Discoloration Kinetics of Carthamus Red in Aqueous Ethanol

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The solubility of carthamus red, red pigment from safflower, in aqueous ethanol largely depended on the ethanol concentration, and it was the highest near 60 and 70% (v/v) ethanol. Discoloration of carthamus red in aqueous ethanol was monitored under temperature-programmed heating conditions where the temperature of test solution raised linearly with time to evaluate the activation energy and frequency factor. The activation energy and frequency factor did not depend on the ethanol concentration, suggesting that ethanol does not affect the discoloration mechanism. The activation energy was 78±5 kJ/mol. The increase in absorbance at 390 nm during discoloration of carthamus red correlated with the decrease in absorbance at 520 nm. This fact also suggests no effect of ethanol on discoloration mechanism of carthamus red.

Keywords: Carthamus red, discoloration, red pigment, safflower, kinetic analysis

1. Introduction

Two pigments are extracted from the dried petals of safflower, which is a highly branched, herbaceous, annual plant. One is a water-soluble carthamus yellow, and another is carthamus red which is poorly soluble in both water and ethanol [1]. Their principal coloring matters of carthamus yellow have been identified as safflorinin A and B, and that of carthamus red as carthamin [1–3]. Carthamus red is used as food colorants as well as cosmetics and dyes [1]. It is also potentially expected to be usable as medicines and pharmaceuticals [4].

Both carthamus yellow and red are susceptible to thermal degradation [1]. It has been reported that thermal degradation of carthamus yellow obeys the first-order kinetics at acidic conditions, but does not at neutral and alkaline conditions [5]. Stability or decomposition of carthamus red in aqueous solution was also investigated under various conditions. Kanehira et al. [6] examined the factors affecting the stability of carthamus red in aqueous solution, such as temperature, pH, light, metal ions and so on. Carthamus red was more readily decomposed to orange-yellow or yellow compounds at higher temperature. The red colorant was unstable on exposure to visible and ultraviolet light. Shin and Yoo [7] also reported that carthamus red in aqueous solution was very unstable to both heat and light. Carthamus red was more stable in aqueous solution at higher pH [8, 9].

As shown later, the solubility of carthamus red in aqueous ethanol largely depends on the ethanol concentration. However, the effect of the ethanol concentration on the discoloration kinetics of carthamus red has not been investigated. In this context, the discoloration behavior was measured at various ethanol concentrations under temperature-programmed heating conditions to evaluate the activation energy and frequency factor for the discoloration.

2. Materials and Methods

2.1 Materials

Carthamus red was a product of Toyo Chem (Tokyo, Japan). Ethanol (purity: >99.5%) was purchased from Junsei Chemical (Tokyo).

2.2 Solubility of carthamus red in aqueous ethanol

Carthamus red was dispersed in an aqueous ethanol, the ethanol concentration of which was in a range of 0 to 100% (v/v), at the concentration of 0.02 g/mL or higher. The suspension was left at the room temperature with occasional stirring for 30 min or longer. Then, the suspension was centrifuged at dial 4 (200×g) for 5 min (Hisiang Tai Centrifuge, As One, Osaka, Japan). The supernatant was diluted 5 times with the aqueous ethanol, and the absorption spectrum from 350 to 650 nm was measured using a spectrophotometer (UV-1280, Shimadzu Corp., Kyoto, Japan). Carthamus red used was not a high-purity carthamin, and the carthamin content...
of the preparation was unknown. The effect of the ethanol content on the absorption coefficient of carthamin was also unknown. Therefore, the absorbance was used as an indication of the solubility.

2.3 Discoloration of carthamus red at a constant temperature

Carthamus red (0.6 g) was dispersed in 30 mL of 60\% (v/v) ethanol and well stirred with a vortex mixer (ZX3 Advanced Vortex Mixer, Velp Scientifica, Usmate, Italy). After centrifugation, 25 mL of the supernatant was mixed with 100 mL of 60\% ethanol. 2.5 mL each of the mixture was placed in a 10-mL amber test tube (inner diameter 13.2 mm; height 105 mm) with a cap (Maruemu, Osaka) (12 tubes), and the tubes were placed in the THB–1 dry block bath (As One) preheated at 75°C. The moment the tube was placed in the block was taken as the start of the discoloration experiment. An amber test tube was used to prevent discoloration by light throughout this study. One of the tubes was removed from the bath over time and immediately cooled to stop the reaction. The absorption spectrum of the solution was measured from 350 to 650 nm with the spectrophotometer. The temperature change during the reaction was measured using a thermocouple thermometer (AD5605H, A & D, Tokyo), the tip of which was located in the approximate center of the liquid part. The thermometer had been calibrated using a digital standard thermometer (SST–100PT, Sansyo, Tokyo).

