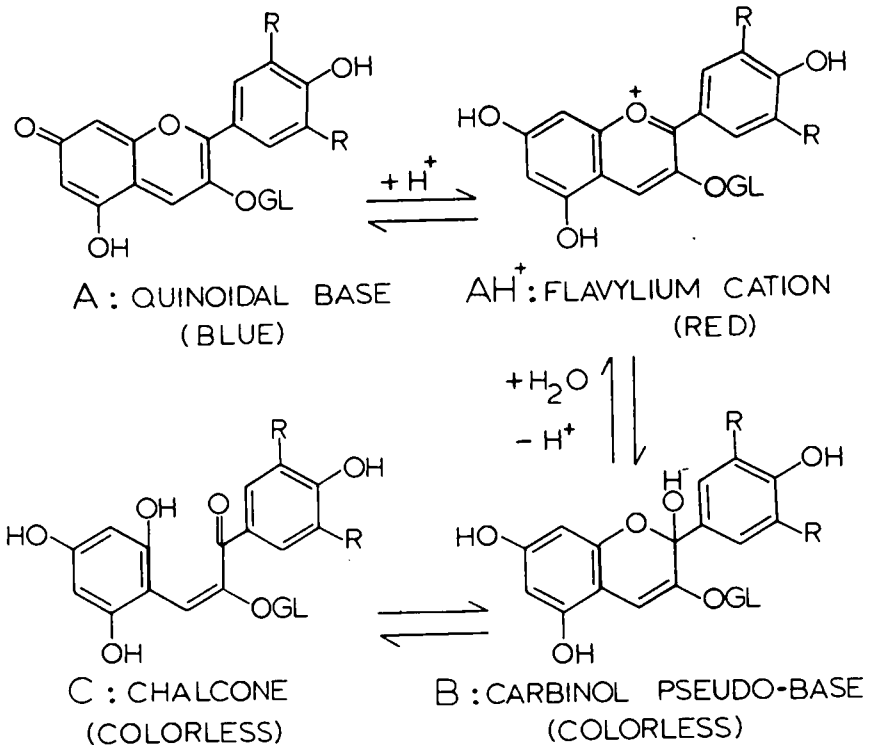


Figure 2. Structural changes in anthocyanins with changes in pH. Source: Francis, 1985.

beverage mixes can be enhanced by elimination of oxygen (Markakis, 1982b; Francis, 1989).

Anthocyanin stability is drastically affected by heat. Logarithmic rise in anthocyanin destruction has been reported as a result of arithmetic increase in temperature (Francis, 1989). The mechanism of anthocyanin degradation with heat is not completely understood, but apparently heated anthocyanins first undergo hydrolysis of the glycosidic bond, followed by the conversion of the aglycon to a chalcone, which subsequently yields an α-diketone. It has also been mentioned that the equilibrium reaction among anthocyanin structures shifts the equilibria toward the chalcone when heated (Markakis, 1982; Francis, 1989).

Light exerts two antithetical effects on anthocyanins, since it favors their biosynthesis but it also accelerates their degradation. Ascorbic acid and anthocyanin also interact resulting in mutual destruction (Markakis, 1982b; Francis, 1989). Two different mechanisms have been proposed for ascorbic acid's destructive effect on anthocyanins: one mechanism involves the anthocyanins oxidation caused by the formation of H_2O_2 when ascorbic acid is oxidized, (Markakis, 1982b; Francis, 1989); the other mechanism suggests the direct condensation of ascorbic acid with anthocyanin pigments (Poei-Langston and Wrolstad, 1981).

The presence of sulfur dioxide can lead to loss of anthocyanin (Parkinson and Brown, 1981). Bleaching of anthocyanins by sulfur dioxide is frequently encountered in the fruit industry. This bleaching may be reversible or irreversible. Fruits preserved by moderate amounts of sulfur dioxide lose their anthocyanin coloration,

by the formation of a colorless SO_2-anthocyanin complex, but color is recovered after they are "desulfured" in acidic media. The irreversible bleaching occurs in the brining of red cherries, which are subsequently used for maraschino, candied, and glacé cherries. The reactions involved in the irreversible sulfur dioxide bleaching are not fully known (Markakis, 1982b).

The chemical instability of anthocyanins, coupled with high cost and low tinctorial strength, has limited the commercial application of anthocyanin pigments (Parkinson and Brown, 1981). However, there is a continuing effort to make anthocyanin colorants that show more stability, and apparently, acylation of the molecule usually lends stability (Shi *et al.*, 1992a; Bassa and Francis, 1987).

Chemical structure

Not all anthocyanins are equal in their resistance to the degrading effect of these various agents. Hydroxyl groups, methoxyl groups, sugars, and acylated sugars have a marked effect on color intensity and stability of anthocyanins. Glycosylation affects the stability of the pigment. Anthocyanins are much more stable than their corresponding anthocyanidins. This is the reason that free aglycons are seldom found in more than trace amounts in foods or food colorants. The diglycosides are usually more stable to discoloration than the corresponding monoglycosides (Mazza and Miniati, 1993). However, it has also been reported that diglycosides hydrate more rapidly and to a larger extent than the corresponding monoglycosides (Dangles *et al.*, 1993). The degree of hydroxylation is also important and anthocyanins with more hydroxyl groups are less stable (Francis, 1989). A group of acylated anthocyanins has

been discovered in recent years which display unusual stability in neutral or weakly acid solutions (Shi *et al.*, 1992a, 1992b; Mazza and Miniati, 1993). Acylated anthocyanin pigments show greater stability during processing and storage (Murai and Wilkins, 1990; Hong and Wrolstad, 1990; Rommel *et al.*, 1992). These acylated pigments also respond differently to pH change than do non-acylated ones (Price, 1991). The presence of acylating groups is believed to protect the oxonium ion from hydration, thereby preventing the formation of the hemiketal (pseudobase) or chalcone (Brouillard, 1981; Francis, 1989). Diacylated anthocyanins have been reported to be more stable due to a stabilization effect by a sandwich-type stacking caused by hydrophobic interactions between the anthocyanidin ring and the two aromatic acyl groups (Mazza and Miniati, 1993; Francis, 1989).

Other natural red colorants

There is considerable interest worldwide in development of food pigments from natural sources. Colorants from microbial sources have been investigated. Traditionally grown on rice or bread, *Monascus* species produce red colorants through solid state fermentation and have been used for years as a general food colorant and medicinal agent in the Orient (Fabre *et al.*, 1993). This colorant is also sensitive to heat, light and pH, and shows low water solubility (Fabre *et al.*, 1993; Wong and Koehler, 1983).

Safety issues

Many consumer advocates rank synthetic dyes as one of the primary additives to subtract from their diet (Anchorage, 1992). Historically, many of the synthetic dyes have been suspected and proven of causing cancer and have been banned. There is even controversy surrounding the currently approved colors. The FDA estimates that between 47,000 and 94,000 Americans are allergic to FD&C Yellow No. 5, the second most important dye used. FD&C Red No. 3 is suspected to be hazardous to humans, since it causes thyroid tumors in rats, and FDA has banned some of its uses and it is possible that it will ban the rest (Anchorage, 1992).

Scientific and public opinions on the safety of food color additives reflect two current but divergent themes in North American science and consumerism. One theme is that safety is not absolute, but relative. The second is that consumers desire absolute safety. They must consider that nothing that we eat can be considered absolutely safe for all consumers under all conditions of use, and that toxicology, although still an inexact science, provides information as to the safety of foods we eat. Although absolute safety is unachievable, we do need a high but realistic degree of relative safety, consistent with the benefits derived from the specific activity. In the case of color additives, where the benefits are primarily psychological, acceptable risk is minimal (Newsome, 1986).

RADISH

China is considered to be the origin of radish (Peirce, 1987) and wild forms exist there today. A number of types were introduced to middle Asia in prehistoric

times. Radishes were a common food in Egypt before the building of the pyramids and they were highly regarded by ancient greeks. Roman writings described several forms and colors of radish at the beginning of the Christian era (Peirce, 1987). Columbus is credited with introducing radishes to the new world. They were seen in Mexico about 1500 and in Haiti in 1565, and were among the first vegetable grown by the colonists in this country (Jensen, 1988). However, the short season radishes, now popular in the USA market, were not grown until after the sixteenth century in Europe. At that time, only the large, late winter types were cultivated (Peirce, 1987). Radishes are presently consumed in a varieties of ways: raw, especially in the USA, but also on a worldwide basis; cooked, brined and fermented (pickled), and dried throughout the Orient (Carlson *et al.*, 1985; Hartman *et al.*, 1988). It is also common practice to peel off the skin of radishes prior to cooking throughout India (Kaur *et al.*, 1978). Radish tops are occasionally cooked as a leafy vegetable in India, but generally they are thrown away or fed to animals (Kaur *et al.*, 1978).

Botanic review

The radish (*Raphanus sativus* L.) is a member of the Mustard family: Brassicaceae (Cruciferae), which refers to the cross form (cruciform) of the 4 diagonally opposed petals (Glimn-Lacy and Kaufman, 1984), and includes both annual and biennial types. The spring and summer radishes (short season types) are annuals; the winter cultivars require up to 2 months to reach edible stage and may be annual or biennial (Carlson *et al.*, 1985; Peirce, 1987; Salunkhe, *et al.*, 1991). The

storage organ of the radish, generally called the root, consists of root and hypocotyl tissue with the latter being predominant (Hall, 1990).

Cultivars

Radish cultivars are often grouped according to their maturation time and growing season, and within each season, by shape and color. Rapid-growing, quick-maturing cultivars are popular with commercial growers and home gardeners for use raw in salads. These are annual plants best known by their small, short, often globe-shaped roots and mild pungency. Although those with red skin, such as "Scarlet Globe" and "Fuego", are the most popular, white and mixed white and red cultivars are also available, as are cultivars with larger, longer, tapered roots. On a worldwide basis the later-maturity radish cultivars are more important. These are commonly called winter radishes, because their firm-fleshed roots, if lifted in autumn, can be stored for winter use without becoming hollow in the center. Winter radishes are biennial plants and are usually more pungent, larger, and well adapted for storing. Size, shape, and even flesh and exterior colors vary considerably. The roots of some cultivars are exceptionally large and/or long. Winter radishes are very popular and are consumed in large quantities in raw, cooked and pickled forms throughout the Orient. Much of their popularity is due to their strong flavor characteristics and their good storage qualities (Hartmann *et al.*, 1988; Harrison *et al.*, 1975; Peirce, 1987).

Composition

Radish composition is summarized in Table 1 (Salunkhe *et al.*, 1991). Radishes are considered a reasonable source of vitamin C, having levels (23 mg/100 g edible portion) similar to onions, beans and tomatoes (26 mg/100 g edible portion) (Salunkhe *et al.*, 1991). Analysis of the chemical constituents in different parts of the plant has shown that ascorbic acid, crude protein, and dry matter are in greater concentration in the leaves, followed by the skin, followed by the peeled roots. Therefore, it is recommended that healthy leaves and skin not be discarded (Kaur *et al.*, 1978). Four kinds of free sugars have been found in radish root: glucose, a major component, abundant at a stage of growth of the root, sucrose, fructose, and xylose. Xylose was detected only at an early stage of growth. In general, the amount of amino acids is greater in the white roots than in the red ones. Valine, leucine, arginine, methionine, asparagine and glutamine are enhanced in the white root, as compared to the red one. On the contrary, aspartic and glutamic acids and tyrosine are higher in the latter. Phenylalanine, an excellent precursor of anthocyanins, is also found in considerable amounts in the white root, but only in traces in the red. Seven different phenolic acids have been identified in radish roots: *p*-coumaric, caffeic, ferulic, phenylpyruvic, gentistic and *p*-hydroxybenzoic acids and an additional unidentified one (Ishikura and Hayashi, 1965), gentistic acid being present in largest amounts.

Members of the family of *Cruciferae* (cabbage, brussels sprouts, cauliflower, turnip and radish) are especially rich in sulfur-containing compounds, which make

Table 1. Composition of Radish. Values per 100 g edible portion. (From: Salunkhe, *et al.*, 1991; * from Jensen, 1988).

Water content (%)	94.8
Energy (Kcal)	17
Protein (%)	0.6
Fat (%)	0.5
Carbohydrate (%)	3.6
Fiber (%)	0.5
Calcium (mg)	12
Manganese (mg)	0.07
Phosphorus (mg)	20*
Iron (mg)	1.0
Vitamin A (IU)	8
Ascorbic Acid (mg)	23
Thiamine (mg)	0.01
Riboflavin (mg)	0.05
Niacin (mg)	0.30

a major contribution to the flavor of these materials (Salunkhe, *et al.*, 1991). The pungent flavor of radish is attributed primarily to the presence of isothiocyanates. These compounds are also volatile, and therefore contribute to characteristic aromas. The flavor compounds are formed through enzymic processes in disrupted tissues and through cooking (Lindsay, 1985; Kim and Rhee, 1993). The fresh flavors of the disrupted tissues are formed by the action of glucosinolases on the precursors of isothiocyanates, thioglycosides (Lindsay, 1985). The compound responsible for the pungency of radish (aroma and flavor character impact compound) has been identified as 4-methylthio-*trans*-3-butenyl isothiocyanate, and the degree of pungency depends on the concentration of this compound (Lindsay, 1985; Salunkhe, *et al.*, 1991; Kim and Rhee, 1993). Thiocyanate ion appears as a result of isothiocyanate decomposition, and causes thyroid enlargement (Tookey *et al.*, 1980). The mechanism of most goitrogenics is to diminish the supply of iodide to the thyroid or interfere with thyroxine production, thus causing the thyroid to enlarge. Compounds formed from glucosinolates, like 3-indolylmethyl-glucosinolate hydrolysis products, also have been found to inhibit carcinogenesis (Carlson *et al.*, 1985). Cruciferous vegetables in the diet have been shown to enhance the *in vivo* detoxification of the carcinogens aflatoxin B_1, polybromobiphenyl (PBB) and 1,2-dimethylhydrazine (Fenwick *et al.*, 1983; Carlson *et al.*, 1985).

Therapeutic value

In a book dedicated to the beneficial health properties of foods, Jensen (1988) refers to the therapeutic value of radishes. He mentions that radishes are strongly

diuretic and stimulate appetite and digestion. The juice of raw radishes is helpful in catarrhal conditions. The mustard oil content of the radish makes it good for expelling gallstones from the bladder (Jensen, 1988). Radish proteins showed significant efficiency when given as supplement with rice and wheat proteins, and showed significant beneficial effect as judged by blood hemoglobin, plasma protein and liver protein levels (Kaur *et al.*, 1978). Therefore, radish could be used as a protein supplement. Since proteins are more concentrated in radish leaves than in the root, it was suggested by Shukla and Sur (1978) that they be used as a supplement for human and animal feed. On comparing the amino acid composition of radish leaf proteins with that of egg protein, Shukla and Sur (1978) found that radish leaves were deficient in phenylalanine and valine, and therefore they could be a good protein supplement for patients suffering from phenylketonuria.

Production of radishes in the USA

Salad radishes are available practically the whole year round. They grow very quickly under suitable conditions and are usually ready for picking within 3 or 4 weeks (Harrison, *et al.*, 1975). Winter varieties, however, take about sixty days to mature (Jensen, 1988). Warm, light, sandy but highly fertile soils are the most satisfactory, and an adequate water supply is essential (Harrison, *et al.*, 1975).

The major commercial acreages in the USA are along the coastal region of Central California and in Florida, but radishes are produced in all states to some extent. California ships 410,000 cwt (18,598 Tons) annually throughout the year. Florida has a winter crop on 24,000 acres (10,000 Ha) of approximately 1,070,000 cwt

(48,535 Tons), and the northeast production supplies the early summer and fall markets. Summer radishes are supplied by Michigan, Minnesota, Ohio, and smaller acreages in several northeastern states. The total USA production is estimated at 1,220,000 cwt (55,339 Tons) (Peirce, 1987).

Some of the factors that will determine the quality of the radish are tenderness, cleanness, smoothness, shape, size, and freedom from pithiness and other defects (Salunkhe, *et al.*, 1991).

Anthocyanins in radish

The aesthetic pleasure that color gives to man is well illustrated by the radish: red or red and white roots of the radish are more popular than white roots (Timberlake and Bridle, 1982). The red coloration of radish is due to the presence of anthocyanins.

The first investigation on the anthocyanins of radish were published in 1910, when Sacher and Schwertschlager reported the potential of these pigments as an acid-base indicator (Mazza and Miniati, 1993). In 1957 anthocyanins from red and purple roots were examined by paper chromatography and the aglycons were identified as pelargonidin and cyanidin, respectively (Harborne, 1958; Harborne and Paxman, 1964). In subsequent work, Harborne identified four different pigments in each variety; they were pelargonidin 5-glucoside-3-sophoroside acylated either with ferulic or with *p*-coumaric acid for the red variety, and the corresponding cyanidin derivatives for the purple variety. Thus, the only chemical difference between the two color types is that the violet roots have one more hydroxyl group than the red

ones (Harborne, 1963). Harborne also showed that the acyl groups are attached to the sugar in the 3-position in all these anthocyanins (Harborne, 1964). Ishikura and Hayashi (1962) assigned the name "raphanusin" for the pelargonidin-5-glucoside-3-sophoroside and found that the red pigment from the root of radish was the mixture of five complex anthocyanins: raphanusin A acylated with *p*-coumaric acid; raphanusin B-1 acylated with ferulic acid; raphanusin B-2 acylated with caffeic acid; raphanusin C-1 acylated with *p*-coumaric acid and ferulic acids; and raphanusin C-2, with the three cinnamic acids attached to the molecule (Ishikura and Hayashi, 1962 and 1963). The same pattern of five acylated anthocyanins was found for the purple variety and these anthocyanins were named rubrobrassicin (cyanidin-5-glucoside-3-sophoroside) A, B-1, B-2, C-1 and C-2 respectively (Ishikura and Hayashi, 1965b). These findings, in combination with the fact that the purple pigment appears in all F1 plants and in 9/16 of the F2 progenies obtained from the crossing between the red and white varieties suggested that there is a gene in radish controlling the hydroxylation of pelargonidin to cyanidin, which is independent of pigment distribution (Harborne and Paxman, 1964; Ishikura *et al.*, 1965). Genetic analyses indicate the participation of two pairs of conditional alleles, for which a factor R contributes to the formation of pelargonidin glucoside and factor E to the formation of a hydroxylating enzyme which is effective for the production of cyanidin derivatives. If we assume the genotype of both parents as RRee for the red and rrEE for the white, the F1 and F2 results may be explained reasonably (Hoshi *et al.*, 1963).

Fuleki resolved 13 pigments from the red skin of radish. All of them were identified as pelargonidin glycosides with one, two, three or no acylations. The acylating groups were once again identified as *p*-coumaric, ferulic and caffeic acids (Fuleki, 1969).

Anthocyanin production in the seedlings of the red varieties is stimulated by the administration of several amino acids such as leucine, phenylalanine, valine, aspartic acid and threonine (Ishikura and Hayashi, 1965). Phenylalanine was the most effective for anthocyanin synthesis, being incorporated into the B-ring and adjacent three-carbon chain. Amino acids, such as valine and leucine were also incorporated into the B-ring of the molecule (Ishikura and Hayashi, 1966a; 1966b).

The anthocyanins in radish are primarily located in the cells of the subepidermal layer of the cotyledons and the hypocotyl axis. Their production and the extent of accumulation is affected by light intensity, quality and duration (Mazza and Miniati, 1993).

MARASCHINO CHERRIES

History

Maraschino cherries, as we know them today, differ considerably from the original product prepared many years ago in Dalmatia. The delicately colored and flavored liqueur was made from fruit, ground pits, bark, and leaves of the marasque cherry tree. This extract was then used to flavor and color cherries similar to the Royal Anne variety cultivated in the USA (Filz and Henney, 1951). The brining

process, as it is known today, was developed at Oregon State University, by Wiegand and coworkers, in the late 1920's (Rose, 1975). The product developed made possible the commercialization of a fruit, otherwise highly perishable, in a very attractive and stable form. Although the maraschino process was inspired by the European product, the brining process provided a higher quality, more uniform cherry, which USA manufacturers colored and flavored to produce the distinctive commercial product.

The cherry-producing districts of Oregon soon indicated an increasing interest in the preparation of cherries for maraschino use. During the 1930 season, approximately 10,000 barrels (1,136 Tons) of cherries were bleached in Oregon. Some of these were shipped to the East coast, furnishing an outlet for a great portion of the Northwest's increasing Royal Ann cherry crop (Bullis and Wiegand, 1931). In 1992, the State of Oregon was the second major producer of sweet cherries (52,000 Tons) in the USA, after Washington (97,000 Tons), from a nation-wide production of 205,400 Tons (OSU Extension Service, 1994). In Oregon, more than 50% of the production (28,000 Tons) was destined for brining.

Maraschino cherry processing

Royal Ann is usually the variety of choice for maraschino cherry processing. Sweet cherries destined for maraschino or similar processing are picked before full ripeness and placed in brine containing between 1 and 1.5% sulfur dioxide and 3000 to 5000 ppm of calcium salts. The brine bleaches the fruit to a pale yellow color, and it also acts as a preservative during subsequent storage. Calcium acts as a

hardening agent, increasing fruit firmness (Wiegand and Bullis, 1930; Beavers and Payne, 1969). After a period of several weeks to two years, the fruit is removed and "finished" into the final product (Anonymous, 1968). Since non uniformly bleached as well as incompletely bleached cherries were frequently obtained, Oregon State University food scientists Beavers and Payne developed a secondary bleaching process that completely eliminated bruise marks and other dark skin discolorations from brined sweet cherries. Cherries are leached in water and placed in acidified sodium chlorite. This means that high quality, brightly colored maraschino and fruit cocktail cherries can be made from fruit otherwise considered undesirable for these products (Anonymous, 1968; Beavers and Payne, 1969; Beavers *et al.*, 1969).

After the bleaching process the cherries are ready for finishing into maraschino cherries. They are removed from the brine, rinsed with water and graded. The fruit is leached in running water to remove most of the sulfur dioxide. At this point of the process cherries are firm, and lack cherry color and flavor. Therefore flavoring (cherry and/or almond food flavors), coloring and sweetening agents are added. Potassium sorbate and sodium benzoate are used as preservatives, and citric acid is used to adjust the pH to acidic conditions (pH usually between 3.4 and 3.8). The cherries are finally bottled and pasteurized (Wiegand and Bullis, 1930; Filz and Henney, 1951).

Coloring of maraschino cherries

FD&C Red No. 4 (Ponceau SX) was used for coloring maraschino cherries for a long time. This synthetic colorant had a brilliant red color with unusual

resistance to the destructive influences of food ingredients and heat (Yang *et al.*, 1966). The use of this colorant was banned in 1976 because of unresolved safety questions (Rumore *et al.*, 1992), and FD&C Red No. 3 and FD&C No. 40 have been used since, FD&C Red No. 40 being the colorant of choice for maraschino cherries because of its solubility properties.

Finding a natural red colorant that can effectively replace FD&C Red No. 40 has proved to be a difficult task, because few natural materials have its bright red color unmixed with other tones (LaBell, 1993). The use of natural colorants as cochineal, concord grape extract, other anthocyanins, and some carotenoids has been studied (McLellan and Cash, 1979; LaBell, 1993; Sapers, 1994). Relatively good stability has been obtained (between 3 and 6 months of storage); however, limitations have been found trying to reproduce the desired hue.

In the present study, we evaluate the feasibility of radish anthocyanins for coloring brined cherries for maraschino cherries, as an alternative to FD&C Red No. 40. We also evaluate color and pigment stability of cherries and syrup colored with radish anthocyanins, compared to FD&C Red No. 40, during a year of storage in the dark at 25°C and the effect of light in color and pigment stability during the storage time.

MATERIALS AND METHODS

Plant Material

Red radishes (*Raphanus sativus* L.) cultivar Fuego, grown by SIRI Produce Inc. (Oregon City, OR.), were obtained September 8, 1993, from Cub Foods (Corvallis). The leaves were cut, the roots were washed with cold water to eliminate extraneus matter and refrigerated at 1°C. The radishes (30 kg) were peeled manually, (ca. 6 kg/day, in 5 batches over a 12 day period) and the epidermal tissue was frozen under liquid nitrogen and stored at -23°C.

Primary (1 gallon) and secondary (3 gallon) bleached cherries in brine were supplied September 8, 1993, by Oregon Cherry Growers Inc. (Salem, OR).

Pigment stability to heat

Radish anthocyanin stability to thermal processing was evaluated by canning radishes in aqueous medium at pH 1.0, 3.8, 4.5 and 6.0. The solutions used were pH 1.0 buffer (125 mL of 0.2N KCl and 385 mL of 0.2N HCl), 40° Brix syrup (pH adjusted to 3.8 with citric acid), pH 4.5 buffer (400 mL of 1M sodium acetate, 240 mL of 1N HCl taken to 1 L with distilled water), and pure distilled water. The cans were immersed in a retort at 115°C for 15 min. Visual appearance of radishes and solutions were recorded before and after heat treatment.

Pigment extraction

The extraction was done following the procedure described by Wrolstad *et al.* (1990) and Hong and Wrolstad (1990). Frozen red radish epidermal tissue was liquid

nitrogen powdered using a stainless steel Waring blender. The powdered samples were blended with ca. 2 L of acetone per kg skin and filtered on a Buchner funnel. The filter cake residue was re-extracted with aqueous acetone (30:70 v/v) until a clear solution was obtained. Filtrates were combined, shaken in a separatory funnel with chloroform (1:2 acetone:chloroform v/v) and stored overnight at 1°C. The aqueous portion (top portion) was collected and placed on a Büchi rotovapor at 40°C (for 5 to 10 min) until all the residual acetone was evaporated. The aqueous extract was made up to a known volume with distilled water.

Monomeric, polymeric anthocyanin content and color density

Monomeric anthocyanin content, polymeric color, and color density were determined using pH differential method and bisulfite bleaching methods, respectively (Wrolstad, 1976). A Shimadzu 300 UV spectrophotometer and 1-cm pathlength disposable cells were used for spectral measurements at 420, 510 and 700 nm. Pigment content was calculated as pelargonidin-3-glucoside, using an extinction coefficient of $\epsilon = 31{,}600$ L cm^{-1} mg^{-1} and molecular weight of 433.2 g L^{-1} (Wrolstad, 1976).

