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Comparison of stability of red colorants from natural sources, roselle and lac in micelles

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Abstract

Natural colorants have been widely used in food and cosmetic products. Nonionic and anionic surfactants are also usually added as solubilizers and stabilizers in the products. Surfactants form a special structure, a so-called micelle. Micelles can alter stabilities and physicochemical properties of other chemicals in the system. The objectives of this study were to determine stabilities in micellar solutions of extracts from roselle calyx (*Hibiscus sabdariffa* Linn.) and lac resin (*Laccifer lacca* Kerr.) providing red colorants. The main constituents of these extracts are two anthocyanins, delphinidin-3-sambubioside and cyanidin-3-sambubioside, and anthraquinones in the form of laccaic acids. The % yields of roselle and lac from the extraction process were 31.96 ± 0.32 and 2.79 ± 0.23, respectively. Roselle colorant faded in the absence and presence of sodium dodecylsulfate (SDS) and Tween80 micelles. The order of stabilities of roselle colorant in solvent systems was buffer ~ Tween80 > SDS. Lac colorant is quite stable in the absence and presence of the surfactants and no significant difference in the % color remaining in the absence and presence of the surfactants was observed.

Introduction

Roselle calyx (*Hibiscus sabdariffa* Linn.) and lac resin (*Laccifer lacca* Kerr.) are sources of natural red colorants used in food and cosmetic products. The major compounds in roselle and lac that produce the red color are anthocyanins and anthraquinones (Francis, 1989; Pouget *et al.*, 1990; Watanabe and Terabe, 2000). These compounds are water soluble and their visual color is pH dependent (Wrolstad *et al*., 2005; Ketmaro *et al*., 2010). The main anthocyanin constituents in roselle calyx are delphinidin-3-sambubioside and cyanidin-3-sambubioside, while the main anthraquinones in lac resin are laccaic acids (Hendry and Houghton, 1992). Roselle and lac colorants are stable in acidic conditions and their apparent pK_a values are 3.00 ± 0.080 and 5.96 ± 0.15, respectively (Ketmaro *et al*., 2010).

Nonionic and anionic surfactants are generally used as solubilizers and stabilizers in food, pharmaceutical and cosmetic products more than cationic surfactants due to toxicity of the cationic surfactants. Surfactants form a special structure, a so-called micelle, at a surfactant concentration greater than the critical micelle concentration, (CMC). Micellar systems can accelerate or decelerate some reactions (Fendler and Fendler, 1975). Compared to the absence of surfactant, the presence of micelles may alter the physicochemical properties and stabilities of the natural colorants in products. The purpose of this study was to determine the effect of surfactants on stability of red colorants from lac resin and roselle calyx using spectroscopy based on absorption in the visible region (380 - 750 nm) and the CIELab system developed by Commission Internationale de l'Eclairage (CIE) to follow up the color change (CIE, 1986; Berns, 2000; Yam and Papadakis, 2004).

Materials and methods

Preparation of lyophilized Roselle and Lac colorants

Ten grams of roselle calyx (Thailand) or grounded stick lac (Thailand) was soaked in 50 ml of water for 2 hr and filtered through Whatman® filter paper number 5 (Whatman International, Maidstone, England). The roselle filtrate was lyophilized until dried powder was obtained using a lyophilizer (Dura-Dry FTS System™, Cambridge, USA). Lac filtrate was centrifuged at 5,000 rpm for 1 hr and further filtered through a cellulose acetate membrane with a pore size of 0.45 µm (Swinnex-25® and MF membrane, Millipore®, Cork, Ireland). The lac filtrate was lyophilized until dried powder was obtained.