2.4 Discoloration of carthamus red under temperature-programmed heating conditions

Carthamus red was dissolved in an aqueous ethanol with a concentration of 0 to 90\% (v/v) to a saturated concentration. After centrifugation, the supernatant was diluted so that the absorbance at 520 nm was 0.7 or less. The diluted solution of 2.5 mL was put into an amber test tube with a screw cap (10 to 12 tubes), and the tubes were placed in the dry block bath. The temperature of the block bath was raised at a constant rate (0.115 to 0.278 °C/min) from 25 °C to 75 °C (for 80\% (v/v) and 90\% ethanol) or 80°C (for other ethanol concentrations). One test tube was taken out with time, stirred well, and the absorption spectrum from 350 to 650 nm was measured using the spectrophotometer.

3. Results and Discussion

3.1 Solubility of carthamus red in aqueous ethanol

Saturated solution of carthamus red in an aqueous ethanol was diluted 5 times with the aqueous ethanol, and the absorption spectrum of the diluent from 350 to 650 nm was measured as an indication of the solubility. The ethanol concentration was in the range of 0 to 100\% in 10\% increments. Figure 1 shows some spectra at different ethanol concentrations. Absorption peaks were observed approximately at 390 nm and 520 nm, which are ascribed to yellow and red, respectively. The absorption around 520 nm was low at ethanol concentrations of
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3.2 Discoloration of carthamus red at a constant temperature

Discoloration of carthamus red dissolved in 60% (v/v) ethanol was monitored by measuring the absorbance at 520 nm of the test solution held at 75°C for predetermined period (Fig. 2). The temperature of the test solution was also measured. Figure 2(A) shows the absorption spectra from 350 to 650 nm of carthamus red solutions stored for different periods. The absorbance at 520 nm decreased and that at 390 nm increased with time.

The change is consistent with the previous report that the carthamus red was decomposed to orange–yellow or yellow compounds [6].

Figure 2 (B) shows the changes in the temperature of test solution and the undiscolored fraction of carthamus red, Y, which is defined by Eq. (1).

\[ Y = \frac{A - A_\infty}{A_0 - A_\infty} \]  

where \( A \) is the absorbance at 520 nm at any time, and \( A_0 \) is the initial \( A \) value. \( A_\infty \) is the absorbance at 520 nm on the baseline of the absorption spectrum, as shown in Fig. 2 (A). The temperature did not reach the desired level immediately after the start of experiment, and it required more than 15 min. Discoloration, that is, a decrease in \( Y \), was observed before the temperature reached the desired level. After the temperature reached the set one, the \( Y \) value decreased linearly on a semi-logarithmic scale with respect to time \( t \). This fact indicated that the discoloration of carthamus red obeyed the first-order kinetics. The phenomenon that the temperature of the test solution does not reach the desired level immediately after the start of experiment is not preferable for the kinetic analysis. However, when the reaction obeys the first-order kinetics, the activation energy and the frequency factor can be obtained in one experiment by increasing the temperature at a constant rate [10, 11]. Therefore, the method was applied to the kinetic analysis for discoloration of carthamus red at different ethanol concentrations.

3.3 Discoloration of carthamus red under temperature-programmed heating conditions

The change in the \( Y \) value for carthamus red dissolved in 80% (v/v) ethanol was measured at three different temperature–rising rates (Fig. 3). In each case, the temperature increased linearly with time, while the \( Y \) decreased more rapidly with increasing temperature. When the discoloration obeys the first-order kinetics and the temperature dependence of the rate constant is expressed by the Arrhenius equation, the following equation holds [10, 11]:

\[ \ln \left( \frac{-\alpha}{RT^2} \ln Y \right) = -\frac{E}{RT} + \ln \left( \frac{k_0}{E} \right) \]  

where \( E \) and \( k_0 \) are the activation energy and frequency factor for discoloration of carthamus red. \( \alpha \) is the temperature–rising rate, \( T \) is the absolute temperature, and \( R \) is the gas constant. When the discoloration obeys the first-order kinetics, the plots of the left side of Eq. (2) against
the reciprocal of the absolute temperature should lie on a straight line independent of the temperature–rising rate and enable us to estimate the $E$ value from the slope of the line. As shown in Fig. 4, the plots for three different temperature–rising rates gave a straight line with a negative slope. This fact indicated that the constant temperature–rising method was applicable to the kinetic analysis of discoloration of carthamus red in aqueous ethanol.

