Original article (postprint): Effect of amphiphilic ionic liquids on the colorimetric properties of polyketides colorants

Effect of amphiphilic ionic liquids on the colorimetric properties of polyketides colorants

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Abstract

Consumers are currently pressuring the food industries to replace synthetic colorants with their natural counterparts. However, natural colorants still represent less than a third of food coloring additives, as they are more difficult to produce and present application issues due to their lower stability. To find surfactants compatible with natural colorants, this study evaluated the effect of amphiphilic ionic liquids (ILs) aqueous solutions on the colorimetric properties of polyketides colorants (yellow-orange and red color) produced by *Talaromyces amestolkiae*. Only the IL *n*-alkyl-3-methylimidazolium bromide ([$C_n$mim]Br with $n = 10$ at 2.5 mM preserved the colorimetric and fluorescence characteristics of the fungal colorants, while the other surfactants (*e.g.*, [$C_n$mim]Br with $n = 12$, 14, and 16, [P_{44414}]Cl, and CTAB) altered them. Fluorescence was more sensitive to the effect of ILs than their color and absorbance. Furthermore, the red colorant was more affected by the ILs than the yellow-orange chromophores. Hence, this work shows that formulations with [C_{10}mim]Br (up to 2.5 mM) can preserve the properties of *T. amestolkiae* polyketides colorants, while the other ILs can be used as additives to modulate their color and fluorescence for biosensing applications.

Keywords: natural colorants, polyketides colorants, colorimetric properties, *Talaromyces amestolkiae*, surfactants, ionic liquids.

1. Introduction

Color is a primary attribute of food, medicines, and cosmetics, acting as an indicator of their safety and quality. [1] Hence, to meet consumers' expectations and attract new customers, industries often add synthetic and natural additives to accentuate or modify the color of their products. [1,2] With the spread of healthier lifestyles, consumers are also paying more attention to the composition and sources of products and materials. [3] For this reason, there was a significant expansion in the market for natural colorants in 2020 (evidenced by an estimated Compound Annual Growth of 8.47 % in the next years), [4] as the public and experts consider them healthier than the synthetic equivalents. [3,5,6] However, natural colorants still represent only 31 % of the total food colorants' market, mainly due to production costs and less stable physicochemical characteristics. [3,7] For instance, their colorimetric properties can be easily affected by small differences in culture medium/formulation and in the processing conditions, which can cause inconsistencies in the color of industrial bioproducts. [3,7] Therefore, it is essential to investigate the effect of solvents/additives used in the production, extraction, and formulation of natural colorants and their relative attributes, helping in the selection of inputs for their preservation, or at least, that allow a reversible change to the desired features after processing. Additionally, these studies can find solvents or additives to enhance or modulate the visuals of products and materials, improving and expanding their applications, such as (bio)sensors and (bio)tracers. With that in mind, this study aimed to evaluate the effect of Ionic Liquids (ILs) with an amphiphilic nature (*i.e.*, cationic surfactants) on the colorimetric properties of natural colorants from the fungus *Talaromyces amestolkiae*.

Natural colorants can be recovered from different sources, including microbes, plants, animals, and minerals. [8] Microbial sources offer advantages compared to other natural systems, namely, better control of the manufacturing process (*e.g.*, removal, or at least, decrease seasonal and geographic variations, decreasing dependence on plants and animal resources) and higher yields and faster processing rates. [8] Furthermore, some microbial colorants may have interesting pharmacological properties. For instance, *T. amestolkiae* colorants have antimicrobial activity against *Staphylococcus aureus* and low cytotoxicity against fibroblast cells. [9] Additionally, *T. amestolkiae* does not produce mycotoxins relevant to human health, appearing as a "suitable source" for the production of natural colorants for food and cosmetics. [7,10] The water-soluble colorants produced by *T. amestolkiae* are mainly red-colored polyketides, which are classified as amino-hexanedioic acid and azaphilone colorants (characteristic chemical structures shown in **Figure 1**). [11]

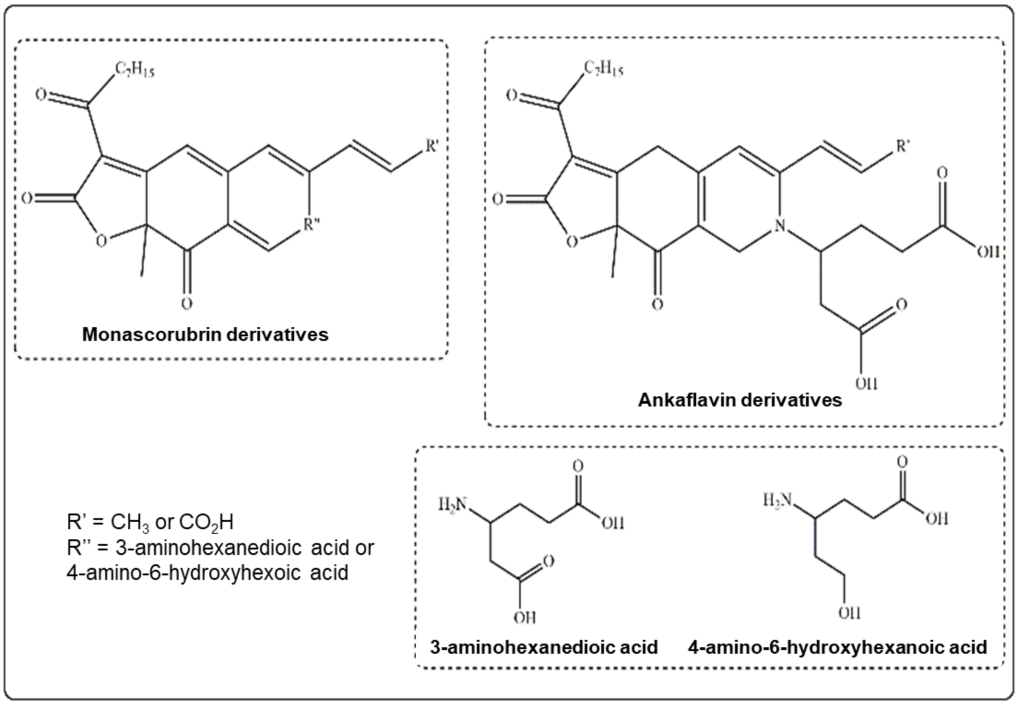

Figure 1. Chemical structures of the derivative compounds detected in *T. amestolkiae* cultivated broth. [11]

Polyketides azaphilone colorants are part of pyranoquinones, a large group of organic molecules characterized by their highly oxygenated bicyclic core, as can be seen in the structures of the monascorubrin and ankaflavin derivatives shown in **Figure 1**. The electron acceptor affinity in the primary ring is responsible for the high oxygenation and vibrant colors of these colorants, but it can also lead to interactions with compounds with amino groups, by exchanging oxygen for nitrogen in the pyran heterocycle. This exchange can change the molecule's characteristic color from yellow-orange to red. [12] Hence, although the high electron acceptor affinity in polyketides allows them to present intense and vibrant colors, they also make these substances more unstable and prone to chemical and visual alterations. [13]

