Stability and properties of the blue colorant indigoidine in different solvents, temperatures and pH

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ABSTRACT

The indigoidine colorant is a sustainable alternative to replace synthetic counterparts in food, cosmetics, and textile industries, offering also antimicrobial and antioxidant properties. Its bluish hue is like indigo, but it stands out for deriving from microbial biomass since it is produced by bacterial strains from the amino acid L-glutamine through bioprocess. For this study the solubility and stability of bacterial indigoidine at different conditions was evaluated. The conducted experiments assessed solubility in distilled water, ethyl acetate, acetone, dimethyl sulfoxide (DMSO), ethanol, ethyl lactate, and methanol, stability at room temperature and in the refrigerator without specific labeling on the solvents. The indigoidine dissolved completely in distilled water, DMSO, ethanol, ethyl lactate, and methanol, and remained stable at temperatures evaluated and pH values, with a greater decrease in its concentration only in the presence of DMSO at room temperature. In conclusion, the indigoidine evaluated showed a good stability in the conditions studied and could be an alternative to other blue synthetic additive in industrial products.

Keywords: Biotechnology 1. Sustainable 2. Stability 3. Solubility 4. Natural Colorant 5.

1 INTRODUCTION

The history of colorants has evolved with milestones such as the accidental discovery of mauveine in 1856 because the synthetic colorants began to domain the market of these compounds[1]. Nowadays, the most well-known blue colorant is indigo, which was initially extracted from plants and became popular in the textile industry but faced challenges of scale and production. By the end of the 20th century, its large-scale chemical synthesis was achieved, driving the textile industry forward[2]. However, the innovative production of synthetic colorants led to significant environmental damage, including water and soil pollution[3].

In this context, attention turned to indigoidine, which offers the colorant sector a sustainable source of microbial and biotechnological production, capable of replacing indigo due to the similarity in their bluish coloration[3]. The hue comes from multifunctional enzymes, Nonribosomal Peptides (NRPs), catalyzing the condensation of two molecules of L-glutamine, a non-essential amino acid[4]. This production from the metabolism of microorganisms meets the need for less environmentally aggressive methods in the coloring process. Additionally, the natural blue colorant may possess antioxidant and antimicrobial properties due to carbon-carbon double bonds conjugated with a carbonyl group, resulting in the ability to eliminate free radicals and cope better with cellular oxidative stress[5]. However, indigoidine lacks extensive literature addressing its molecular properties and behavior. Therefore, it is necessary to study the properties and stability of the colorant to enrich the scientific understanding of indigoidine, allowing its identification as a potential substitute for synthetic colorants, particularly, indigo. To promote more knowledge about this molecule, this work evaluated the solubility of microbial indigoidine as its stability in the presence of solvents in different temperature levels and pH value.

2 MATERIAL & METHODS

The indigoidine natural Blue Dye (CAS 2435-59-8) food grade (99.5% of purity) was purchased from Shanghai Dekang Medical Technology Limited, Hong Kong. The indigoidine solubility test was performed with dimethyl sulfoxide (DMSO), ethyl lactate, hexane, ethanol, ethyl acetate, distilled water, acetone, and methanol. The experiments were carried out adding 1 mL of an indigoidine solution in the concentration of 500 µg/mL in a Falcon tube The solution was prepared with each solvents described. If a complete solubilization did not occur, an additional 0.5 mL of the reagent was added. After the process, the final samples underwent scanning (300-700 nm) in a UV-visible spectrophotometer in duplicate, and the absorbance values at 612 nm were used for analysis.

First, the stability of indigoidine at temperature was carried out with two solutions of 0.1 g/L in DMSO, one maintained at room temperature and the other in the refrigerator without specific temperature control. The experiment lasted at least 60 days and the indigoidine concentration was measured twice a week. A stability curve was generated for each sample result. In the second stage, the solutions had a concentration of 500 µg/mL in distilled water, ethanol, methanol, and ethyl lactate, maintained only at room temperature, undergoing weekly indigoidine measurement. Stability curves were also plotted for each of the samples. This test lasted approximately 40 days. In the third stage, the experiment was standardized for the solvents used in the first and second stages, at a concentration of 500 µg/mL, under the same previous analyses, for 4 weeks. The three stages were analyzed in duplicate.

The stability of indigoidine at pH was tested using a range of pH 2.0 to 11.0, with McIlvaine buffer solutions for solutions whose pH was 2.0 to 8.0 and solutions with sodium carbonate buffer-sodium bicarbonate for solutions with pH 9.0 to 11.0, both prepared using Milli-Q water. The concentration of solubilized indigoidine was 1 mg/mL, under indigoidine measurement and colorimeter analysis in the first hour of the experiment, 24 hours, and after 1 week of the experiment.

