UV SPECTROSCOPIC CHARACTERIZATION AND STABILITIES OF NATURAL COLORANTS FROM ROSELLE CALYX, LAC RESIN AND GARDENIA FRUIT

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Abstract: This research intended to study natural water-soluble colorants from three sources; roselle calyx, lac resin and gardenia fruit.Their UV spectroscopic characteristics and acid dissociation constants were determined. Stabilities of the colorants under various conditions (water, acidic, alkaline, low and high temperatures, oxidizing and UV light) were monitored using spectroscopy. Aqueous solutions of roselle, lac and gardenia colorants showed maximum UV absorption wavelengths (λ_{max}) at 520, 490 and 450 nm, corresponding to visualized color, i.e. red, red and yellow, respectively. The observed pK_a values of roselle and lac colorants were determined using spectrophotometry to be 3.00 ± 0.08 and 5.96 ± 0.15, respectively. Roselle and lac colorants were very stable under acidic condition while gardenia colorant was stable under alkaline condition. Temperature had no effect on the color of lac colorant. Light and oxidizing agent accelerated color bleaching of roselle, lac and gardenia colorants.

Keywords: roselle, lac, gardenia, colorant, stability

INTRODUCTION: Coloring agents have been used in many industries such as food, pharmaceutical and cosmetics. Natural colorants are preferred over synthetic ones due to their superior safety. However, instability of natural colorants limits their use. Popular natural colorants in Thailand are red from roselle calyx *(Hibiscus sabdariffa* L., *Oryza sativa* L.) and lac resin *(Laccifer lacca* Kerr.) and yellow from gardenia fruit *(Gardenia jasminoides* Ellis. or cape jasmine). These colorants have been widely utilized as coloring agents in Thai cultural food and cosmetics. Major compounds in roselle calyx, lac resin and gardenia fruit colorants are anthocyanins, laccaic acids and crocin, respectively, as shown in Figure 1[1, 2]. Roselle and lac colorants are very water soluble and their colors are pH dependent. The main anthocyanins in roselle calyxs are delphenidine-3-sambutioside and cyanidine-3-sambutioside[3]. The presence of glycosides on anthocyanins increases their stability during storage[2]. Coloring agents in lac resin consist of laccaic acid A, B, C and E which are anthraquinone colorants. Laccaic acid A is the major component[4]. Crocin in gardenia fruit is classified as a carotenoid colorant. Crocin is a digentiobioside ester of dicarboxylic carotenoid (crocetin)[5]. Gentiobiose is a diglucoside with a beta 1-6 linkage making the molecule water soluble[2]. Although, these natural colorants have been used for centuries, comparisons of their stability have never been report. This research focused on UV spectro-scopic characteristics, acid dissociation constants and stabilities under various conditions of the colorants. This information will be employed in order to choose suitable conditions for applying these natural colorants in food, pharmaceutical and cosmetic products.

MATERIAL AND METHODS:

Chemicals

Sodium hydroxide was purchased from Merck (Germany). Hydrochloric acid was purchased from AnalaR® (England). Phosphoric acid was purchased from Mallinckrodt chemical (USA).

Extraction and preparation of lyophilized colorant powder

Roselle calyx and lac resin were harvested from Nongkai and Srisaket provinces, respectively, in Thailand. Gardenia fruit was purchased from Vejpong Pharmacy (Hock Ann Tung) Co., LTD., Bangkok, Thailand. Colorants were isolated from dried crude materials using an