Considering that consistency and stability are fundamental in industrial settings, the flick character of natural colorants can limit their commercial application. For example, Mapari *et al.* showed that light exposure and pH changes could alter the color pattern of red-orange and yellow fungal colorants, particularly the red components. [13] Furthermore, the changes in the color of natural colorants with the addition of surfactants, or other amphiphilic substances, are also common, which have a particular impact on the development of formulations such as cosmetics and foods. Piyarat *et al.* found that the red colorant from roselle calyx turned yellowish within 12 days of exposure to aqueous solutions of anionic surfactants, namely, Sodium dodecyl sulfate (SDS) and Polysorbate 80 (Tween 80). [14] Interestingly, these authors observed that, in contrast, the red extract of lac resin maintained its characteristic color properties. [14] Other examples show the stability of natural colorants in solutions of anionic surfactants, such as β-carotene in Tween 20-nanoemulsions [15] and natural anthocyanins in SDS micellar systems. On the contrary, there are still few works that evaluated the effects of ionic surfactants on natural colorants. Although some studies have used imidazolium-based ILs of amphiphilic nature in the extraction natural from colorants *T. amestolkiae*, [16,17] the impacts of these substances on the colorimetric properties of the colorants have not been properly evaluated.

Cationic surfactants include amphiphilic ILs with longer/larger ionic alkyl chain lengths that can form micelles (or similar) structures above their critical micellar concentration (CMC). In addition to these amphiphilic ILs, other conventional alkylimidazolium salts can be also included, for instance, cetyltrimethylammonium bromide (CTAB). Among the families of ILs that may exhibit amphiphilic behavior are alkylimidazolium ILs, which consist of imidazolium cations combined with either organic or inorganic anions. [18] One of the most representative classes is 1-alkyl-3-methylimidazolium bromide ([$C_n$mim]Br), ILs that are characterized by presenting an imidazolium head group linked to an alkyl chain and a bromide counterion. With alkyl chains with more than eight carbons, the [$C_n$mim]Br ILs can show amphiphilicity similar to traditional cationic surfactants. [19] The CMCs in water for [$C_n$mim]Br with n = 10, 12, 14 and 16 are 41.0, 9.8, 2.5, and 0.061 mM, respectively. [20] Another well-known amphiphilic IL is tributyl-tetradecylphosphonium chloride [P_{44414}]Cl, a bulky phosphonium IL that has a CMC of 3.3 mM in water. [21] The CMC values of amphiphilic ILs are in line with CTAB that has a CMC of 0.9 mM in water. [22] CTAB is a quaternary ammonium salt and cationic bactericidal detergent, widely used as topic antiseptic and buffering for DNA extraction. [23,24] The chemical structures of these cationic surfactants are shown in **Figure 2**.

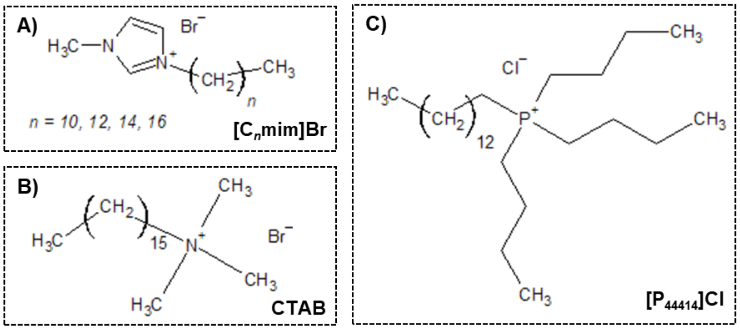

Figure 2. Chemical structures of: A) 1-alkyl-3-methylimidazolium bromide ILs ([C$_n$mim]Br, n = 10, 12, 14 and 16), B) hexadecyltrimethylammonium bromide (CTAB), and C) tributyl-tetradecylphosphonium chloride ([P$_{44414}$]Cl).

The self-assembly properties of cationic surfactants (ILs or salts) make them promising for the preparation of micellar formulations [19,21,25] for food, pharmaceutical, and cosmetic products with natural colorants. However, it is still necessary to evaluate the effect of these ionic compounds on the stability and color properties of natural colorants to allow their commercial application. With this aim, here we evaluated the effect of ILs of amphiphilic nature, namely [C$_n$mim]Br (n = 10, 12, 14, 16) and [P$_{44414}$]Cl, and compared with a common cationic surfactant (CTAB), on colorimetric properties of natural polyketide colorants from the cultivated broth of *T. amestolkiae*. To achieve this goal, we assessed the impact of each IL/CTAB aqueous solution (at 2.5 mM) on the color (chroma and hue angle), absorbance spectrum, and three-dimensional fluorescence spectrum of the natural colorants. With this information, we seek to gain insights into the effect of ILs of amphiphilic nature as a function of the increase of alkyl chain length on polyketide colors, aiming at future applications of cationic surfactants in the processing and formulation of foods, medicines, and cosmetics with natural colorants.

2. Experimental

2.1. Materials

The 1-bromohexadecane, 1-bromoteradecane, 1-bromododecane, 1-bromodecane, and 1-methylimidazole were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetone and ethyl ether (HPLC) were purchased from Vetec (Brazil) and used without prior purification. The 1-alkyl-3-methylimidazolium bromide ILs [1-decyl-3-methylimidazolium bromide ($[C_{10}mim]Br$), 1-dodecyl-3-methylimidazolium bromide ($[C_{12}mim]Br$), 1-tetradecyl-3-methylimidazolium bromide ($[C_{14}mim]Br$), and 1-hexadecyl-3-methylimidazolium bromide ($[C_{16}mim]Br$), purity < 99%, determined by NMR] were synthesized by the Center for Natural and Exact Sciences, the Federal University of Santa Maria – UFSM, RS, Brazil, following the methodology previously described in the literature. [26,27] The ILs were obtained in a single step from a nucleophilic substitution reaction between the methylimidazole and the respective alkyl bromide at a 1:1 ratio, heating acetonitrile (70 ◦C) for 24 h. Then, the ILs were washed successively with ethyl ether and filtered under vacuum in a Kitassato. Finally, the ILs were submitted to vacuum in a pump (10^{-3} mbar) for 24 h at 25 °C. All ILs were characterized by electrospray ionization mass spectrometry (ESI-MS), ^{1}H and ^{13}C nuclear magnetic resonance (NMR) in D_2O and data are following previous reports. [26,27] The tributyl-tetradecylphosphonium chloride ($[P_{44414}]Cl$) was kindly donated by Cytec (WoodlandPark, NJ). The ionic surfactant salt cetyltrimethylammonium bromide (CTAB) was purchased from Sigma-Aldrich® (St. Louis, MO, USA). All other reagents were from analytical grade and used as received. The chemical structures of ILs and CTAB are shown in **Figure 2**.

