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Isolation of ionizable red Monascus pigments after extractive fermentation in nonionic surfactant micelle aqueous solution

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ABSTRACT

Production of red *Monascus* pigments can be enhanced by extractive fermentation in a nonionic surfactant micelle aqueous solution. Using excess MSG (monosodium glutamate) as the sole nitrogen source, several kinds of red *Monascus* pigments, including red *Monascus* pigments with MSG residue as major components, were produced during the fermentation process. However, the corresponding downstream processing, i.e., isolation of red *Monascus* pigments from the nonionic surfactant aqueous solution, remains unsolved. In the present work, a novel ionic liquid-nonionic surfactant aqueous two-phase system, such as coacervate phase: ionic liquid ([Bmim]Cl) = 1:3 (W/W) and temperature = 60 °C, was developed for transferring of the anionic red *Monascus* pigments with MSG residue from nonionic surfactant micelle aqueous solution into ionic liquid aqueous solution. Thus, the challenging isolation of red *Monascus* pigments from nonionic surfactant aqueous solution was converted to the relatively easy separation between red *Monascus* pigments and water-miscible ionic liquid. By screening various organic solvents, water-chloroform two-phase system was selected for recovery of red *Monascus* pigments from the water-miscible ionic liquid. Finally, the isolated red *Monascus* pigment with MSG residue was confirmed by UPLC–MS analysis.

1. Introduction

Solubilization is defined as "preparation of a thermodynamically stable isotropic solution of a substance normally insoluble or barely soluble in a given solvent by an introduction of additional amphiphilic compounds or components" [1]. In most cases, nonionic surfactant micelle aqueous solution is utilized for solubilization of molecular solutes (neutral compounds). The mechanism about solubilization of organic compounds in nonionic surfactant micelle aqueous solution has been studied extensively, such as a hydrocarbon solubilized in the micelle hydrophobic core, a more polar material oriented in the palisades region, a polar additive solubilized in the region of the hydrated layer, and an additive directly associated with the electrical double layer [2]. Furthermore, nonionic surfactant micelle aqueous solution separates spontaneously into a surfactant-rich (coacervate phase) phase and a dilute phase above a certain temperature. This two-phase system, which is known as cloud point system, has been applied for extraction of metal ions, organic compounds, and bio-macromolecules (cloud point extraction). One of the interesting applications about solubilization of solutes in a nonionic surfactant micelle aqueous solution or cloud point system in the biotechnology field is extractive fermentation [3–5] as well as bioremediation [6,7].

There are many reports about cloud point extraction of weak organic acids in their molecular state under acid condition while back-extraction of them in their anionic state under alkaline condition [8–10]. On the contrary, it is reported recently that some solutes in their ionized state can also be solubilized in the nonionic surfactant micelle aqueous solution [11,12]. This peculiar character may be attributed to the formation of mixed-micelles between the nonionic surfactant and the ionized solutes with relatively weak polarity, which is similar to the formation of mixed micelles between the nonionic surfactant and the ionic surfactant. Different from the extraction of the neutral state of solutes by the conventional organic solvent-water two-phase system, extractive fermentation of the cationic prodigiosin [13], as well as the anionic red *Monascus* pigments [14,15], has been carried out successfully in nonionic surfactant micelle aqueous solution. *Monascus* pigments are traditional food additives of East Asia. The major pigment components are usually divided into yellow *Monascus* pigments, orange ones, and red ones. It is believed that red *Monascus* pigments are originated from the chemical modification of the

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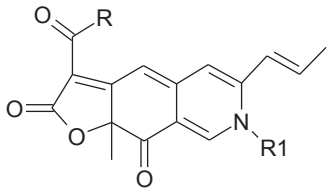

Fig. 1. Molecular formula of red *Monascus* pigments with different amine residues. R_1 = hydrogen, amino acid residues, and glucosamine residues, respectively

corresponding orange *Monascus* pigments. As shown in Fig. 1, R1 = hydrogen corresponds to red *Monascus* pigment rubropunctamine ($R = C_5H_{11}$) or monascorubramine ($R = C_7H_{15}$) [16] while R1 = amino acid residues [17] or glucosamine residues [18] correspond to red *Monascus* pigment derivates. Red *Monascus* pigments exist as cation, neutral molecule, and anion depending on their molecular structure of amine residue as well as their environmental condition (such as pH). The solubilization of ionized red *Monascus* pigments in a nonionic surfactant micelle aqueous solution makes extractive fermentation of red *Monascus* pigments become possible [14]. During extractive fermentation in a nonionic surfactant micelle aqueous solution using excess MSG (monosodium glutamate) as the sole nitrogen source, the production of red *Monascus* pigments is directly related to the MSG concentration [15].