Anthocyanin purification

The aqueous extract was passed through a C-18 Sep-Pak cartridge (Waters Associates, Milford, MA), previously activated with methanol followed by 0.01% aqueous HCl (Wrolstad *et al.*, 1990). Anthocyanins (and other phenolics) were adsorbed onto the mini- column; sugars, acids and other water soluble compounds

were eluted with 2 volumes of 0.01% aqueous HCl and anthocyanins were subsequently recovered with methanol containing 0.01% HCl (v/v). The methanolic extract was then concentrated using a Büchi rotovapor at 35°C and the pigments were dissolved in deionized water containing 0.01% HCl. This purified fraction is referred as radish anthocyanin extract (RAE).

Alkaline hydrolysis of anthocyanins

Approximately 10 mg of purified pigment was hydrolyzed (saponified) in a screw-cap test tube with 10 mL of 10% aqueous KOH for 8 min at room temperature in the dark, as described by Hong and Wrolstad (1990). The solution was neutralized using 2N HCl, and the hydrolysate was purified using a C-18 Sep-Pak cartridge, as previously described.

Acid hydrolysis of anthocyanins

Fifteen mL of 2N HCl was added to ca. 1 mg of purified saponified pigment in a screw-cap test tube, the contents of which were flushed with nitrogen and capped. The pigment was hydrolyzed for 45 min at 100°C, then cooled in ice bath. The hydrolysate was purified using a C-18 Sep-Pak cartridge, as previously described (Hong and Wrolstad, 1986).

HPLC separation of anthocyanidins and anthocyanins

The anthocyanin profile of radish epidermal tissue was determined using High Performance Liquid Chromatography (HPLC). The pigments, which had been partially purified by solid phase extraction with C-18 Sep-Pak cartridge, were filtered

through a 0.45-μm Millipore filter type HA (Millipore Corp., Bedford, MA). A High Performance Liquid Chromatograph Perkin-Elmer Series 400, equipped with a Hewlett-Packard 1040A photodiode array detector, Hewlett-Packard 9000 computer was used. Simultaneous detection at 280, 320 and 520 nm was used. The spectra (detection wavelengths from 250 to 600 nm) were recorded for all peaks.

Columns and mobile phase:

System I. Columns: Polymer Labs PLRP-S (5 micron) 250 x 4.6 mm id (Polymer Labs, Amherst, MA), fitted with a Polymer Labs, 1.5 cm x 4.6 mm id guard column. Solvent: (A) 4% phosphoric acid (40 mL concentrated phosphoric acid, made up to 1 L with deionized water), filtered through a 0.45-μm Millipore filter type HA (Millipore Corp., Bedford, MA) and (B) 100% HPLC grade acetonitrile.

System II. Column: Supelcosil LC-18 (5 micron), 250 x 5 mm id (Supelco, Inc., PN), fitted with a ODS-10, 4 cm x 4.6 mm id, Micro-Guard column (Bio-Rad Laboratories). Solvents: (C) 100% HPLC grade acetonitrile and (D) 1% phosphoric acid, 10% acetic acid, 5% acetonitrile and water (10 mL concentrated phosphoric acid, 100 mL acetic acid, 50 mL acetonitrile, made up to 1 L with deionized water), filtered through a 0.45-μm Millipore filter type HA (Millipore Corp., Bedford, MA).

Conditions for anthocyanin analysis

For the separation of acylated radish anthocyanins a PLRP-S column was used. The program used was: linear gradient from 15 to 20% A (from 85 to 80% B) in 40 min, at a flow rate of 1 mL/min. For separation of anthocyanins obtained after

the saponification of the pigments a Supelcosil LC-18 column was used. The program used was a linear gradient from 0 to 30% C (100 to 70% D) in 30 min and a flow rate of 1 mL/min. The injection volume was 50 μL.

Conditions for anthocyanidin analysis

Anthocyanidins, obtained from the acid hydrolysis of saponified pigment, were separated using a Supelcosil LC-18 column. The program used a linear gradient from 0 to 30% C (100 to 70% D) in 30 min, flow rate 1 mL/min. Injection volume of 50 μL.

Identification of the aglycon

Pelargonidin-3-glucoside was obtained from strawberry juice concentrate, anthocyanins were purified using solid phase extraction on C-18 Sep-Pak cartridge, and hydrolyzed with 2N HCl (procedure previously described). The retention time and spectra for the pelargonidin were compared to the aglycon from radish anthocyanins.

HPLC separation of the acyl groups

Phenolic acids, obtained from the saponification of radish pigments, were separated using High Performance Liquid Chromatography (HPLC).

The solution was filtered through a 0.45-μm Millipore filter type HA (Millipore Corp., Bedford, MA). The elution of peaks was monitored with simultaneous detection at 280 and 320 nm. The spectra (detection wavelengths from 250 to 600 nm) were recorded for all peaks.

Phenolic acids, obtained by the saponification of radish pigments, were separated using the two different systems previously described. With System I, the PLPR-S column was used, and the program used was a linear gradient from 10 to 15% A (90 to 85% B) in 15 min, a linear gradient from 15 to 25% A (85 to 75% B) in 15 min and 5 min isocratic at 25% A (75% B). With System II, the Supelcosil LC-18 column was used, running isocratic at 5% C (95% D).

Identification of the acyl groups

Phenolic acid standards: ferulic, caffeic, and *p*-coumaric acid, were obtained from Sigma Chemical Co. (St. Louis, MO). The identity of the acyl groups was confirmed by the use of the two different HPLC systems. Retention times and spectra were compared. Samples of acyl groups obtained from radish, spiked with the standards, were also analyzed.

Preparation of maraschino cherries

The procedure used for the preparation of maraschino cherries is shown in Figure 3. One gallon of primary bleached cherries and 3 gallons of secondary bleached cherries were used. The cherries were first washed with running cold water for 5 to 6 hr. Then they were placed in a kettle with fresh water, heated to boiling, maintained at boiling temperature for 15 min, and drained. The boiling procedure was performed two more times (using fresh water each time), to reduce the SO_2 level. The cherries were drained and syrup (15° Brix) containing 0.1% potassium sorbate (w/v), 0.1% sodium benzoate (w/v) and 0.25% citric acid (w/v) was added.

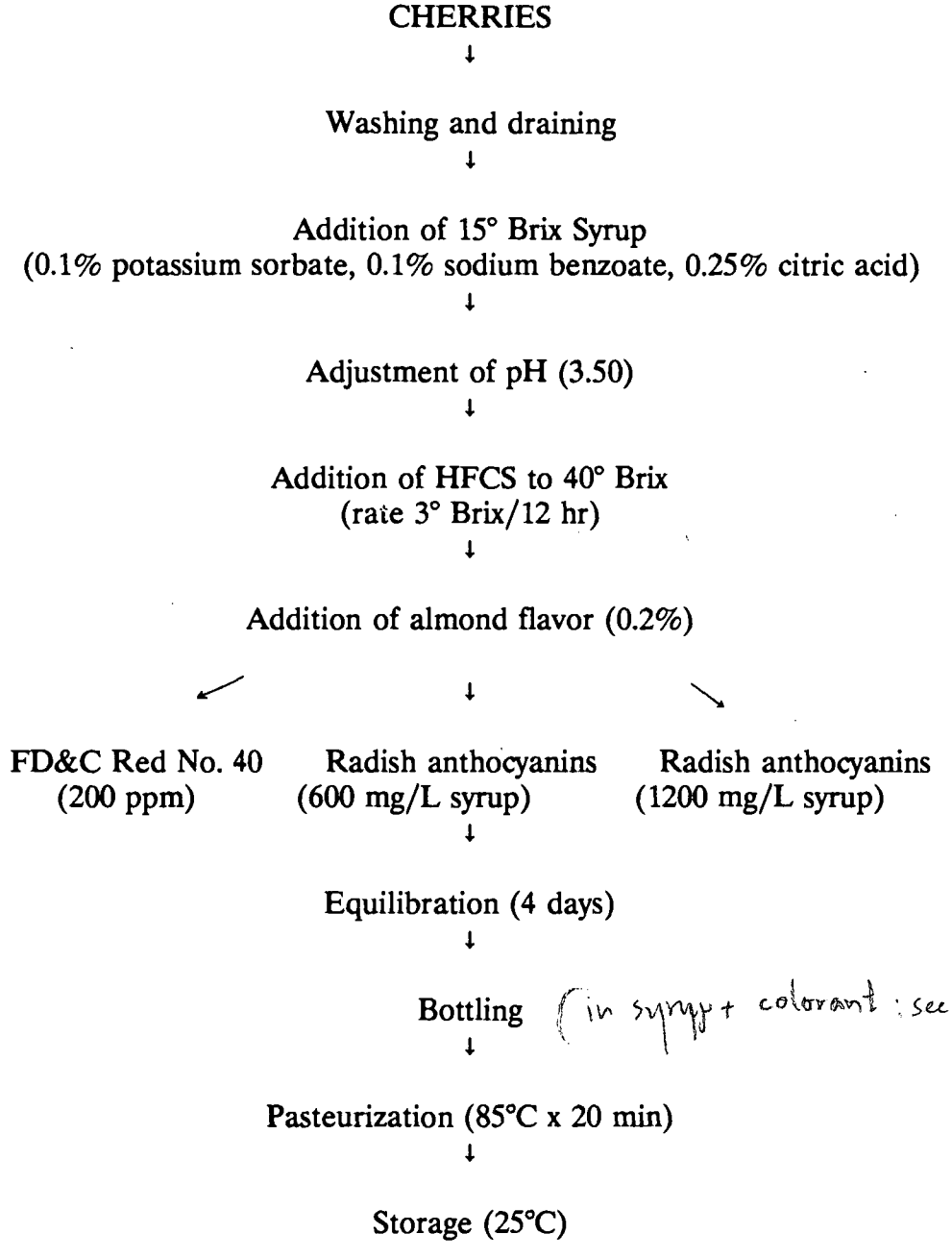


Figure 3. Maraschino cherry processing

High fructose corn syrup (HFCS) (IsoSweet 100, Staley A.E., Staley Manufacturing Co. IL.) was used as the sweetener. Cherries were placed in a 40°C temperature room, and HFCS was added with gentle stirring increasing the °Brix of the syrup in 3° increments. This procedure was continued increasing the °Brix at a rate of 3° Brix every 12 hr, to reach equilibrium at 40° Brix. Then, almond extract (Mutual Flavors, Los Angeles, CA) (0.2% v/v), the flavoring agent, was added.

Color treatments:

Cherries and syrup were separated into 6 containers. Three color treatments were used: 200 ppm of FD&C Red No. 40, 600 mg RAE/L syrup and 1200 mg RAE/L of syrup. The pH of the syrup was adjusted to 3.5 with citric acid (if higher than 3.5) or sodium bicarbonate (if below 3.5). Potassium sorbate and sodium benzoate were also added to restore the 0.1% (v/w) level. Color and flavor were allowed to equilibrate for 4 days at 40°C. During this time the solutions were manually stirred every 12 hr. Processing trials were replicated.

Bottling and storage:

Colored cherries and syrup (primary and secondary bleached cherries, after the different coloring treatments) were placed in 320-mL glass jars, passed through a steam tunnel, capped and pasteurized in water at 85°C for 20 min. Small vials (20 mL capacity), containing syrup only were prepared, flushed with nitrogen, capped and pasteurized under the same conditions. Colored secondary bleached cherries and syrup were stored in the dark, at 25°C. The storage experiments were originally

planned for 10 months of storage (40 weeks) and complete analyses were done for samples of all the treatments during this time period. However, samples of cherries and syrup colored with radish anthocyanins were available to complete the stability study for a year time period, and an extra measurement is provided for these samples, corresponding to the 53rd week of storage. Vials containing syrup only, were stored both under dark and continuous fluorescent light conditions at 25°C for 12 months.

Determination of pH

A Brinkmann 605 pH-meter (Methrohm Herisan, Switzerland) was used. The instrument was calibrated with buffers pH 4.0 and 7.0 and the pH was directly measured in all syrup samples.

Determination of °Brix

An Auto Abbe refractometer 10500 (Reichert-Jung, Leica Inc., NY, USA) was used. The instrument was set up to measure % soluble solids with temperature compensated mode.

Determination of free SO_2 level

The free sulfur dioxide (SO_2) level was determined by iodine titration method (Beavers *et al.*, 1970) with a modification using a standard iodine solution 0.02N (instead of 0.156 N suggested). Free SO_2 level was measured in cherries, brine and syrup, before and after pasteurization of maraschino cherries.

Color measurements

Color characteristics (Hunter CIE L*, a*, b*) were measured using a ColorQuest Hunter colorimeter (HunterLab, Hunter Associates Laboratories Inc., Reston, VA). The equipment was set up for either reflectance (for color measurements in cherries) or transmittance (for syrup), with specular included, Illuminant C, 10° observer angle. Drained cherries were placed in a 5 cm pathlength optical glass cell (Hellma, Germany) and reflectance measurements were obtained from the average of 4 readings for each sample. Samples of syrup were placed in a 1 cm pathlength optical glass cell (Hellma, Germany) and C.I.E. L*, a*, b* values along with percent haze were measured in the transmission mode. Chroma $(a^{*2} + b^{*2})^{1/2}$ and hue angle ($\tan^{-1}$ a*/b*) were calculated from tristimulus values Hunter CIE a* and b*. Chroma provides a measure of the intensity of the color, while the hue angle (0° = red-purple, 90° = yellow, 180° = bluish- green, and 270° = blue) indicates the sample color itself (McGuire, 1992).

Statistical analysis

The results for color measurements were submitted to one-way analysis of variance and Tukey multiple comparison for a 95% confidence. Rates of anthocyanin and color degradation were obtained from linear regression analysis (95% confidence interval). All statistical analyses were done using Statgraphics 5.0 software.

RESULTS AND DISCUSSION

Effect of thermal processing at different pH on radish pigments

A preliminary study was conducted to test radish pigment stability with heat, by thermally processing radishes in aqueous media at different pH. The cans were opened and visual observations were recorded. Radish anthocyanins appeared to be stable with heat, as evidenced by the presence of red color after treatment. No evidence of browning was found. Radishes processed in aqueous medium at pH 1.0 exhibited an intense orange-red color; radishes processed at pH 3.8 and pH 4.5 exhibited an intense red hue, while radishes processed at pH 6.0 exhibited a red-pink hue, lighter in intensity. Radish pigments were highly soluble in aqueous media, diffusing with thermal processing from the epidermal tissue into the flesh and the liquid media. The radishes (skin and interior flesh) and the aqueous media showed a homogeneous color.

Characterization of radish anthocyanins

A total of 9 g of anthocyanin was extracted and purified from the epidermal tissue of 30 kg of red radish. The average yield of epidermal tissue was 203 g/kg of radish root. The monomeric anthocyanin content of the radish was found to be 154 ± 13 mg/100 g of epidermal tissue as pelargonidin-3-glucoside (pg-3-glu), representing an average yield of 300 ppm (anthocyanin/root weight, w/w).

The monomeric anthocyanin content in the epidermal tissue decreased during the refrigerated storage of radishes at 1°C (Table 2), with a yield of 169 mg

Table 2. Yield of monomeric anthocyanin from red radish epidermal tissue. (Day No.= number of days that radishes were kept at refrigerated storage).

Batch	Day No.	Total g skin	mg Acn/100 g skin
I	1	1,207.5	170
II	5	800.0	163
III	7	1,261.8	152
IV	9	1,193.3	147
V	12	1,553.2	138

monomeric anthocyanin/100 g skin from the first extraction batch (day 1) to 137 mg monomeric anthocyanin/100 g skin for the fifth batch (day 12). This reduction in the anthocyanin content may be due to anthocyanin degradation and/or anthocyanin diffusion from the skin into the flesh during the refrigerated storage.

Anthocyanins from radish were separated by HPLC (Figure 4). Two anthocyanins (peaks 5 and 6) represented ca. 70% of the total area, and two others (peaks 3 and 4) represented ca. 20%. Four other minor peaks were also detected (Peaks 1, 2, 7 and 8), each one accounting for 1 to 3% of the total area. The UV-visible spectra for all peaks are shown in Figure 5. Some important structural properties can be obtained from the spectral data, including the presence of acylation by hydroxylated aromatic organic acids (Hong and Wrolstad, 1990). The spectra of all the peaks obtained from radish anthocyanins extract showed a maximum absorbance in the 310 nm region, corresponding to the presence of acylating groups. Harborne (1958) found that the ratio of absorbance at the acyl maximum wavelength

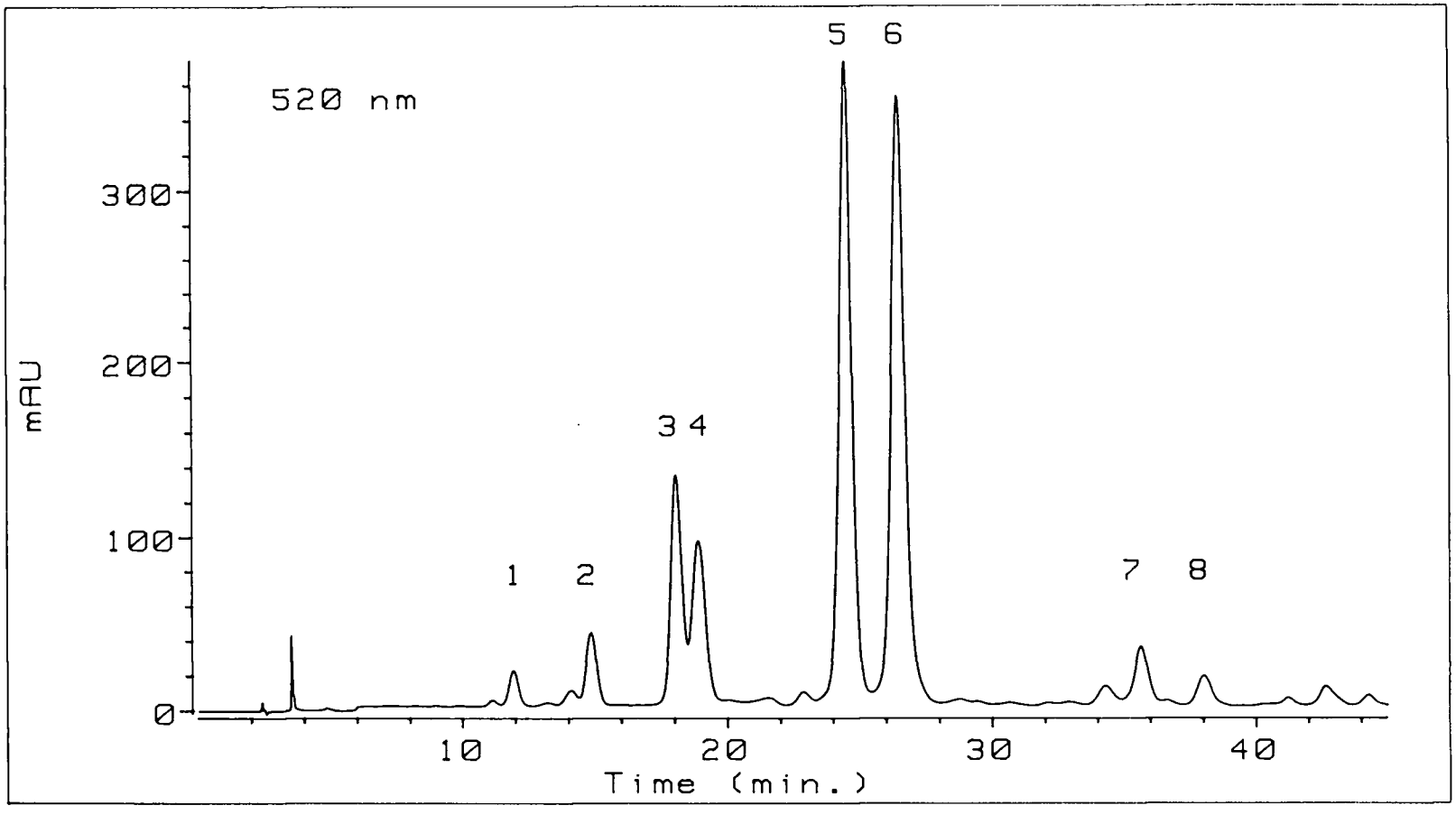


Figure 4. HPLC separation of red radish anthocyanins. Column, Polymer Labs PLRP-S, 250 x 4.6 mm i.d. Solvents A: 4% phosphoric acid, and B: 100% HPLC grade acetonitrile. Linear gradient from 15 to 20% A (85 to 80% B) in 40 min. Flow rate: 1 mL/min. Injection volume: 50 μL.

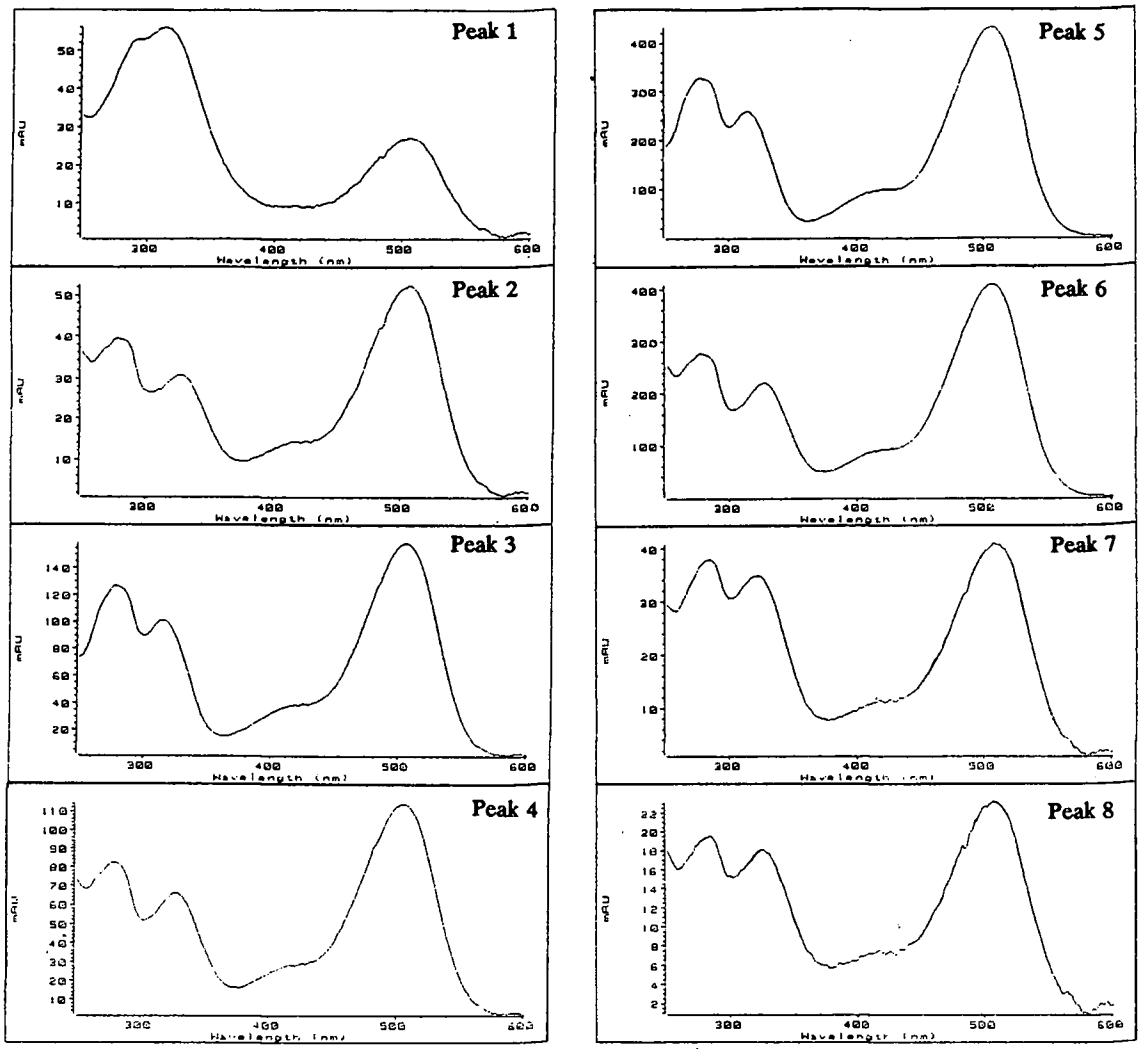


Figure 5. Spectra of anthocyanins separated from red radish (HPLC conditions in Figure 4)

to the absorbance at the visible maximum wavelength (A_{max} acyl/ A_{max} visible ratio) is a measure of the molar ratio of the cinnamic acid to the anthocyanin. In acidified methanolic solution, a ratio of 48 to 71% is indicative of 1/1 molar ratio, while a ratio of 83 to 107% is characteristic of 2/1 molar ratio of cinnamic acid to anthocyanin (Harborne, 1958). The ratios of A_{max} acyl/A_{max} visible found for the radish anthocyanins were 207, 60.8, 62.5, 58.3, 58.1, 52.4, 83.3, and 80.4% for peaks 1, 2, 3, 4, 5, 6, 7, and 8, respectively. These ratios suggest that all major anthocyanins found in the radish epidermal tissue (peaks 3, 4, 5 and 6), and one minor peak (peak 2), were acylated in a 1/1 molar ratio of the cinnamic acid to the anthocyanin. It also suggests the presence of two cinnamic acids per mole of anthocyanin in peaks 7 and 8. The other minor peak (peak 1) appears to be acylated with more than two cinnamic acids (Figure 5).