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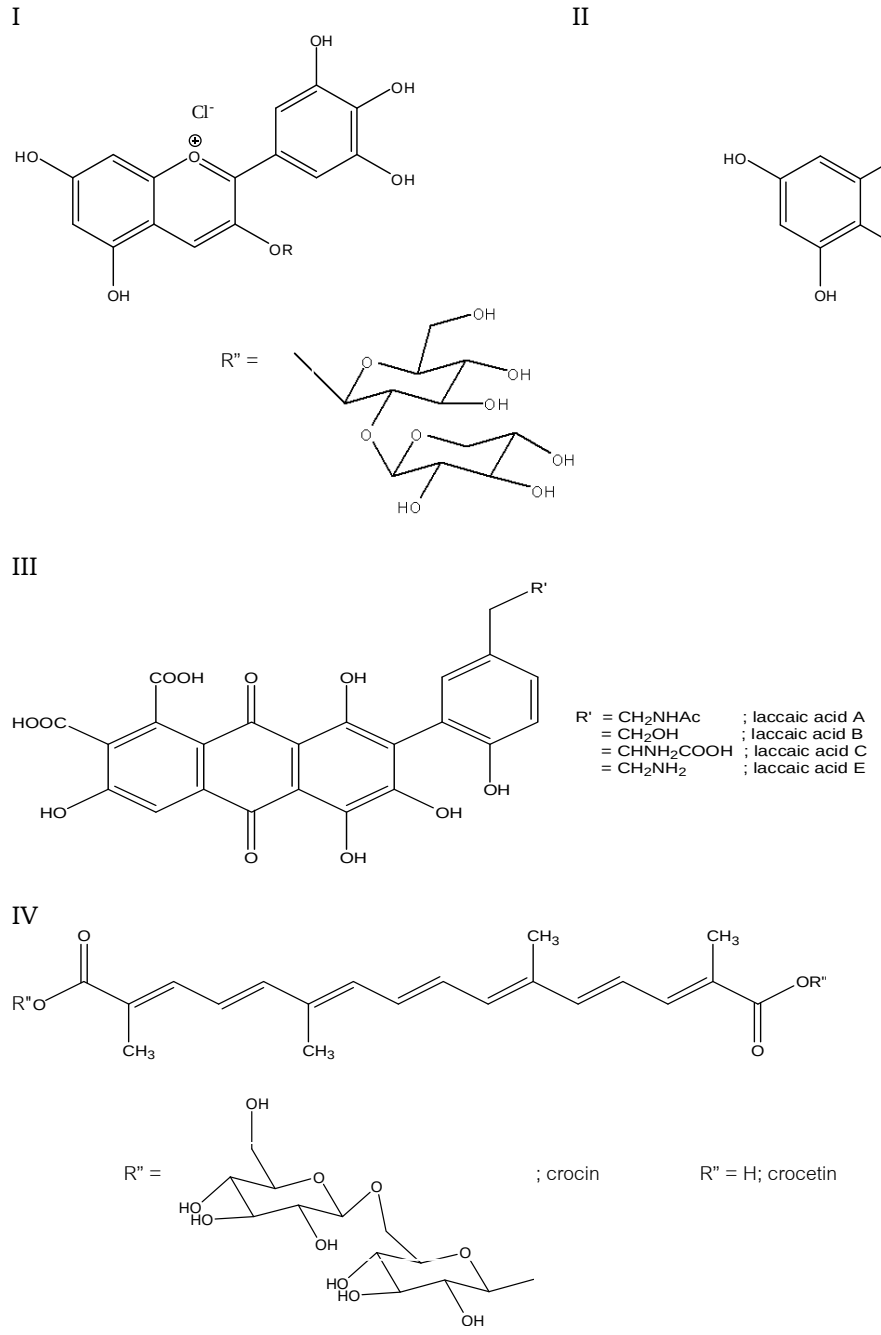


Figure 1 Major compounds in roselle calyx (delphinidin-3-sambubioside, I, and cyaniding-3-sambubioside, II), lac resin (laccaic acids, III) and gardenia fruit (crocin and crocetin, IV).

aqueous extraction technique. Ten grams of the crude material, i.e. roselle calyx, lac resin or gardenia fruit, was separately ground and soaked in water (60 ml) for 2 hours. The mixture was then decanted and filtered through Whatman® filter paper (No. 5). The filtrate was lyophilized and percent yield was calculated as grams of lyophilized colorant per gram of crude (n=3).

UV spectroscopic characterization of the colorants in various pH solutions and pK_a determination of roselle and lac colorants

The lyophilized colorant (2.25 mg/mL roselle, 0.25 mg/mL lac and 0.25 mg/mL gardenia) was dissolved in 3 different media, 0.1N hydrocholoric acid (HCl), water or 0.1 N sodium hydroxide (NaOH).

Absorption spectra of the solutions were collected over the visible wavelength range (400-700 nm) using a Shimadzu UV-vis spectro-photometer UV-1601.