In most cases, cloud point extraction of the neutral state of ionizable solutes can be back-extracted by adjustment of pH [8–10]. However, the solubilization of ionized red *Monascus* pigments in the nonionic surfactant micelle aqueous solution makes the back-extraction of red *Monascus* pigments from the nonionic surfactant aqueous solution by adjustment of pH become impossible. Winsor II microemulsion (also known as reversed micelle system), where a water-in-oil microemulsion phase coexists with an excess oil phase, is always utilized for isolation of ionic compounds [19–21] as well as proteins [22] from the ionic surfactant micelle aqueous solution. However, the high solubilization of ionized compounds in the nonionic surfactant micelle aqueous solution also leads to the low efficiency by stripping ionic compound into the excess water phase via Winsor II microemulsion extraction. Thus, isolation of the ionized solutes with a relatively weak polarity from a nonionic surfactant micelle aqueous solution is challenging [23]. Ionic liquid has been applied as a novel separation medium, which has been studied extensively [24]. The water-miscible ionic liquids have been applied as cationic hydrotrope for solubilization of anionic compounds [25] as well as co-surfactant of cloud point system for extraction of ionized solutes [26]. The binary mixtures involved in a water-miscible ionic liquid-nonionic surfactant-water ternary system can be completely miscible at room temperature. Very interestingly, there is an immiscible window (similar to an island) in this ternary system. The island region separates into a novel ionic liquid-nonionic surfactant aqueous two-phase system, in which one is a nonionic surfactant-rich aqueous phase with very high nonionic surfactant content and the other one is an ionic liquid-rich aqueous phase with very high ionic liquid content [27–29]. Comparison to the conventional cloud point system, the ionic liquid-nonionic surfactant aqueous two-phase system has introduced ionic liquid into the dilute phase while the surfactant-rich phase remains unchangeable. The introduction of ionic liquid, i.e., a cationic hydrotrope, in the dilute phase is beneficial for extraction of anionic organic compounds via electrostatic interaction. Examination of dye partitioning in the novel ionic liquid-nonionic surfactant aqueous two-phase system also confirms that the anionic dyes, despite the partitioning coefficient is strongly related to the dye character, are preferred to partition into the ionic liquid-rich aqueous phase [30]. Thus, the novel ionic liquid-nonionic surfactant aqueous two-phase system may provide a way for stripping of the anionic compound from nonionic surfactant micelle aqueous solution.

Setting the production of multicomponent of red *Monascus* pigments by extractive fermentation in the nonionic surfactant (Triton X-100) micelle aqueous solution as a model [15], the downstream processing for isolation of the anionic red *Monascus* pigments with MSG residues from the nonionic surfactant micelle aqueous solution was carried out in the present work. The basic procedure includes cloud point extraction of *Monascus* pigments into the coacervate phase by keeping the fermentation broth above a certain temperature, then transferring the anionic red *Monascus* pigments from the coacervate phase into ionic liquid by ionic liquid-nonionic surfactant aqueous two-phase system, and finally recovery of pigments from ionic liquid by chloroform-ionic liquid aqueous solution two-phase system.

2. Materials and methods

2.1. Materials

Ionic liquids, 1-butyl-3-methylimidazolium chloride ([Bmim]Cl), was purchased from Shanghai Chemical Technology Co Ltd. On the basis of information provided by the supplier, the purity of [Bmim]Cl (solid) was more than 98%. *Tert*-octylphenoxy poly(oxyethylene) ethanol nonionic surfactants, Triton X-100, was purchased from Sigma. Those agents were used without further purification. *Monascus anka* CICC 5013 was collected from China Center of Industrial Culture Collection.

2.2. Phase diagram of ternary system

The tie-line of the nonionic surfactant (Triton X-100)-ionic liquid ([Bmim]Cl) aqueous two-phase system was determined following the same procedure as our previous work [30]. Briefly, ternary systems consisting of various fractions of the ionic liquid, nonionic surfactant, and water were prepared. Each ternary system (5 ml) was put into a 10-ml glass tube with scale and incubated in a thermostat water bath (30 °C) for at least 10 h. In some cases, phase separation occurred in the ternary systems, which separated into a surfactant-rich aqueous phase (upper) and an ionic liquid-rich aqueous phase (bottom). In those cases, the volume of each phase was recorded. Sample with determined volume and weight was fetched from each phase. The sample was diluted with gradient dilution. Then, the concentration of nonionic surfactant/ionic liquid in the diluted sample was determined by HPLC (high-performance liquid chromatography) analysis (SHIMADZU installed with InertSustain C18, Japan). Ionic liquid [Bmim]Cl was detected with wavelength 230 nm at 3 min by running with acetonitrile/water (2:8) at 1 ml/min while nonionic surfactant Triton X-114 was detected with wavelength 270 nm at 4.2 min by running with acetonitrile at 1 ml/min. The water content in each phase was achieved according to mass conservation. Based on the weight fraction of every component in the surfactant-rich aqueous phase and ionic liquid-rich aqueous phase, tie-line was determined. A series of tie-line was utilized to estimate the two-phase region of the ternary system.

2.3. Extractive fermentation of red Monascus pigments

Extractive fermentation of red *Monascus* pigments in the Triton X-100 micelle aqueous solution was conducted with a similar procedure as our previous work [15]. *Monascus anka* was maintained on potato dextrose agar (PDA) medium (potato 200 g, glucose 20 g and agar 15–20 g, per liter of tap water) at 4 °C. Inoculum culture was conducted in a 250 ml Erlenmeyer flask with 50 ml of working volume at 30 °C and 200 rpm for 30 h. The medium composition of inoculum culture was glucose 20 g, $(NH_4)_2SO_4$ 4 g, peptone 10 g, KH_2PO_4 4 g, KCl 0.5 g, and $FeSO_4{\cdot}7H_2O$ 0.01 g (per liter of tap water). One milliliter of inoculum culture was added into 250 ml Erlenmeyer flask containing 50 ml of work volume. The extractive fermentation medium consisted of glucose 50 g, MSG 30 g, KH_2PO_4 2.4 g, $CaCl_2$ 0.1 g, $FeSO_4{\cdot}7H_2O$ 0.01 g, and Triton X-100 70 g, per liter of tap water. The initial pH was