2.2. Microorganism and natural colorants production by T. amestolkiae

Talaromyces amestolkiae DPUA 1275 was kindly provided by the DPUA Culture Collection, from the Mycology Laboratory of the Federal University of Amazonas (Manaus, AM, Brazil). The cultures were preserved in sterile distilled water and reactivated in PDA-YE (gL^{-1}): Potato Dextrose Agar (30 g.L^{-1}) supplemented with yeast extract (5 g.L^{-1}) and maintained at 30 °C for 168 h.

For the production of natural colorants, the inoculum was prepared in Petri plates (90 mm x 10 mm) containing 10 mL of PDA-YE medium by transferring the reactivated microorganism to the center of the plate and keeping at 30 °C for 168 h. Erlenmeyer flasks (250 mL) containing 50 mL of the culture medium were prepared and then inoculated with 10 mycelium agar discs (8 mm in diameter). The composition of the culture medium was (g.L^{-1}): glucose (10), monosodium glutamate – MSG (25), $MgSO_4$ (0.012), Fe_2SO_4 (0.010) and $CaCl_2$ (0.015). [28] The pH of the culture medium was adjusted to a pH value of 5.0 and sterilized at 121 °C for 15 min. The Erlenmeyer flasks were then incubated in an orbital shaker (New Brunswick Innova 40R, Eppendorf, Inc., USA) at 30 °C for 168 h and 150 rpm. At the end of the cultivation, the cultivated broth was filtered first with an 80 gm^{-2} paper filter (Whatman, UK) and subsequently with a 0.45-µm filter (Millipore, Bedford, MA, USA). The clarified cultivated broth was stored at -80 ºC for further stability studies.

2.3. Studies of the effect of amphiphilic ionic liquids on the colorimetric properties and fluorescence of natural colorants

This study evaluated the effect of aqueous solutions of the cationic surfactants [$C_n$mim]Br (n = 10, 12, 14, and 16), [P_{44414}]Cl, and CTAB on the colorimetric properties and fluorescence of the colorants from the cultivated broth of *T. amestolkiae*. Each surfactant was mixed with the cultivated broth to reach a concentration surfactant of 2.5 mM, a total volume of 12 mL, and an absorbance at wavelength 500 nm (Abs_{500nm}) of 1.0 units of absorbance (UA), which corresponds to the maximum absorbance of the red colorant. [29] Final adjustments of concentration and volume were performed with McIlvaine buffer solution, pH 6.5. To evaluate the stability of the colorants, the solutions were incubated for 24 h in a thermoregulated bath at 25 ± 1 ºC, with sampling at 0, 1, 3, 6, and 24 h for spectroscopy analysis, and at 0 and 6 h of incubation for colorimetry and spectrofluorometry measurements.

2.4. Analytical methods

The concentration of extracellular colorants was estimated by spectrophotometric analysis by reading the absorbance of the supernatant at 420, 470, and 500 nm, [29–31] which corresponds to the maximum absorbance for yellow, orange, and red colorants, respectively. The measurement was performed using an EnSpire® Alpha Plate Reader spectrophotometer (PerkinElmer®) and considering the dilution factor of each sample. The results were expressed in UA.

The three-dimensional fluorescence spectra of the colorants were carried out on a spectrofluorometer (RF-6000 - Shimadzu®), scanning at excitation wavelength with intervals of 2 nm from 220 to 600 nm and emission of 1 nm from 370 to 650 nm. The scan speed was 6000 $nm.min^{-1}$, excitation bandwidth of 10 nm and emission of 1 nm, and high sensitivity. [32,33] Data presented in fluorescence intensity (FI) in unities of fluorescence (UF).

The quantitative description of the color was performed by colorimetric analysis. The L*, a*, and b* color coordinates were measured by a Chromameter with the CIELAB color system (Hunterlab ColorQuest XE). These values were then used to calculate chroma (C*) and hue angle (H°) values according to **Eq. 1** and **Eq. 2**, respectively. L* indicates lightness from 0 (black) to

100 (white). Positives and negatives in a* represent red and green, respectively, whereas positives and negatives in b* represent yellow and blue, respectively. Chroma values denote the saturation or purity of the color. Values close to the center at the same L* value indicate dull or gray colors, whereas values near the circumference represent vivid or bright colors. Hue angle values represent 0 for redness, 90 for yellowness, 180 for greenness, and 270 for blueness. [28]

$$C = \sqrt{\frac{a^2}{b^2}} \quad \textbf{(Eq. 1)} \qquad\qquad H^\circ = arctg\left(\frac{b^*}{a^*}\right) \quad \textbf{(Eq. 2)}$$

3. Results and discussion

3.1. Effect of amphiphilic ionic liquids on the color properties of colorants from T. amestolkiae

The main colorants produced by *T. amestolkiae* were previously characterized by us, and five polyketides azaphilone compounds derived from the monascorubrin (orange) and ankaflavin (yellow) colorants were identified (*cf.* **Figure 1**). [11] Hydrophilic colorants derived from ankaflavin yellow colorants are ionizable compounds with the insertion of 3-aminohexanedioic acid in place of the pyranoid oxygen of ankaflavin (R'') and differences in their methyl or carboxyl radicals (R'). As for monascorubrin orange colorant derivatives, pyranoid oxygen is replaced by 3-aminohexanedioic acid or 4-amino-6-hydroxyhexanoic acid residues, with additional differences in their methyl or carboxyl radicals (R'). These colorants can exist as cations, anions, or neutral molecules depending on the structure of amine residue and environmental conditions (*e.g.*, pH). Furthermore, these colorants have been reported to be stable from pH 3 to 11, precipitating at pH 1 due to the hydrolysis of key chemical groups, and losing their red color at pH 13 as a result of a negative halochromism [11] (*i.e.*, the cation absorbs at a shorter wavelength than the neutral molecule). [34] Considering the pH stability range of the colorants, in this study, the pH of all solutions was maintained at pH 6.5. To avoid any temperature degradation effect, all assays were also performed at 25 ºC.

The first assay evaluated the effect of different solutions of cationic surfactants (*i.e.*, [$C_n$mim]Br (n = 10, 12, 14, 16), [P_{44414}]Cl, and CTAB at 2.5 mM) on the color of the natural colorants (extract of 1.0 Abs_{500nm} in McIlvaine buffer at pH 6.5) produced by *T. amestolkiae*. A negative blank using Mcllvaine buffer pH 6.5 without colorant and surfactant, and a sample buffer using the colorant extract in McIlvaine buffer pH 6.5 without surfactant were also evaluated as standard controls. To infer the impact of each cationic surfactant on the color profile of natural colorants, samples were collected after 0 and 6 h of incubation for evaluation of their colorimetric parameters. The colorimetric parameters after 0 and 6 h of incubation are shown in **Figures 3.A** and **3.B**, while the visual aspect of all solutions is shown in **Figure 3.C.** Raw colorimetric data are detailed in **Table S1** from the **Supporting Information (SI)**.