Identification of Roselle and Lac extracts using HPLC and LC-MS

HPLC for roselle colorant was conducted on a Shimadzu LC20® (Japan) connected to a C18 column (5µm, 250 x 4.6 mm; Phenomenex®, USA) at 40°C

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using a UV detector set at an analytical wavelength of 530 nm. Mobile phase A consisted of 5% formic acid (Carlo Erba Reagent, Italy) in distilled water and mobile phase B was 100% acetonitrile (Lab-scan Analytical Sciences, Ireland). The elution profile had a linear gradient with 5% B to 20% B from 0 - 10 min, 20% B to 80% B from 10 - 13 min, isocratic elution at 80% B from 13 - 17 min, and finally linear elution from 80% B to 5% B from 17 - 20 min at a flow rate of 0.8 mL/min. LC-MS was performed on an Agilent 1100 HPLC® system (USA) and a Bruker Daltonic "Esquire 300+" Iontrap Mass Spectrometer with an API-ESI source (USA) using the same separation conditions for HPLC. ESI-MS data were acquired in the positive mode using a data-dependent LC-MS method. The ESI voltage, capillary temperature, and sheath gas pressure were 4000 V, 300°C, and 30 psi, respectively. The methods were modified from those in Comesky *et al.* (2009).

The HPLC system used for Lac colorant characterization included a detector for monitoring at 280 nm and a mobile phase of acetonitrile:distilled water (1:4 v/v) running at a flow rate of 0.8 ml/min. LC-MS was performed using separation under the same HPLC conditions and a mass spectrometer with an electrospray source operating in the negative-ion mode. A gas sheath flow of 50 psi nitrogen, electrospray voltage of 4000 V, and a capillary temperature of 300°C were utilized. The methods were modified from those in Oka *et al.* (1998).

Determination of CMC for sodium dodecylsulphate (SDS) and polysorbate 80 (Tween80)

A series of SDS (Carlo Erba Reagent, Rodano, Italy) or Tween80 (Sigma Aldrich Inc., St. Louis, USA) samples at various concentrations were prepared in 0.2 M succinate buffer (Ajax Fine Chemical, Seven Hills, Australia) at pH 4.5 with addition of preservatives (0.1% methyl paraben and 0.01% propyl paraben) and the ionic strength was adjusted to 0.25M using sodium chloride (Merck, Germany). Surface tension of the solutions was measured using a tensiometer (Dataphysics DCAT11, Germany) equipped with a Wilhelmy plate at 26 ± 1°C. The CMC of each surfactant was determined from the profile of surface tension and surfactant concentration. All experiments were done in triplicate.

Stability of Roselle and Lac color extracts in surfactant solutions

Roselle solution (5.2 mg/ml) and lac solution (0.25 mg/ml) were dissolved in 0.2 M succinate buffer (pH 4.5) with preservatives in the presence or absence of SDS or Tween 80 micellar systems. The concentration of the surfactants was varied (1-, 5-, 10-, and 15-fold the CMC values) and ionic strength was adjusted to 0.25 M using sodium chloride. The solutions were placed in well-sealed amber glass vials and kept in a stability chamber at 30ºC for 2 months or a half-life period. Samples were taken and determined at appropriate times.

Spectra of the samples were measured using a UV spectrophotometer (Shimadzu UV-1601, Japan) at wavelengths of 400 - 700 nm. Absorbances at absorption maxima at the initial time ($A_{\lambda max,\ t0}$) and time t ($A_{\lambda max,\ t}$) were determined and the percentage of color remaining was calculated using Eq. 1 (Fan *et al.*, 2008).

$$\% \text{ color remaining} = A_{\lambda max,\ t}/A_{\lambda max,\ t0} \times 100 \quad \text{Eq. 1}$$

The CIELab system was also used to monitor the color change. This system was developed to identify colorants in three-dimensional space. The L-axis is the lightness, which ranges from 0 (black) to 100 (white). The two coordinates a and b depict redness (positive value of a) to greenness (negative value of a) and yellowness (positive value of b) to blueness (negative value of b), respectively (Gonnet, 1998). Other color parameters, chroma (C), hue (h), ΔH and Euclidean distance (ΔE), were calculated using Eqs. 2-5, respectively. ΔH is the difference in hue between two points and ΔE provides information regarding color change in three-dimensions between two points.