Since major components in roselle and lac colorant are ionizable, pK_a determination was carried out on them using the UV-spectroscopic titration technique. The lyophilized powder of roselle or lac colorant (1.26 or 0.32 mg/ml, respectively) was dissolved in 0.05 M phosphoric acid solution. The colorant solution (50 mL) was transferred to a jacketed vessel equipped with a magnetic stirrer and controlled at a temperature of 26 ± 1 °C. The solution was pumped from the vessel through a flow-through cuvette using a peristaltic pump (Imatec, Switzerland). The solution was gradually titrated from pH 2 to 9 using 20 M NaOH. After each increment of NaOH, pH value and UV absorption spectra were taken using a pH meter attached with pH probe (model 420 A, Orion, MA, USA) and a UV-vis spectro-photometer. The observed pK_a values of roselle and lac colorants were determined using Equation (1).

$$pK_a = pH + \log\left[\frac{A_{HA} - A_{obs}}{A_{obs} - A_{A^-}}\right] \qquad \text{Equation (1)}$$

where A_{HA} is absorbance of protonated form, A_{A^-} is absorbance of unprotonated form and A_{obs} is absorbance of colorant at any pH[6].

Stability of natural colorants from roselle calyx, lac resin and gardenia fruit

Stabilities of the colorants were investigated under various conditions; i.e., acidic, alkaline, oxidation, water, UV light and elevated temperature. The sample solutions (2.25 mg/mL roselle, 0.25 mg/mL lac and 0.25 mg/mL gardenia) were prepared in different media, 0.1N HCl, 0.1N NaOH or 3% hydrogen peroxide (H_2O_2) corresponding to acidic, alkaline, or oxidative conditions, respectively[7]. Samples were kept in small sealed vials and placed in a dark chamber at 30°C. At appropriate times, samples were taken and UV absorption spectra over the visible wavelength range were collected.

To investigate hydrolysis and photostability of the colorants, sample solutions (2.25 mg/mL roselle, 0.25 mg/mL lac and 0.25 mg/mL gardenia) were prepared in ultra purified water, filled in small sealed vials and placed in a dark chamber or in a UV light chamber (photostability cabinet, APT Engineering, Thailand) at 30°C. At appropriate times, samples were taken and UV absorption spectra over the visible wavelength range were collected.

To determine stability at elevated temperature, the colorants were dissolved in ultra purified water. Sample solutions (2.25 mg/mL roselle, 0.25 mg/mL lac and 0.25 mg/mL gardenia) were filled in small sealed vials and kept at 4, 30 and 72 °C in the dark. At appropriate times, samples were collected and UV absorption spectra over the visible wavelength range were collected.

The percent color remaining of samples was calculated based on absorbance at maximum absorption wavelengths (λ_{max}) using Equation (2) and plotted as a function of time.

$$\%\ \text{remaining} = \frac{\text{absorbance at sampling time}}{\text{absorbance at initial time}} \times 100 \qquad \text{Equation (2)}$$

RESULTS AND DISCUSSION:

Extraction and preparation of lyophilized colorant powder

Aqueous extracts of roselle calyx, lac resin and gardenia fruit were pink-red, dark-red and yellow, respectively. Appearance of the roselle and lac extracts was clear while that of gardenia extract was translucent colloidal.

Physical appearance of lyophilized colorants from roselle, lac and gardenia are pink-red bulky powder, dark-red bulky powder and yellow fiber-like cake, respectively. Percent yields from the aqueous extraction processes were 31.98 ± 0.32, 1.99 ± 1.47 and 9.59 ± 2.52 for roselle calyx, lac resin and gardenia fruit, respectively.

UV spectroscopic characterization of the colorants in various pH solutions and pK_a determination of roselle and lac colorants

UV spectroscopic characteristic of roselle, lac and gardenia colorants in 0.1 N HCl, water and 0.1 N NaOH were shown in Figure 2. In the pH