### 3.4 Effects of ethanol concentration of activation energy and frequency factor for the discoloration

The discoloration of carthamus red dissolved in aqueous ethanol with various ethanol concentrations was measured under temperature–programmed heating conditions to evaluate the activation energy, $E$, and frequency factor, $k_0$ (Fig. 5). Since the oxygen concentration, which may affect the discoloration of carthamus red, in the test solution depends on both the ethanol concentration and the temperature, the estimated values would be apparent ones. Both the values of $E$ and $k_0$ were slightly scattered, but hardly depended on the ethanol concentration. The $E$ value was $78 \pm 5$ kJ/mol, and log $k_0$ one was $8.6 \pm 0.8$ in the units of s$^{-1}$ for $k_0$. Kim and Paik [9] reported the activation energy for discoloration of carthamin in aqueous solution of pH 5 to 12 was in the rage of 65 to 70 kJ/mol, which were almost the same as those estimated in this study. This fact suggests that ethanol has a large effect on solubility of carthamus red, but does not affect its discoloration mechanism.

### 3.5 Relationship between red decrease and yellow increase

As mentioned above, the absorbance at 520 nm decreased and that at 390 nm increased during the temperature–rising process at any ethanol concentration. The absorbance at 520 nm and 390 nm are the indicators of carthamus red and orange–yellow or yellow compounds, respectively. Figure 6 illustrates the relationship between the decrease in absorbance at 520 nm, $\Delta A_{520}$, and the increase in absorbance at 390 nm, $\Delta A_{390}$ during discoloration processes at all the ethanol concentrations. All the plots lie on a straight line passing through the origin. This fact also indicates that the carthamus red is decomposed to yellow compounds through the same
mechanism at any ethanol concentration.

Acknowledgments

We thank Riken Vitamin for the supply of carthamus red, and Mr. Y. Nakae for his technical assistance.

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含水エタノール中でのベニバナ赤色素の退色動力学

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食用色素（着色料）は、食品の見栄えや保存性に影響する添加物である。ベニバナの花弁から抽出される色素は天然色素の1つであり、黄色と赤色の色素を含む。黄色の色素（ベニバナ黄）の主体は水溶性のsafflomin AとBであり、赤色色素（ベニバナ赤）のそれは水またはエタノールにも溶けにくいcarthaminである[1-3]。ベニバナ赤は食品、化粧品などで使用される[1]とともに、医薬品としての利用も期待されている[4]。

ベニバナ黄とベニバナ赤はともに熱により分解しやすい[1]。ベニバナ黄の熱分解は、酸性条件では1次反応速度式に従うが、中性やアルカリ性条件ではそうでない[5]。水系でのベニバナ赤の安定性に及ぼす因子については詳細な研究がなされており、高温では橙黄色または黄色の化合物に分解する[6]。ベニバナ赤は熱とともに、光に対しても不安定であるが[7]、pHが高いと比較的安定である[8]。

ベニバナ赤は水にもエタノールにも溶けにくいが、水とエタノールの混合液（含水エタノール）には比較的よく溶ける（Fig. 1）。ときに、エタノール濃度が50～80%（v/v）ではよく溶ける。このように、ベニバナ赤の溶解度はエタノール濃度に大きく依存するが、退色動力学に及ぼすエタノール濃度の影響については報告がない。そこで、ベニバナ赤の退色過程に対する活性化エネルギーと頻度因子を検討した。

ベニバナ赤溶液を入れた試験管を、所定の温度に設定したヒートブロックに入れても、反応液はすぐにその温度にならない（Fig. 2）。しかし、ベニバナ赤の退色は比較的速い反応であるため、反応液の温度が上昇する間も、退色反応が進行し、速度解析を難しくする。一方、反応液の温度が所定の値に達したのは、ベニバナ赤の最大吸収波長である520 nmにおける吸光度は片対数紙上で時間に対して直線的に低下する（Fig. 2）。したがって、ベニバナ赤の退色過程は1次反応速度式に従うと仮定した。また、昇温過程でも退色が進行することを逆に利用し、反応液の温度を時間に対して直線的に上昇させると定常昇温法により、退色反応に対する活性化エネルギーと頻度因子を一度の実験で決定することを考えた[10,11]。

まず、80%（v/v）に溶解したベニバナ赤の退色過程を異なる昇温速度で観察し（Fig. 3）、1次反応を仮定した定常昇温法が適用できることを検証した（Fig. 4）。つぎに、種々のエタノール濃度におけるベニバナ赤の退色過程を測定し、活性化エネルギーおよび頻度因子を算出した。ややバラツキはあるものの、ベニバナ赤の退色過程に対する活性化エネルギーと頻度因子はエタノール濃度には依存しなかった（Fig. 5）。

ベニバナ赤の退色過程では520 nm付近の吸光度（赤色）が減少するとともに、390 nm付近の吸光度（黄色）が増加する（Fig. 2）。そこで、種々のエタノール濃度での退色過程に対し、520 nmにおける吸光度の減少ΔA_{520}と390 nmにおける吸光度の増加ΔA_{390}をプロットすると、エタノール濃度に依存せず、1本の直線となった（Fig. 6）。

これらの結果より、ベニバナ赤の溶解度はエタノール濃度に大きく依存するものの、その退色機構はエタノール濃度には依存しないことが強く示唆された。