The indigoidine concentration was analyzed at absorbance measurement at 612 nm using EnSpire multimode plate reader (PerkinElmer™). The color analysis was performed using a CM-5 Spectrophotometer (Konica Minolta Sensing Americas Inc.) through the variables “L”, “a”, “b”, “c” and “h”, which, respectively, represent the difference between light (+) and dark (-), the difference between red (+) and green (-), the difference between yellow (+) and blue (-), the difference in chroma between lighter (+) or more opaque (-) and finally, the difference in hue.

3 RESULTS & DISCUSSION

At the end of the indigoidine solubility experiment, complete solubilization occurred only in DMSO, ethanol, distilled water, methanol, and ethyl lactate. Acetone exhibited partial solubility, while ethyl acetate and hexane did not solubilize the molecule. Following, the final samples underwent scanning (300-700 nm) in a spectrophotometer (Figure 1). The octanol-water partition coefficient (K_{ow}) of the solvents used is log k_{ow} (-1.35), log k_{ow} -0.30, log k_{ow} -0.74, 0,06, log k_{ow} -0.24, log k_{ow} 0.73, log k_{ow} 4.0 respectively. So, it can be observed that indigoidine is more soluble in polar compounds with positive coefficients. In Solvents with K_{ow} values above 0.1 (hexane and ethyl acetate), there is no indigoidine solubilization. Additionally, the curves without absorbance peaks, especially in the range of 600 nm, were those in which the solvents were unsuccessful in the indigoidine solubility test – again, ethyl acetate and hexane.

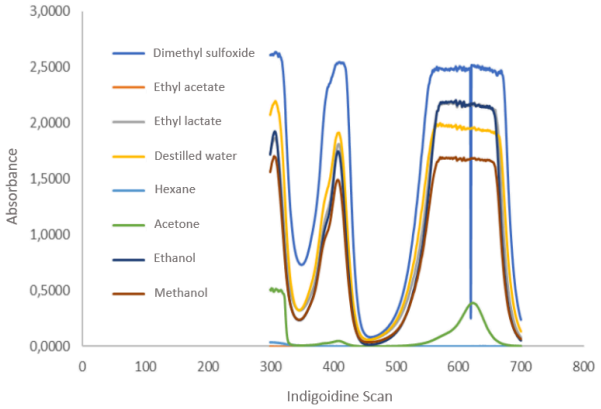

Figure 1 Indigoidine scan in the range of 300-700 nm on a spectrophotometer in the solvents DMSO, ethyl acetate, ethyl lactate, distilled water, hexane, acetone, ethanol, and methanol.

About the stability of commercial indigoidine in DMSO at a concentration of 0.1 g/L, the results are shown in Figure 2. It is observed that over the course of 60 days of the experiment, the solution kept in the refrigerator experienced a smoother decline compared to the sharp drop exhibited by the solution kept at room temperature in the first 10 days, along with a lesser loss of color. Meanwhile, the stability of indigoidine in the other solvents (distilled water, ethanol, ethyl lactate, and methanol) at a concentration of 500 µg/mL only at room temperature exhibited similar behavior in all the reagents for that temperature, and the color remained blue and dark until the end. The studies lasted for approximately 40 days, and the absorbance decreased more abruptly in the first 5 days and then remained stable. The stability results were standardized in another experiment using the two temperatures analyzed for DMSO and are presented in Figure 3A and 3B.

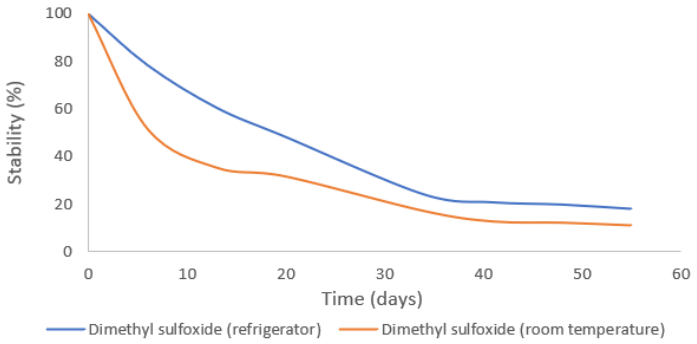

Figure 2 Stability of indigoidine in DMSO at a concentration of 0.1 g/L in a 60-day duration assay, each solution kept at room temperature and in the refrigerator.