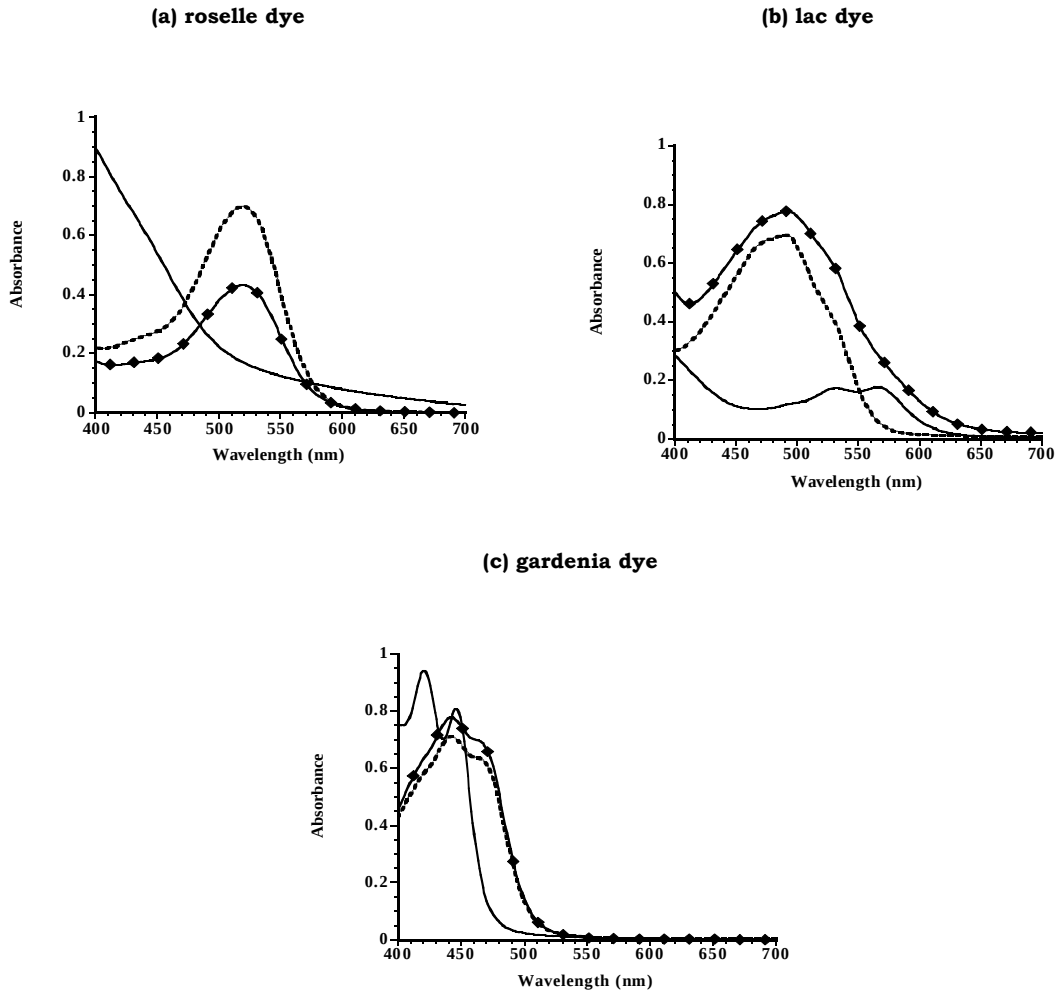


Figure 2 UV-visible spectra of roselle (a), lac (b) and gardenia dyes (c) in 0.1N hydrochloric acid (-----),water (—◆—) and 0.1N sodium hydroxide (——).

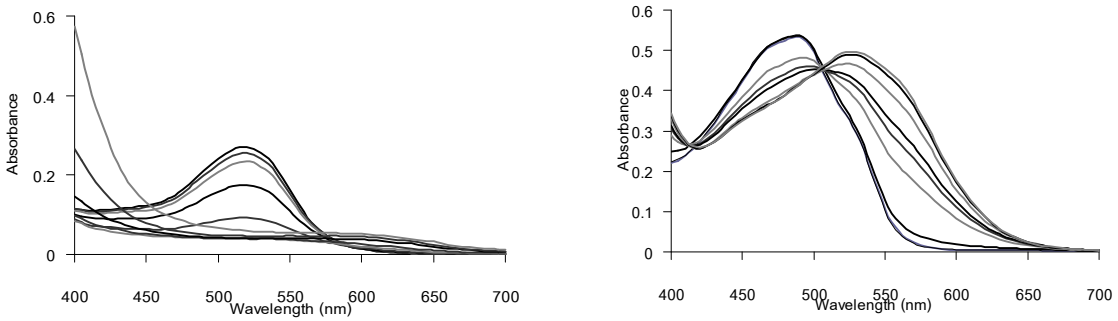


Figure 3 UV-visible spectra of roselle (a) and lac (b) colors at various pH (2-9) for pK_a determination.

range of 2-9, UV absorption spectra of roselle and lac colorants were greatly affected by pH of the media since both anthocyanins in roselle calyx and laccaic acids in lac resin possess ionizable functional groups (Figure 1). The observed color of roselle colorant solution changed from pink to yellowish when pH of the solution was changed from acidic to alkaline. Color of lac colorant solution was orange, red and violet in 0.1 N HCl, water and 0.1 N NaOH, respectively.