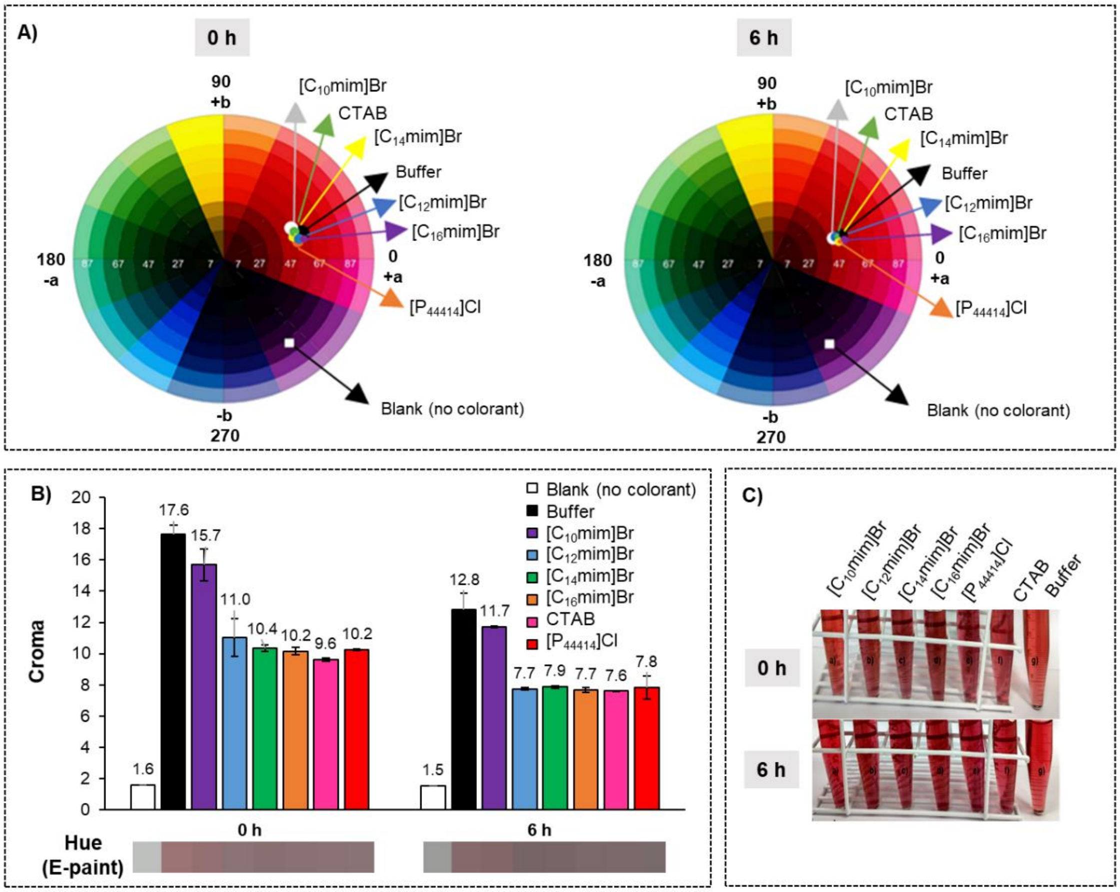

Figure 3. (A) Colorimetric parameters in the color wheel (average of a duplicate) of solutions composed of colorant and McIlvaine buffer (pH 6.5) [blank (no colorant) and buffer] and in the presence of aqueous solutions of cationic surfactants at 2.5 mM of [C_{10}mim]Br, [C_{12}mim]Br, [C_{14}mim]Br, [C_{16}mim]Br, [P_{44414}]Cl, and CTAB, after 0 h and 6 h of incubation. **(B)** Average and standard deviation (duplicate) of the colorimetric parameters of the solutions at 0 h and 6 h. **(C)** Visual aspect of the solutions (representative of the duplicate).

The analysis of color and tonality was investigated immediately after the surfactant addition (0 h) and after 6 h of incubation at 25 ºC. As visually detected in **Figure 3.C [a** and **g],** and in terms of hue angle (H°) and chroma (C*) parameters in **Figure 3.A** and **3.B,** only the [C_{10}mim]Br solution exhibited a colorimetric profile similar to the buffer control. In contrast, all other surfactant solutions immediately changed the color of the cultivated broth (**Figure 3.A** and **3.B**, and **Table S1** from the **SI**). A decrease in H° and C* values towards the violet colors was observed, that is, the addition of cationic surfactants caused a chroma shift with lower saturation than the control. Concerning luminosity (L*), all solutions had an initial brightness equivalent to 50.87 (on average), which was reduced to 45.83 after 6 h of incubation. From an analysis of the three chromatic coordinates L* a* b*, it is clear that increasing the exposure time up to 6 h of the solutions led to a reduction of these coordinates to $\Delta L^* = 5.04 \pm 0.91$, $\Delta a^* = 3.06 \pm 0.95$ and $\Delta b^* = 0.53 \pm 0, 36$. The addition of cationic surfactants to the cultivated broth reduced the a* value over time (**Table S1** from the **SI**). Higher values of a* represent proximity of red color, so a decrease of this coordinate represents a decrease in redness.

From a general analysis, it is clear that $[C_{10}mim]Br$ was the amphiphilic IL that caused the smallest color change in the *T. amestolkiae* extract. It is important to note that any surfactant in water at a concentration above the critical micelle concentration (CMC) form aggregated structures, commonly called micelles. [16] According to the literature, [20] the CMC of the amphiphilic ILs are classified as: $[C_{10}mim]Br$ – 41.0 mM < $[C_{12}mim]Br$ – 9.8 mM < $[C_{14}mim]Br$ – 2.5 mM < $[C_{16}mim]Br$ – 0.61 mM. Under the conditions of this study (*i.e.*, a surfactant concentration of 2.5 mM), $[C_{10}mim]Br$ is the surfactant with the shortest cationic alkyl chain length and at a concentration far from the CMC value, *i.e.*, the higher is the alkyl chain the closest is the solution to the CMC. This result indicates a relationship between the formation of micelles and the decoloring effect of the surfactant. Therefore, it appears that the surfactants with longer chains are forming micelles and the colorant is being encapsulated and, consequently, changing the chroma profile of the natural colorant. Further experimental studies such as Nuclear Magnetic Resonance (NMR), Dynamic Light Scattering (DLS), and Small-Angle X-ray Light Scattering

SAXS, are necessary to clarify the structures at the molecular level. If the main purpose of using natural colorant is aesthetic, *i.e.*, wishing to prioritize the coloring properties, [C_{10}mim]Br is the most suitable to be used as an additive in extraction and formulation processes. However, using more dilute solutions of other amphiphilic ILs, far below their CMC values, could be considered to lessen the negative effect on the colorimetric properties of natural colorants.