Saponification of the radish anthocyanins revealed the presence of two major acylating groups (Figure 6a, peaks 3 and 4), accounting for 49 and 41% of the total area at 280 nm detection for peaks a3 and a4, respectively. The comparison of their retention times and spectra with the pure standards (Figure 6b and 7) permitted the identification of the acylating acids as *p*-coumaric (peak a3) and ferulic acids (peak a4). Caffeic acid, which has been previously reported as an acylating acid in radish anthocyanins (Ishikura and Hayashi, 1963; Fuleki, 1969), was not found, evidenced by comparing the retention time of the pure standard (16 min) with the 280 nm chromatogram obtained from the saponified RAE (Figure 6, with no peaks at 16 min retention time). Peak 1 corresponded to the saponified anthocyanin. Another peak

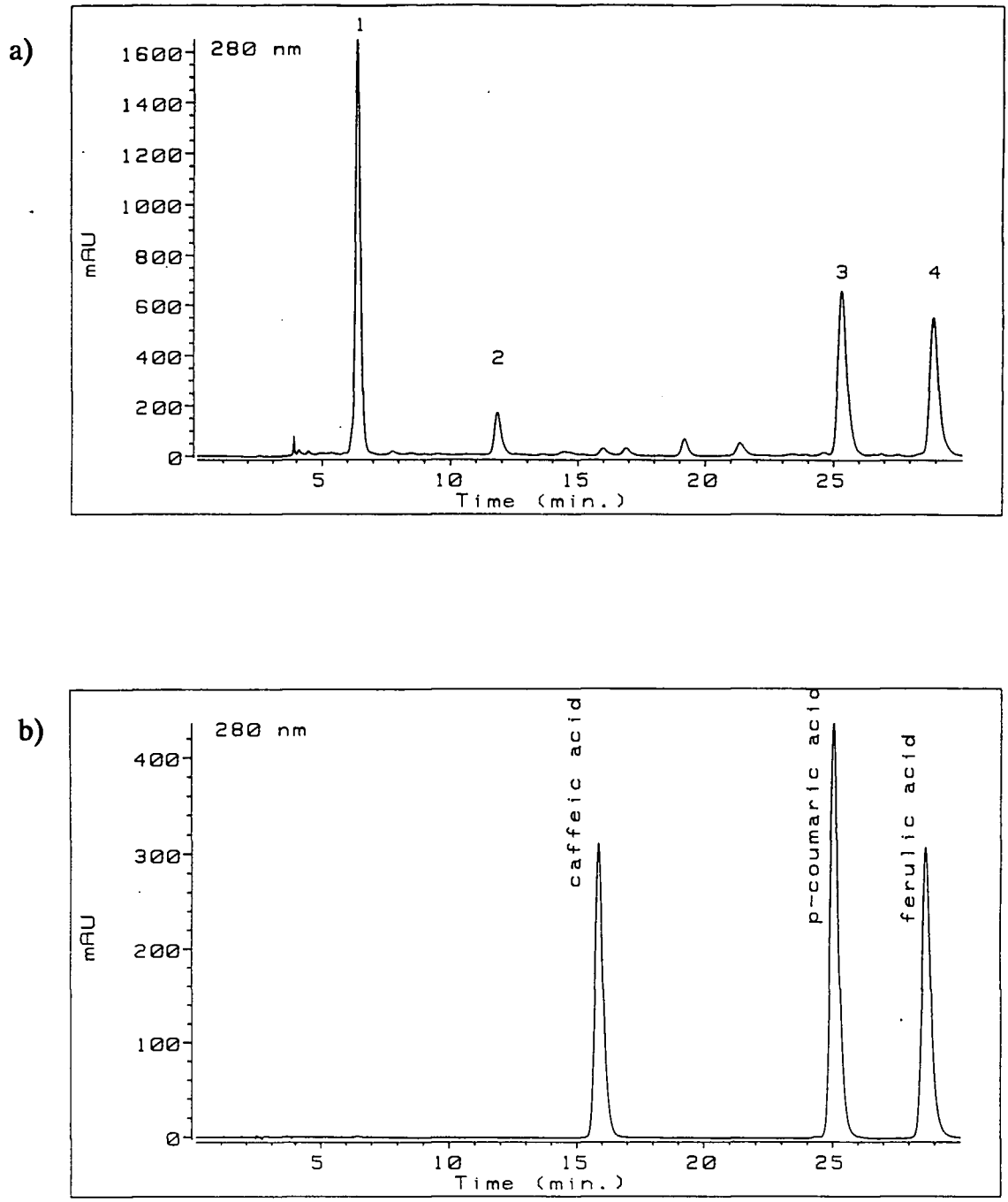


Figure 6. HPLC separation of phenolic acids. (a) saponified radish anthocyanin (peak 1) and acylating groups (peaks 2-4). (b) pure standards. Column, Supelcosil LC-18, 250 x 5 mm i.d. Solvents C: 100% HPLC grade acetonitrile and D: 1% phosphoric acid, 10% acetic acid, 5% acetonitrile, and water. Gradient: isocratic at 5% C (95% D). Flow rate: 1 mL/min. Injection volume: 50 μL.

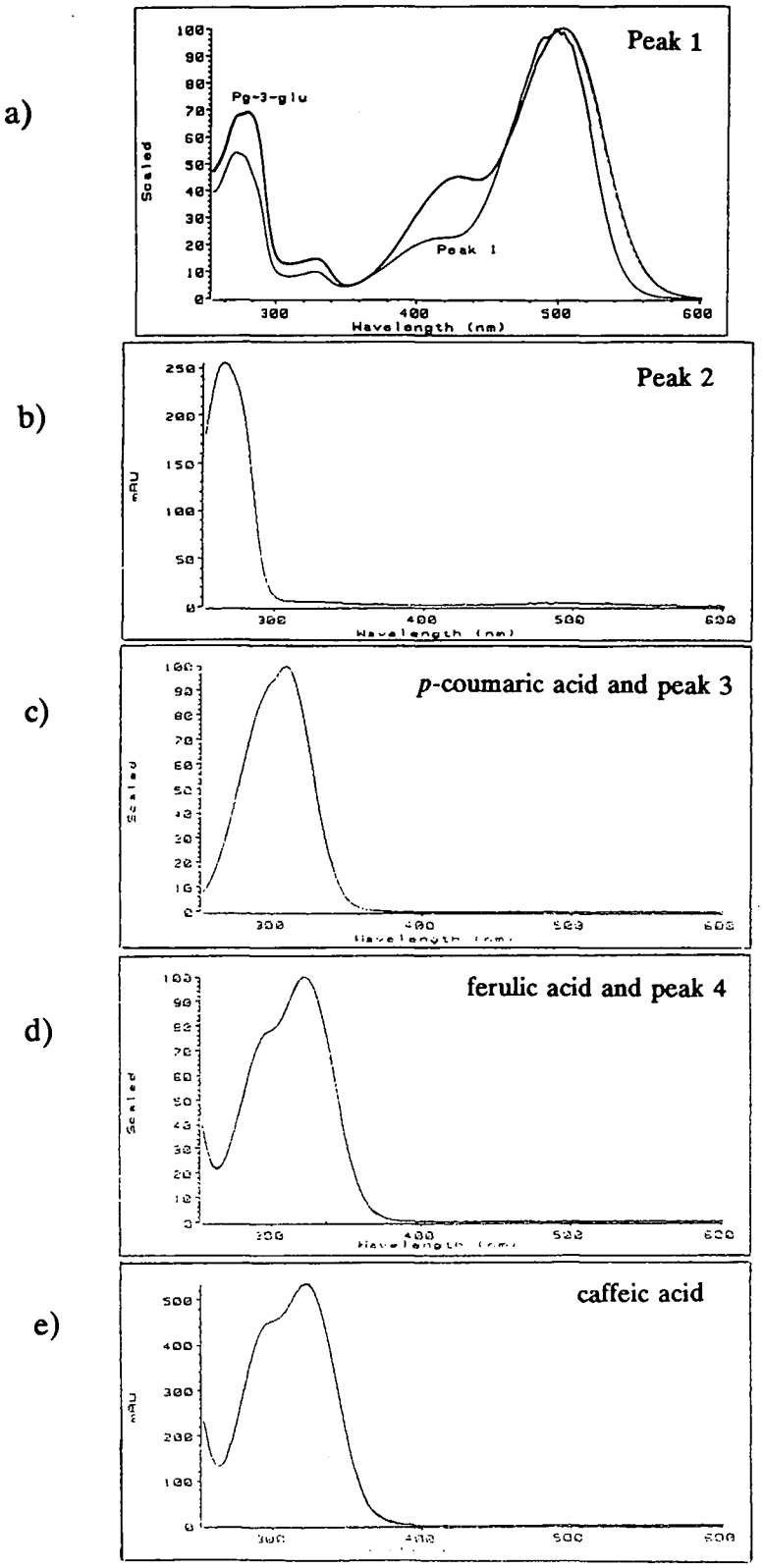


Figure 7. Comparative spectra of (a) saponified radish anthocyanin and pelargonidin glucoside (from strawberry), (b) acylating peak 2, (c) acylating peak 3 and *p*-coumaric acid, (d) acylating peak 4 and ferulic acid, and (e) pure caffeic acid (same HPLC conditions as for Figure 6).

(Peak 2) was detected, but its identity was not determined. The spectrum and maximum absorbance wavelength of this undetermined peak were similar to those of hydroxybenzoic acid; however, their retention times did not match. Saponification of radish anthocyanins produced only one peak detected at 520 nm (Figure 8). The retention time of the saponified anthocyanin was shorter than that of pelargonidin-3-glucoside (pg-3-glu) from strawberry, suggesting the presence of more glycosidic substitutions. A comparison of the spectra of this saponified pigment with the spectrum of pg-3-glu (Figure 7a), shows a similar maximum absorbance wavelength but a great difference in the 440 nm shoulder. Harborne (1967) reported that the spectra also give information about the glycosidic substitutions in the anthocyanin molecule. He found that the 3-glycosides exhibit ratios of absorbance at 440 to absorbance at visible maximum wavelength that is about twice as large as for the 3,5-diglucosides. The ratio obtained for pg-3-glu was approximately twice that obtained for the radish anthocyanin. These results are in agreement with the findings of Ishikura and Hayashi (1962), who determined that the pigment in red radish epidermal tissue is a mixture of different acylated anthocyanins with a common basic component, pelargonidin-3-sophoroside-5-glucoside (pg-3-soph-5-glu), raphanusin. Fuleki (1969) also reported the presence of pelargonidin-monoglucosides in the variety French Breakfast, but these pigments were not found in our experiment. This can be explained by varietal differences; however, it is also possible that the methods of extraction used may have caused partial hydrolysis of the raphanusin moiety, yielding pg-3-glu or pg-5-glu.

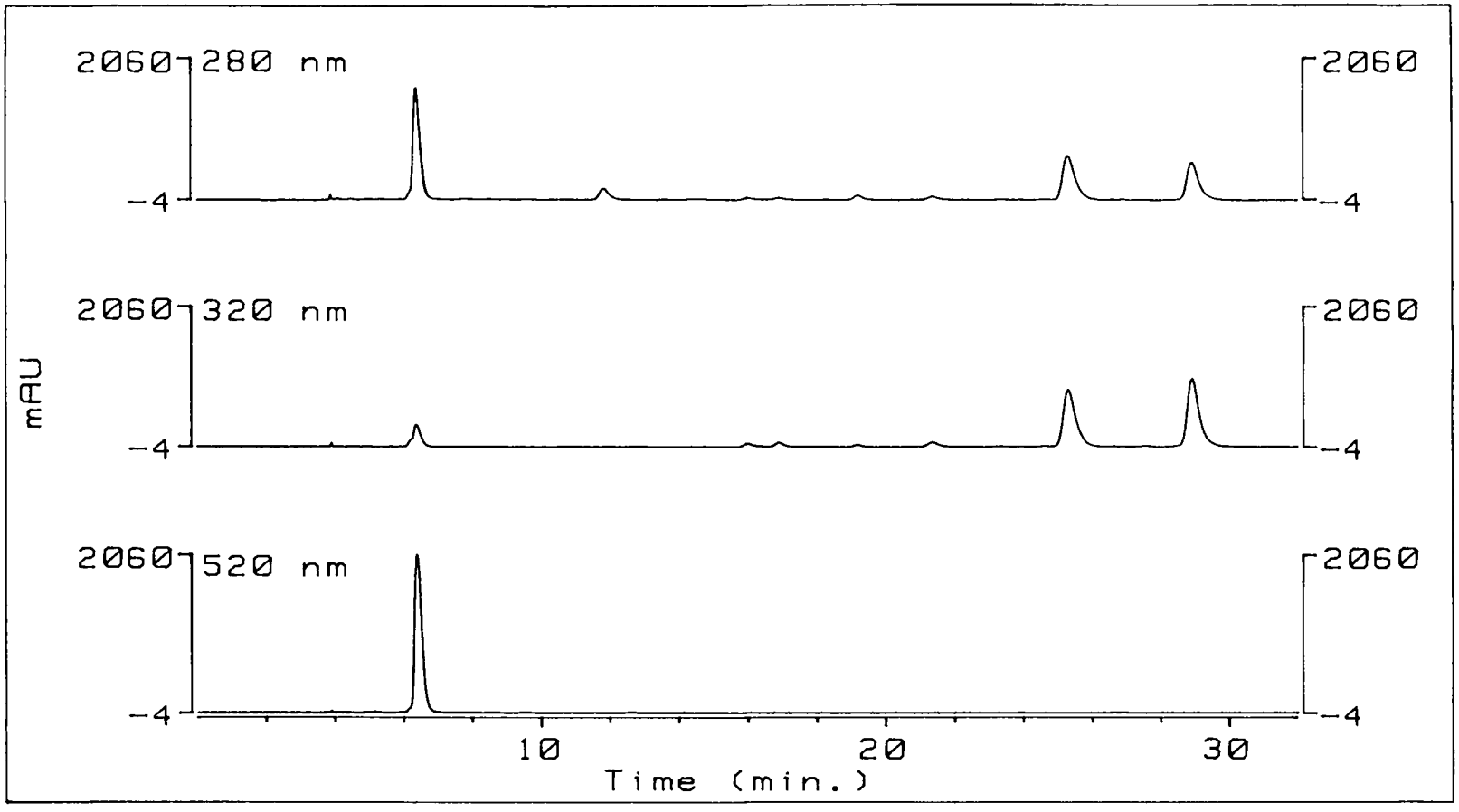


Figure 8. HPLC separation of saponified radish anthocyanins at different wavelengths: 280, 320 and 520 nm (same HPLC conditions as for Figure 6).

Acid hydrolysis of the saponified radish anthocyanins resulted in only one anthocyanidin (Figure 9). The retention time and spectrum of the hydrolyzed pigment matched that of pelargonidin obtained from strawberry, confirming previous identification of radish anthocyanins as pelargonidin derivatives (Ishikura and Hayashi, 1962; Harborne, 1963; Fuleki, 1969).

The combined information obtained from HPLC data of RAE, the saponified pigments and acid hydrolyzed pigments showed that all the anthocyanins present were pg-3-soph-5-glu with one or more acylating groups. The two major pigments (Figure 4, peaks 5 and 6) were identified as pg-3-soph-5-glu, one acylated one *p*-coumaric acid and the other with ferulic acid in a 1/1 molar ratio. Since the retention time of *p*-coumaric acid is shorter than that of ferulic acid under these HPLC conditions, we believe that peak 5 corresponds to pg-3-soph-5-glu acylated with *p*-coumaric acid, while peak 6 corresponds to pg-3-soph-5-glu acylated with ferulic acid. Peaks 3 and 4 showed the same composition (pg-3-soph-5-glu acylated with *p*-coumaric and ferulic acid, respectively); however, the different retention times obtained (with respect to peaks 5 and 6) suggest that the acylation may be in a different position, or that these pigments are geometric isomers of the major ones.

Coloring maraschino cherries with radish anthocyanins

Before coloring the cherries, the SO_2 level was drastically decreased by successive washing procedures (Table 3). The purpose of this process was to eliminate undesirable flavor and aroma characteristics due to the presence of SO_2,

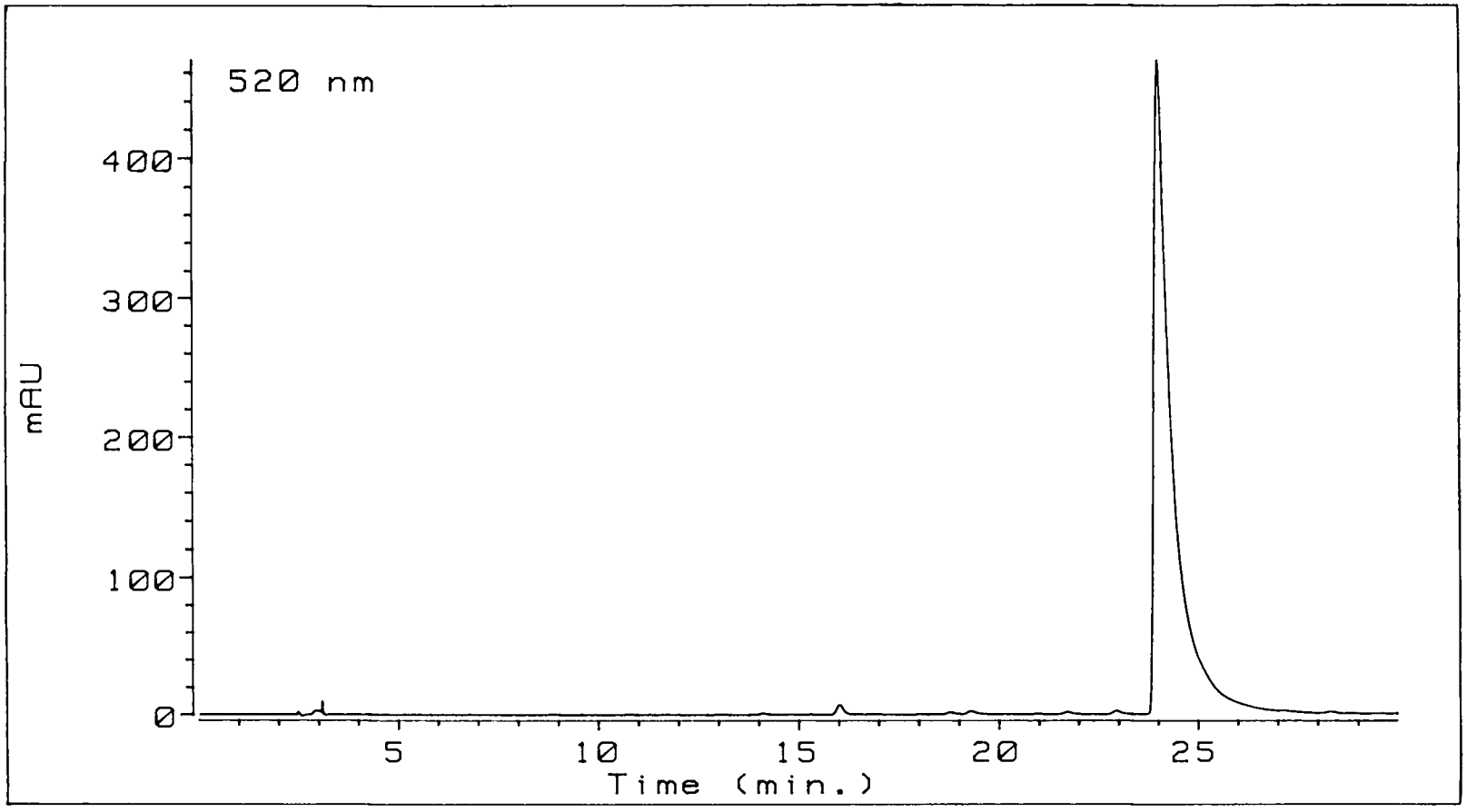


Figure 9. HPLC separation of hydrolyzed saponified radish anthocyanins. Column, Supelcosil LC-18, 250 x 5 mm i.d. Solvents C: 100% HPLC grade acetonitrile and D: 1% phosphoric acid, 10% acetic acid, 5% acetonitrile, and water. Gradient: linear gradient from 0 to 30% C (100 to 70% D) in 30 min. Flow rate: 1 mL/min. Injection volume: 50 μL.

Table 3. Free SO_2 level in primary and secondary bleached cherries

	Primary Bleached	Secondary Bleached
Brined cherries	3,500 ppm	2,000 ppm
After washing	480 ppm	145 ppm
After processing	26 ppm	18 ppm

reducing destruction of the coloring agent by sulfite bleaching and decreasing the risk of allergic reactions to sulfites by consumers (Nordlee *et al.*, 1985).

Color measurements of cherries and syrup before and after coloring and processing are presented in Table 4. Color measurements (Hunter CIE L* (lightness), a* (redness) and b* (yellowness)), and the calculated color intensity (c) and hue angle (Figure 10) showed that the radish anthocyanin extract (RAE), in the two different concentrations used (600 mg/L of syrup, RAE C1, and 1200 mg/L of syrup, RAE C2) imparted red color to the cherries extremely close to that of FD&C Red No. 40. The results were even better in reproducing the color of FD&C Red No. 40 in the syrup (Figure 11), where hue angle and color intensity, given by the angle form by the vector and the X axis, and by the length of the vector, respectively, almost matched. No statistical differences (multiple comparison analysis of variance) were found among the L*, a*, b*, and c values of the secondary bleached cherries and syrups colored with RAE C1, RAE C2 and FD&C Red No. 40 (2-sided p-values higher than 0.1). The statistical evidence also suggests (2-sided p-value 0.07) that there were no significant differences among the hue angles of the secondary bleached

Table 4. Color measurements of cherries (top) and syrup (bottom) samples before and after coloring and processing. Reflectance, specular included, 10° observer angle. PB: primary bleached cherries; SB: secondary bleached cherries.

	Reflectance specular excluded					Reflectance specular included				
	L*	a*	b*	chroma (c)	hue angle	L*	a*	b*	chroma (c)	hue angle
PB in brine	47.32	-0.13	32.78	32.78	90.23	55.44	0.19	26.78	26.78	89.59
PB in syrup	35.18	1.85	25.69	25.76	85.88	44.25	1.41	17.10	17.16	85.29
SB in brine	49.67	-2.14	2.43	3.24	131.37	56.33	-1.77	1.59	2.38	138.07
SB in syrup	35.28	-1.10	3.11	3.30	109.48	42.64	-0.77	1.62	1.79	115.42
PB RAE C1	22.52	20.50	10.73	23.13	27.66	35.05	13.24	5.58	6.77	11.48
	(1.66)	(1.14)	(0.38)	(1.18)	(0.47)	(0.17)	(0.52)	(0.55)	(0.70)	(1.19)
PB RAE C2	18.20	20.00	8.47	21.71	22.96	33.15	11.36	3.67	11.93	17.89
	(0.24)	(0.96)	(0.56)	(1.10)	(0.38)	(0.57)	(0.67)	(0.29)	(0.73)	(0.35)
PB FDC R40	18.00	24.35	12.13	27.20	26.47	33.60	13.34	4.75	14.15	19.57
	(0.57)	(2.75)	(2.37)	(3.51)	(1.91)	(0.12)	(1.26)	(0.59)	(1.39)	(0.56)
SB RAE C1	15.27	16.21	5.21	17.03	17.82	31.83	7.83	1.77	8.03	12.70
	(0.55)	(1.81)	(1.16)	(2.08)	(1.88)	(0.11)	(1.20)	(0.46)	(1.27)	(1.32)
SB RAE C2	14.42	12.76	3.41	13.20	14.95	31.33	5.51	0.96	5.59	9.83
	(0.77)	(0.19)	(0.37)	(0.28)	(1.31)	(0.47)	(0.06)	(0.15)	(0.08)	(1.36)
SB FDC R40	16.38	19.91	8.99	21.84	24.29	32.37	9.99	3.13	10.47	17.40
	(0.73)	(0.97)	(0.80)	(1.22)	(0.88)	(0.19)	(1.16)	(0.67)	(1.31)	(1.63)

	Reflectance specular excluded					Reflectance specular included				
	L*	a*	b*	chroma (c)	hue angle	L*	a*	b*	chroma (c)	hue angle
PB in brine	77.42	-1.79	3.83	4.23	115.05	71.35	-2.05	3.68	4.21	119.00
PB in syrup	79.03	-1.11	1.39	1.78	128.61	73.05	-1.28	1.65	2.09	127.80
SB in brine	77.84	-1.40	1.50	2.05	133.03	72.56	-1.64	1.60	2.29	135.00
SB in syrup	78.97	-0.80	1.59	1.78	116.00	72.63	-0.90	1.83	2.04	116.00
PB RAE C1	40.50	42.96	25.97	50.20	31.15	31.82	50.06	43.58	66.37	41.04
	(0.16)	(0.11)	(0.23)	(0.21)	(0.16)	(0.16)	(0.01)	(0.21)	(0.15)	(0.13)
PB RAE C2	34.11	36.45	16.57	40.03	24.44	23.37	47.54	33.05	57.89	34.81
	(0.04)	(0.04)	(0.05)	(0.05)	(0.05)	(0.01)	(0.07)	(0.13)	(0.12)	(0.06)
PB FDC R40	31.92	54.17	47.61	72.11	41.31	31.92	54.17	47.36	72.11	41.31
	(0.23)	(0.21)	(0.02)	(0.17)	(0.10)	(0.23)	(0.21)	(0.02)	(0.17)	(0.10)
SB RAE C1	70.70	43.16	26.25	50.51	31.31	31.93	50.13	43.58	66.42	41.00
	(0.21)	(0.09)	(0.20)	(0.19)	(0.14)	(0.18)	(0.09)	(0.04)	(0.09)	(0.03)
SB RAE C2	34.92	37.53	17.64	41.47	25.17	24.16	48.03	34.29	59.01	35.52
	(0.55)	(0.53)	(0.60)	(0.74)	(0.45)	(0.48)	(0.23)	(0.68)	(0.58)	(0.40)
SB FDC R40	41.52	47.52	29.35	55.85	31.70	32.64	54.04	48.77	72.79	42.07
	(0.09)	(0.01)	(0.17)	(0.09)	(0.15)	(0.25)	(0.00)	(0.26)	(0.17)	(0.15)

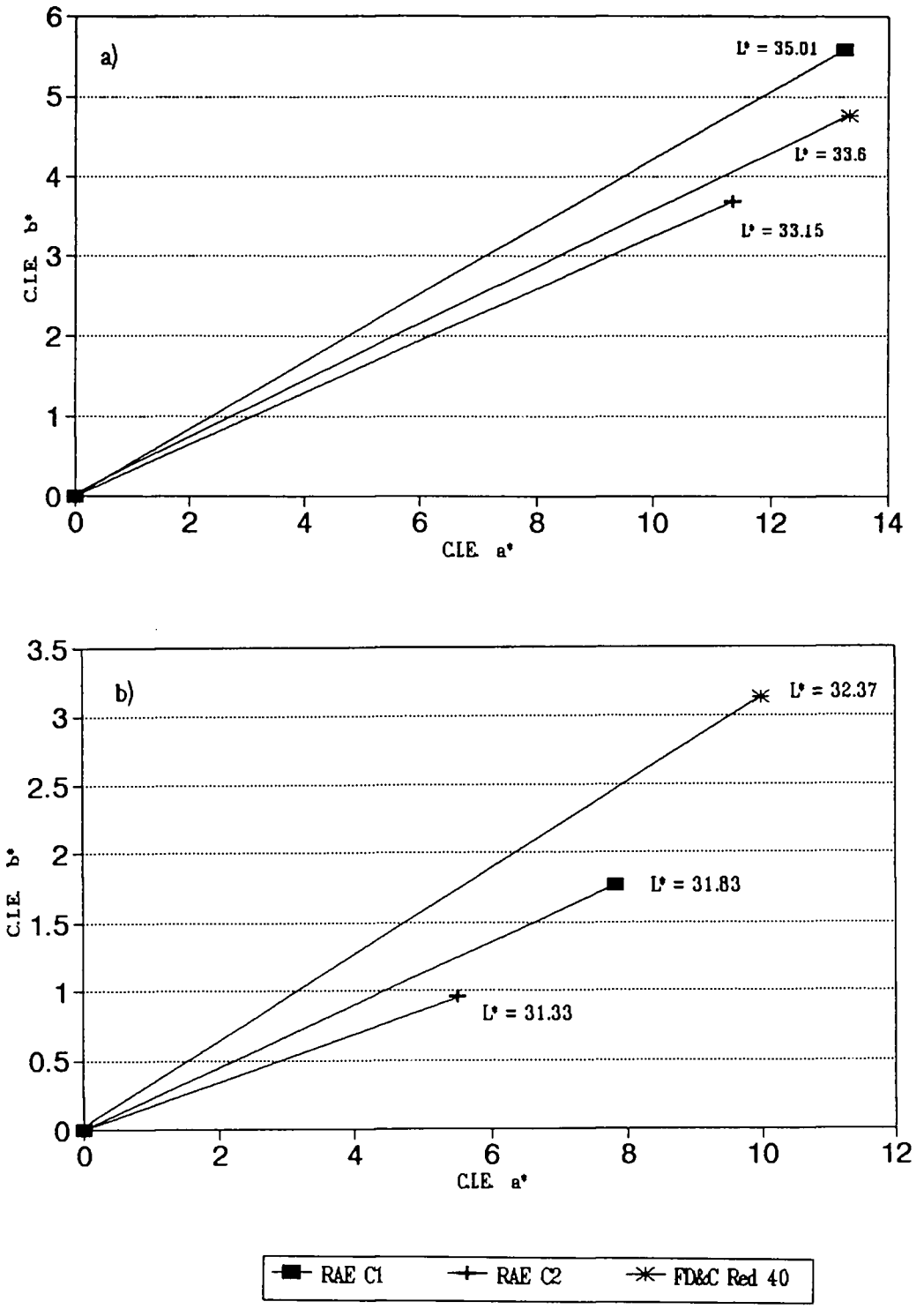


Figure 10. Color of primary (a) and secondary (b) bleached cherries after the different coloring treatments. Reflectance, specular included, 10° observer angle.

