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Photochemical and Thermal Stability of Carmoisine, Sunset Yellow, and Tartrazine Food-Coloring Dyes in Commercial Beverages

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Abstract: In many developing countries, displaying beverages under sunlight has been customized. The photostability and dark degradation of the titled color dyes used in the commercial beverages Fanta® and Mirinda® have therefore been investigated in the presence of food acids citric and ascorbic acids. The degradation products have been identified in the case of Sunset Yellow as sulfanilic acid and the 2-sulfonic acid-5-amino-6-hydroxyl naphthalene sodium salts. The degradation products of Carmoisine are naphthionic acid (1-sulfonic acid sodium salt-4-amino-naphthalene) and 1-naphthalene sulfonic acid-3-amino-4-hydroxyl naphthalene sodium salts. A third food coloring dye, Tartrazine, has also been studied for comparison. The dark reactivity of the studied dyes in citric or ascorbic acids was confirmed to obey the second-order kinetic model. The stability of the dyes goes in the order Tartrazine > Sunset Yellow > Carmoisine with evaluated energies of activation (E_a) of 19.9, 16.5, and 14.8 kJ mol^{-1}, respectively. The photochemical quantum yield ((c) for the three dyes was determined, showing that Carmoisine, in the presence of ascorbic acid, has the highest photostability compared with the other two dyes.

Keywords: sunset yellow E110; Tartrazine E102; carmoisine E122; food-coloring dyes; photostability; dark reaction.

1. Introduction

In the increasing pursuit of an improved quality of life, the demands for food quality are much higher. In order to enhance the appearance, flavor, taste, and storage periods of food, food additives are commonly used in the food industry [1]. Food additives are classed according to their function and usage. They may be preservatives, antioxidant colors, flavors, sweeteners, emulsifiers, acidulates, vitamins, and minerals [2-4]. According to the US Food and Drug Administration (FDA), there are two classes of color additives: uncertified (or listed) and certified ones. Uncertified color additives have met safety standards and are called "natural colors". They are obtained from vegetables, plants, animals, and mineral sources or are synthetic duplicates of naturally existing colorants. These colors are exempted from certification.

The FDA does not recognize the description "natural" for these colors but refers to them as "uncertified color additives." On the other hand, certified color additives are well-known artificial colors. Several scientists and consumer organizations have raised the question of whether artificial food colors should not be allowed in our food as they serve no useful nutritional purpose, and the improper use of some of them has led to potential threats to human health [5-9]. Moreover, due to improper operation, food additives will flow into the environment during production, processing, packaging, transportation, and storage [10]. Safety concerns have reduced the number of artificial dyes permitted as food colorants. Those remaining are under close scientific scrutiny, and their future is uncertain. The main advantages of synthetic colors are their cost efficiency and good thermal, light, and chemical stability [11-15].

Much attention has been given to developing natural pigments as colorants. This process has been more expensive. Color stability is a major concern when natural pigments are used as colorants. Heat, oxygen, pH, and light are among the factors to affect pigment stability [16]. The term "coal-tar dyes" is sometimes used in some texts to refer to artificial colorants. The coal-tar designation comes from the presence of

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aromatic rings. Some coal-tar dyes structurally resemble many known carcinogens in these rings and the diazo (─N═N─) link.

On the other hand, the natural colors must be processed before they can be added to food. At processing, they may become contaminated. There is no assurance that it is pure just because a color is obtained from animal or vegetable sources.

In this article, the photochemical stability and dark degradation were studied for two common color dyes, namely Carmoisine and Sunset Yellow (Fig.1), which are used in commercial beverages using fluorescence and electronic absorption spectra, HPLC, thin layer chromatography as well as photochemical quantum yield (φ_c) determination. A third food coloring dye, Tartrazine, has also been studied for comparison.

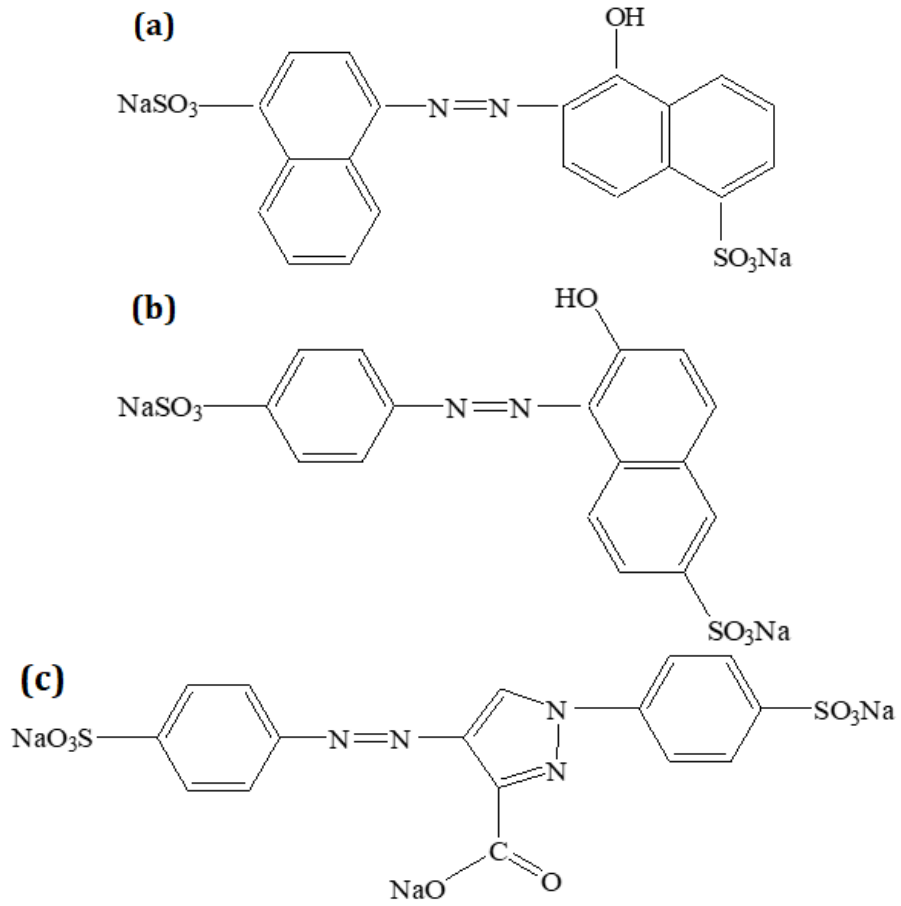


Figure 1. Chemical structures of (a) Carmoisine, (b) Sunset Yellow, and (c) Tartrazine.