3.2. Effect of amphiphilic ionic liquids on the absorbance of colorants from T. amestolkiae

To obtain further information about the impact of cationic surfactants on colorants characteristics, in the second part of this work, the Ultraviolet-Visible (UV-Vis) spectra of all solutions at 0 and 24 h of incubation were also measured, with the respective spectra shown in **Figure 4.A** and **4.B**, respectively. Additionally, **Figure 4.C** presents the maximum absorbance peak (λ_{max}) of the yellow-orange peak (peak 1) and red peak (peak 2) after 0 and 24 h of exposure to the different surfactant solutions. To infer the stability during 24 h, the λ_{max} at 500 nm (Abs_{500nm}) was also determined and presented as the relative Abs_{500nm} (%) in **Figure 4.D**. The Abs_{500nm} of the colorant in the buffer control at 0 h was used as a reference for 100 %. The image with the raw Abs_{500nm} values of **Figure 4.D** is presented in **Figure S1** from the **SI**.

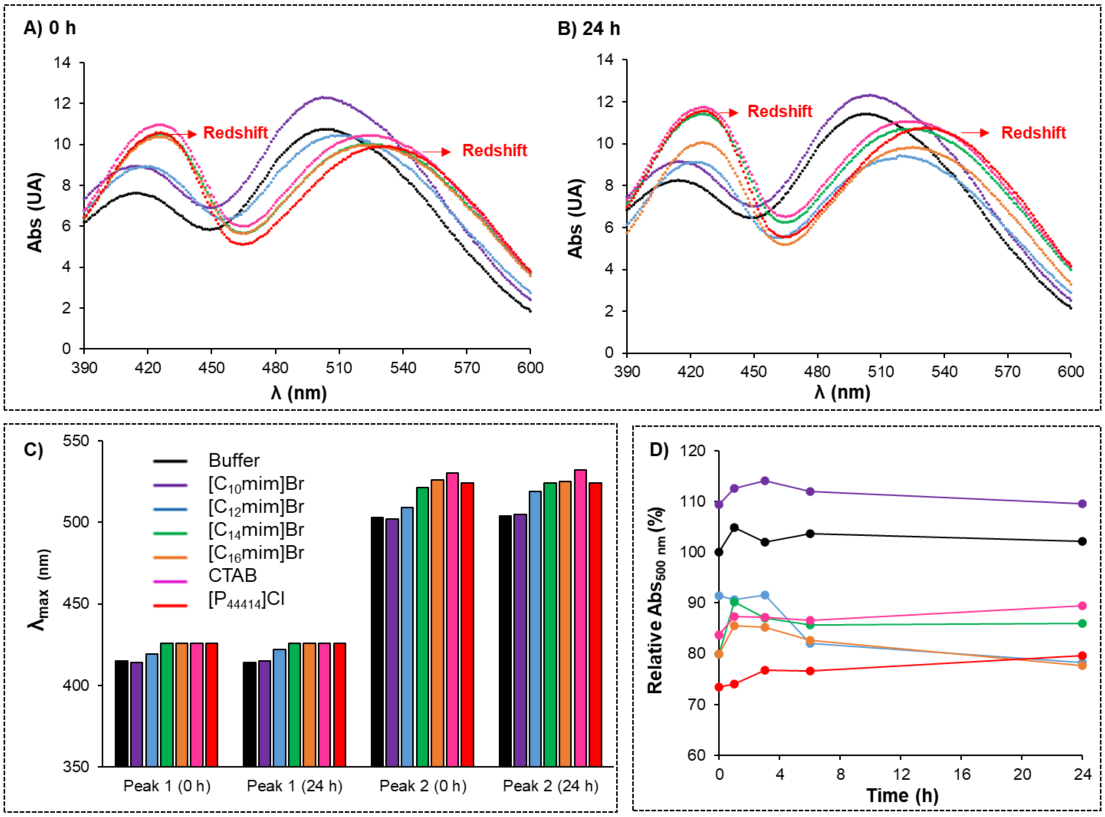

Figure 4. Absorbance spectra of solutions composed of colorant and McIlvaine buffer (pH 6.5) with or without the addition of cationic surfactant aqueous solutions (at 2.5 mM) of [C_{10}mim]Br, [C_{12}mim]Br, [C_{14}mim]Br, [C_{16}mim]Br, [P_{44414}]Cl, and CTAB at **(A)** 0 h and **(B)** 6 h. **(C)** Maximum absorbance peak [λ_{max} (nm)] of yellow-orange chromophores (peak 1) and red chromophores (peak 2) at 0 and 24 h of exposure to the different solutions. **(D)** Relative absorbance at 500 nm [Relative Abs_{500nm} (%)] of the solutions over time (24 h). Abs_{500nm} of the colorant in buffer at 0 h was the reference for the 100 % for the Abs_{500nm} (%) calculations.

As depicted in **Figure 4.A** and **4.B**, the clarified cultivated broth has two λ_{max} peaks, namely: 1) a first peak in the wavelength range of 390 and 460 nm – associated with yellow-orange colorants with λ_{max} at 420 nm; and 2) the second peak in the range from 460 to 600 nm – associated with red colorants, which has a λ_{max} value around 500 nm. The analysis of UV-Vis spectra (**Figure**

4.A and 4.B) confirms the previous results of the colorimetric assay (**Figure 3**) as when adding most cationic surfactants immediate shifts of λ_{max} to longer wavelengths for both the first peaks (from 415 to 425 nm) and the second peak (from 500 to 530 nm) were observed, characterizing a bathochromic shift (redshift). Overall, the bathochromic shift was more pronounced with surfactants with longer alkyl chains, as confirmed by the shift of the maximum absorbance peak (λ_{max}) of the yellow-orange (peak 1) and red peak (peak 2) shown in **Figure 4.C**. For [C_{12}mim]Br, the shift was time-dependent, which initially showed only a minor bathochromic shift for the two peaks with a further intensification after 24 h of incubation. However, for the other amphiphilic ILs, the impact of surfactants on the relative absorbance of red colorant absorbances was instantaneous, *i.e.*, the bathochromic shift or hyperchromic and hypochromic effects occurred immediately after the addition of the surfactant, and the absorbance profile remained stable throughout the entire 24 h. The time-dependent behavior can be confirmed in **Figure 4.D**, where, except [C_{12}mim]Br, the effect profile of cationic surfactants on the red colorant absorbance ate 500 nm remained similar from 4 h to 24 h of the study.

Although amphiphilic ILs caused bathochromic shifts for both peaks, their effects on the absorbance values were opposite, namely, causing an increase in peak 1 absorbance and a decrease in peak 2 (**Figure 4.A** and **4.B**). All ILs caused a hyperchromic effect for peak 1, that is, the addition of IL increased the absorbance at 500 nm of the colorant. On the other hand, most ILs caused a decrease in the absorbance value for peak 2 (hypochromic effect). The combined severe increase of absorbance for peak 1 and decrease for peak 2 can suggest there may be a shift in color from red to yellow-orange for the red chromophores with the addition of most cationic surfactants. Interestingly, [C_{10}mim]Br was an exception, causing a hyperchromicity for both peaks. The moderate hyperchromicity in both peaks for [C_{10}mim]Br is likely associated with changes in the microenvironment around the chromophore.