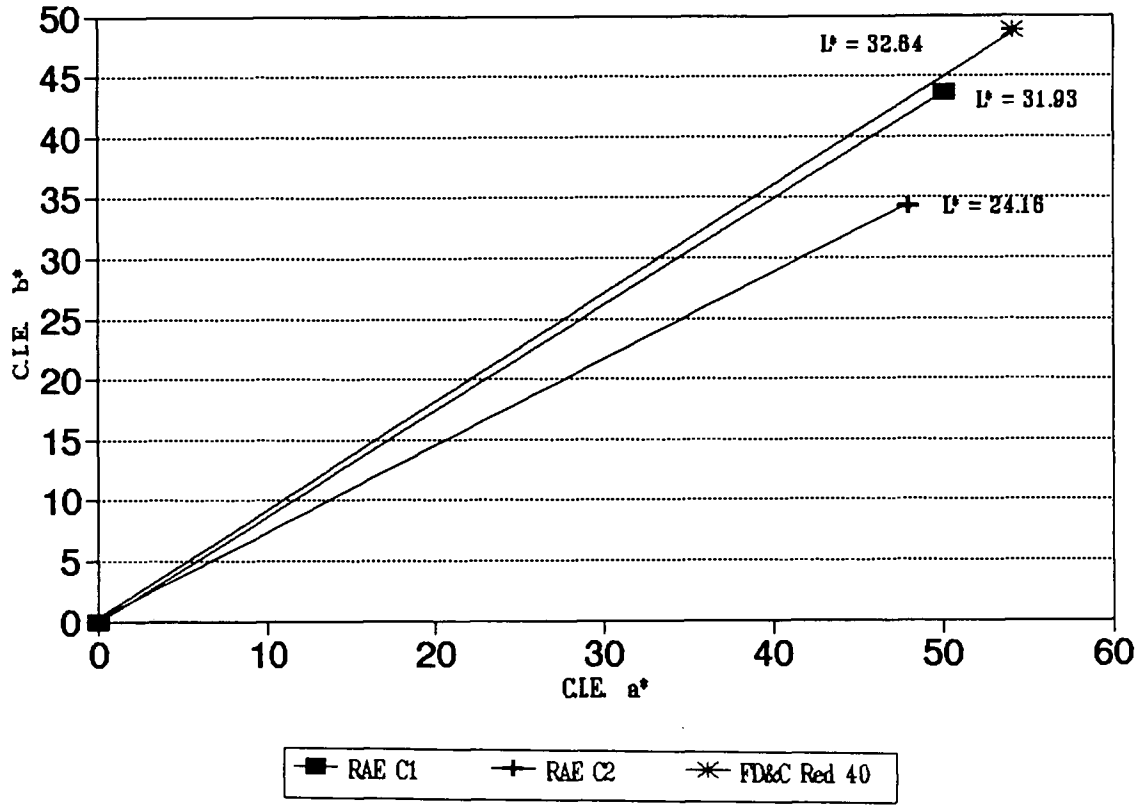


Figure 11. Color of syrup after the different coloring treatments. Reflectance, specular included, 10° observer angle.

cherries and syrup colored with the different treatments. However, small visual differences were detected among the treatments (Figure 12), especially when comparing the cherries colored with RAE C2 with the other two treatments. A concentration of 1200 mg of radish anthocyanins per L of syrup (RAE C2) gave the primary bleached cherries the closest color characteristics (L^* = 18.20, a^* = +20.00, b^* = +8.47) to FD&C Red No. 40 (L^* = 18.00, a^* = +24.35, b^* = +12.13), but the statistical evidence showed significant differences between the treatments (2-sided p-values < 0.0001). In the case of the secondary bleached cherries (Figure 10b), a concentration of 600 mg/L syrup (RAE C1) gave the cherries the closest color characteristics (L^* = 15.27, a^* = +16.21, b^* = +5.21) to FD&C Red No. 40 (L^* = 16.38, a^* = +19.91, b^* = +8.99), with no statistical differences between them.

Changes in cherry color during storage

The changes in color of secondary bleached cherries with time are presented in Figures 13, 14, 15, 16 and 17. The statistical analysis of the data showed that time (2-sided p-values 0.003 and 0.005) and treatment (2-sided p-values < 0.0001) had a significant effect in L^* and a^* values. The multiple comparison analysis (Tukey) showed no significant changes in L^* or a^* values during the first 40 weeks of storage (Figure 13 and 14). After this time, the L^* value started to show a significant increase (cherries started to become lighter), while the a^* value started to decrease in the cherries colored with RAE. No significant changes (2-sided p-value 0.54) with time were found on L^* nor a^* values on samples colored with FD&C Red No. 40. Time did not significantly affect the yellowness of the samples (b^* values), in any of

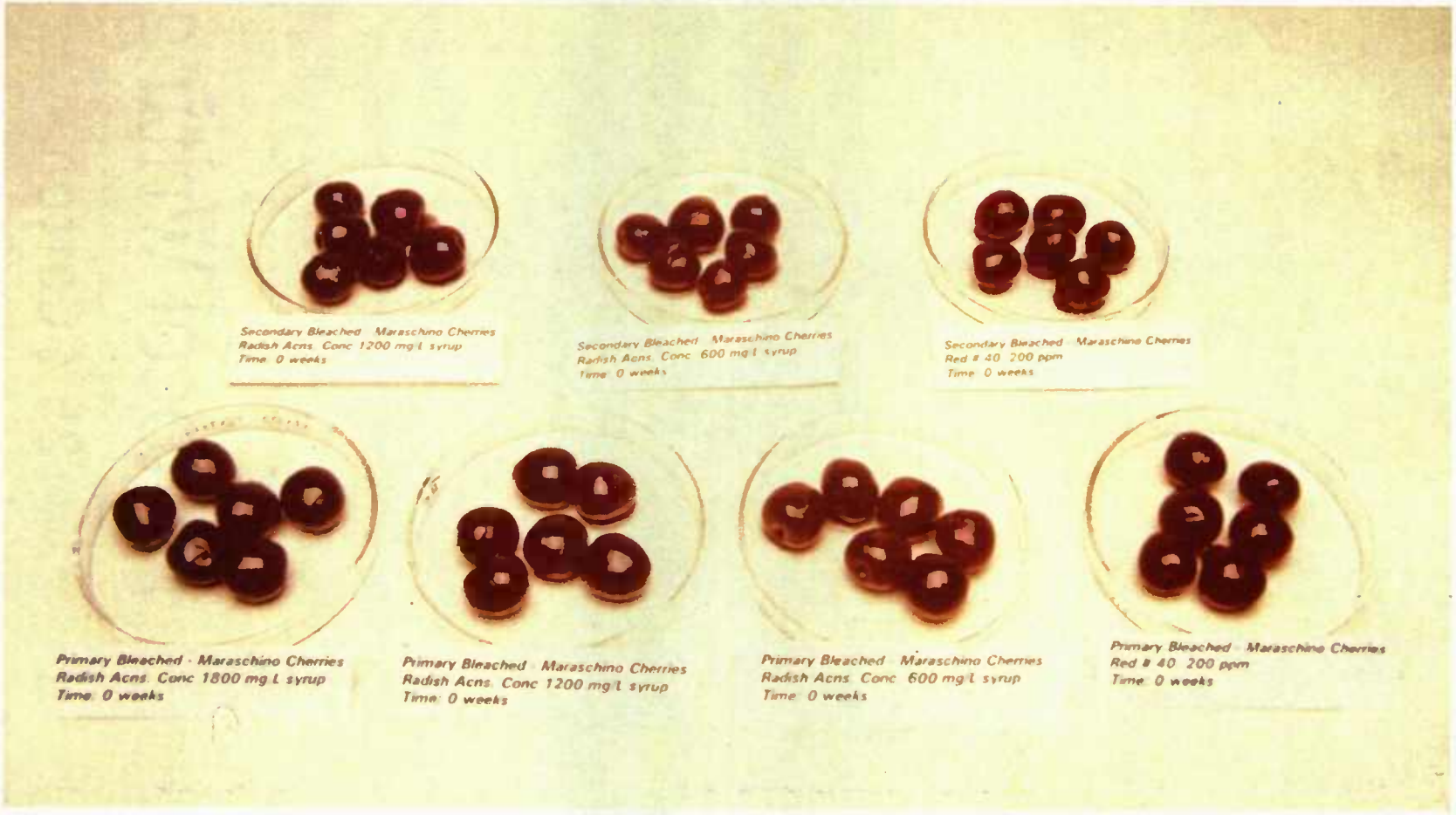


Figure 12. Photograph of cherries colored with the different treatments. On top: secondary bleached cherries colored with RAE C2, RAE C1, and FD&C Red No. 40. On bottom: primary bleached cherries colored with RAE (1800 mg/L), RAE C2, RAE C1, and FD&C Red No. 40 (from left to right).

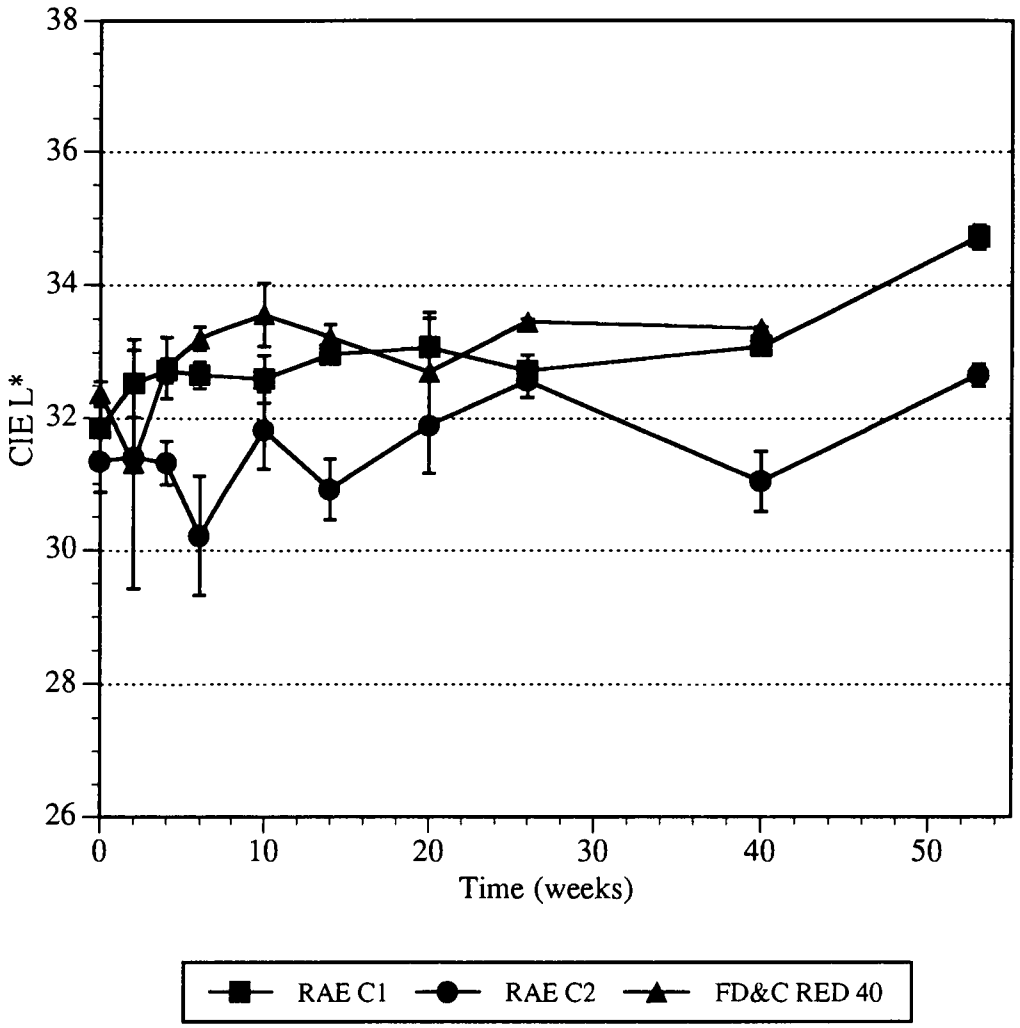


Figure13 . Color measurements in secondary bleached cherries. Changes of Hunter CIE L* values with time.

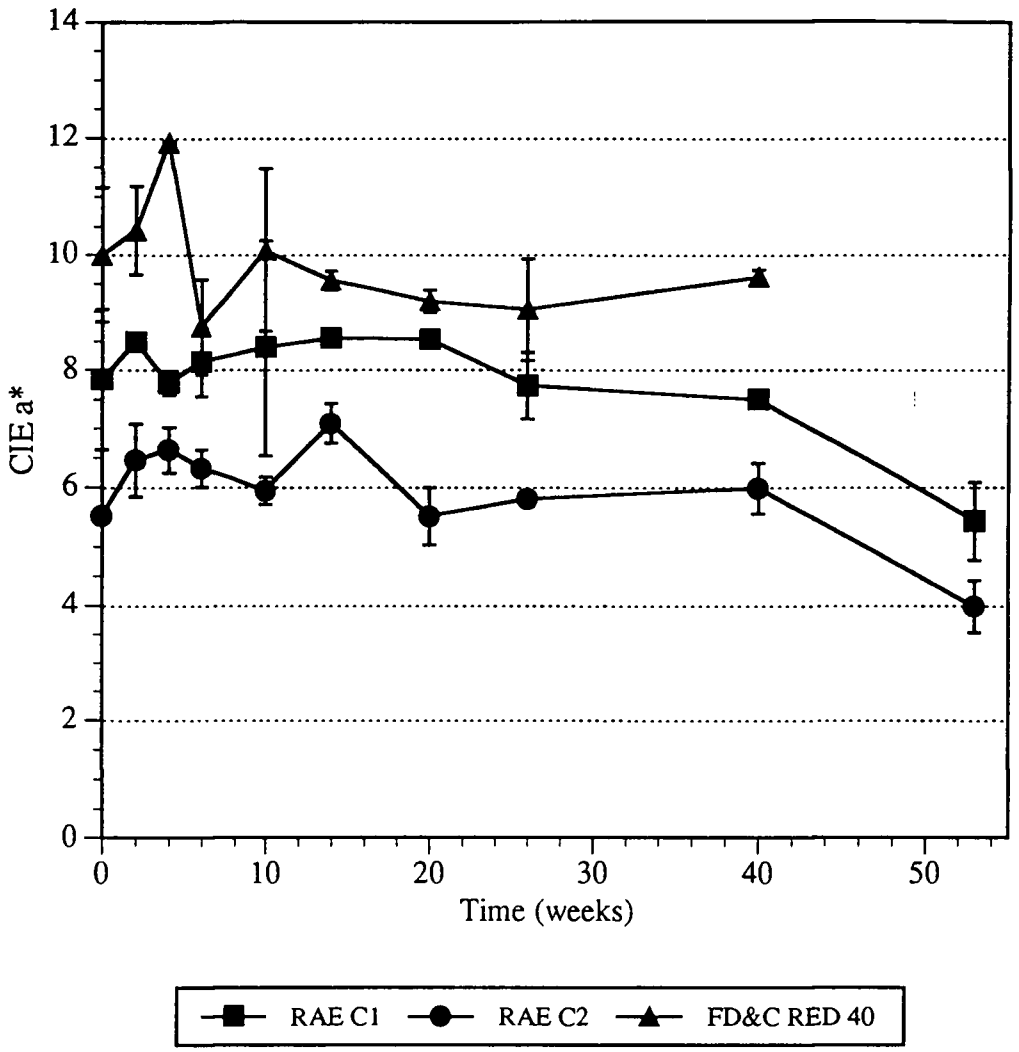


Figure 14. Color measurements in secondary bleached cherries . Changes of Hunter CIE a* values with time.

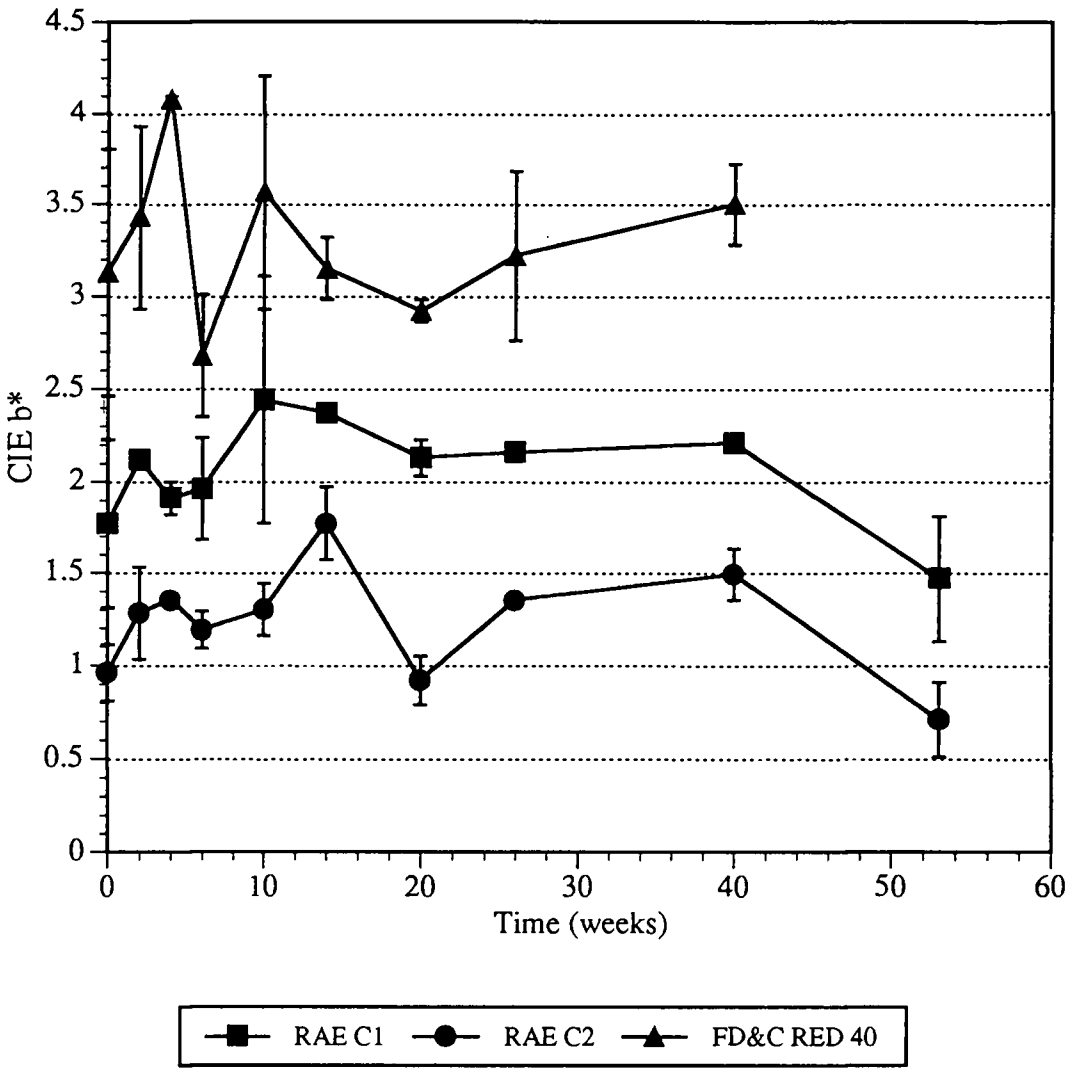


Figure 15. Color measurements on secondary bleached cherries. Changes of Hunter CIE b* values with time

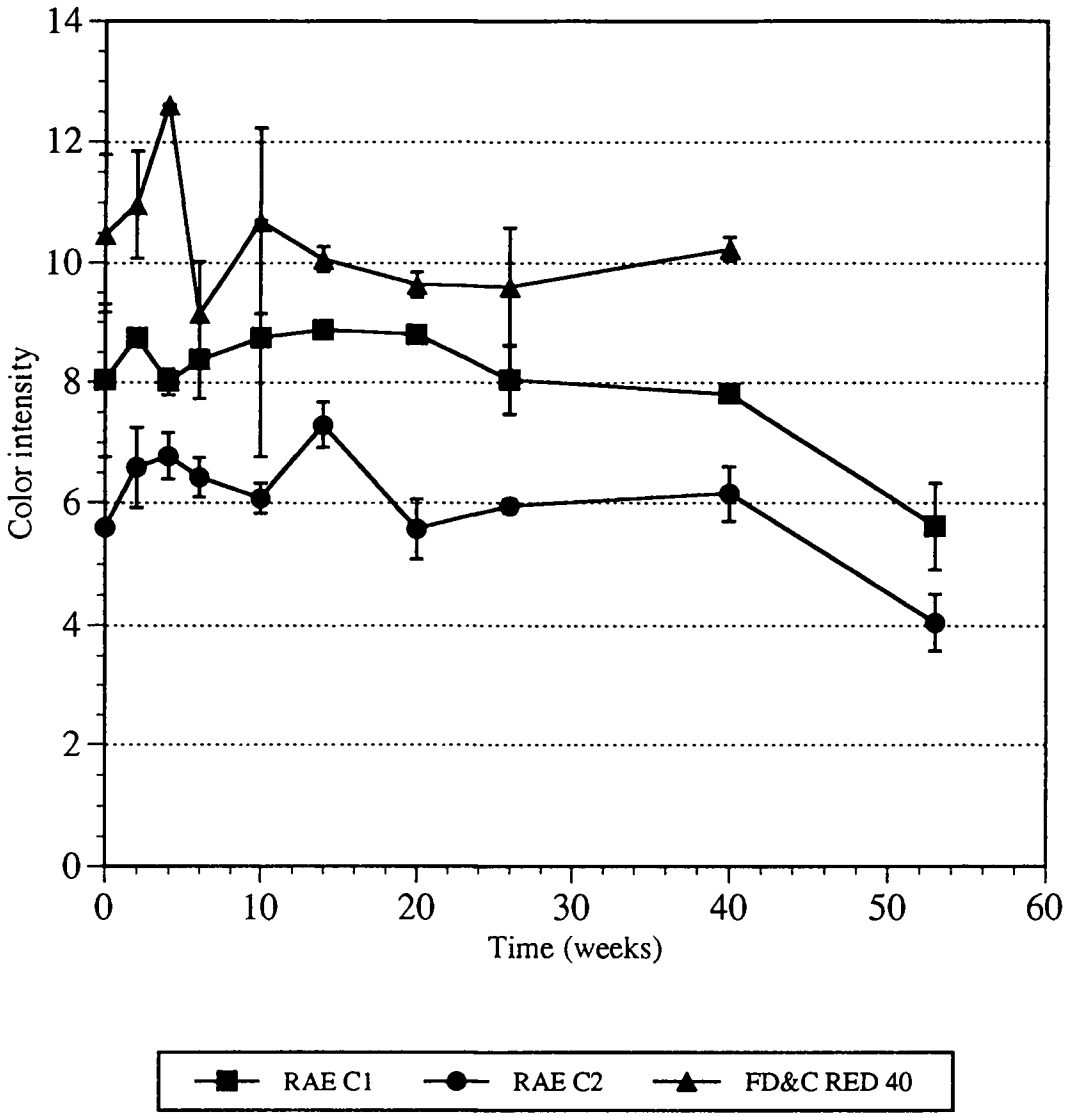


Figure 16. Color measurements in secondary bleached cherries. Changes of color intensity with time.