2. Experimental

Food, drug, and cosmetic (FD and C) dyes were either obtained from Delta Aromatic Co. or kindly supplied by Prof. Dr. Guy Duportail of Louis Pasteur University. The three dyes used are FD and C yellow No. 6 (Sunset Yellow E110), FD and C yellow No. 5 (Tartrazine E102), and FD and C Red No. 2 (Carmoisine E122). Each dye has a concentration of 85%, with 15% NaCl used as a carrier. Beverage products (Mirinda® and Fanta®) were taken randomly from

the popular Egyptian market. All the solvents used in this study's spectral runs and extraction were of analytical grades. The buffer used was acetate buffer of pH 3.1, where 15.62 ml of 1 M acetic acid was mixed with 12.5 ml sodium acetate and diluted to 250 ml. The pH was adjusted to 3.1 using acetic acid and a pH meter. UV-visible absorption spectra were measured by using a Shimadzu FL60 spectrophotometer. Fluorescence spectra were measured by using a Shimadzu RF 510 Spectrofluorometer. A pH meter (model Crison, pH/mv meter digit 501) was used for pH adjustment and measurement. HPLC (Hewlett Packard HP 1100) with Hypercil ODS C_{18} 4.6 mm × 250 mm 5 µm column was utilized. The mobile phase is a mixture of methanol and acetonitrile (80:20 v/v). The mobile phase was filtered by suction through a membrane filter with a pore diameter of 0.45 µm. The eluent flow rate was kept constant at 1.5 $mL.min^{-1,}$ and the injection volume was set at 20 µL. All experiments were performed at room temperature. The diode array detector is programmed to monitor the dyes at a wavelength of 245 nm. The chromatographic system was initially conditioned by passing the mobile phase through the column until a stable baseline signal was obtained.

Photochemical quantum yields (φ_C) were measured by measuring the disappearance of starting material using spectrophotometry according to the method

developed by A. J. Lees and summarized elsewhere [17-19]. This method accounts for the change in absorbance at irradiation wavelength during the photoreaction. Photoproducts such as amines, sulfanilic acid, and naphthionic acid were determined according to the procedure described [20]. The degradation of Carmoisine and Sunset Yellow by a potent reducing agent has been done according to the method described earlier [21].

Separation of color dyes from Mirinda® and Fanta® was carried out using solvent extraction. Acid dyes were extracted from aqueous solutions under acid conditions using higher alcohols, particularly isobutanol and n-butanol. 100 ml beverage product, 2 ml concentrated sulfuric acid, and 25 ml n-butanol were put in a stopped cylinder. Vigorous shaking was undertaken using a shaker; then, the n-butanol was decanted. Extraction was repeated until the color disappeared entirely from the aqueous beverage product. The extract was passed through 1 cm diameter, 20 cm long plastic tubes packed well with basic alumina. Upon elution with n-butanol, different zones from each color formed. Each zone was cut alone, and each fraction was poured into a small beaker containing 5-10 ml of 30% ethyl alcohol. After alumina precipitation, the supernatant liquor was taken, and the UV-visible absorption spectra were measured and compared with those of pure dyes in the same solvent.

Mirinda® shows only one orange zone that matches the UV-visible absorption spectrum of Sunset Yellow. A fragile yellow zone also appeared above the orange zone but could not be identified. Fanta® shows two zones. The upper one is orange, which matches the Sunset Yellow absorption band. The lower one has a faint pink color. Fanta® contains Sunset Yellow and Carmoisine, according to the (Egyptian Organization for Standardization and Quality Control). Elution by 40% aqueous ammonium sulfate shows that the orange zone elutes first. The pink one i.e., reverses the order of formation of colored zones compared with n-butanol elution.

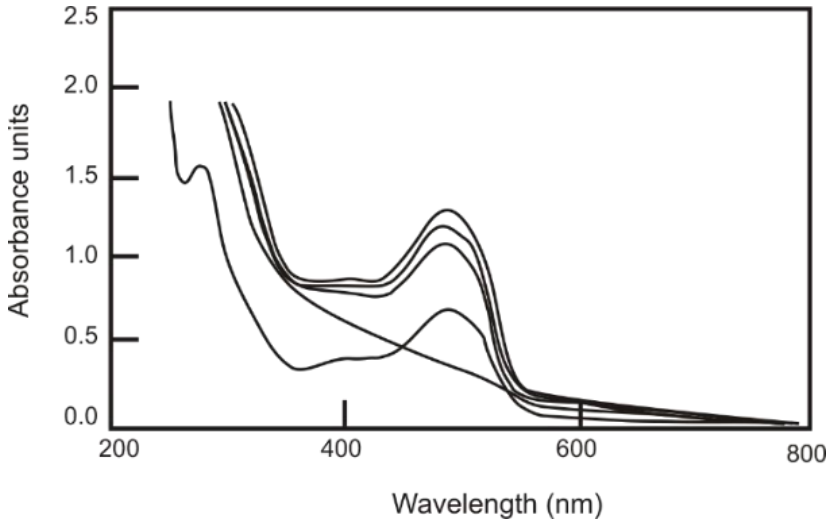


Figure 2. The change in absorption spectra of Mirinda® solution as a result of sunlight irradiation.

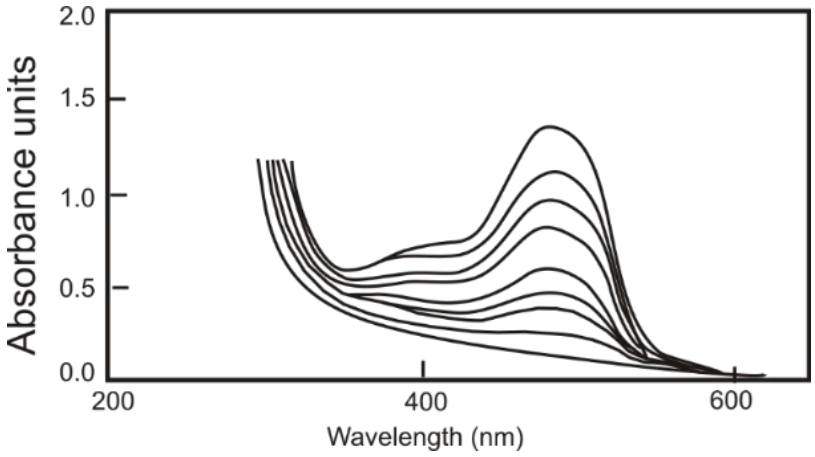


Figure 3. The changes in absorption spectra of Fanta® solution result from sunlight irradiation.

3. Results and Discussion

3.1. Effect of Direct Sunlight on Mirinda® and Fanta®

Sunset Yellow and Carmoisine in Mirinda® and Fanta® undergo Photodegradation upon subjecting these beverages to sunlight. This is evident from changes in UV-visible absorption spectra shown in Fig. 2 and 3. As shown in Fig. 2, the irradiation times at decreasing absorbance at 500 nm are 0, 5, 9, and 13 days. Distilled water was taken as a reference. The absorption spectrum of totally degraded color is also shown (the minimum absorption at 500 nm). In Fig. 3, the irradiation times at decreasing absorbance are 0, 4, 8, 11, 15, 18, 21, and 28 days, taking distilled water as a reference. The degraded sample absorption is also shown.

Photodegradation is also associated with increased sample fluorescence, as shown in Fig. 4 and 5. As shown in Fig. 4, the irradiation times at increasing emission intensities at 440 nm are 3, 7, 20, and 29 days. The excitation spectrum of the third sample (λ_{em} = 440 nm) is shown at a shorter wavelength. As shown in Fig. 5, the irradiation times at increasing emission intensities at 440 nm are 3, 18, 25, 28, 35, and 39 days.