As this study shows, the use of cationic surfactants of a more hydrophobic nature (*i.e.*, with bulkier and longer alkyl chains) as additives in aqueous solutions results in more colorimetric alterations in colorants, particularly in red colorants. The second peak (red chromophore) had a more pronounced bathochromic shift than the first peak (yellow-orange chromophore), showing that the red colorant is more sensitive to the addition of cationic surfactants to the medium (**Figure 4.C**). These results are in line with those from Mapari *et al.*, [13] which also revealed that red colorants from fungi from the genera *Penicillium* and *Epicoccum* are less stable than yellow chromophores to light and pH changes. Youn-Sook and Dong-II also reported that red colorants are less stable than yellow colorants from safflower, both in powder or in aqueous solutions under light exposure or long-term storage. [35]

The increase of λ_{max} with more hydrophobic surfactants confirms that they are likely associated with changes in the chromophore microenvironment. According to a previous report by Patra *et al.*, cationic surface-active ILs can affect red hydrophilic colorants by electrostatic and hydrophobic interactions, depending on the properties of the ILs and colorants. [36] These researchers used 1-decyl-3-methyl imidazolium chloride, [C_{10}mim]Cl, (an amphiphilic IL with a CMC of 51.7 mM) at different concentrations (2.39 to 112.77 mM) to evaluate the interaction of the IL with two ionic synthetic red colorants, namely, safranine T (cationic) and congo red (anionic). Similar to [C_{10}mim]Br at 2.5 mM (in our study), the addition of [C_{10}mim]Cl had no impact on the absorbance of the red colorants at very low concentrations (*e.g.*, 2.39 mM). For the cationic colorant safranine T, even with the addition of higher concentrations of the IL, there were only small changes in the intensity and maximum absorbance of the colorant. However, for Congo red anionic colorant, increasing the concentration of [C_{10}mim]Cl considerably altered the absorbance of characteristic peaks of the synthetic colorant, *i.e.*, decreasing the absorbance of the red peak around 500 nm and increasing the yellow-orange peak around 340 nm. [36] Patra *et al.* [36] stated that electrostatic repulsive interactions are occurring between the amphiphilic IL and the colorant with the same charge (both cationic), but these are weaker than the interactions between cationic surfactants and anionic colorants with different charges. The authors also confirmed that the IL effect is concentration dependent, particularly significant at the concentration close to CMC of the [C_{10}mim]Cl. [36] Therefore, in addition to the self-assembling (amphiphilic) and hydrophobicity effects of the cationic surfactant, the IL concentration and nature of the dye will play a key role in the formation of specific IL-colorant interactions, which can also impact the colorimetric properties of the colorant.

3.3. Effect amphiphilic ionic liquids on the fluorescence of colorants from T. amestolkiae

As previously reported by our research group, the *T. amestolkiae* extract shows three fluorescence regions in the 3D fluorescence spectra. [11] They include a red region between excitation wavelengths (λ_{ex}) 470-480 nm and emission wavelengths (λ_{em}) 515-525 nm, and two yellow-orange regions around λ_{ex} 470-480 nm, λ_{em} 515-525 nm and λ_{ex} 440-460 nm, λ_{em} 420-440 nm. The 3D spectra of the extract in McIlvaine buffer pH 6.5 are depicted in **Figure S2.A** from the **SI**. As fluorescence is particularly sensitive to changes in the fluorophore microenvironment, [32,33] the effect of different cationic surfactants in the fluorescence intensity (FI) and maximum fluorescence wavelengths of the three peaks using a 3D fluorescence spectra at 0 and 6 h was evaluated (*cf.* **Figure S2** and **S3** from the **SI**). From the 3D spectra, the emission spectra for the maximum λ_{ex} (260, 326, and 478 nm) of each peak and solution were subtracted (at 0 and 6 h) and the respective results are presented in **Figure 5**. The excitation spectra for the maximum λ_{em} (435, 440, 520 nm) of the systems at 0 and 6 h are also reported in **Figure S4** from the **SI**.

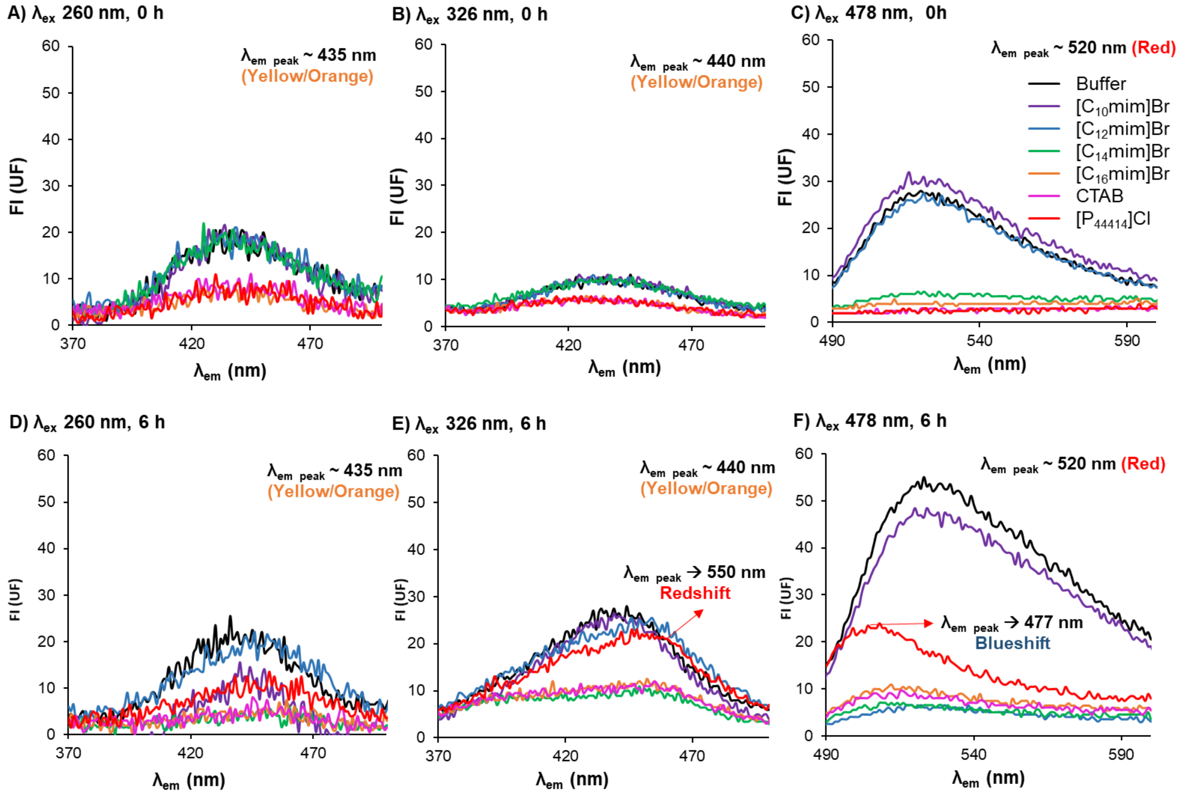

Figure 5. Emission spectra at λ_{ex} **(A)** and **(D)** 260 nm, **(B)** and **(E)** 326 nm, and **(C)** and **(F)** 478 nm of solutions composed of colorant and McIlvaine buffer (pH 6.5) with or without of the addition cationic surfactant solutions (2.5 mM), [C_{10}mim]Br, [C_{12}mim]Br, [C_{14}mim]Br, [C_{16}mim]Br, [P_{44414}]Cl, and CTAB at **(A), (B),** and **(D)** at 0 h and **(C), (D),** and **(E)** at 6 h. Data are presented as the average of a duplicate as fluorescence intensity (FI) in unities of fluorescence (UF).