$$C = (a^2 + b^2)^{1/2} \quad \text{Eq. 2}$$

$$H = \tan^{-1}(b/a) \quad \text{Eq. 3}$$

$$\Delta H = (\Delta a^2 + \Delta b^2 - \Delta C^2)^{1/2} \quad \text{Eq. 4}$$

$$\Delta E_{ab} = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2} \quad \text{Eq. 5}$$

The samples were transferred into closed cuvettes that were later scanned using a HP scanjet G4010. The scanned pictures were saved as TIFF files and these digital files were loaded into Adobe® Photoshop® CS version 8.0. The software was set for determining Lab color mode ($L^*a^*c^*$) in Info Palette (Yam and Papadakis, 2004). The color parameters were calculated from average values of eight measurements of each picture. All experiments were done in triplicate.

Statistical analysis

Statistical tests were determined using ANOVA.

The statistical tests were two-tailed tests and a value of $p \leq 0.05$ was considered significant. Data are presented as mean values with standard deviation.

Results and Discussion

Preparation of lyophilized Roselle and Lac colorants

Lyophilized colorants from roselle and lac were pink-red and dark red bulky powders, respectively. The % yield of roselle from the extraction process was 31.96 ± 0.32 and that of lac was 2.79 ± 0.23.

Identification of Roselle and lac extracts using HPLC and LC-MS

The HPLC chromatogram and LC-MS data for roselle colorant revealed two major compounds at retention times of 8.9 and 9.5 min corresponding to delphinidin-3-sambubioside and cyanidin-3-sambubioside, respectively (Figure 1). The theoretical molecular weight of delphinidin-3-sambubioside is 597.3 and that of cyanidin-3-sambubioside is 581.2. The presence of mass to charge ratios of 303.0 and 287.0 corresponding to delphinidin and cyanidin was due to fragmentation of delphinidin-3-sambubioside and cyanidin-3-sambubioside during the ionization step, consistent with previous reports (Hsieh *et al.*, 2008).

The HPLC chromatogram of lac dye showed three major peaks at 8.9, 14.7, and 16 min (Figure 2). The peak at 16 min was due to coelution of compounds with retention times of 16.4 and 16.7 min. LC-MS data showed that the compounds at retention times of 8.9, 16.4, and 16.7 min corresponded to laccaic acids C, A, and B with theoretical molecular masses of 539, 537, and 496, respectively (Oka *et al.*, 1998). The peak at a retention time of 14.7 min was an unidentified compound with a mass to charge ratio of 519.9.

Determination of the CMC of sodium dodecylsulphate (SDS) and polysorbate 80 (Tween80)

As the concentrations of SDS and Tween80 were increased, surface tension decreased and reached a plateau at high surfactant concentrations. The inflection point of the log-concentration of surfactant vs. surface tension profile was defined as CMC. The apparent CMCs of SDS and Tween80 in the buffer solution were $5.00 \times 10^{-4} \pm 2.82 \times 10^{-5}$ and $1.85 \times 10^{-5} \pm 8.32 \times 10^{-6}$ M, respectively.

The CMCs of SDS and Tween80 in buffer solutions were compared to the values in water, which have been found to be 8×10^{-3} M (at 37°C) (Kodama *et al.*, 1972) and 1.2×10^{-5} M (at 23°C),