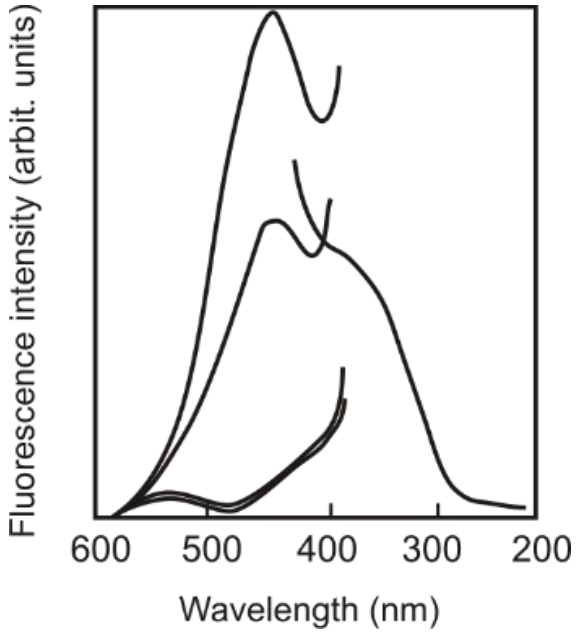


Figure 4. The changes in emission spectra (λ_{ex} = 366 nm) of Mirinda® solutions result from sunlight irradiation.

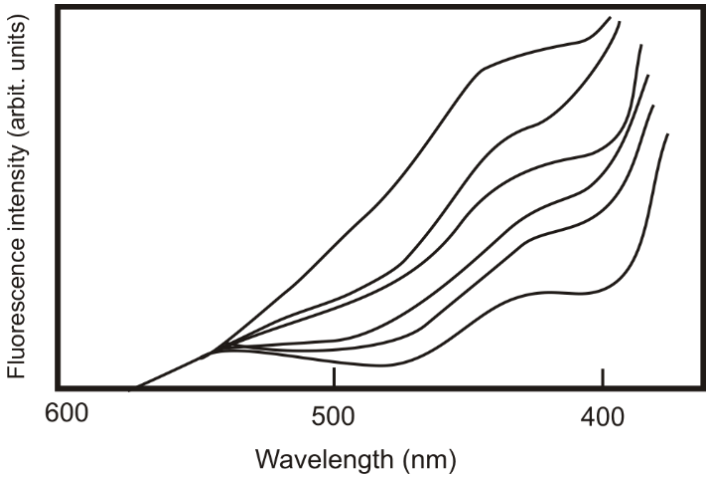


Figure 5. The changes in emission spectra (λ_{ex} = 366 nm) of Fanta® as a result of sunlight irradiation.

3.2. Effect of Food Acids on the Stability of Coloring Dyes

The photostability of the three coloring dyes has been studied in food acids such as ascorbic and citric acid. The study was carried out at a pH of 3.1 to mimic the condition of beverages, and sunlight was used in photo irradiation. The extent of dye fading was expressed as the fractional change (α), which is based on the absorbance at the absorption maxima as shown in Eq. 1:

$$\alpha = (A_0\text{-}A)/A_0 \qquad \text{Eq. 1}$$

Where A_0 and A are the absorbances at irradiation times *zero* and *t*, respectively. The results are shown in Fig. 6.

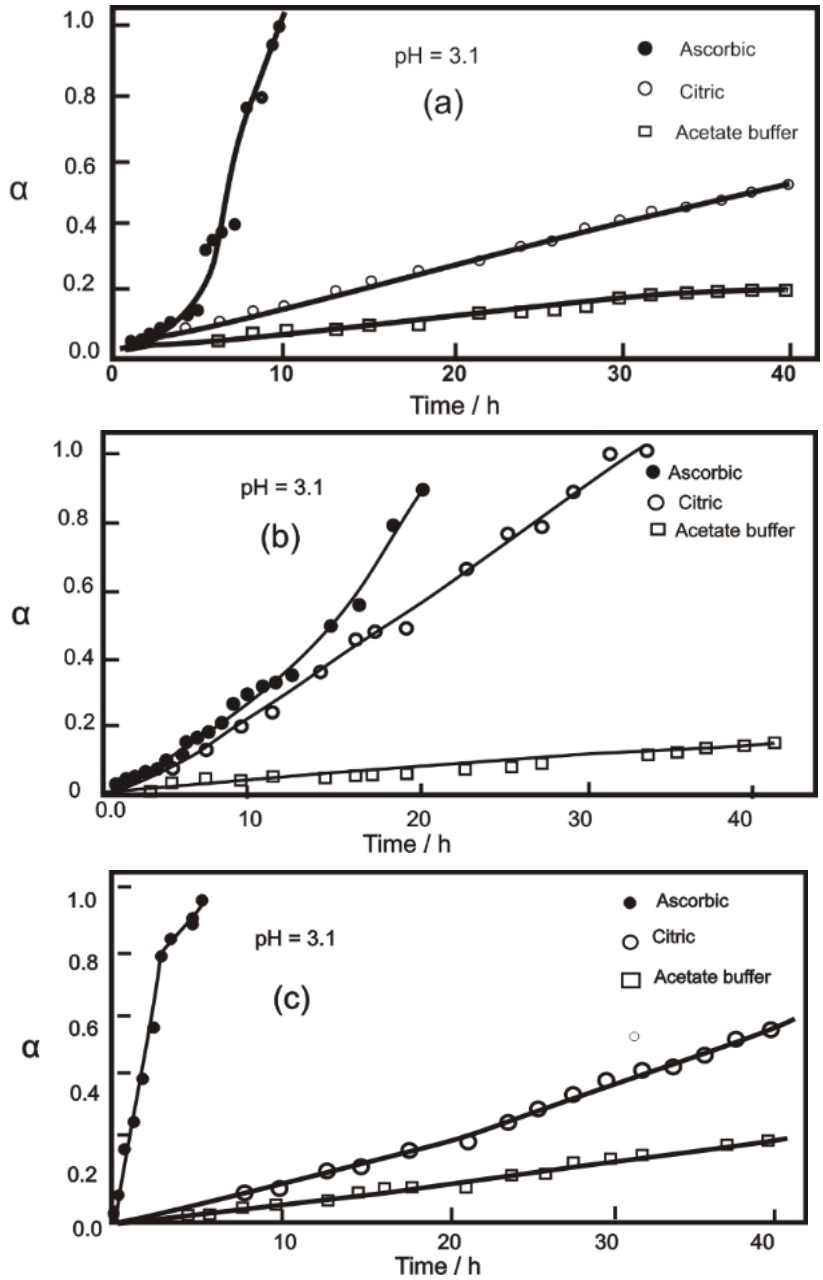


Figure 6. Fractional changes (α) of (a) Sunset Yellow, (b) Tartrazine, and (c) Carmoisine as a function of sunlight irradiation time in the presence and absence of food acids.

The dark stability of the coloring dyes has also been studied at a pH value of 3.1 (acetate buffer) in the presence of ascorbic acid and citric acid. The fractional change (α) was calculated after ten days in the dark and listed in Tables 1 and 2. The three food dyes under consideration show relatively higher stability in the presence of citric acid than ascorbic acid.

Table 1. Dark stability of the coloring dyes in the presence of ascorbic acid.

Samples	Carmoisine		Sunset Yellow		Tartrazine	
	ABS. at 517 nm	Fractional change (α)	ABS. at 482 nm	Fractional change (α)	ABS. at 427 nm	Fractional change (α)
Fresh Sample	1.332	0.376	1.363	0.176	1.321	0.112
After 10 Days in the Dark	0.831		1.122		1.172	

Table 2. Dark stability of the coloring dyes in the presence of citric acid.