Similar to the previous experiments, more surfactant ILs had a greater impact on the fluorescence of both yellow-orange and red colorants of *T. amestolkiae* extract at both 0 h and 6 h. For the yellow-orange colorants presented in **Figures 5.A**, **5.B**, **5.D**, and **5.E**, there was only a minor redshift for the ILs with lower CMC (namely, [C_{14}mim]Br, [C_{16}mim]Br, [P_{44414}]Cl, and CTAB) for peak 2 at 6h, from λ_{em} 440 to 550 nm. However, these more surfactant ILs quenched peaks 1 and 2 FI, particularly after 6 h of exposition. For the red colorants, these same ILs reduced the FI of peak 3 for both 0 and 6 h; however, [C_{12}mim]Br also quenched the fluorescence of the red colorant after 12 h. Contrarily to the redshift on peak 2, the more surfactant ILs caused a blueshift (from λ_{em} 520 to 4770) on the fluorescence of the red colorant. This is evident for [P_{44414}]Cl but subtle for [C_{14}mim]Br, [C_{16}mim]Br, and CTAB, considering they almost completely extinguished the fluorescence of the red colorant. Interestingly, at 0 h, [P_{44414}]Cl almost extinguished the fluorescence of all three peaks, but after 6 h, the FI was partially recovered for peaks 2 and 3 but with a redshift for the first and a blueshift for the latter. This phenomenon could indicate time-dependent structural alterations of the colorants. Finally, once again [C_{10}mim]Br overall had no impact on the properties of the colorant at 0 h and 6 h, and [C_{12}mim]Br only quenched the fluorescence of the red colorant (peak 3) after 6 h of exposure. Regardless of the red and blueshifts for the emission of the dyes in the different IL solutions, there were no visible shifts for the maximum excitation of the colorants in the surfactants in their excitation spectra, only alterations in FI (**Figure S4** from the **SI**).

Finally, to better demonstrate the impact of the ILs on the FI of the colorants, **Figure 6** presents the Relative FI (%) of each of the three peaks at 0 h and 6 h. The reference for 100% for each peak is the FI at 0 h in the buffer control. The absolute values for the FI are presented in **Figure S5** from the **SI**.

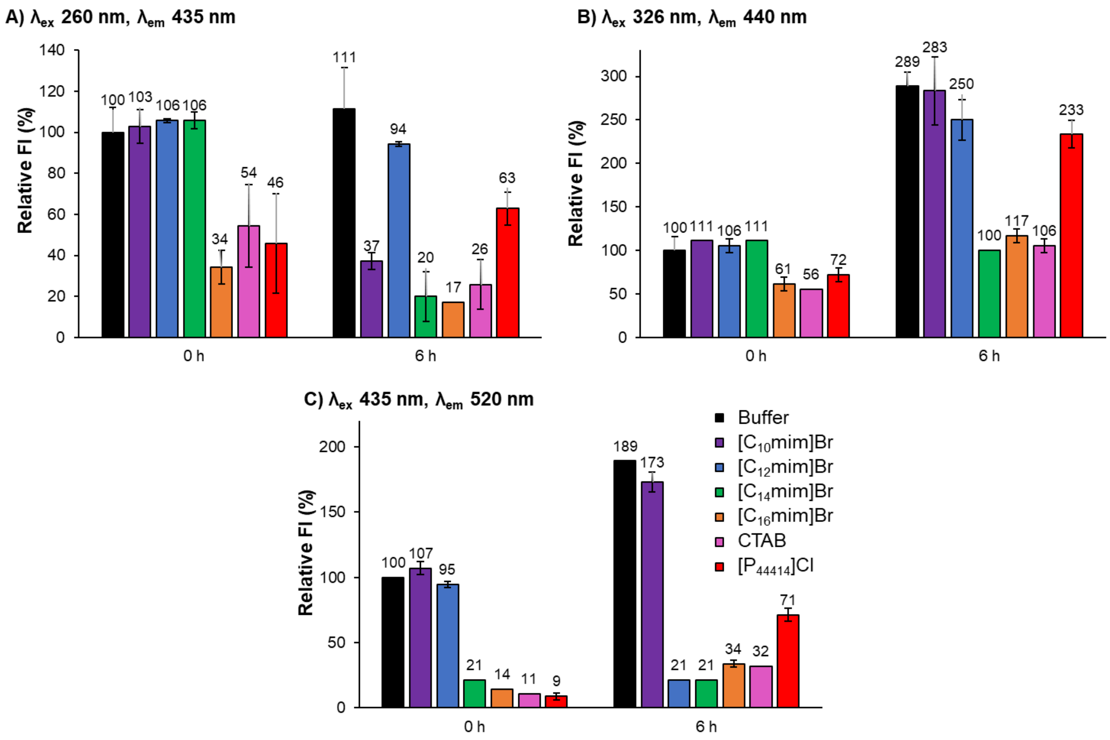

Figure 6. Relative fluorescence intensity [Relative FI (%)] at the maximum FI for the three peaks of the colorants solutions in McIlvaine buffer (pH 6.5) with or without the addition of cationic surfactant solutions (at 2.5 mM) ([C_{10}mim]Br, [C_{12}mim]Br, [C_{14}mim]Br, [C_{16}mim]Br, [P_{44414}]Cl, and CTAB). The three peaks correspond to **(A)** λ_{ex} 260 nm, λ_{em} 435 nm (yellow-orange), **(B)** λ_{ex} 326 nm, λ_{em} 440 nm (yellow-orange), and **(C)** λ_{ex} 435 nm, λ_{em} 520 nm (red) at 0 and 6 h. Results are presented as the average of a duplicate with respective standard deviations. [Relative FI (%)] was calculated considering the FI of the colorants in buffer solution at 0 h as the reference for 100%.