The last and standardized temperature stability study showed significant color stability in the presence of distilled water, ethanol, methanol, and ethyl lactate for both temperature evaluated. However, once again, the sample of DMSO at room temperature exhibited the sharpest decline in its absorbance peak at 612 nm, while the refrigerated sample showed better stability, albeit still lower compared to the other solvents. These data are displayed in Figure 3A for the refrigerated sample and in Figure 3B for the sample kept at room temperature.

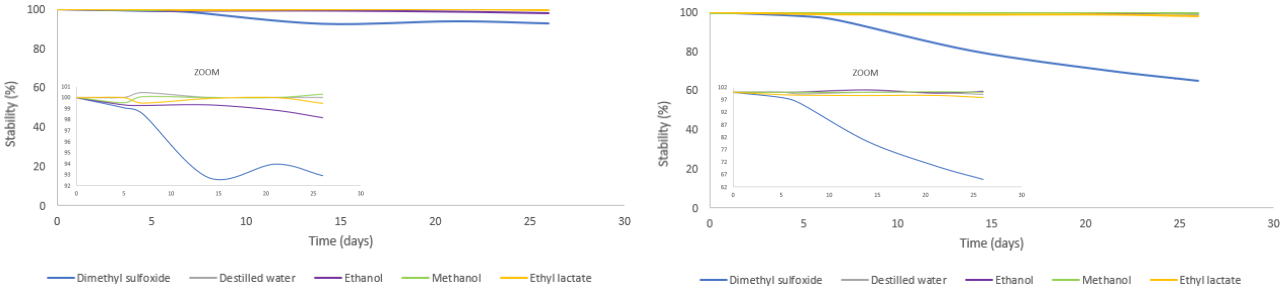

Figure 3 Stability of commercial indigoidine (500 µg/mL) in DMSO, ethanol, methanol, distilled water, and ethyl lactate in a 4-week duration test with solutions kept in the refrigerator (A) and at room temperature (B), respectively.

Although small, the more abrupt stability drop for the solutions in DMSO, for both temperatures, can be based on the properties of the solvent compared to the others. The chemical structure, the thermal stability of DMSO, and the conditions under which the solvent is most sensitive can interact unfavorably with indigoidine, leading to its faster degradation.

Regarding the properties of the colorant at different pH values, it can be seen that the indigoidine exhibits notable stability at both acidic and alkaline pH extremes. In all solutions, ranging from pH 2.0 to pH 11.0,the amount of indigoidine was higher than 95% after 500 hours of study. Furthermore, the coloration, analyzed by colorimeter, was similar across all samples, as they exhibited stable coefficients such as "L", "a", "b", "c", and "H" remained stable between hour 0 and hour 504 of the experiment, and showed close proximity among all tested pH samples. The stability results of indigoidine in several pH values is presented at Figure 4.

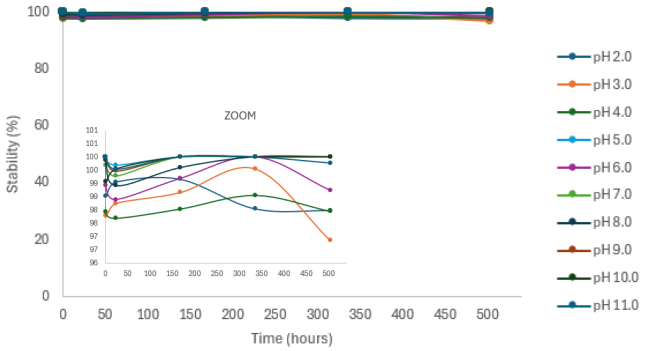

Figure 4 Stability of indigoidine (500 µg/mL) at pH from 2.0 to 11.0 in buffer solution during 3 weeks with measurements in the zero hour and after 1, 24, 168, 336 and 504 hours after the experiments beginning.

4 CONCLUSION

In conclusion, it is appropriate to emphasize the importance of the sustainable and biotechnological replacement of synthetic colorants with natural sources such as natural indigoidine. Furthermore, fostering the enrichment of literature regarding natural blue colorants is essential for shaping this scenario. In this study, it was understood that indigoidine better solubilizes in polar organic solvents with octanol-water partition coefficient (K_{ow}) values lower than 0.1 and negative, preferably. The results of the solubility test guided the selection of solvents for the temperature stability tests, which showed that indigoidine was stable in the presence of all solvents - DMSO, distilled water, ethyl lactate, ethanol and methanol - and pH from 2.0 to 11.0 under the conditions evaluated.

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