Samples	Carmoisine		Sunset Yellow		Tartrazine	
	ABS. at 517 nm	Fractional change (α)	ABS. at 482 nm	Fractional change (α)	ABS. at 427 nm	Fractional change (α)
Fresh Sample	0.536	0.041	0.486	0.013	0.403	0.012
After 10 Days in the Dark	0.514		0.480		0.397	

The extent of Photodegradation of coloring dyes under investigation has been studied as a function of food acid concentrations.

Solutions were irradiated in sunlight under similar conditions and for the same period (4 h). As shown in Fig. 7 and 8, the relative stability of coloring dyes in the presence of ascorbic and citric acid goes in the order Tartrazine > Sunset Yellow > Carmoisine. The same order is shown in the dark stability of these dyes.

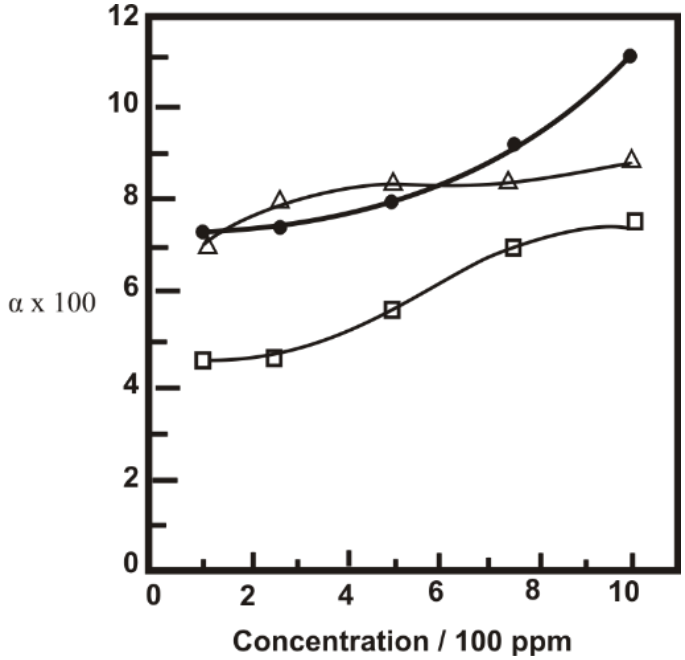


Figure 7. Fractional change (α) of Sunset Yellow (Δ), Tartrazine (ϒ), and Carmoisine (•), as a function of sunlight irradiation in the presence of different concentrations of citric acid.

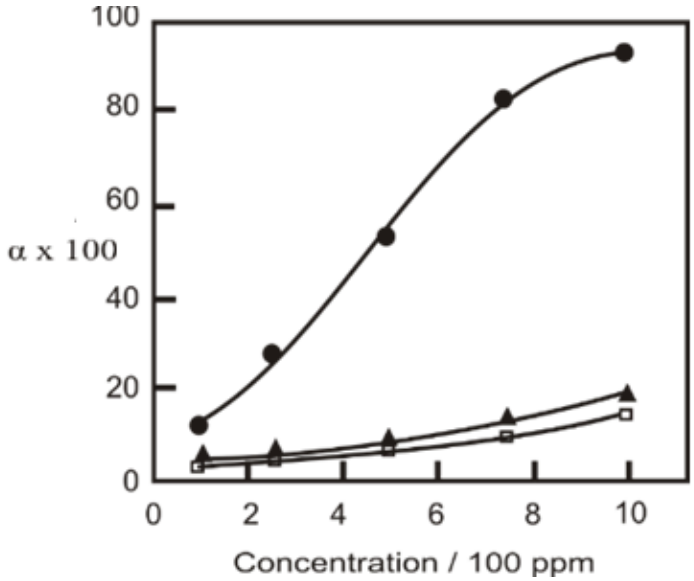


Figure 8. Fractional change (α) of Sunset Yellow (Δ), Tartrazine (ϒ), and Carmoisine (•), as a function of sunlight irradiation in the presence of different concentrations of ascorbic acid.

3.3. Emission Study of Coloring Dyes Degradation

Food acids, e.g., citric and ascorbic acids, in beverages largely enhance the reductive degradation process to aromatic amines, constituting a health hazard. Many aromatic amines have been reported to be potent carcinogens, mutagens, and/or hemotoxicants [22]. The changes in emission spectra due to sunlight irradiation of Carmoisine and Sunset Yellow in the presence of food acids have been studied. Fig. 9 and 10 show the changes in fluorescence spectra of Carmoisine and Sunset Yellow as a function of sunlight irradiation time. The excitation spectra of the emitting species are also shown.

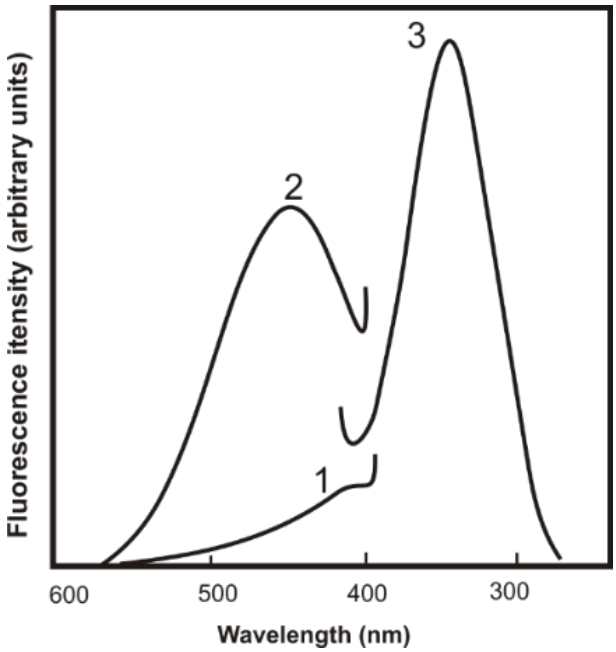


Figure 9. The fluorescence spectra (λ_{ex} = 366 nm) of Sunset Yellow in the presence of ascorbic acid, (1) before and (2) after sunlight irradiation. (3) is the excitation spectrum (λ_{em} = 450 nm) of sample (2).

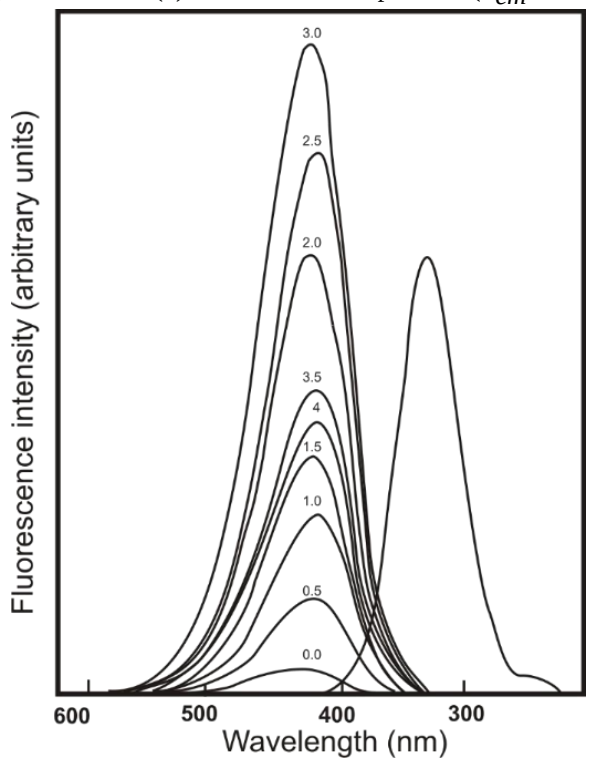


Figure 10. Changes in emission spectra (λ_{ex} = 366 nm) of Carmoisine solution / pH 3.1 / 1000 ppm ascorbic as a function of sunlight irradiation. The irradiation times in hours are quoted on the spectra. The excitation spectrum (λ_{em} = 420 nm) is also shown at shorter wavelengths.