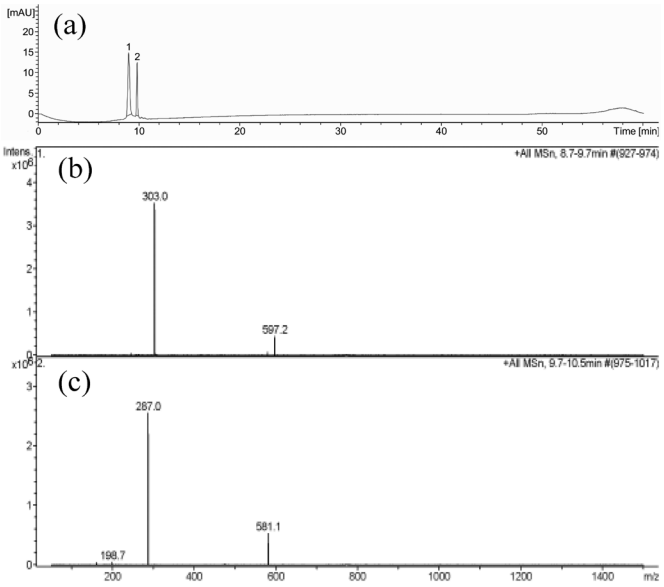

Figure 1. LC-MS of roselle colorant and HPLC chromatogram of roselle colorant (a), mass spectra of the peak at retention time of 8.9 minutes (b), and mass spectra of the peak at retention time of 9.5 minutes (c)

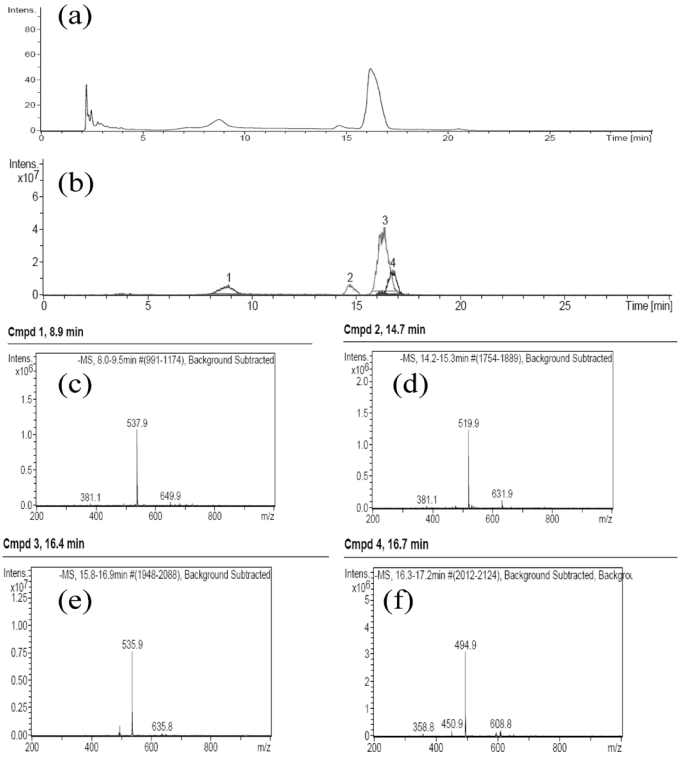

Figure 2. LC-MS of lac colorant and HPLC chromatogram of lac colorant (a and b), mass spectra of the peak at retention time of 8.9 minutes (c), mass spectra of the peak at retention time of 14.7 minutes (d), mass spectra of the peak at retention time of 16.4 minutes (e), and mass spectra of the peak at retention time of 16.7 minutes (f)

(Zheng and Obbard, 2002) respectively. The CMC of SDS, an anionic surfactant, in the buffer system was lower than that of SDS in water because the presence of electrolytes in the buffer reduced repulsion forces