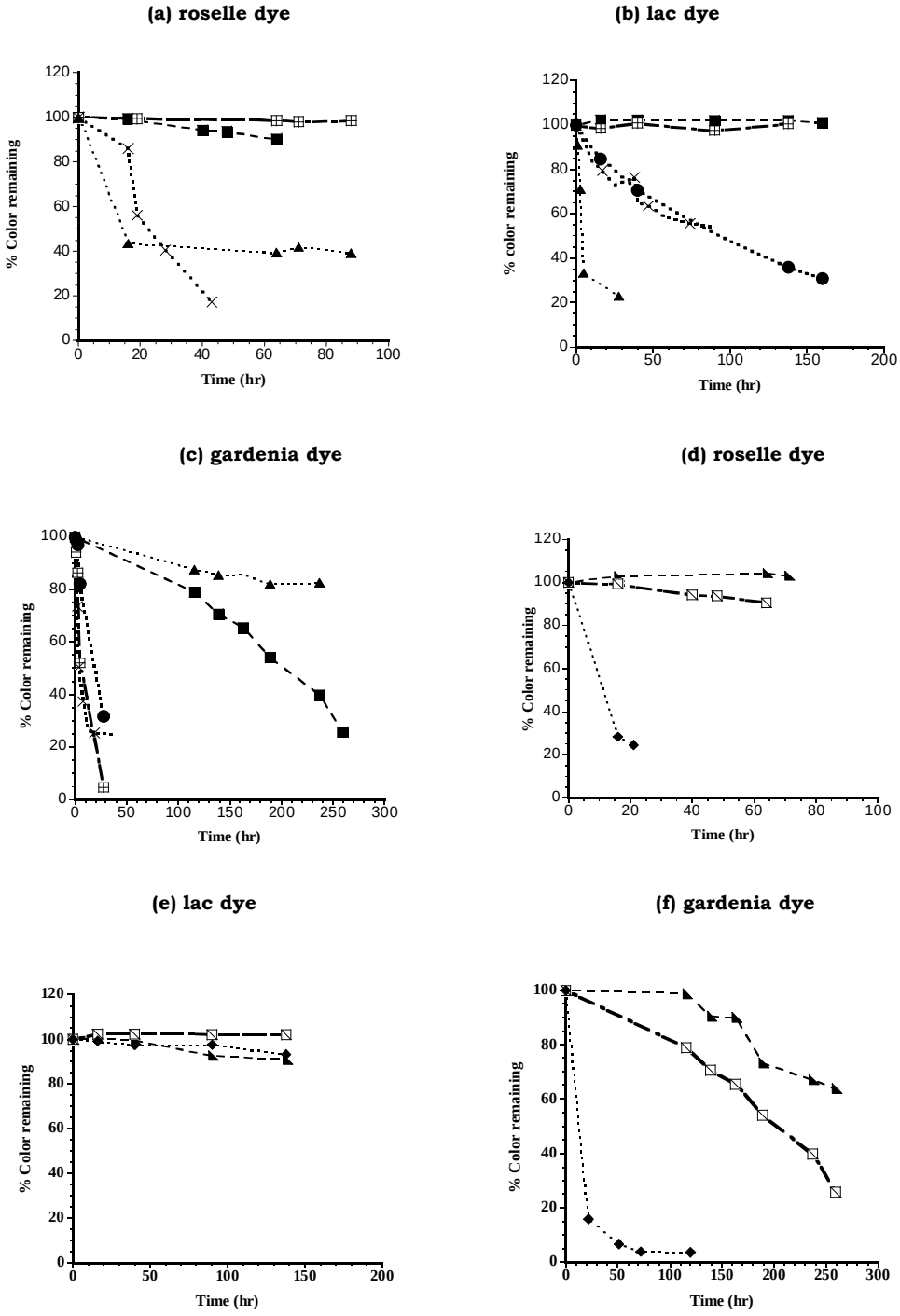


Figure 4 Percent color remaining of roselle dye (a), lac dye (b) and gardenia dye (c) in five conditions: water (■), acid (⊞), alkaline (▲), UV light (×), oxidation (•) and temperature effect on roselle dye (d), lac dye (e) and gardenia dye (f) at 4 ˚C (◣), 30 ˚C (⧅) and 72 ˚C (◆).