The corresponding changes in the absorption spectra of Carmoisine food dye are shown in Fig. 11.

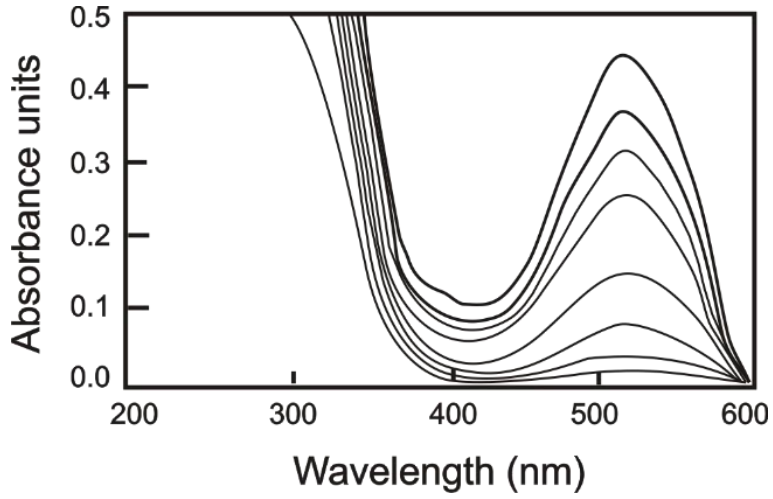


Figure 11. The changes in absorption spectra of Carmoisine in the presence of 1000 ppm ascorbic acid at pH 3.1 resulted from exposure to sunlight for 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 4.5 h.

As the Photodegradation of the color dye proceeds, the emission intensity at 420 nm initially increases and then decreases, as shown in Fig. 12.

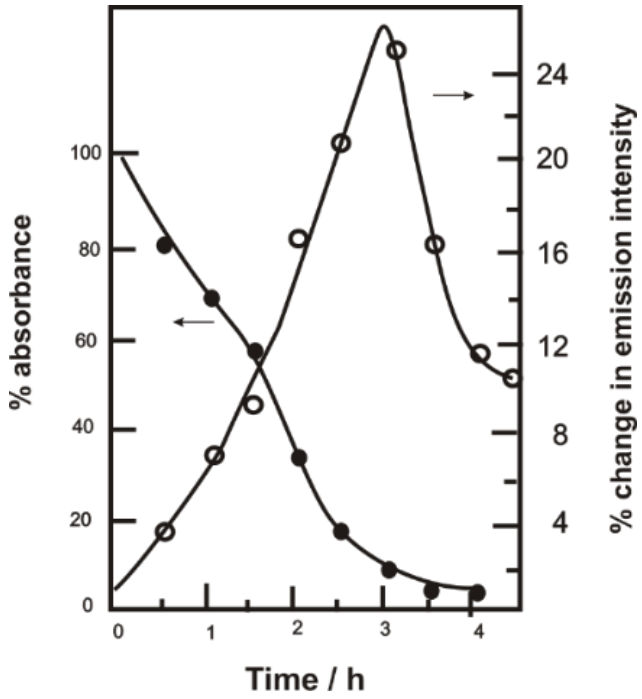
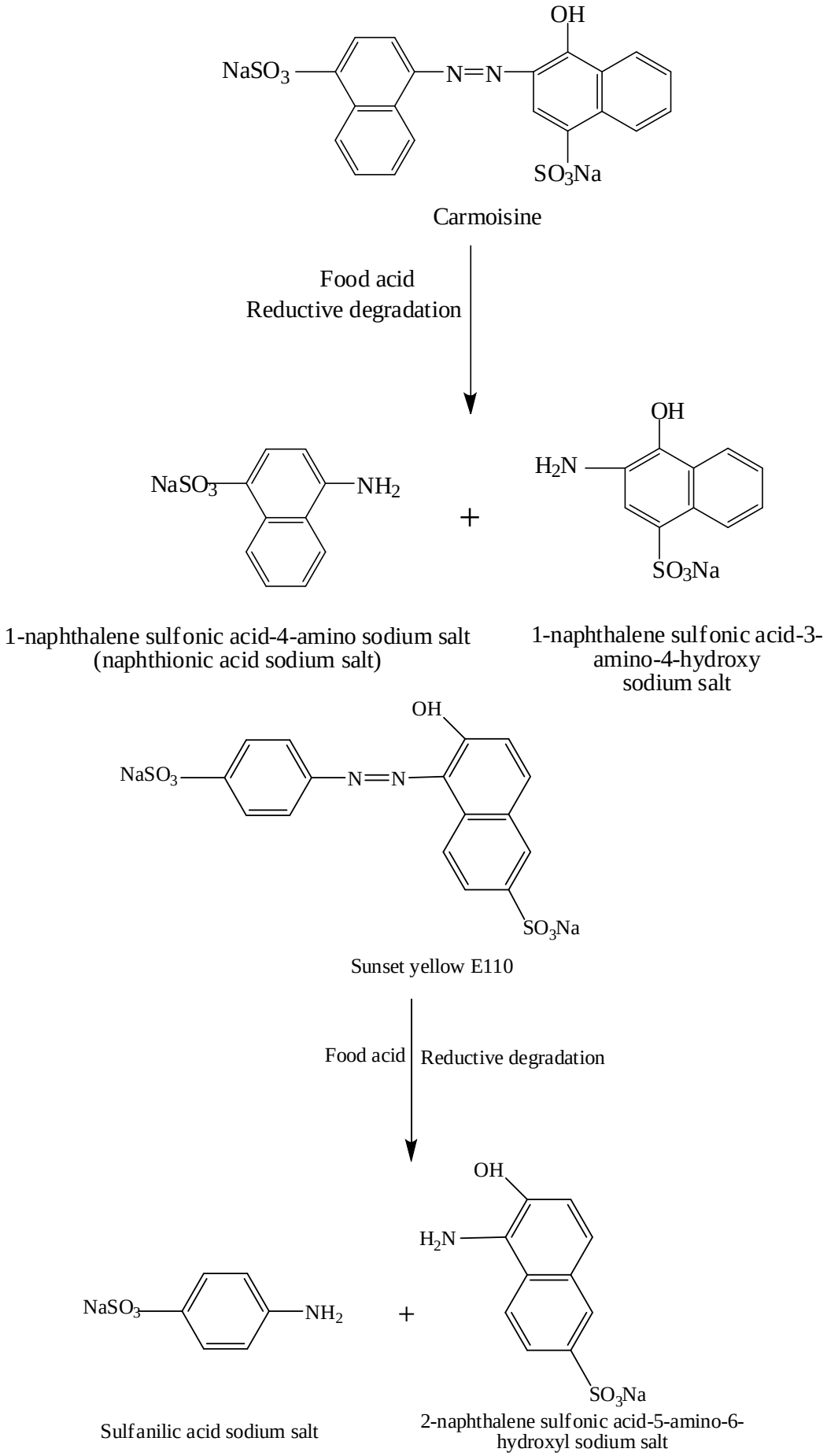


Figure 12. The percentage of absorbance and emission changes of Carmoisine (pH 3.1, 1000 ppm ascorbic acid) is due to sunlight irradiation time.