As can be seen in **Figure 6**, independently of the incubation time (0 or 6 h), the cationic surfactants with longer alkyl chain lengths caused a greater decrease in the relative FI of the three peaks. As discussed before, [P_{44414}]Cl initially quenched the FI of the three peaks but partially restored it after 6 h of the incubation. It is worth mentioning that the surfactants had a more intense effect on the fluorescence of the colorants than on their absorbance. In **Figure 4.D**, the colorants retained more than 70 % of their absorbance at 500 nm for all surfactant solutions, while up to 91 % of their fluorescence was quenched under the same conditions (*cf.* **Figure 6**). Fluorescence is overall more sensitive to structural and microenvironmental changes than absorbance. [32] Although the presence of cationic surfactants can be an obstacle for applications that require the maintenance of the fluorescence properties of the colorants, there are certainly promising opportunities in the use of these substances as fluorescence modulators in (bio)sensor applications. [33]

3.4. Association between CMC and the effects of amphiphilic ionic liquids on colorants from T. amestolkiae

To obtain an overview of the different results of this work, **Table 1** compiles the effects of the cationic surfactant solutions on the colorimetric and fluorescent properties of the natural colorants of *T. amestolkiae* extract. The surfactants solutions are ordered according to their CMC (from lowest to highest values) and the numbers from 1 to 6 indicate the position of the surfactant regarding the intensity of its effect on the properties of the colorants.

Table 1. Compilation of the effects of the different ILs on the colorimetric and fluorescent properties of the natural colorants produced by *T. amestolkiae*. The ILs are presented in order of their critical micelle concentration (CMC) from lowest to highest, and the numbers from 1 to 6 show the position of the IL regarding the intensity of its effect on the colorants.

Lowest to highest CMC	**[C$_{16}$mim]Br**	**CTAB**	**[C$_{14}$mim]Br**	**[P$_{44414}$]Cl**	**[C$_{12}$mim]Br**	**[C$_{10}$mim]Br**
CMC (mM)	0.061 [20]	0.9 [22]	2.5 [20]	3.3 [21]	9.8 [20]	41.0 [20]
↓Croma	2	1	4	3	5	6
↑ Peak 1 λ_{max}	1 to 4				5	6
↑ Peak 2 λ_{max}	2	1	3	4	5	6
↓Abs$_{500\ nm}$ (3 h)	2	3	4	1	5	6
↓FI at 6 h (λ_{ex} 260 nm, λ_{em} 435 nm)	1	3	2	5	6	4
↓FI at 6 h (λ_{ex} 326 nm, λ_{em} 440 nm)	2	1	3	4	5	6
↓FI at 6 h (λ_{ex} 435 nm, λ_{em} 520 nm)	3	4	1 to 2	5	1 to 2	6
Average position*	2	2	3	4	5	6

*Average of the positions for each IL, rounded for zero decimal places.

Table 1 reveals the main trends previously discussed in each of the individual studies, *i.e.*, larger and bulkier cationic surfactants with higher surfactant nature (lower CMC) have a more intense effect on the color and fluorescence properties of natural colorants from *T. amestolkiae* extract. As can be seen in **Table 1**, [C$_{16}$mim]Br and CTAB (two surfactants with lowest CMC values) are linked as the cationic surfactants with the highest impact on colorants properties, being the relative impact following the CMC ranking (from lowest to highest) of each amphiphilic IL. Therefore, there is an evident association between the self-assembly aptitude of amphiphilic ILs and the intensity of their effects on the colorimetry, absorbance, and fluorescence of the natural fungal colorants.

4. Conclusions

In this work, we studied the effects of amphiphilic ILs on the color, absorbance, and fluorescence of natural polyketides colorants presented in the cultivated broth of *T. amestolkiae*.

The red colorants were more sensitive than their yellow-orange counterparts to the effects of amphiphilic ionic liquids. Furthermore, there was a decrease in the chroma, hue angle, luminosity, and redness of the colorants with the addition of the ILs. The ILs with bulkier and longer cationic alkyl chains, those with strong surfactant behavior (*i.e.*, lower CMC), have the highest impact on the properties of the natural colorants. These ILs also caused a redshift in the absorbance of the yellow-orange and red peaks but resulted in hyperchromicity for the yellow-orange peak and hypochromic effect for the red chromophore. This phenomenon may suggest a shift in color from red to yellow-orange for red chromophores. In the case of [C_{10}mim]Br, this phenomenon was responsible for hyperchromicity in both peaks. In the fluorescence studies, only [P_{44414}]Cl caused an evident redshift in the emission for peak 2 (yellow-orange) and a blueshift for peak 3 (red). The fluorescence of the colorants was more sensitive to the addition of the cationic surfactants than their color and absorbance. Overall, [C_{10}mim]Br at 2.5 mM maintained the properties of natural colorants, appearing to be suitable for applications that aim to preserve the visuals of colorants. However, unlike the other ILs, [C_{10}mim]Br was in a concentration considerably below its CMC. Thus, it is still necessary to evaluate the other ILs at lower concentrations to infer if they can be used as additives below their CMC without changing the colorant's characteristics. This work has provided new insights into the effects of ILs on natural fungal colorants and may help select surfactants to preserve the properties of colorants on food, cosmetic, and pharmaceutical formulations.

Credit authorship contribution statement

Nathalia V. Veríssismo: Investigation, Formal Analysis, Writing - original draft, Writing - review & editing. Cecília Naomi Nakamura: Data curation, Formal analysis, Investigation, Methodology. Fernanda Oliveira: Investigation, Formal Analysis, Writing - original draft, Writing - review &

editing. Bruna L. Kuhn: Investigation. Clarissa P. Frizzo: Conceptualization, Writing - review & editing. Jorge F. B. Pereira: Investigation, Writing - original draft, Writing - review & editing. Valéria C. Santos-Ebinuma: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supporting information (SI)

Additional data and experiments for the colorant in buffer or surfactant solutions: colorimetric properties (Table S1), absorbance at 500 nm over 24 h (Figure S1), 3D fluorescence spectra (Figures S2 and S3), excitation spectra (Figure S4), the fluorescence intensity of the three chromophores (Figure S5).

Abbreviations

[$C_n$mim]Br, 1-alkyl-3-methylimidazolium bromide; [C_{10}mim]Br, 1-decyl-3-methylimidazolium bromide; [C_{12}mim]Br, 1-dodecyl-3-methylimidazolium bromide; [C_{14}mim]Br, 1-tetradecyl-3-methylimidazolium bromide; [C_{16}mim]Br, 1-hexadecyl-3-methylimidazolium bromide; [P_{44414}]Cl, tetradecylphosphonium chloride; Abs_{500nm}, absorbance at wavelength 500 nm; C*, chroma; CMC, critical micellar concentration; CTAB, cetyltrimethylammonium bromide; DLS, dynamic light scattering; FI, fluorescence intensity; H°, hue angle; ILs, ionic liquids; NMR, nuclear magnetic resonance; SAXS, Small-Angle X-ray Light Scattering; SDS, sodium dodecyl sulfate; SI, supporting information; *T. amestolkiae*, *Talaromyces amestolkiae*; UA, units of absorbance; UF, unities of fluorescence (UF); UV-Vis, ultraviolet-visible; λ, wavelength; λ_{em}, emission wavelengths; λ_{ex}, excitation wavelength; λ_{max}, maximum absorbance peak.

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