Observed pK_a values were calculated based on UV-absorbance at 520 nm for anthocyanins in roselle colorant and at 560 nm for laccaic acids in lac colorant (Figure 3) using Equation (1). The observed pK_a values of colorants from roselle and lac were 3.00 ± 0.08 and 5.96 ± 0.15, respectively. These observed pK_a values of anthocyanins from roselle calyx agree well with reported values of anthocyanins from other sources which are in the range of 3.0-4.5 depending on types and sources of anthocyanins[8].

Stability of natural colorants from roselle calyx, lac resin and gardenia fruit

Stability of roselle, lac and gardenia colorants under various conditions are shown in terms of percent color remaining as a function of time (Figure 4). The order of stability under studied conditions is as follows, roselle colorant in acid > in water at 4ºC > in water at 30 ºC > in alkaline ~ under UV light ~ in water at 72 ºC > in H_2O_2 solution; lac colorant in acid ~ in water (4, 30, 72ºC) > in H_2O_2 solution ~ under UV light > in alkaline; gardenia colorant in alkaline > in water at 4 ºC > in water at 30 ºC > in water at 72 ºC ~ in acid ~ in H_2O_2 solution ~ under UV light.

Roselle colorant was stable in water at 4˚C and acidic conditions. When pH is less than 3.0, the major component, delphinidin, exists as flavylium cation. Under proton rich environment, delphinidin cannot undergo deprotonation and further degradation to the carbinol pseudobase (colorless) and chalcone (yellowish) is not favorable[9]. Thus, delphinidin was very stable under acidic condition. On the contrary, delphinidin is very labile at high pH values giving rise to formation of degradation products [2,10]. The reaction was also accelerated at high temperature (72 ºC) and in the presence of UV light, which is in agreement with a previous study[11]. Moreover, in 3% H_2O_2, color of roselle changed from red to colorless within 5 minutes. The colorless products are speculated to be hydroquinone derivatives resulting from oxidation at the delphinidin phenol group[2,12].

Laccaic acids, components in lac colorant, were stable in water and in acidic solution. As suggested, lac pigments in ethanol, propylene glycol and glycerol could be stabilized by the addition of citric, malic, acetic, lactic or hydrocholoric acid[2]). Temperature elevation did not accelerate lac color fading. The oxidizing agent, H_2O_2, induced the loss of color in lac colorant. The reaction likely takes place at the phenolic functional groups giving rise to colorless products. In the same manner, the ionized form of laccaic acid at pH higher than 6.0 facilitates formation of colorless products.

Crocin in gardenia colorant was unstable under all condition. However, color of gardenia is the most stable in alkaline solution among the three colorants studied. The degradation of crocins from saffron and gardenia fruit were reported to increase with light, decreasing pH and increasing temperature [13-15]. The crocins became colorless probably due to the loss of conjugation in their molecules[15]. Anthocyanins and crocin consist of sambutiosyl and gentiobiosyl connected to their core structures, delphenidin and crocetin, respectively, through ester bonds. Therefore, both compounds undergo acid-base catalyzed ester hydrolysis resulting in color fading. Anthocyanin and crocin colorants changed from their initial colors to brown at high temperature and in the present H_2O_2 because the disaccharides may undergo Millard reaction and oxidation.

CONCLUSIONS: The visualized colors of ionizable natural colorants, roselle and lac colorants, depend on the major species which exist in acid-base equilibrium in the medium. Thus, their colors are greatly affected by pH of media. Roselle and lac colorants are very stable in acidic medium while gardenia colorant is rather stable in alkaline medium. At high temperature, lac colorant is very stable while roselle and gardenia colorants are labile. Light and oxidizing agents accelerate bleaching of roselle, lac and gardenia colorants.

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