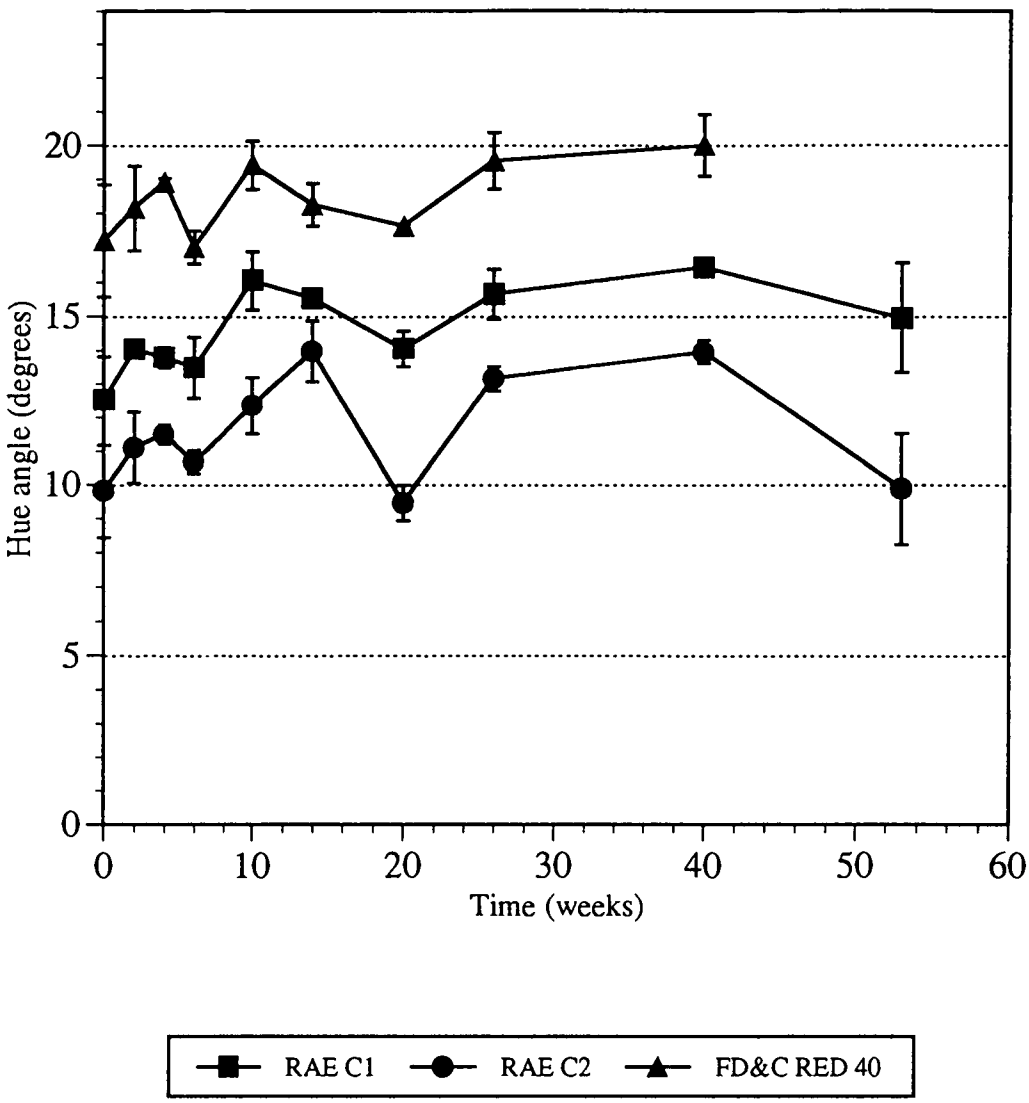


Figure 17. Color measurements in secondary bleached cherries. Changes of hue angle with time.

the color treatments (Figure 15). Significant changes (2-sided p-value < 0.0001) in the color intensity (c) of cherries were detected on the 40th week of storage on the samples colored with RAE and FD&C Red No. 40 (Figure 16). No significant changes in the hue angle of the cherries (Figure 17) were detected during the storage time for RAE C1 and FD&C Red No. 40 (2-sided p-value 0.10, and 0.28, respectively). The statistical findings also suggest that the hue angle was not significantly affected by time for the cherries colored with RAE C2 (2-sided p-value 0.03), and the tendency with time was not clear. Visual changes started to be detected at the 20th week of storage. These observations, and the high standard deviation obtained for the color measurements suggest that the methods were not entirely adequate. A limitation of the methodology was that the cherries were not completely flush with the flat surface of the cell leaving air spaces which affected the measurements. The results suggest that the efforts made to take several measurements in order to increase accuracy were not sufficient to compensate for this limitation. This most likely accounts for the high standard deviations (up to 22% of the measurements) which were obtained for most points. It is also possible that visual differences in cherry color were not due to changes in the three basic components of color (a*, b* and L*), but to gloss, which was not measured. The color measurements for the cherries did show that the main color changes during storage time were due to decreases in color intensity (chroma) rather than changes in hue angle.

Changes in syrup color during storage

Changes in syrup color were evident during storage and these changes correlated better with visual appearance (Figures 18, 19, 20, 21 and 22). There was statistical evidence that the lightness (L*) of the syrups colored with RAE increased during storage time (2-sided p-values < 0.01) (Figure 18). Significant differences were detected from the 26th week, for the syrup colored with RAE C1, and from the 40th week for the syrup colored with RAE C2. No significant effect of time on the L* value of syrup colored with FD&C Red No. 40 was detected (2-sided p-value 0.93). The degree of redness (a*) decreased markedly in the sample colored with FD&C Red No. 40 during the first 4 weeks of storage (Figure 19). Comparing this result with Figure 14, we can observe that there was also an increment in redness of the cherries during this time. These changes suggest that the cherries colored with FD&C Red No. 40 were still absorbing color from the syrup during the first 4 weeks of storage at 25°C, and that was after that point in time that the equilibrium was reached. This phenomenon was not observed in the cherries that were colored with RAE, suggesting that the solubility and diffusion rate of RAE into the cherries were higher that those of FD&C Red No. 40. After the fourth week of storage, however, the a* value for cherries colored with FD&C Red No. 40 showed no significant changes with time (2-sided p-value 0.56), during the 40 weeks of storage. Regression analyses showed that the level of redness in the cherries colored with RAE decreased significantly with time, and that the rate of redness loss was not the same for the two different treatments (Figure 19). The a* value decreased at a faster rate (0.331 units

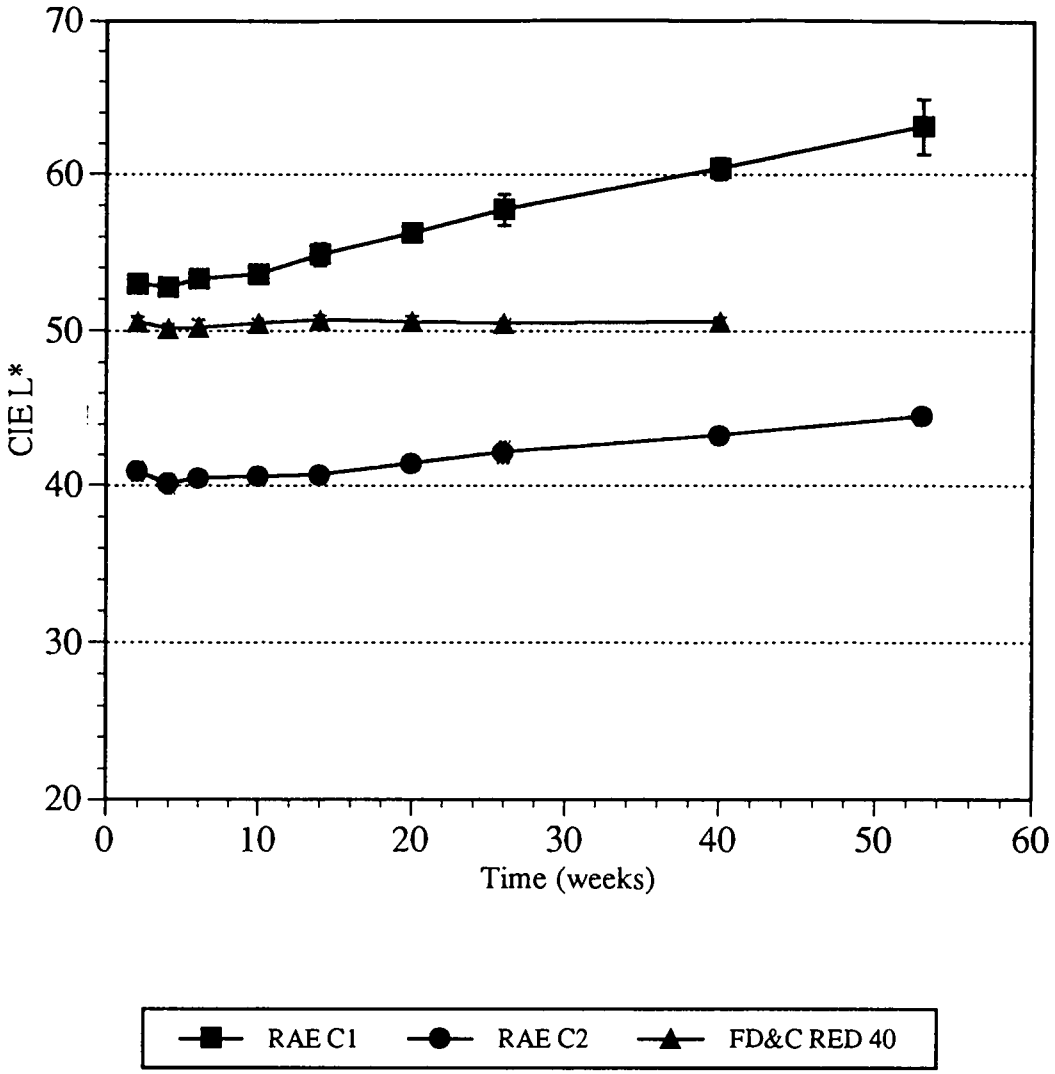


Figure 18. Color measurements in syrup. Changes of Hunter CIE L* values with time

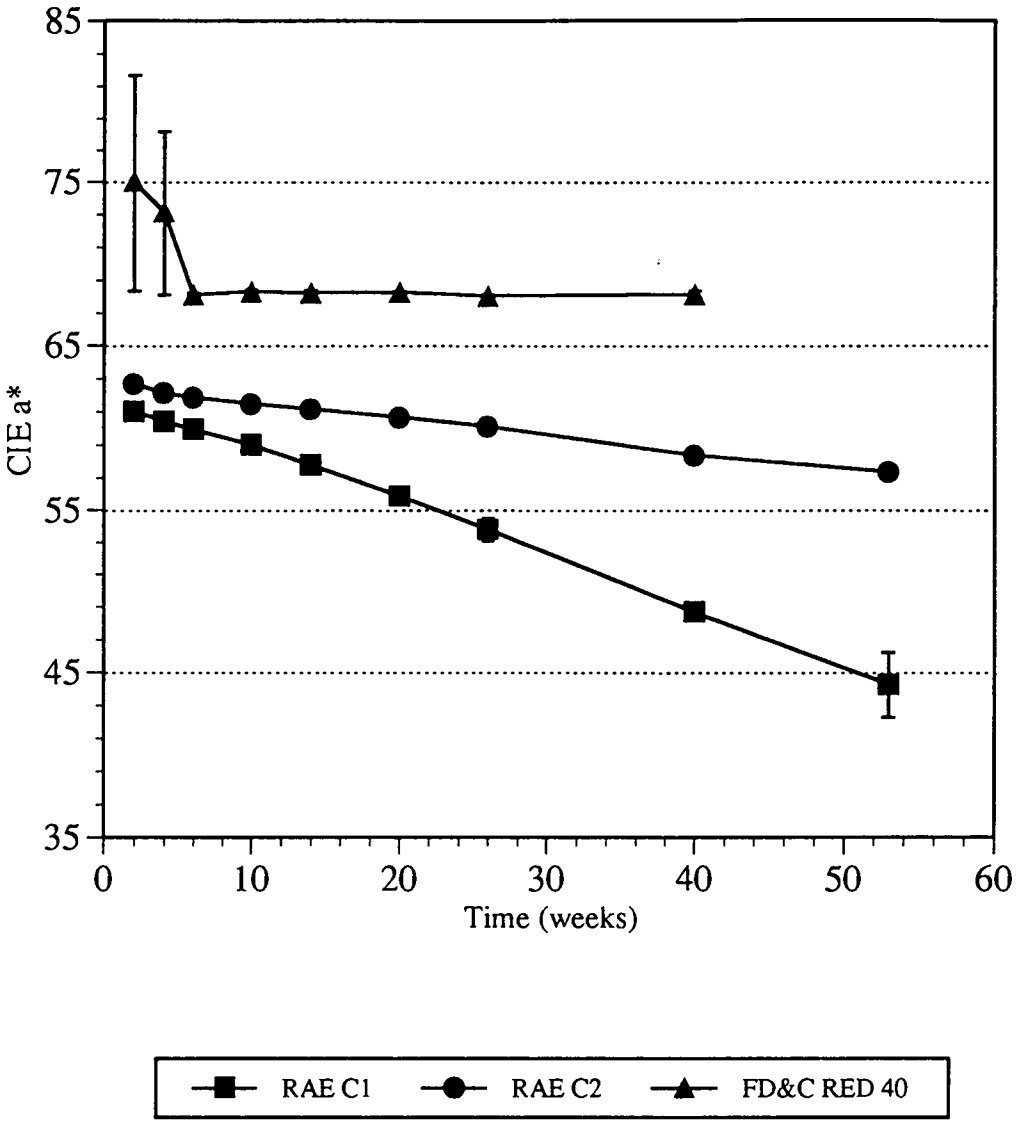


Figure 19. Color measurements in syrup. Changes of Hunter CIE a* values with time.

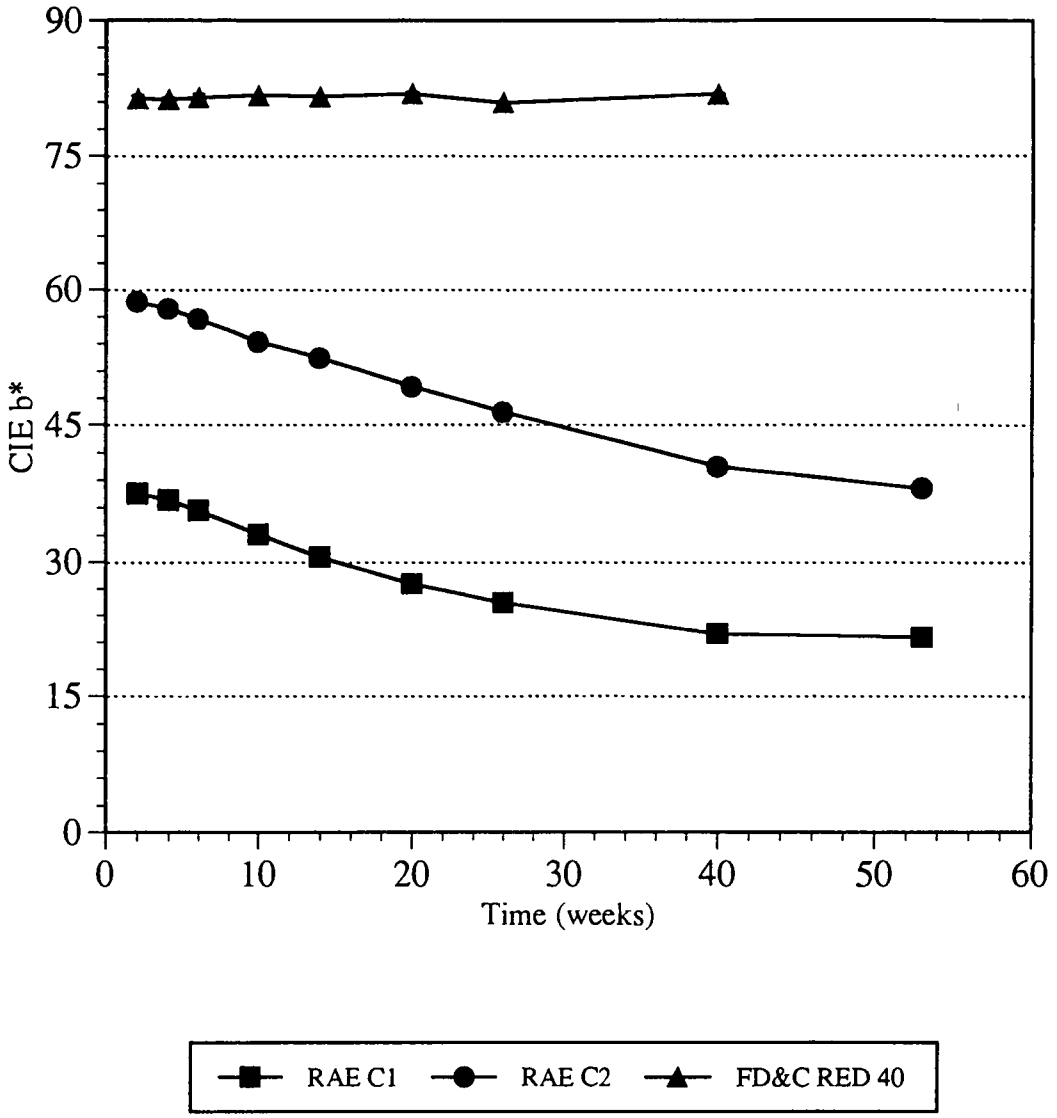


Figure 20. Color measurements in syrup. Changes of Hunter CIE b* values with time.

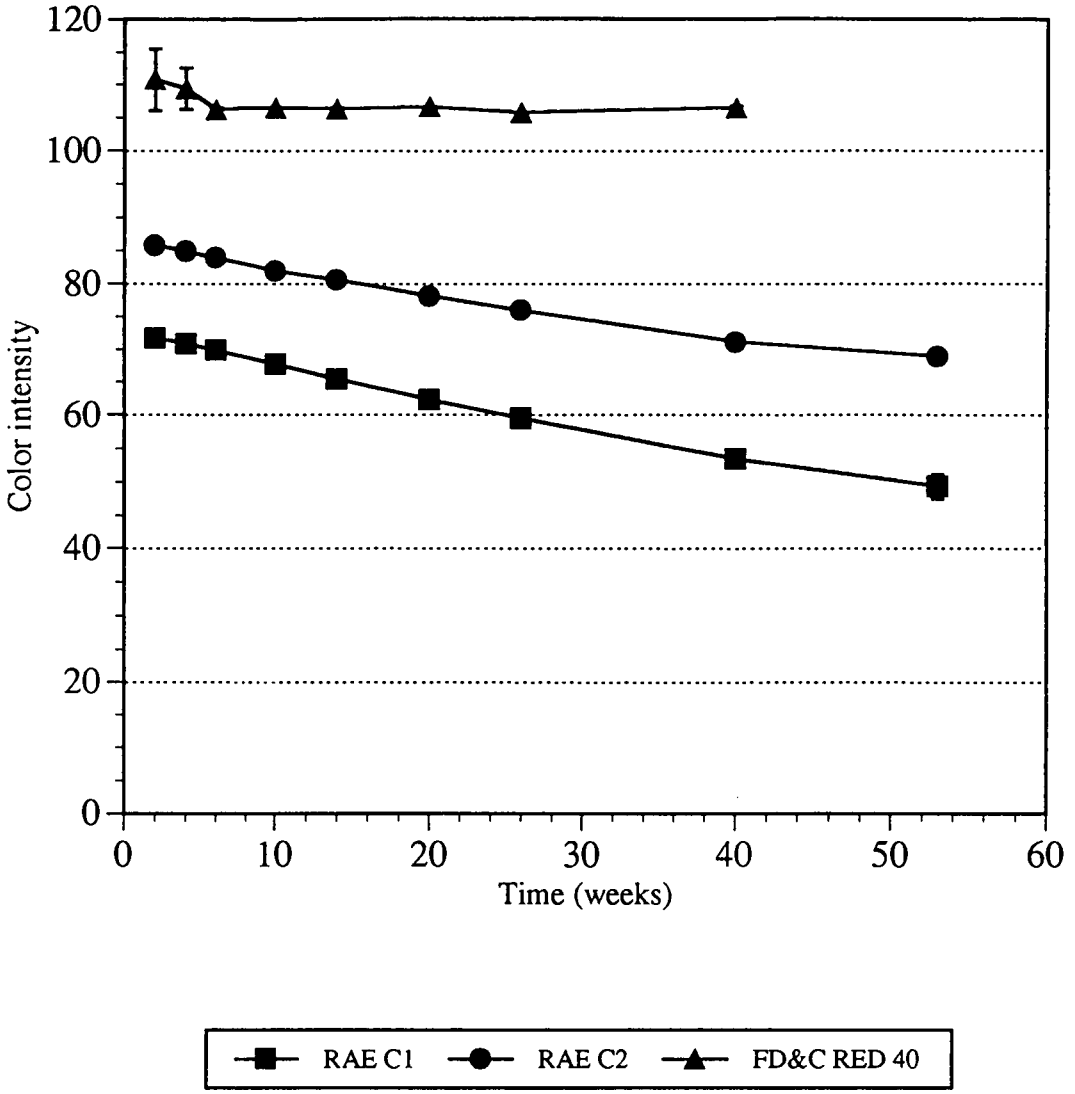


Figure 21. Color measurements in syrup. Changes of color intensity with time.

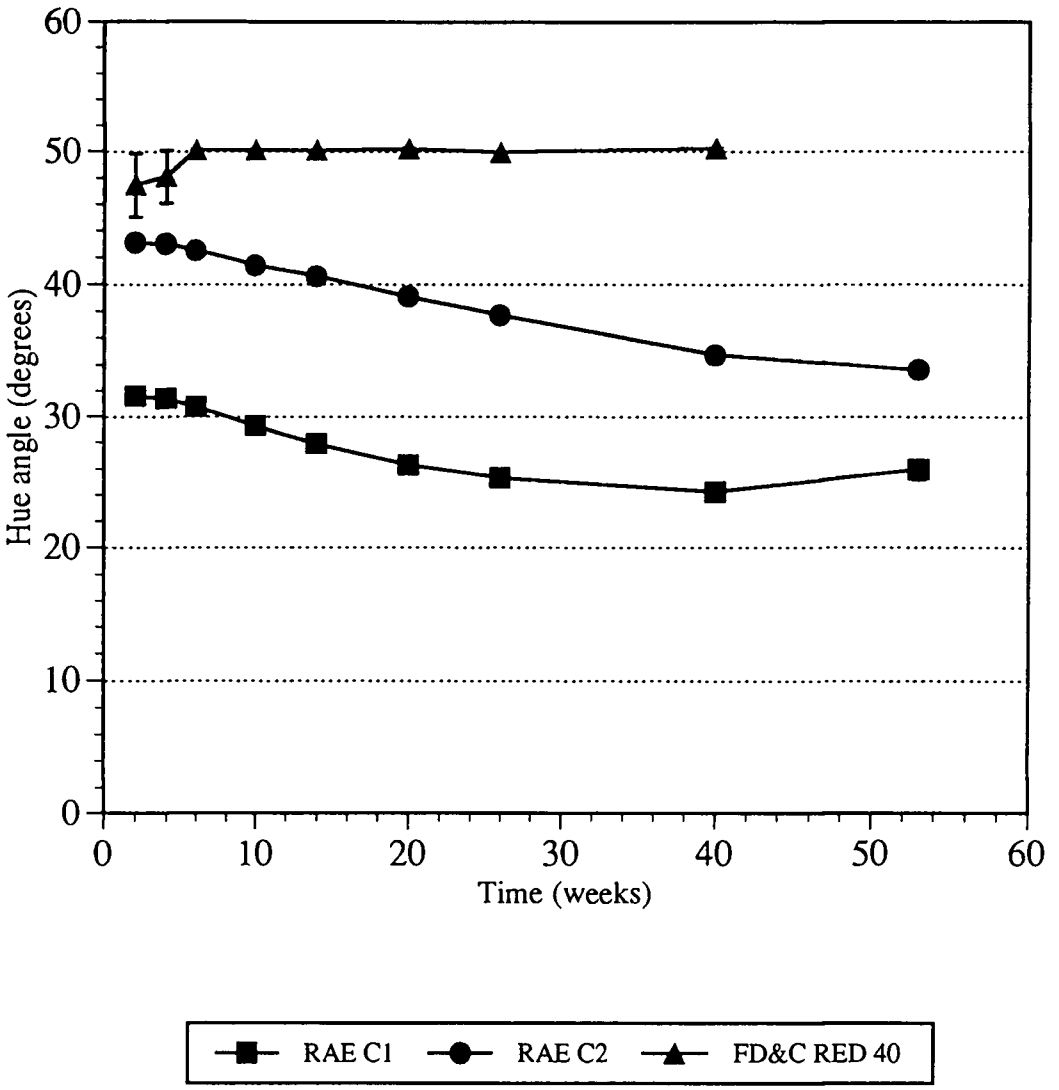


Figure 22. Color measurements in syrup. Changes of Hue angle with time

per week) in the samples colored with RAE C1 (regression analysis, 2-sided p-value < 0.0001) than in those colored with RAE C2 (0.101 units per week) (2-sided p-value < 0.0001). These results agree with the findings of Skrede and coworkers (1992), who showed that color stability of strawberry syrup was dependent upon total anthocyanin concentration, with greatly improved color stability when the anthocyanin concentration was increased. Significant changes with time were found in the b* values of the syrups (Figure 20) colored with RAE (2-sided p-value < 0.0001) and FD&C Red No. 40 (2-sided p-value 0.09). In the samples colored with RAE, significant reductions in b* values were detected from week 10. The b* values of the syrups colored with FD&C Red No. 40 started to show statistical differences at week 40. Color intensity and hue angle were affected significantly by time in the samples colored with RAE (2-sided p-value < 0.0001), but not in the case of syrup colored with FD&C Red No. 40 (2-sided p-value 0.16). Statistical evidence indicated that color intensity (c) decreased at a higher rate (0.452 units per week) in the sample colored with RAE C1 (regression analysis, 2-sided p-value < 0.0001), than in syrup colored with RAE C2 (0.344 units per week) (2-sided p-value < 0.0001) (Figure 21). These results indicate that the color of these syrup samples became lighter (less intense) during the storage time, specially in the ones colored with RAE C1. The hue angle of the syrup showed significant reduction from the 10th week of storage for samples colored with RAE C1 with a tendency to stabilize after the 20th week, while significant reductions were observed from the 14th week for samples colored

with RAE C2 (Figure 22). These changes indicate that the color of these syrup samples changed from a bright orange-red towards a more blueish based red color.

Changes in syrup pigment content during storage

At time zero (immediately after processing), the monomeric anthocyanin content was 15.8 ± 0.4 mg/L and 30.7 ± 0.9 mg/L for the syrup colored with RAE C1 and RAE C2, respectively. These values are lower than the initial concentration added to the syrup (60 mg/100 mL and 120 mg/100 mL syrup), and is explained by the diffusion of the anthocyanins into the cherries. It is also possible that some anthocyanin degradation may have occurred, due to the residual SO_2 and processing conditions.

The monomeric anthocyanin content decreased significantly during storage at 25°C (Figure 23). The loss of food quality for most foods can be represented by a mathematical equation of the following form:

$$dA/d\theta = kA^n$$

where A is the quality factor measured, in our case, monomeric anthocyanin concentration; θ represents time; k is a constant, which depends on temperature and water activity; and n represents a power factor called the order of the reaction, which defines whether the rate is dependent on the amount of A present (Labuza, 1982). If the changes in monomeric anthocyanin content of syrup with time fit a linear model, that would indicate that the rate of degradation of monomeric anthocyanins with time was constant at this temperature, modeled as zero-order kinetics (n = 0).