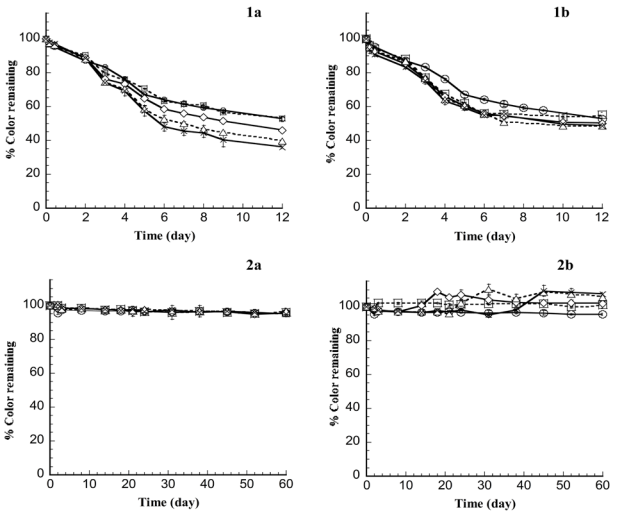

Figure 3. Percentage of color remaining of roselle colorant in SDS (1a) or Tween80 (1b) micellar systems and lac colorant in SDS (2a) or Tween80 (2b) micellar systems; succinate buffer pH 4.5 (—O—), 1CMC (--□--), 5 CMC(—◊—), 10 CMC (--△--), and 15 CMC (—X—).

among the polar head groups; therefore, formation of SDS micelles was facilitated in the buffer. In contrast, the CMC of Tween80, a non-ionic surfactant, in the buffer system was similar to that of Tween80 in water. The surfactant concentrations were prepared at CMC, and 5, 10 and 15 times CMC (5CMC, 10CMC and 15CMC, respectively) for the subsequent stability study.

Stabilities of Roselle and Lac color extracts in surfactant solutions

The roselle and lac colorants have been found to be very stable in acidic conditions (Ketmaro *et al*., 2010). Therefore, this stability study was conducted in succinate buffer solution at pH 4.5. Roselle color extract in the absence and presence of SDS or Tween80 faded overtime, with UV absorbance decreasing at 528 nm and increasing at 420 nm. This result was consistent with the observed color change from red to yellowish within 12 days. The lac colorant was more stable than the roselle colorant, since the color of lac remained visually unchanged in the presence or absence of surfactants over the 2 months of the study.

UV absorption maxima (λ_{max}) of roselle and lac colorants occurred at 528 and 490 nm, respectively. The % color remaining in the roselle and lac solutions was determined as the % absorbance at time t compared to that at the initial time (Figure 3). The % color remaining of the roselle colorant in SDS at 15-fold CMC had the lowest value, corresponding to the reduced absorbance of roselle solution at 528 nm. Compared to the absence of SDS, the presence of SDS at high concentration caused significant difference in

Table 1. CIE Lab parameters of roselle and lac solution over the storage time

Parameters		SDS					Tween80			
		Buffer	1 CMC	5 CMC	10 CMC	15 CMC	1 CMC	5 CMC	10 CMC	15 CMC
	Roselle									
ΔH	6 days	31.4	32.1	29.9	25.1	23.2	40.6	38.3	38.1	35.7
	12 days	51.1	52.6	50.3	42.5	42.6	52.3	51.2	49.6	49.6
ΔE	6 days	32.8	33.7	32.7	28.5	26.0	42.7	40.8	39.8	38.9
	12 days	52.9	53.8	52.5	44.9	44.9	54.5	53.5	51.4	52.1
	Lac									
ΔH	31 days	8.0	13.6	13.3	13.7	8.3	8.3	10.9	11.7	10.0
	60 days	10.9	16.1	14.1	16.6	10.1	10.1	13.3	14.5	14.5
ΔE	31 days	9.8	16.2	16.2	16.7	10.0	10.0	12.7	14.2	12.3
	60 days	13.0	19.2	16.8	19.5	12.6	12.6	15.9	18.6	19.0