The emission at 420 nm is not attributed to the color dyes themselves because they occur at shorter wavelengths than the absorption bands of these dyes. The emission is attributed to the degradation products. In the presence of food acids, reductive Photodegradation of the color dyes occurs at the azo center (–N ═ N–), giving amino products, e.g., sulfanilic acid sodium salt and other naphthalene sulfonate amino product as shown in Scheme 1.



Scheme 1. Reductive degradation products of Carmoisine and Sunset Yellow food coloring dyes.

The production of amines in the degradation process of color dyes was confirmed by diazotization procedure [20].

N-1-naphthyl ethylene diamine dihydrochloride (NEDD) was used as an aromatic amine that forms a diazonium amino compound with the degradation of amine products. Sulfanilic acid sodium salt undergoes diazotization coupling with the subsequent appearance of a new absorption band at 545 nm, as shown in Fig. 13.

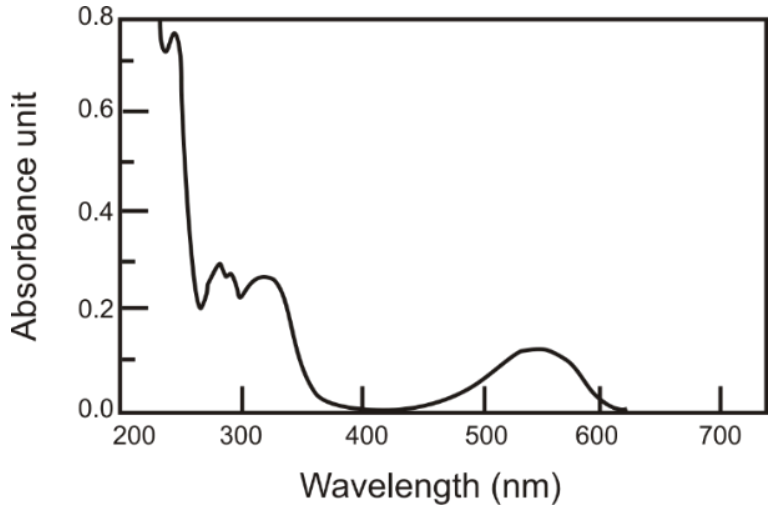


Figure 13. Absorption spectrum of the diazotized sodium salt of sulfanilic acid of concentration 2.0×10^{-5} mol dm^{-3} with NEDD.

A Beer-Lambert plot corresponding to the increase in absorbance of the diazotization product of sulfanilic acid sodium salt and NEDD as a function of increasing sulfanilic acid sodium salt concentration is shown in Fig. 14. The molar absorptivity (ε) of the diazotized derivative is calculated as 1.86×10^4 dm^3 mol^{-1} cm^{-1}.

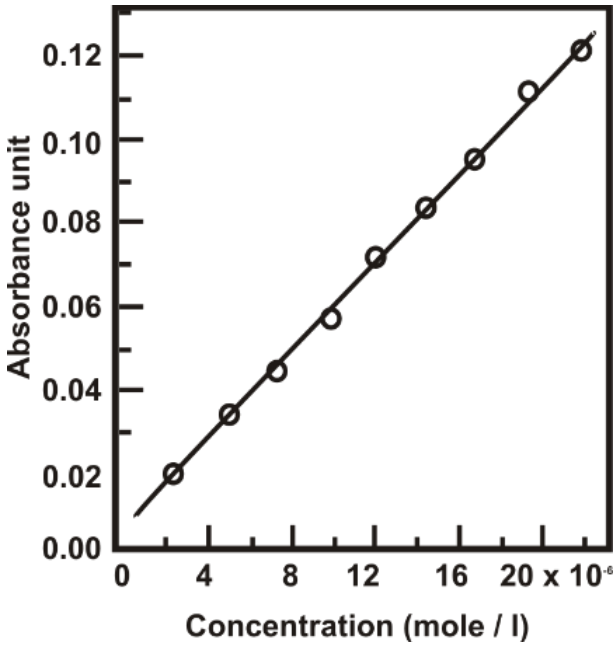


Figure 14. The linear relation between the diazotized sulfanilic acid sodium salt concentration and absorbance at 545 nm.

In a beverage sample (Fanta®), which was subjected to sunlight until color bleaching, diazotization resulted in the appearance of an absorbance band at 545 nm, as shown in Fig. 15.

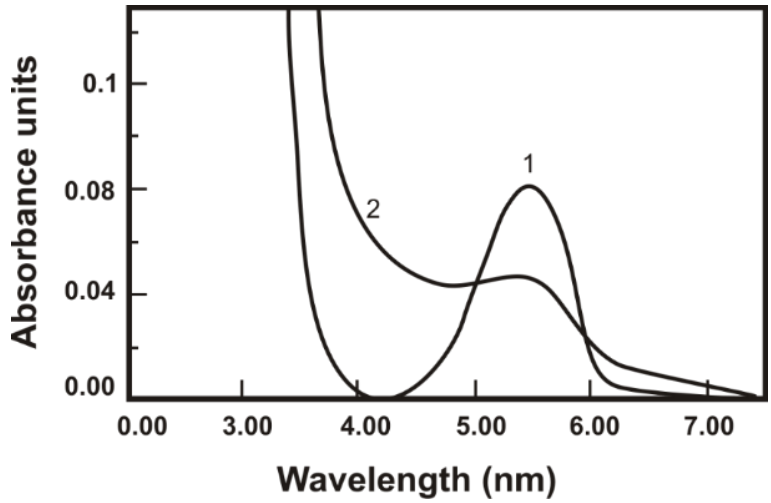


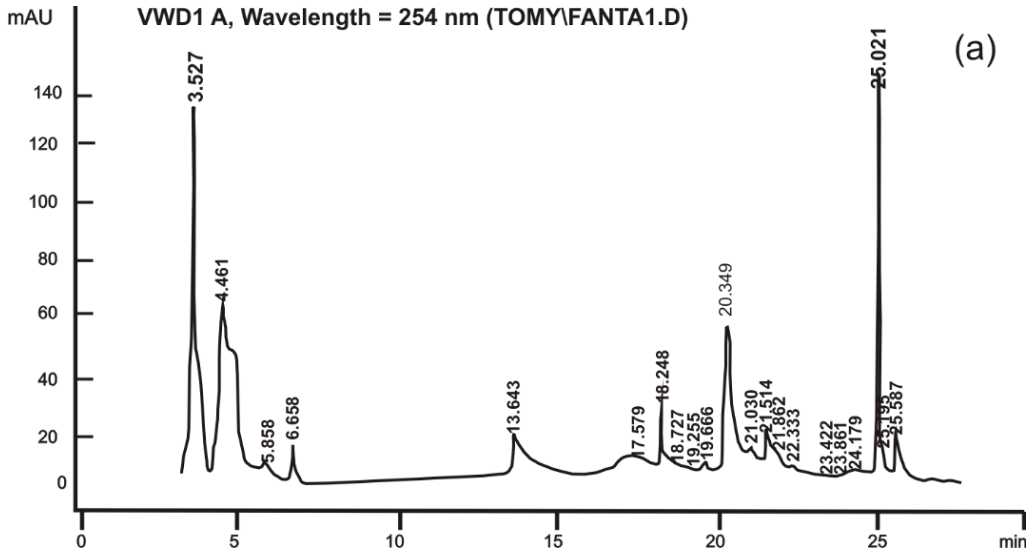
Figure 15. The absorption bands of (1) diazotized sulfanilic acid sodium salt and (2) diazotized Fanta® product following sunlight degradation.