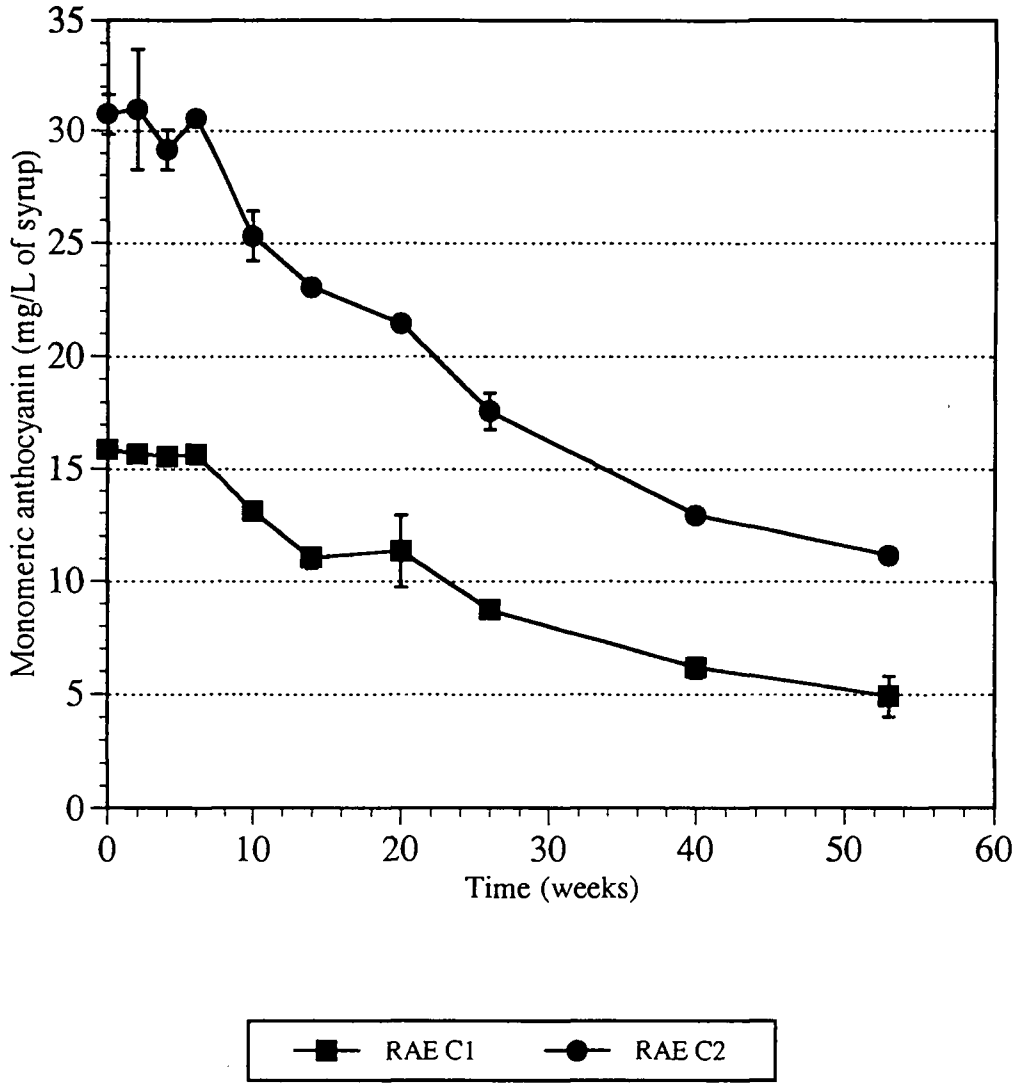


Figure 23. Changes in syrup monomeric anthocyanin (mg/100 mL syrup) with time.

Regression analyses showed that the rate of degradation depended on the anthocyanin concentration (2-sided p-value <0.0001) and was determined to be 0.228 mg/week and 0.413 mg/week for the syrup colored with RAE C1 and RAE C2, respectively (2-sided p-values < 0.0001). The equations that describe the changes in monomeric anthocyanin at 25°C with time (for zero-order kinetics) are:

Monom. Acn = 15.76 - 0.228 (time)
SE (0.377) (0.016) $r^2 = 0.92$

Monom. Acn = 30.49 - 0.413 (time)
SE (0.651) (0.027) $r^2 = 0.93$

where Monom. Acn. represents mean monomeric anthocyanin content (in mg/100 mL), and SE represents Standard Error. Fifty percent of the anthocyanin (half life) was estimated to be destroyed after 34.6 weeks and 36.9 weeks of storage at 25°C for syrup samples colored with RAE C1 and RAE C2, respectively. However, a zero-order kinetics (n = 0, therefore $A^n = 1$), would indicate that anthocyanin degradation did not depend on the concentration of the substrate (anthocyanins), and the fact that two different linear fits were found for the two different anthocyanin concentrations represents a contradiction to the model.

By plotting the changes of the logarithm of the monomeric anthocyanin content with time (Figure 24), it also followed a first-order kinetics (2-sided p-value < 0.0001), and the r^2 obtained indicates a better fit for this model. Therefore, the

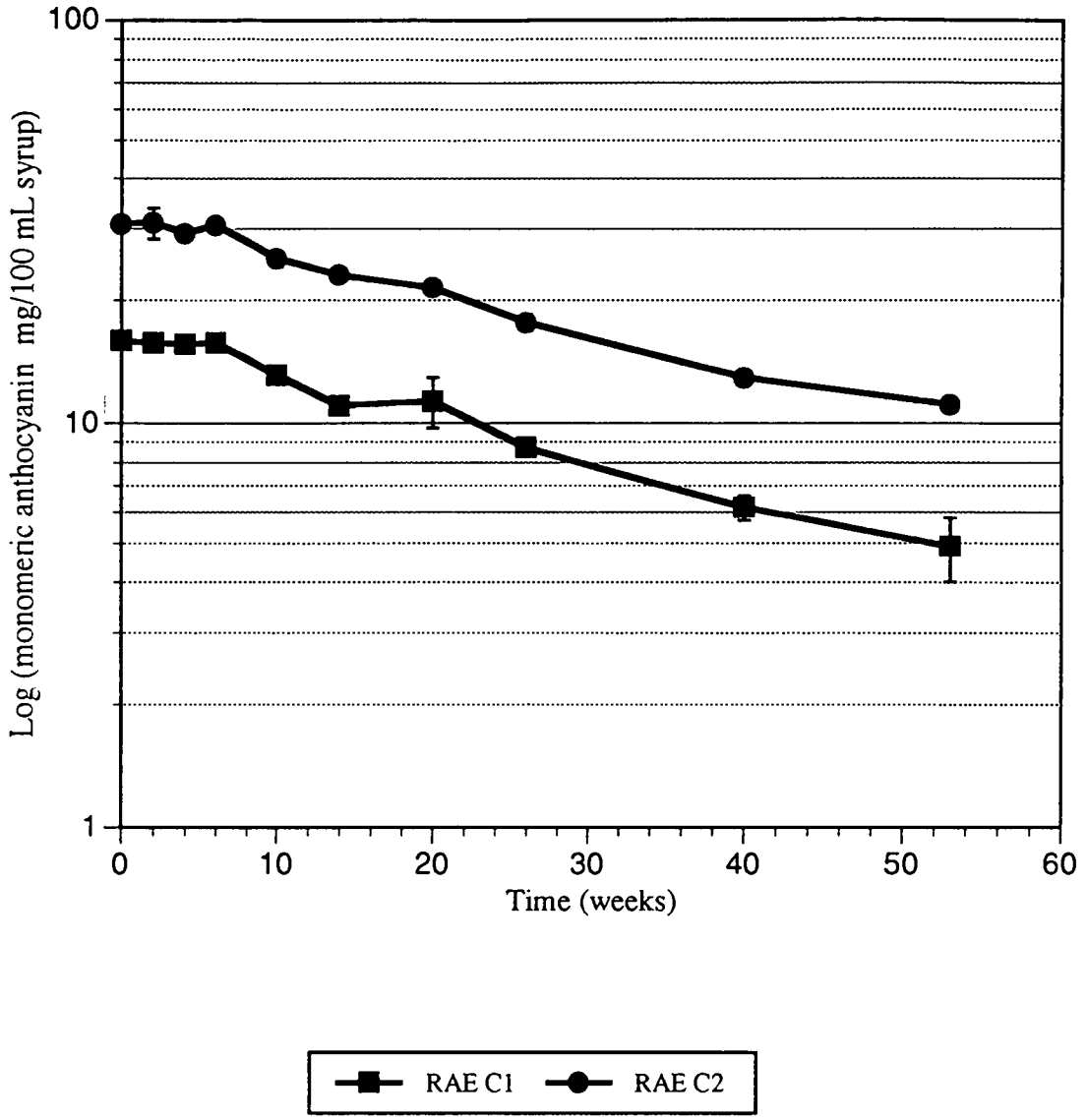


Figure 24. Changes in syrup monomeric anthocyanin with time

rate of anthocyanin degradation depended on the concentration of the substrate (Monom. Acn.), and as the concentration of anthocyanins decreased, the rate of degradation also decreased. The equations that describe the monomeric anthocyanin degradation on syrup samples colored with RAE C1 and RAE C2 (for first-order kinetics) with time are:

Log (Monom. Acn) = 2.81 - 0.024 (time)
SE (0.032) (0.001) $r^2 = 0.95$

Log (Monom. Acn) = 3.46 - 0.021 (time)
SE (0.020) (0.001) $r^2 = 0.97$

According to the first-order of degradation kinetics, the half life (50% degradation) of the anthocyanin at 25°C was calculated to be 28.8 and 33.0 weeks, for syrup samples colored with RAE C1 and RAE C2, respectively. Studies on degradation kinetics of anthocyanins have indicated a first-order reaction for sour cherries anthocyanins (Cemeroglu *et al.*, 1994), black raspberry (Daravingas and Cain, 1968) concord grape, red cabbage, and ajuga anthocyanins (Baublis *et al.*, 1994). However, Baublis and coworkers (1994) also found that the degradation of *tradescancia* anthocyanins, which showed higher stability than other anthocyanins studied when stored at room temperature and exposed to light, showed linearity (zero-order reaction). Labuza and Riboh (1982) pointed out that most quality related reaction

rates are either zero or first-order reactions and statistical differences between the two types may be insignificant.

Mazza and Miniati (1993) indicated that the half life (50% destruction) of a typical anthocyanin, cyanidin-3-rutinoside, at room temperature in an acidic (pH 2.8) medium, is about 65 days (about 9 weeks). McLellan and Cash (1979) reported that dark tart cherry juice may be used for coloring maraschino cherries, and estimated a relative stable period of 6 to 9 months, depending on variations from ambient temperature. However, in their experiment 50% of the anthocyanin was destroyed during the first 12 weeks of storage at 18°C. Radish anthocyanins showed much higher stability (between 29 and 33 weeks), although the temperature used in our experiment was higher (25°C). In recent years acylated anthocyanins which display unusual stability in neutral or weakly acid solutions have been found (Francis, 1989). Stabilization of the colored species of anthocyanins, especially the quinonoidal base, seems to be conferred by the presence of acyl groups linked to the sugar moiety of the pigment molecule, and is attributed to stacking of the aromatic residues of the acyl groups with the pyrylium group of the anthocyanin molecule (Jackman and Smith, 1991). However, many studies (Francis, 1989; Lu *et al.*, 1992; Stringheta *et al.*, 1992; Dangles *et al.*, 1993; Davies and Mazza, 1993) indicate that monoacylated anthocyanins do not display the color stability of di- or polyacylated anthocyanins, indicating that at least two constituent acyl groups are required for good color stability/retention in neutral or acidic media. These studies suggest that acylation with one aromatic acid does not necessarily improve color stability (Lu *et al.*, 1992;

Stringheta *et al.*, 1992). However, all of them agree that acylation improves color and pigment stability. In our study of color and pigment stability of cherries colored with RAE, we found that one acylating group gave higher stability to the pigment than any other anthocyanin previously recommended for coloring cherries. It has also been reported (Dangles *et al.*, 1993) that acylation with aromatic acids causes a bathochromic shift to higher wavelength of maximum absorbance. In the case of pg-3-soph-5-glu, we found that the maximum absorbance (in pH 1.0 buffer) was 500 nm, while the maximum absorbance of pg-3-soph-5-glu with one acylating group was 505 nm. Dangles and coworkers (1993) found a 20 nm bathochromic shift by increasing from 0 to 4 caffeyl acylating groups in a pg-3-soph-5-glu moiety. They attributed this change to a reduction in the polarity of the environment of the flavilium chromophore caused by its hydrophobic association with the caffeoyl residues. They also found that this bathochromic shift caused differences in the color of the different pg-derivatives when placed in solution under the same conditions, changing towards a more red-purple hue. In this research we found that the presence of one acylating moiety, *p*-coumaric or ferulic acid, attached to the pg-3-soph-5-glu moiety gave a red color extremely close to that of FD&C Red No. 40 under the conditions used.

Changes in syrup pigment were also monitored by spectral measurements of color density and polymeric color. Color density was calculated by adding the absorbance at 420 nm and the absorbance at 510 nm. Statistical evidence was found that the color density decreased significantly during the storage time (2-sided p-value

< 0.0001) (Figure 25). Color density was expected to decrease with the decrease in monomeric anthocyanin content, since anthocyanins are the compounds responsible for the absorbance in this wavelength range, providing the colored characteristics to the product. Percentage of polymeric color was measured, as an index of the degree of anthocyanin polymerization (Figure 26). The statistical analysis provided convincing evidence that time affected the degree of polymerization, and that these changes were dependent on the anthocyanin concentration (2-sided p-value < 0.0001). Figure 26 shows that the percent polymeric color increased during the first 40 weeks of storage. After that, the percent polymeric color decreased (week 53). This could be explained by the precipitation of the polymerized material. Bakker *et al.* (1986) reported the increase in absorbance of polymeric color during the first weeks of storage of wine samples, reaching a plateau, and slowly decreasing thereafter. This decrease was attributed to the precipitation of large polymeric molecules and/or the reduced color intensity of the larger polymers (Bakker *et al.*, 1986). Sediment was not observed at week 40; however, it became visually evident after 50 weeks of storage.

Changes in chromatographic profile during storage time

Chromatographic analyses were performed on syrups colored with radish anthocyanins during storage time. Changes in the anthocyanin profile with time were evident (Figure 27). Comparing these chromatograms with those of freshly extracted radish anthocyanins (Figure 4), we observe that the proportional amounts of the two main peaks (peaks 5 and 6, in Figure 4) decreased with time. At the time of coloring the cherries, peaks 5 and 6 represented ca. 70% of the total anthocyanins, while

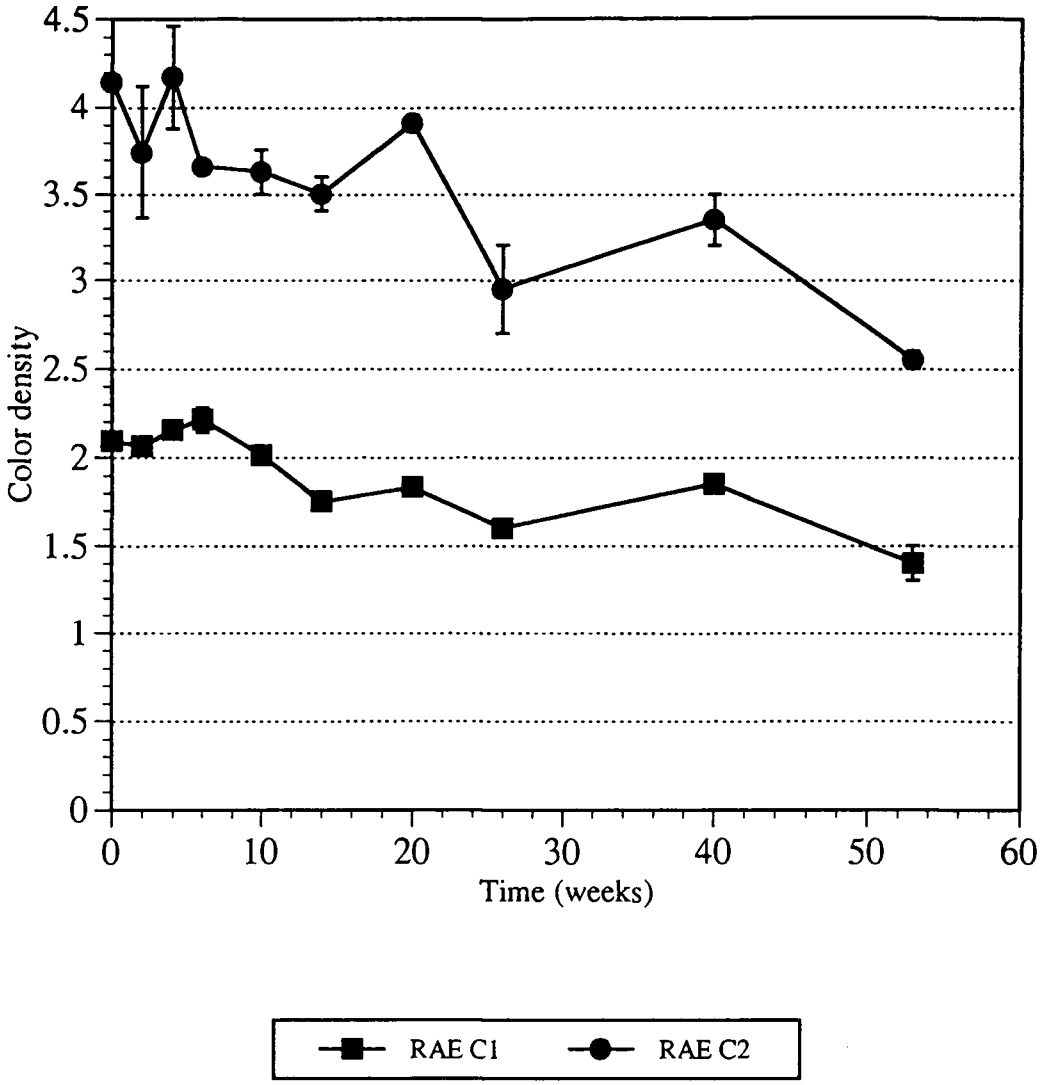


Figure 25. Changes in syrup color density with time

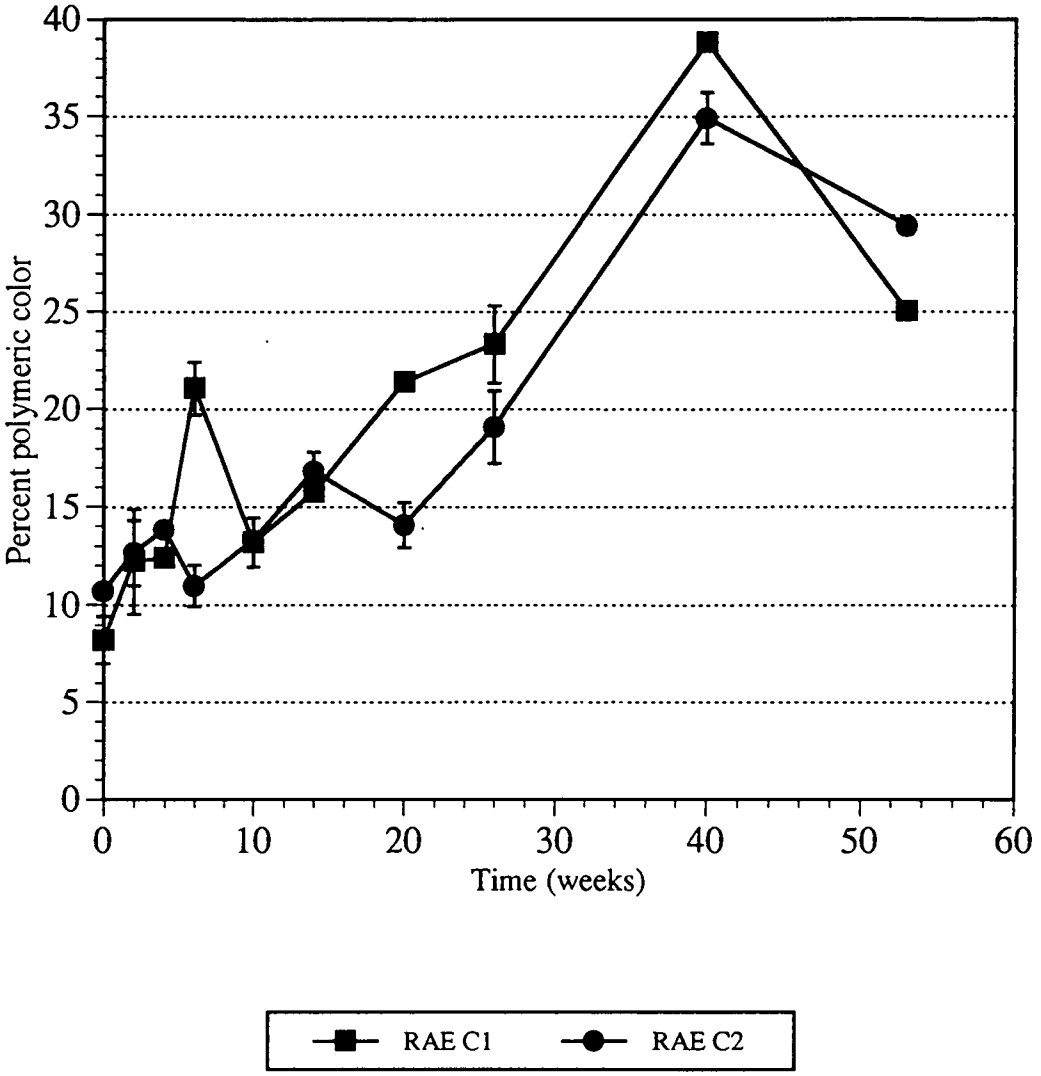
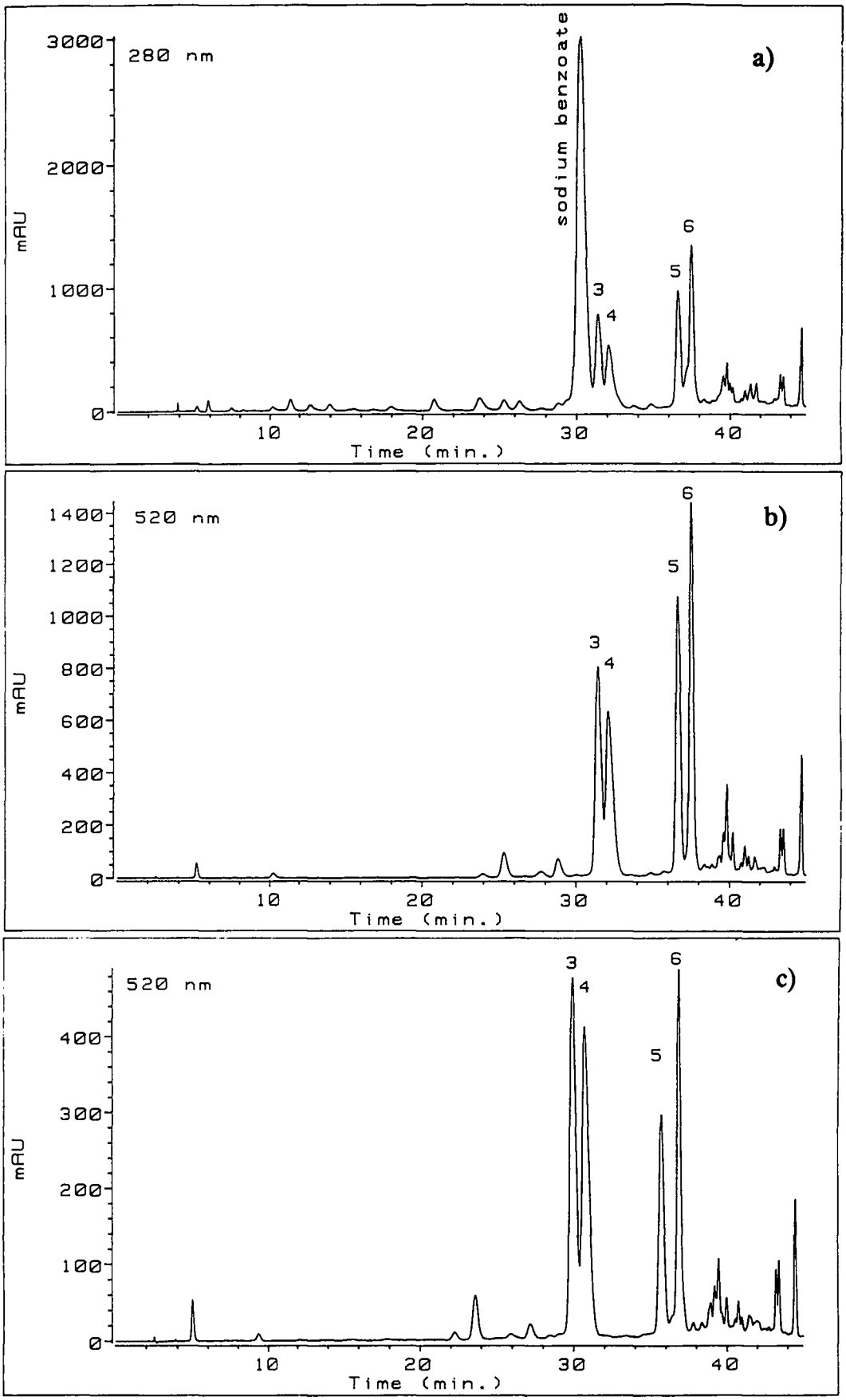
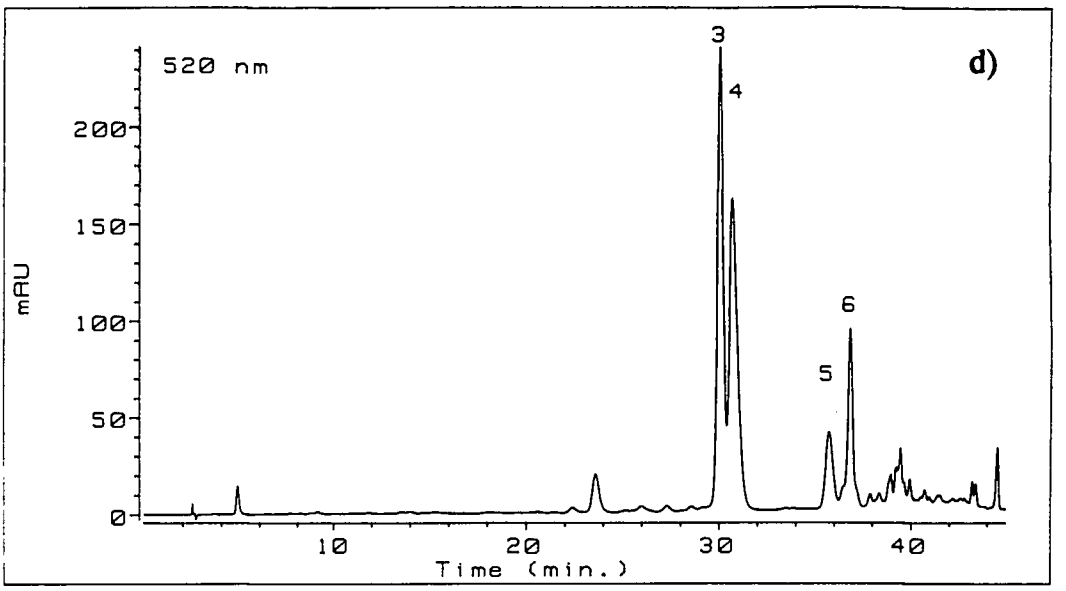
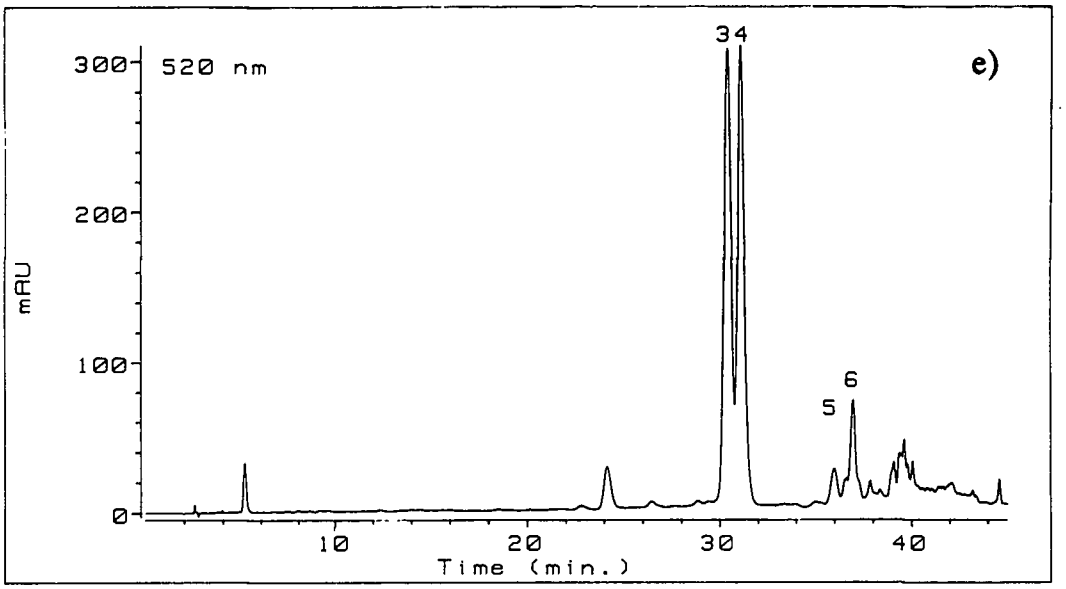