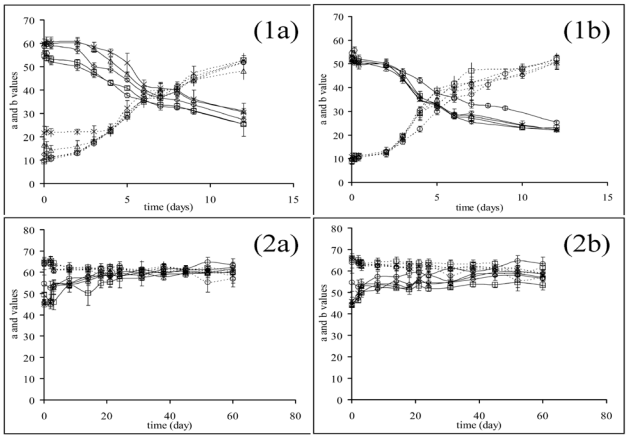

Figure 4. The a (bold line) and b (dash line) values of roselle colorant in SDS (1a) or Tween80 (1b) micellar systems and lac colorant in SDS (2a) or Tween80 (2b) micellar systems; succinate buffer pH 4.5 (-O-), 1CMC (--□--), 5 CMC(—◊—), 10 CMC (--△--), and 15 CMC (—X—).

% color remaining (ANOVA, $p < 0.05$). The values for % color remaining of roselle colorant in Tween80 micelles were similar to the results obtained for roselle colorant in buffer solution. For lac solution, there was no significant difference in the % color remaining in the absence and presence of micelles (ANOVA, $p > 0.05$).

CIELab parameters for roselle and lac solutions were calculated using Eqs. 3-6. Profiles of a and b values vs. time are shown in Figure 4 and other parameters are reported in Table 1. The values of a and b for the roselle solution showed a change of color from red (high values of a) to yellow (high value of b). An effect of SDS on roselle colorant was observed, with higher absorbance and stronger chroma when SDS was added in a buffered solution of roselle colorant due to the interaction of SDS and the colorant. Furthermore, ΔH and ΔE for roselle in buffer solution implied more color change. ΔH and ΔE represent redness, greenness, blueness and yellowness, while % color remaining was determined at a particular wavelength representing a color. Thus, the redness of roselle decreased more in the presence of SDS micelles, but the overall color of the solution lasted longer.

Changes in a, b, C, ΔH and ΔE for lac did not differ significantly in the absence and presence of surfactants (ANOVA, $p > 0.05$) and these values were rather stable throughout the experiments. These results support the data from UV spectroscopy. The roselle colorant faded when dissolved in solvent in the following order: SDS solution > Tween80 solution ~ buffer solution. Higher SDS concentrations induced greater color fading, but this color change did not depend on the Tween80 concentration.

A weak interaction between the flavylium cation in roselle colorant and non-ionic micelles (Tween80) was expected; therefore, the stability of roselle colorant in the presence of Tween80 was comparable to that in the absence of micelles. This color change of roselle solution was mainly due to delphinidin and cyanidin degradation. The lac colorant is rather stable in the absence or presence of micellar systems as determined using both UV spectrophotometry and CIELab. The surfactants had no influence on lac stability. Our results show that surfactants affect color stabilities to a different extent depending on the properties of the surfactants and the colorants. SDS, a negative surfactant, is likely to have more influence on the color than Tween80, a neutral surfactant. As a pseudophase micellar system, SDS micelles may provide a microenvironment that facilitates roselle color fading.

This work is useful for cosmetic and food formulations which usually contain surfactants as solubilizers or stabilizers of other ingredients such as organic compounds. Micelles are formed when concentrations of the surfactants are above their critical micelle concentrations. In the formulations, organic ingredients solubilized or stabilized by the surfactants reside in non-polar environment of the micellar structure and/or interact with polar region of the micelles depending on polarities and chemical structures of the organic ingredients. Since the natural colorants widely added to increase esthetic value in these products are affected by the micelles as presented in this study, both concentration and type of the surfactants used in the products should be considered.

Conclusion

The % yields of roselle and lac from the extraction process were 31.96 ± 0.32 and 2.79 ± 0.23, respectively. Spectroscopy data and CIELab values were used for assessment of color changes. Lac colorant, which contains anthraquinolones, was more stable than roselle colorant, which contains anthocyanins, in the absence and presence of SDS and Tween80. Roselle solution changed from pink to yellowish within 12 days, and the order of stabilities of roselle colorant in the solvent systems was buffer ~ Tween80 > SDS. Lac colorant was quite stable in the absence and presence of surfactants.

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