Reductive degradation of the two coloring dyes, Carmoisine and Sunset Yellow was also performed chemically using metallic zinc and HCl. A thin layer chromatogram of the degradation products and sulfanilic acid sodium salt and naphthionic acid sodium salt were obtained as reference substances.

The chromatogram clearly shows that sulfanilic acid sodium salt is a degradation product of Sunset Yellow and naphthionic acid sodium salt is a degradation product of Carmoisine. The other degradation product of Sunset Yellow, 2-naphthalene sulfonic acid-5-amino-6-hydroxyl sodium salt, did not appear visually on the chromatogram due to color criteria.

3.4. HPLC Study of Photodegradation of Coloring Dyes in Mirinda® and Fanta®

HPLC analysis in the present study has encountered some complications because of the many chemical substances in beverage samples. However, some features were observed when irradiating beverage samples. HPLC chromatograms of irradiated Mirinda® sample and sodium salt of naphthionic acid are shown in Fig. 16.



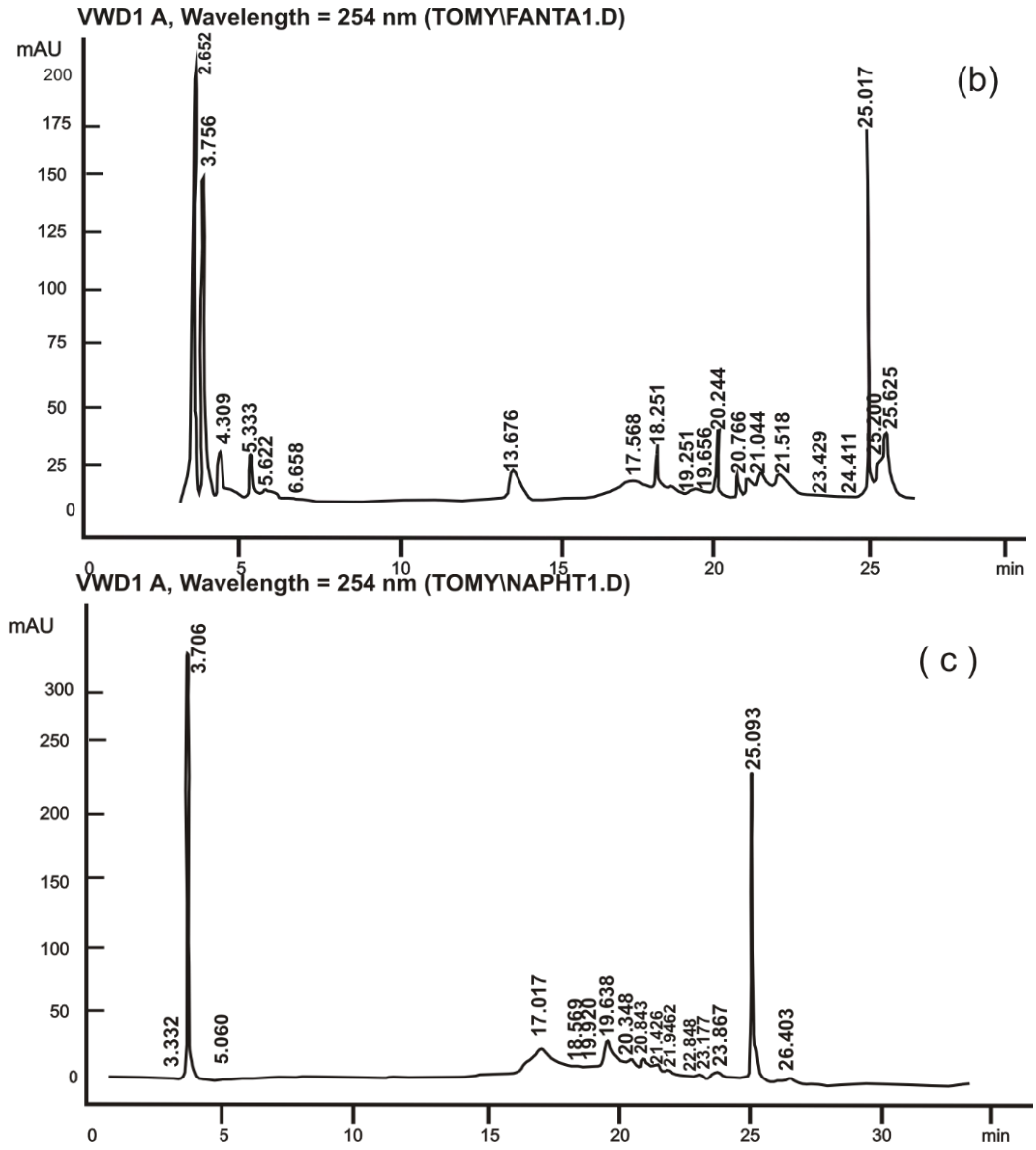


Figure 16. HPLC chromatogram of (a) 1-day and (b) 4-days sunlight irradiated Mirinda® sample, and (c) naphthionic acid sodium salt.

A new retention time peak at 3.756 min develops upon sunlight irradiation of Mirinda®. This peak coincides with the central peak given by naphthionic acid sodium salt at a retention time of 3.706 min, as shown in Fig. 16(c).

Upon sunlight irradiation of Fanta®, two new peaks of retention times 3.749 and 3.617 min develop. The peak at 3.749 min coincides with the peak given by sulfanilic acid sodium salt at a retention time of 3.749 min. These chromatograms are shown in Fig. 17. The second developing peak at a retention time of 3.617 min is assigned to naphthionic acid, whose central peak occurs at 3.706 min. As shown in Fig. 17(c). It has been demonstrated that Fanta® contains both Carmoisine and Sunset Yellow as coloring dyes. Reductive degradation of these two dyes gives sulfanilic acid and naphthionic acid sodium salts, respectively.