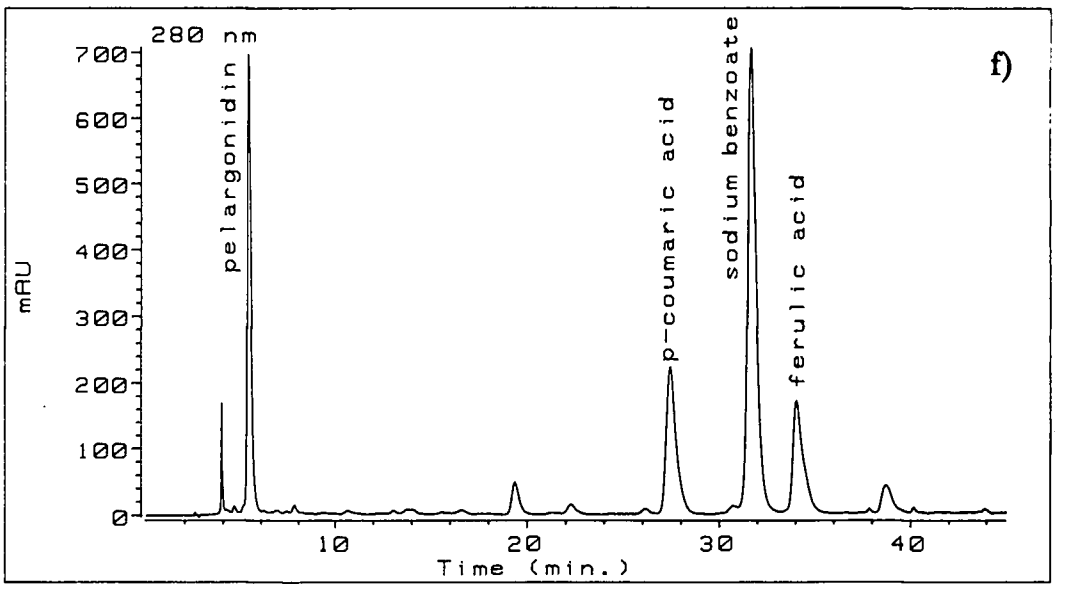
Figure 26. Changes in percent polymeric color in with time

Figure 27. Chromatographic separation of red radish anthocyanins from maraschino syrup samples colored with RAE C1. (a) shows the 280 nm chromatogram of a 2 weeks old sample with the presence of the preservative agent. (b), (c), (d), and (e) show the 520 nm chromatograms after 2, 10, 26 and 40 weeks of storage in the dark at 25°C, respectively. (f) shows a 280 nm chromatogram of the saponified pigment from week 10 (same HPLC conditions as for Figure 4).


a)
280 nm
sodium benzoate
3
4
5
6
mAU
Time (min.)
b)
520 nm
c)
520 nm


520 nm
d)
3
4
5
6
mAU
200
150
100
50
0
10
20
30
40
Time (min.)


520 nm
e)
34
5
6
mAU
300
200
100
0
10
20
30
40
Time (min.)


280 nm
f)
pelargonidin
p-coumaric acid
sodium benzoate
ferulic acid
mAU
700
600
500
400
300
200
100
0
10
20
30
40
Time (min.)

peaks 3 and 4 represented only ca. 20% of the total area. After 10 weeks of storage at 25°C, peaks 3 and 4 had become the major anthocyanins present. After 40 weeks of storage, peaks 5 and 6 represented ca. 9% of the total area (at 520 nm detection) while peaks 3 and 4 represented ca. 80%. The remaining percent area corresponded mainly to the presence of polymeric anthocyanins, which can be observed in Figure 27 as small, and not well defined peaks, with long retention times. The area of the sodium benzoate peak (Figure 27a), compound used as a preservative, was considered constant over time, and used as a reference. The relative percent of peaks 5 and 6 decreased from ca. 40% of the sodium benzoate peak area, for week two, to 19.6, 6.1, and 2.7% for weeks 10, 26 and 40, respectively. The percent of peaks 3 and 4, initially ca. 38% of the benzoate peak area, decreased to 33.78, 20.86, and 22.6% of the reference area for weeks 10, 26 and 40 weeks of storage at 25°C, respectively. The acylation pattern was tested again after 40 weeks of storage, resulting in *p*-coumaric and ferulic acids (Figure 27f). This tendency of anthocyanin degradation with time could suggest that peaks 3 and 4 were more stable to the storage conditions. However, since all these peaks were identified as pg-3-soph-5-glu acylated with *p*-coumaric or ferulic acids, similar stability is expected for all of them, and it is more likely that the degradation of peaks 5 and 6 involved the formation of peaks 3 and 4 as intermediate steps.

Changes in haze during storage

After processing, the color of syrup samples was bright and clear, with no evidence of haze formation. However, during the first week of storage, the

appearance of haze was observed in all the samples colored with RAE, and was monitored during the storage time (Figure 28). A regression analysis showed convincing evidence that the percentage haze did depend on the treatment, but did not depend on the storage time (2-sided p-value 0.13). The average percent haze was 3.66, 9.49 and 14.93 for syrup colored with FD&C Red No. 40, RAE C1 and RAE C2, respectively.

Color loss as well as haze formation during storage of red raspberry are problems that commercial producers commonly encounter (Rommel *et al.*, 1990). Color loss and haze formation, related to increased percent of polymer color has been reported during the storage of red raspberry (Rommel *et al.*, 1990), and processing and storage of strawberries (Abers and Wrolstad, 1979). Since the haze formation was much greater in the samples colored with anthocyanins, it is very likely that it was caused by anthocyanin polymerization.

Color and stability of anthocyanins stored under dark and light conditions

The comparative color stability of syrup samples stored under light and dark conditions over a year of storage at 25°C is shown in Figures 29, 30, 31, 32 and 33. Statistical evidence showed that b*, c and hue angle values were not significantly affected by the light exposure (2-sided p-value > 0.10), for all color treatments. Changes on L* and a* values were significantly affected by light exposure (2-sided p-value 0.0001 and 0.0073, respectively) but in different degree for the different treatments. Light accelerated the changes on L* and a* values over time in greater extent in samples colored with RAE C1 and RAE C2 than in samples colored with

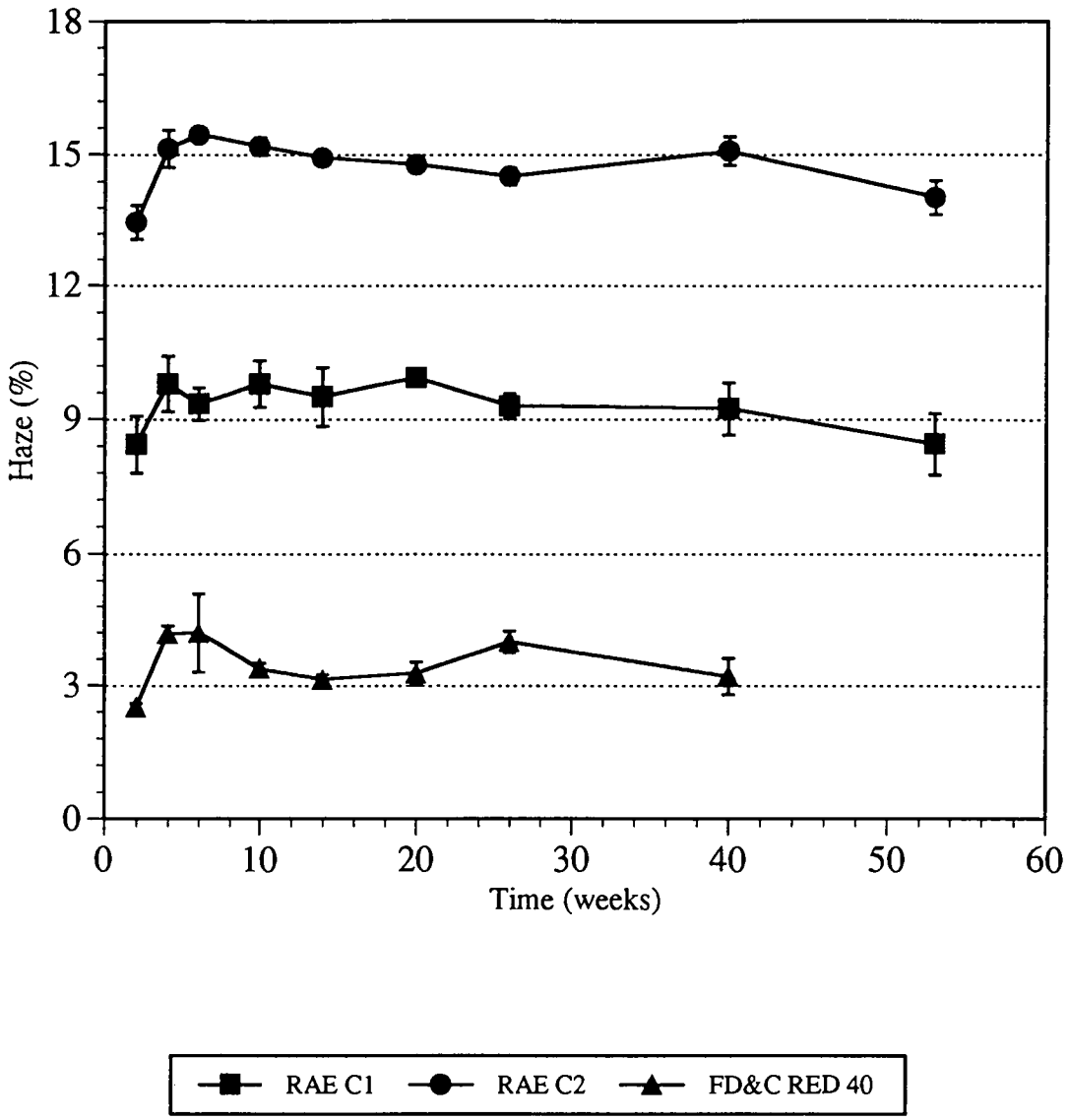


Figure 28. Changes in syrup percent haze with time

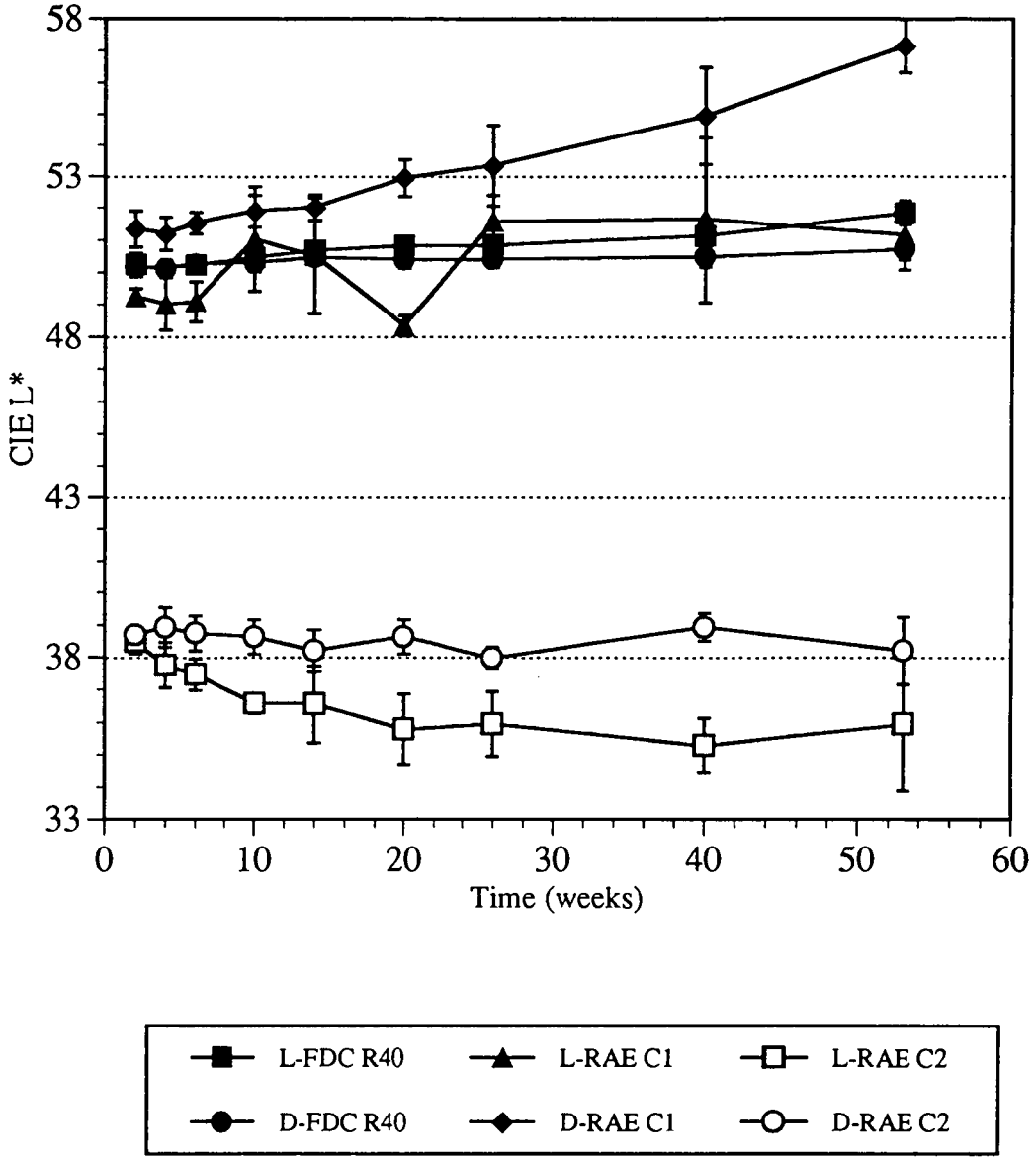


Figure 29. Comparison of syrup color between light and dark exposure. Changes of Hunter CIE L* values with time.

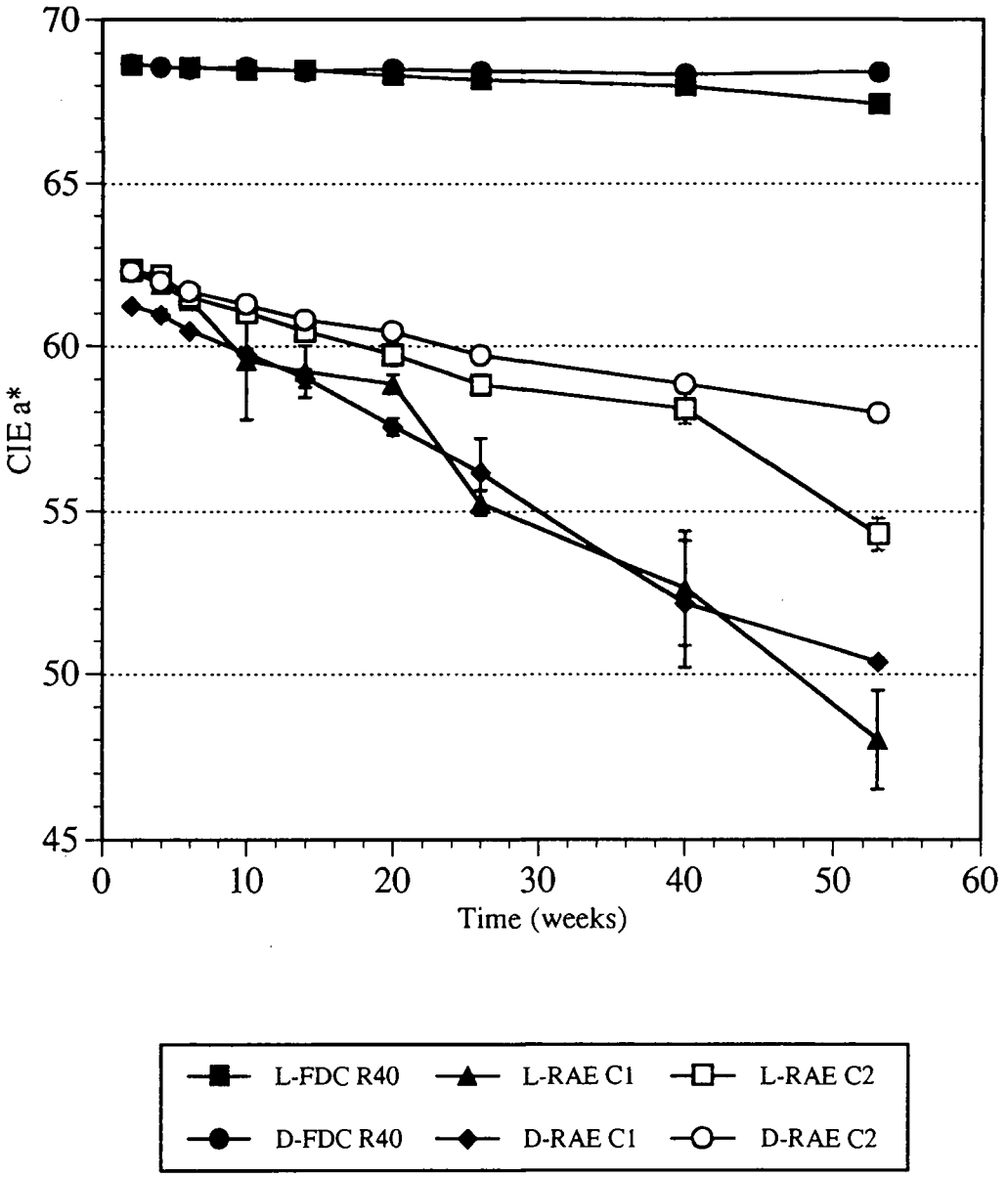


Figure 30. Comparison of syrup color between light and dark exposure. Changes of Hunter CIE a* values with time.

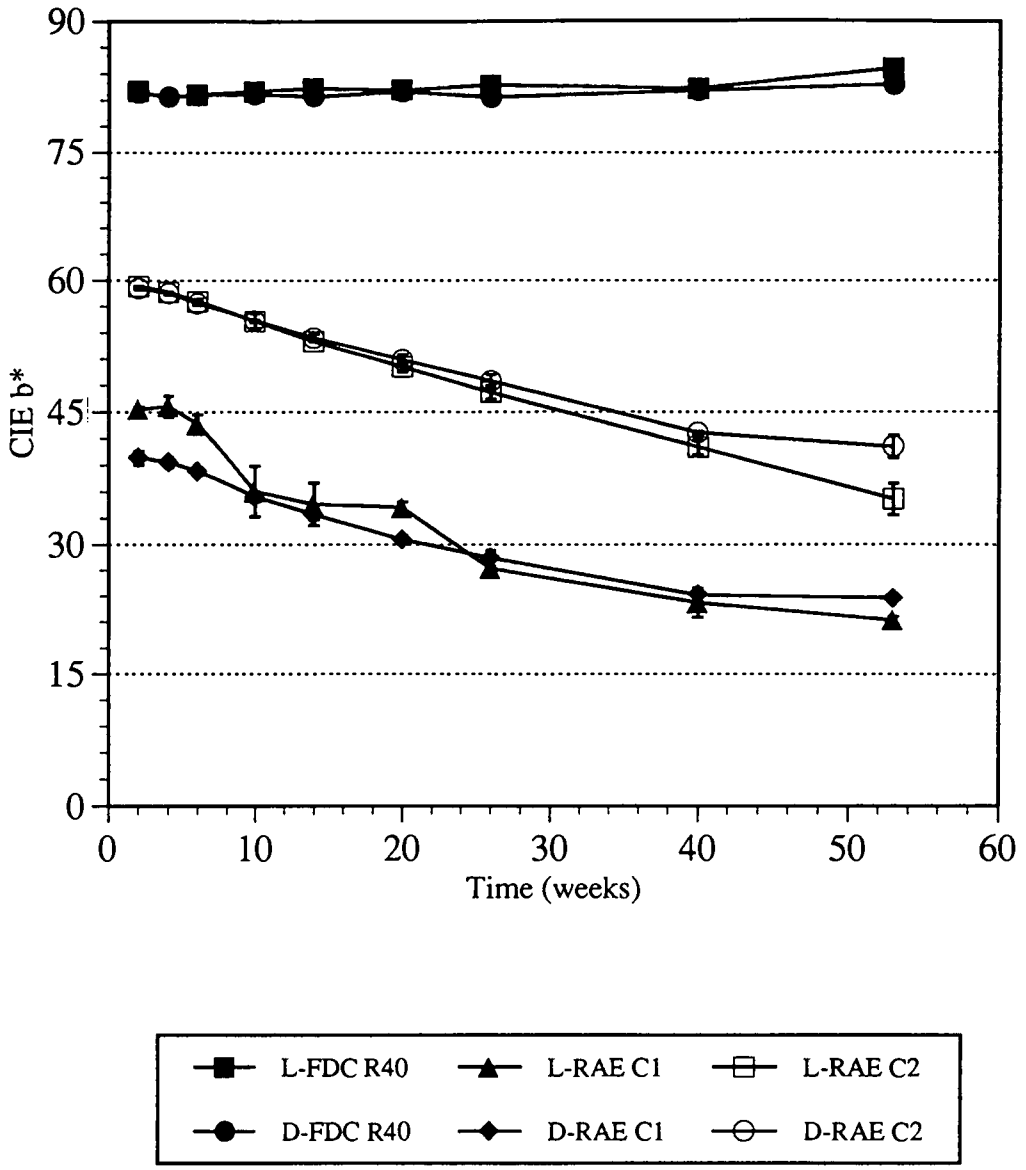


Figure 31. Comparison of syrup color between light and dark exposure. Changes of Hunter CIE b* with time

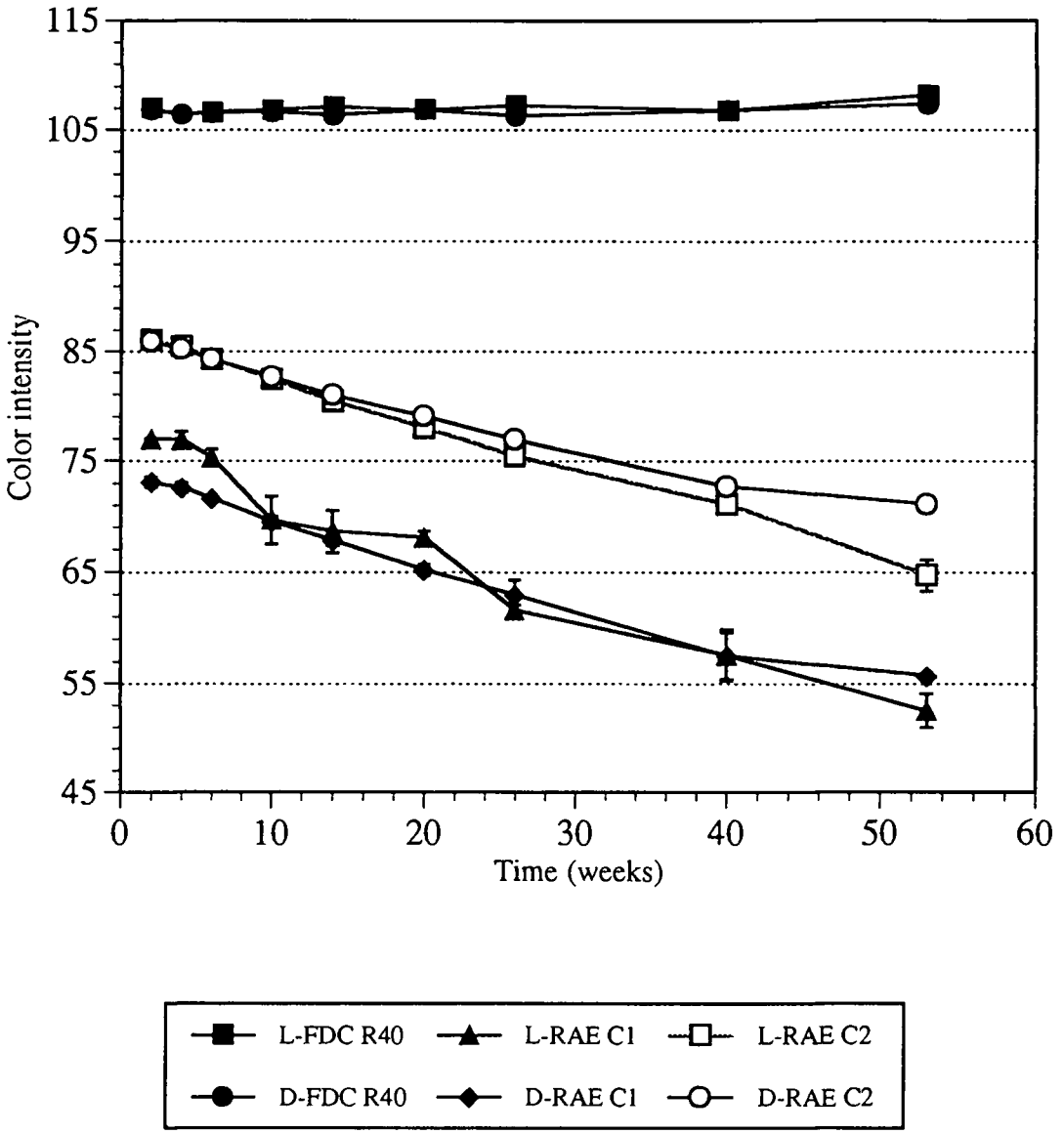


Figure 32. Comparison of syrup color between light and dark exposure. Changes of color intensity with time.

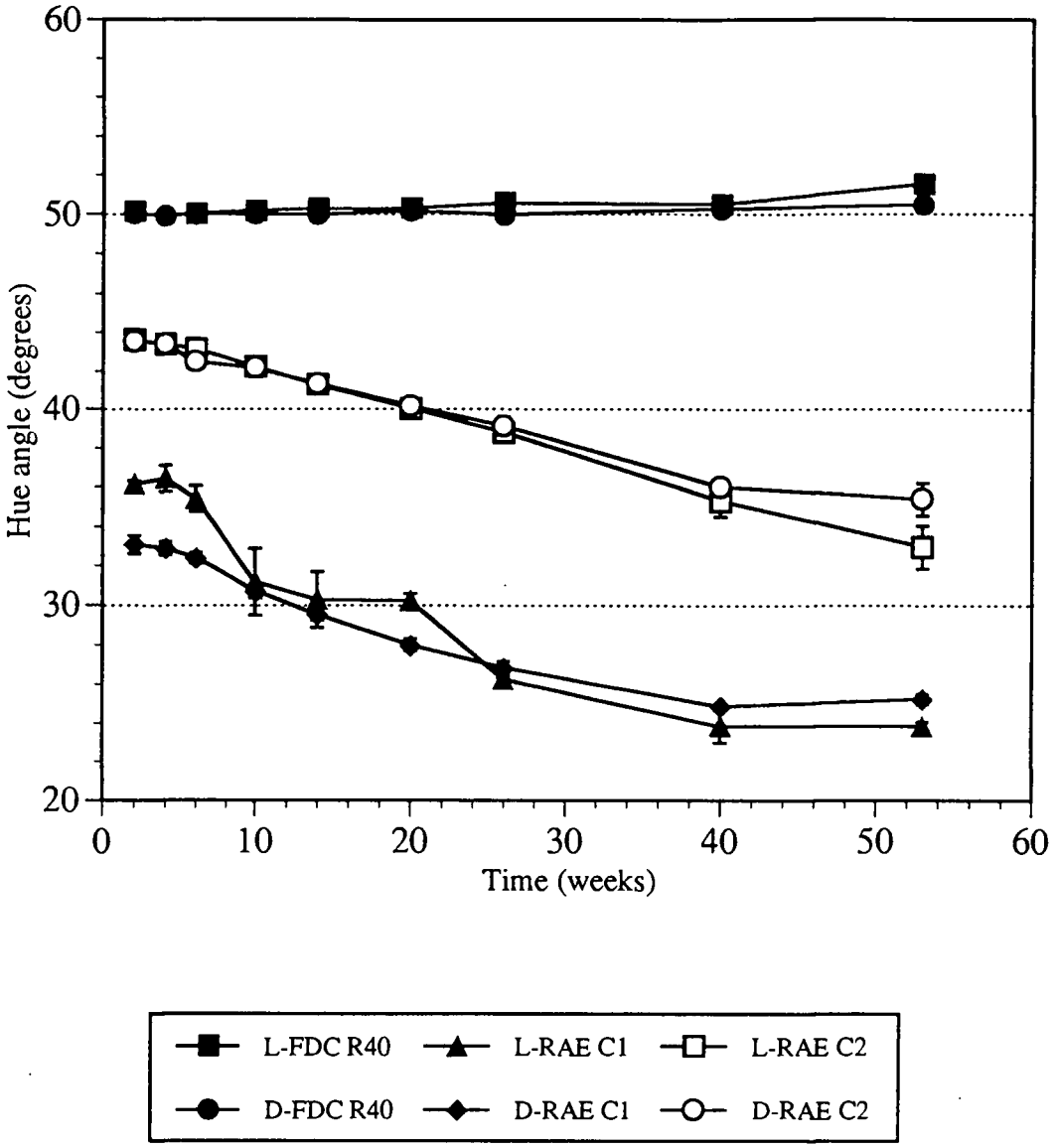


Figure 33. Comparison of syrup color between light and dark exposure. Changes of hue angle with time.

FD&C Red No. 40. We also found convincing evidence that the effect of light on L* and a* degradation became greater when the storage time increased, for all the different treatments. The effects of light on preparations containing anthocyanins are usually deleterious (Francis, 1989). Monoglycosides are usually the least stable, diglycosides being intermediate in stability. The most light-stable anthocyanins in wine have been reported to be acylated diglucosides (Francis, 1989). The light stability of samples colored with radish anthocyanins was surprisingly high, specially for color intensity and hue angle.

The comparative anthocyanin stability of syrup samples stored under light and dark conditions over a year of storage at 25°C is shown in Figures 34, 35 and 36. The anthocyanin content was significantly affected by light, time and treatment (2-sided p-values < 0.0001). Statistical evidence showed that the monomeric anthocyanin content for samples colored with RAE C1 decreased at the same rate (0.21 mg/week) for the samples that were stored under light and dark conditions during time; the samples that were exposed to light had a lower anthocyanin concentration (on average, 1.79 mg) than the samples stored in the dark during the storage time (2-sided p-value < 0.0001). In the syrup samples that had the higher anthocyanin concentration (RAE C2), the statistical evidence showed that the monomeric anthocyanin content decreased at a higher rate (0.36 mg/week) in the samples that were exposed to light (2-sided p-value 0.0001) than in the ones stored under dark conditions (0.23 mg/week). Palamidis and Markakis (1975) reported that the half life of anthocyanins extracted from grape pomace and incorporated into a carbonated

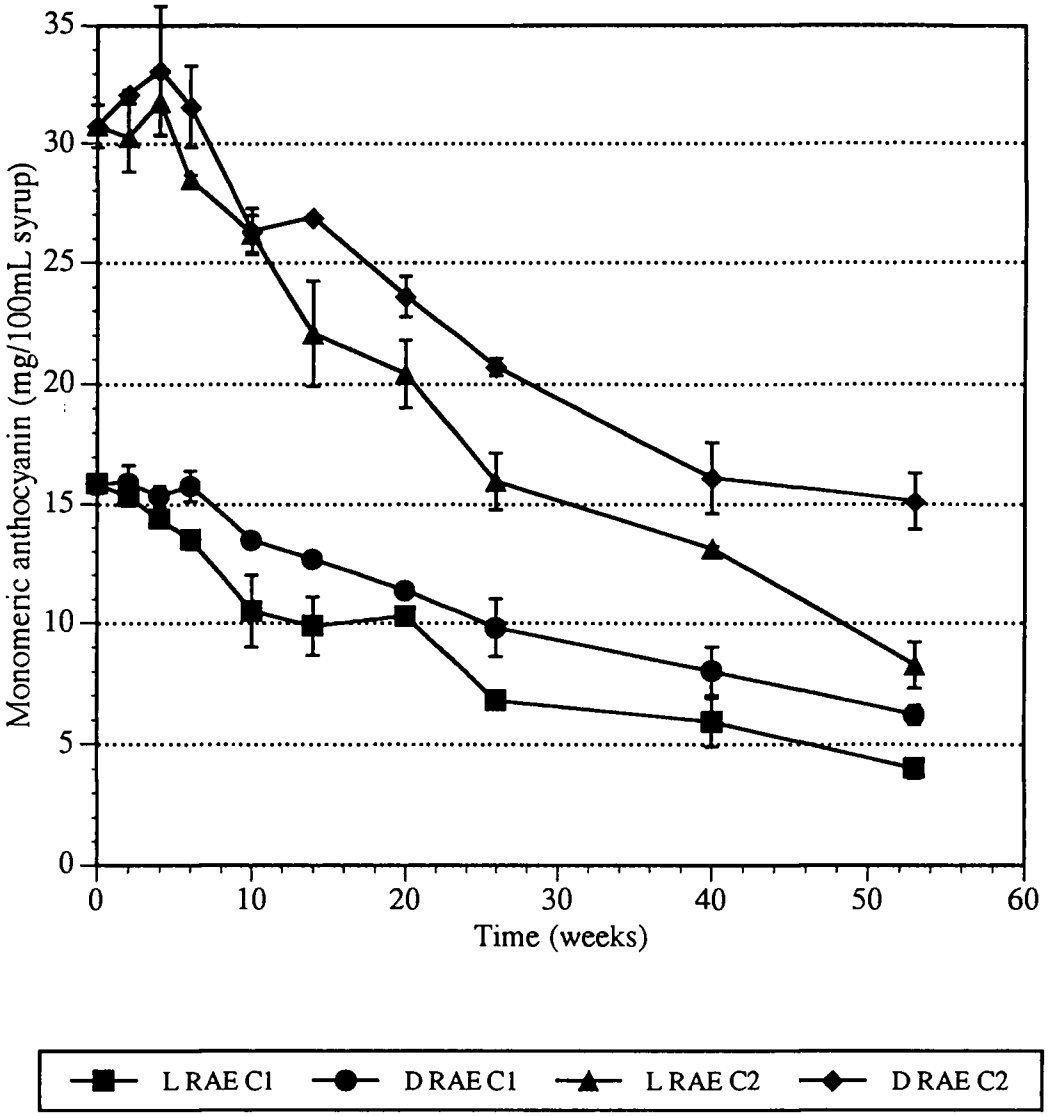


Figure 34 . Comparison between light and dark exposure. Changes of syrup monomeric anthocyanin content (mg anthocyanin/100 mL syrup) with time.

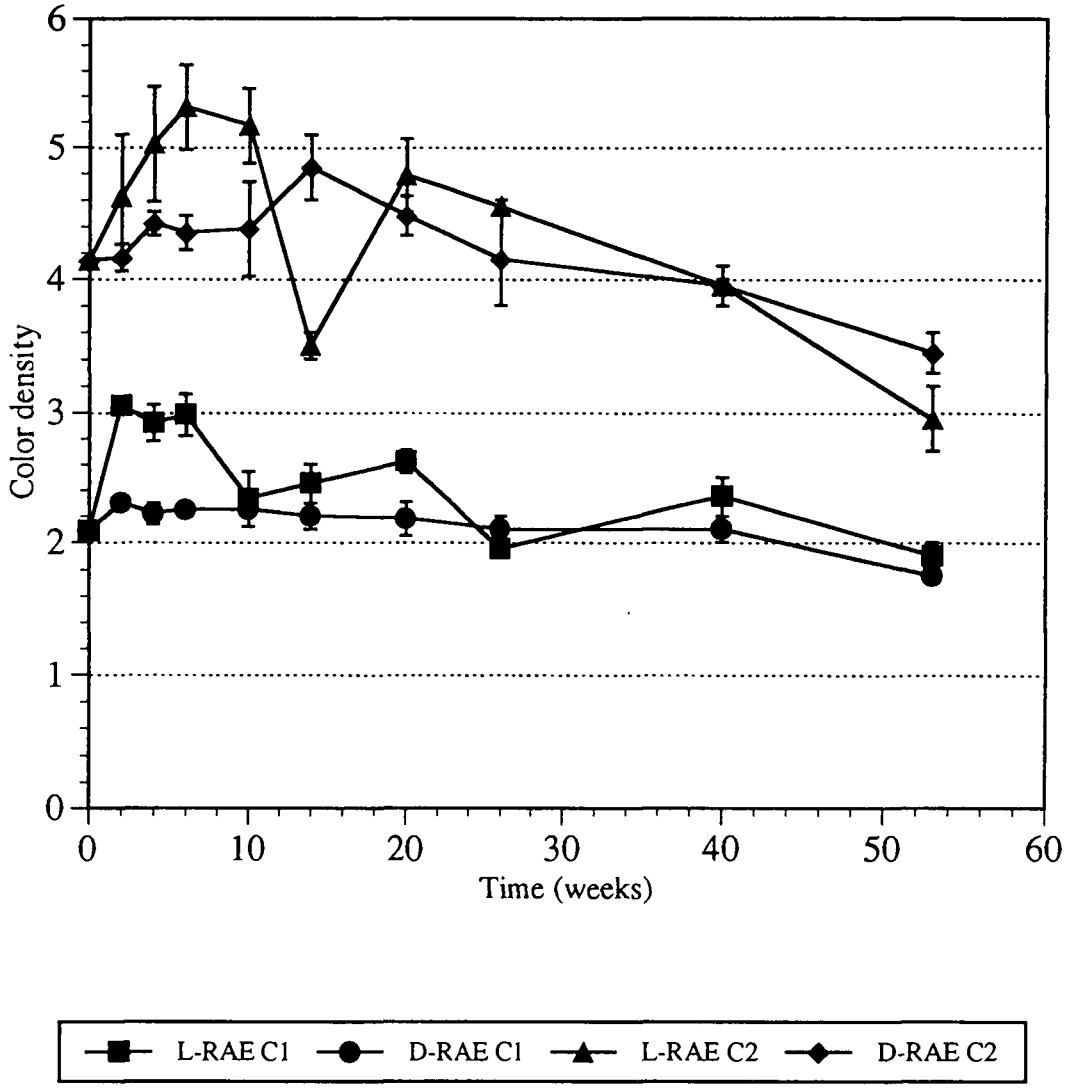


Figure 35. Comparison between light and dark exposure. Changes of syrup color density with time

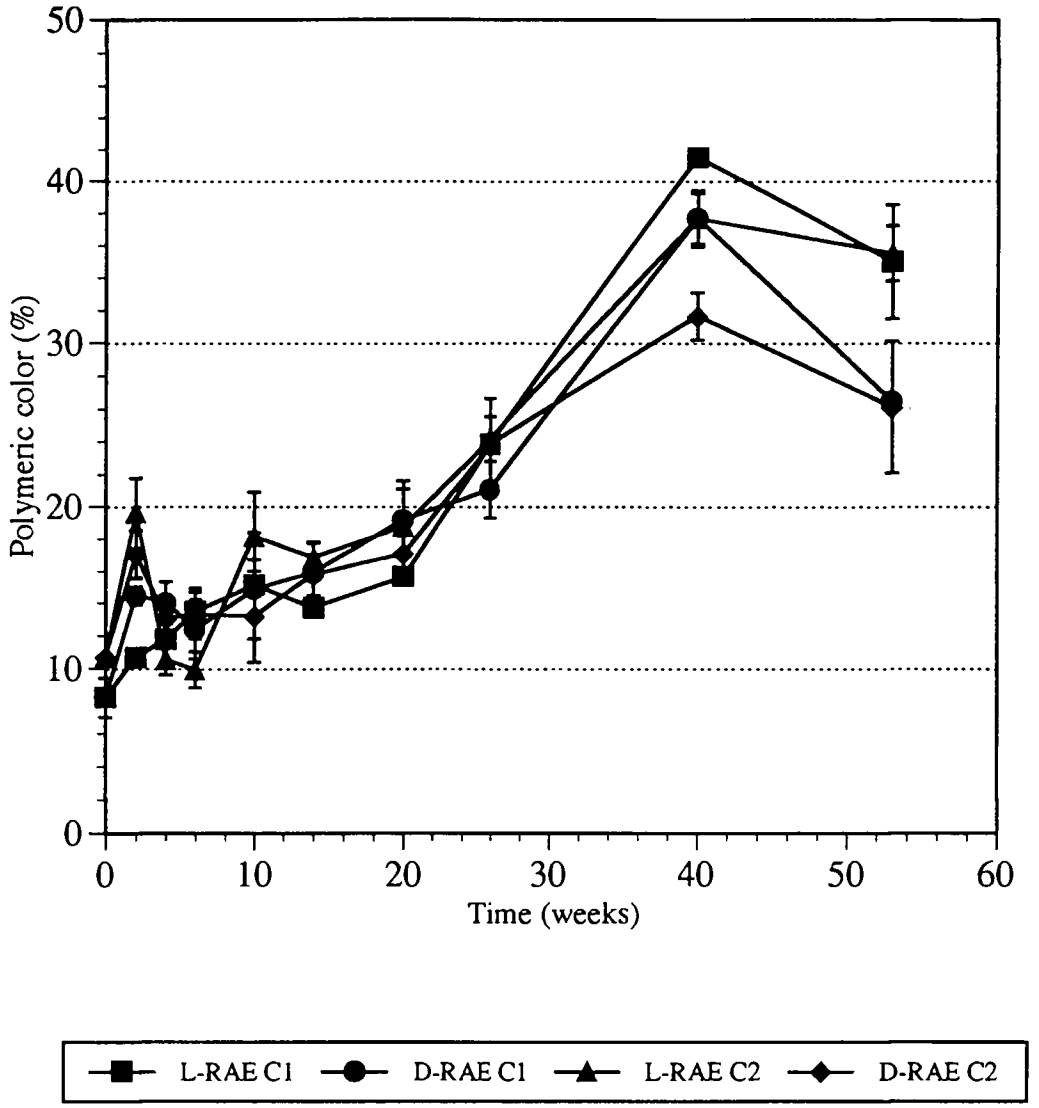


Figure 36. Comparison between light and dark exposure. Changes of syrup polymeric color during time.

beverage at 22°C and exposed to light was 3.3 times shorter than the half life when stored in the dark at 20°C. RAE proved to be very stable to light compared to the grape anthocyanins, since the half life was only 1.5 times shorter for RAE C2 than the half life when stored in the dark. In addition, the statistical analysis did not show changes in the half time of RAE C1 when comparing dark versus light exposure. Polymeric color formation was not significantly affected by light exposure (2-sided p-value 0.87). However, in the light-exposed samples a precipitated material appeared at the 50th week of storage, which did not occur in the samples stored in the dark. It is possible that light affects polymerization in a way that promotes the formation of polymers with higher molecular weight, favoring the precipitation of the material.

Light did not significantly affect (2-sided p-value 0.39) haze formation (Figure 37). However, very high standard deviations were obtained from the 40th week of storage for the light exposed samples. This could be related to the fact that polymerized material was precipitating in these samples, reducing the percent haze. Additional experiments were performed to try to eliminate the already formed haze. Centrifugation up to 10,000 rpm for 10 min was not efficient precipitating the haze. However, filtration (through a filter paper Whatman No. 4) was very efficient in removing the suspended material. After filtration, percent haze was measured (Table 5). No significant differences were found in the percent haze among the three different coloring treatments after filtration. Since haze formation did not depend

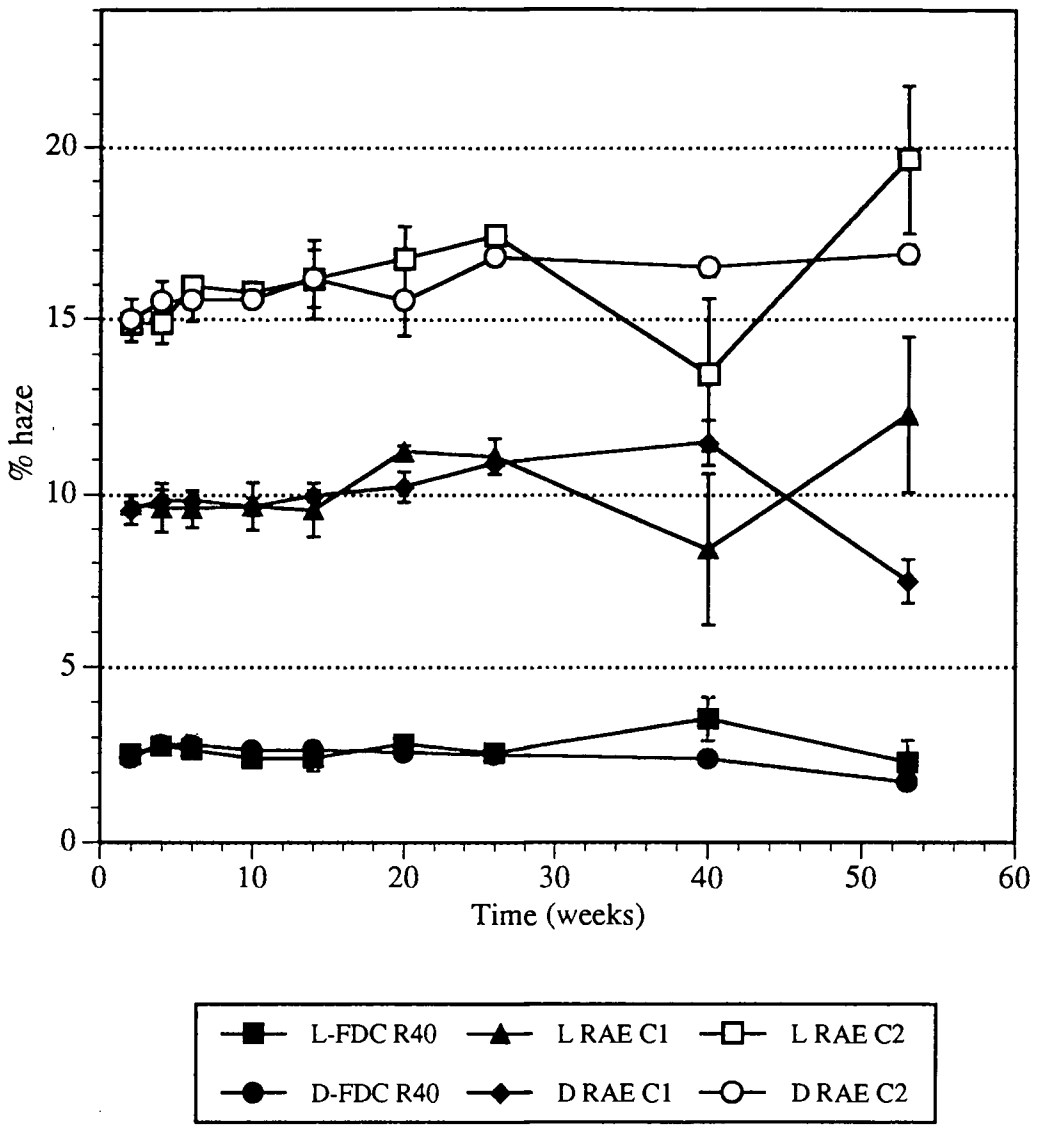


Figure 37 . Comparison between light and dark exposure. Changes of syrup transmission haze (%) with time.

Table 5. Transmission haze (%) in syrup samples stored at 25°C for 53 weeks, before and after filtration (L = exposed to light; D = stored in the dark).

Treatment	Haze (%) before filter	Haze (%) after filter
L - RAE C1	12.26 (0.09)	0.49 (0.08)
D - RAE C1	7.46 (2.03)	3.13 (0.57)
L - RAE C2	19.63 (0.62)	0.93 (0.14)
D - RAE C2	16.87 (1.06)	2.00 (1.58)
L - FD&C Red 40	2.27 (0.32)	1.51 (0.44)
D - FD&C Red 40	1.71 (0.16)	1.08 (0.40)

on time, it may be possible that this problem could be solved by filtering the colored solutions, before adding it to the cherries.

SUMMARY

Thermal processing of radishes (cultivar Fuego) in aqueous media at different pH showed that the pigments were stable to heat. Processed radishes showed red hue and no evidence of browning. Radish pigments were highly soluble in the aqueous media, diffusing from the epidermal tissue into the flesh and solution.

HPLC and spectral analyses of radish anthocyanins confirmed the previous identification of the pigments as pg-3-soph-5-glu acylated with p-coumaric and/or ferulic acids. Caffeic acid, previously reported as acylating group in radishes, was not present. Eight anthocyanins were separated, four of which represented ca. 90% of the total area. They were identified as pg-3-soph-5-glu monoacylated with p-coumaric and ferulic acid, respectively. They probably differ in the position of the acylating group or correspond to different geometric isomers.

RAE imparted red color to secondary bleached cherries and syrup extremely close to that of FD&C Red No. 40. The color obtained with RAE on primary bleached cherries was slightly darker and not as bright as the one obtained with FD&C Red No. 40.

High stability of color and pigment was obtained for all secondary bleached cherries and syrup samples colored with RAE and stored in the dark at 25°C. Monomeric anthocyanin destruction followed first-order kinetics. The half life (50% destruction) of monomeric anthocyanins was 29 and 33 weeks for samples colored with RAE C1 and RAE C2, respectively. This high stability is attributed to the presence of acylating groups, making the molecule more resistant to degradation. A

higher anthocyanin concentration exerted a protective effect on color stability. Haze formation was observed in all syrup samples colored with RAE, which was attributed to the formation of anthocyanin polymers. Filtration of the syrup effectively removed the haze.

Light accelerated L* and a* degradation in all syrup samples. Light also accelerated the monomeric anthocyanins' rate of degradation in samples colored with RAE. The effect of light was dependent on color treatment and storage time. Light exposure did not affect b*, color intensity, nor hue angle during storage.

From the results obtained and visual observations of the product, we consider that secondary bleached cherries and syrup colored with RAE C1 showed appearance comparable to that of FD&C Red No. 40 for at least 6 months of storage at 25°C. During this time, light did not exert visual detrimental effects in color appearance.

The evaluation of different radish cultivars is being currently conducted at OSU Food Science and Technology laboratories looking for varieties with high anthocyanin content. Future research will involve the development of a procedure for extraction and concentration of radish anthocyanins as aqueous extract, without the use of organic solvents. In this study, we will try to develop a pilot plant procedure that could be amenable to use by the industry.

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