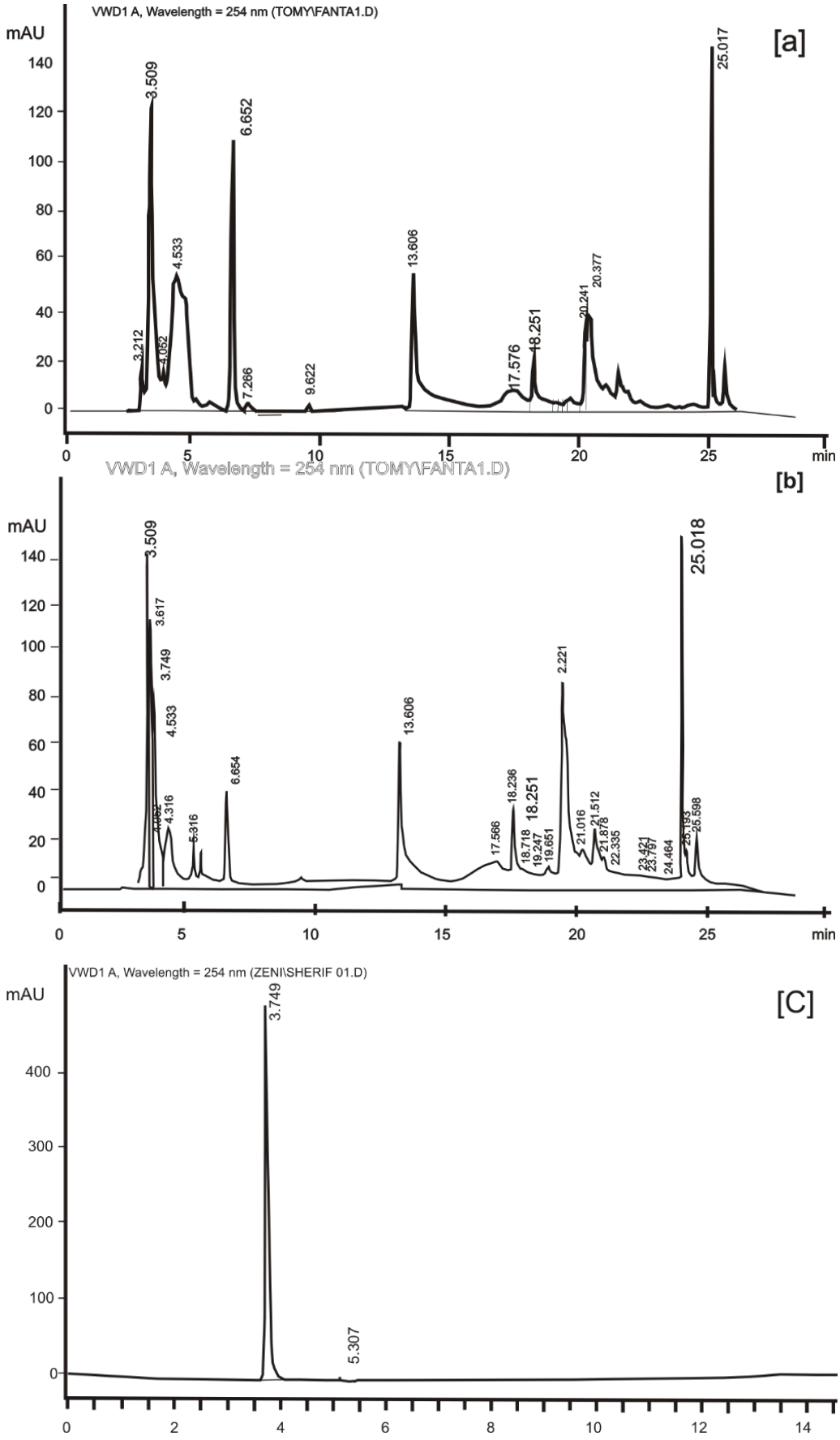


Figure 17. HPLC chromatograms of (a) fresh and (b) 4-day sunlight irradiated Fanta® samples and (c) sulfanilic acid sodium salt.

3.5. Photochemical Quantum Yields (φ_C) of Dyes

The quantum yield, or quantum efficiency, shows how efficient a photochemical reaction is. It is the ratio of the number of photons emitted to the number of photons absorbed. The three studied food dyes' photochemical quantum yields (φc) have been measured using sunlight irradiating dye solutions containing 1000 ppm ascorbic or citric acids at a pH of 3.1. In the same silica cell in which light intensities were measured, 3 ml of coloring dye solution was exposed to sunlight for 1 h, then the absorbance changes ($A_0 - A_t$) were measured. The corresponding changes in number of moles per liter (Δn) were calculated from absorbance changes. The Photochemical quantum yields (φ_C) were then calculated using Eq. 2:

$$\varphi_C = \Delta n / I_0 \qquad \text{Eq. 2}$$

For each sample, three calculations were made for which the average value was taken. Table 3 summarizes the φ_c data for Carmoisine, Sunset Yellow, and Tartrazine in 1000 ppm ascorbic or citric acid. The φ_c values of food dyes increase in the order Tartrazine < Sunset Yellow < Carmoisine. This means that the order of stability goes as Tartrazine > Sunset Yellow > Carmoisine.

Table 3. Summary of φ_c values of the coloring dyes in ascorbic and citric acid presence.

Coloring Dyes	Irradiation	Average φc
Carmoisine	1000 ppm ascorbic	1.15×10^{-5}
	1000 ppm citric	0.19×10^{-5}
Sunset Yellow	1000 ppm ascorbic	0.18×10^{-5}
	1000 ppm citric	0.17×10^{-5}
Tartrazine	1000 ppm ascorbic	0.10×10^{-5}
	1000 ppm citric	0.10×10^{-5}

3.6. Kinetics of the Thermal Degradation of Dyes

The kinetics of thermal degradation of coloring dyes have been studied by applying absorbance changes at their absorption maxima [23-25]. The degradation process obeys a second-order kinetic model as in Eq. 3:

$$k_t = \frac{1}{A_t} - \frac{1}{A_0} \qquad \text{Eq. 3}$$

where k is the second-order rate constant, A_0 and A_t are the absorbances of the dye at time $t = 0$ and $t = t$ (min), respectively. From the second-order kinetic equation plots and Arrhenius plots for dye degradation, the activation energies were determined as E_a = 14.8, 16.5, and 19.9 kJ mol^{-1} for Carmoisine, Sunset Yellow, and Tartrazine.

4. Conclusion

In this study, HPLC and diazotization techniques established the reductive degradation of Sunset Yellow and Carmoisine. Other less specific techniques, e.g., molecular fluorimetry and electronic absorption spectroscopy, also proved the degradation of both food dyes. Food acids, e.g., citric and ascorbic acids in beverages, essentially enhance aromatic amines' reductive degradation process, which constitutes a health hazard. The presence of food acids, which also function as antioxidants, minimizes the role of singlet oxygen as a photodegradation route in such azo-compounds, as Adams et. al. 26 described.

The effect of food acids, ascorbic and citric acids, on the stability of the coloring dyes has been studied in some detail. Both dark and sunlight degradation were observed, and the relative dark stability of the studied dyes was determined by Tartrazine > Sunset Yellow > Carmoisine. This order corresponds with the activation energies for the 19.9, 16.5, and 14.8 kJ mol-1 degradation processes for Tartrazine, Sunset Yellow, and Carmoisine. Thermal degradation of the coloring dyes obeys the second-order kinetic model in dye concentration in the presence of ascorbic or citric acids. For the three food coloring dyes, the following order of efficiency in degradation was observed: ascorbic acid > citric acid > acetate buffer

The relative sunlight stability in the presence of citric or ascorbic food acid goes in the order Tartrazine > Sunset Yellow > Carmoisine as deduced from photochemical quantum yield (φ_C) values for the coloring dyes.

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