Arch. Pharm. Res. Vol.20, No.6, pp. 643-646, 1997

Stability of Carthamin from *Carthamus tinctorius* in Aqueous Solution: pH and Temperature Effects

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(Received August 7, 1997)

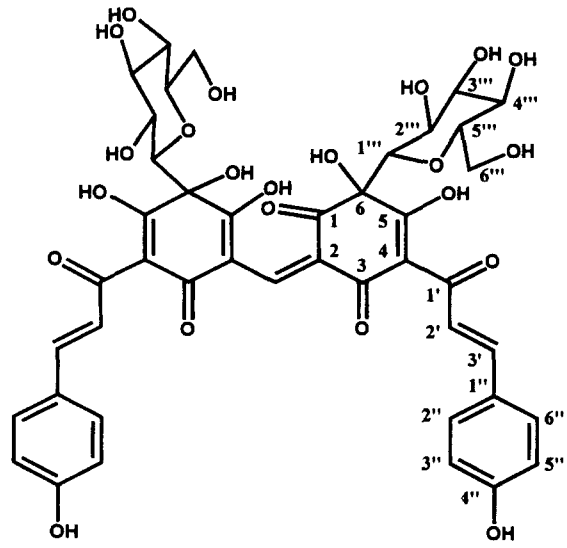
Thermal stability of a red pigment, carthamin, from *Carthamus tinctorius* was investigated to explore possible applications as natural color additives for foods, cosmetics, and nutraceuticals. Degree of degradation reactions of carthamin at acidic, neutral and alkaline conditions were determined with UV/Vis spectral measurements. Decomposition half lives of carthamin at 25°C were 4.0 h, 5.1 h, and 12.5 h at pH 5.0, pH 7.0, and pH 12.0, respectively, indicating that carthamin is much more stable at alkaline pH than acidic or neutral conditions. The activation energies of carthamin at pH 5.0, pH 7.0, and pH 12.0 were 15.6, 15.7 and 16.8 kcal/mol, respectively.

Key words : *Carthamus tinctorius*, Compositae, Safflower, Carthamin, Thermal stability

INTRODUCTION

In recent years, natural colorants have been reexamined from the point of safety, especially in the field of food and cosmetic industry (Hendry and Houghton, 1996). Flower petals of *Carthamus tinctorius* contain red-colored and yellow-colored components (Harbone, 1994). The red or yellow color from safflower had been used traditionally for dyeing fabrics, food colorings, cosmetics and paintings in Korea and other Oriental countries. The stability of the red color from safflower origin is good enough to endure the sun light and several washings when it is dyed to cotton or silk. The extracts of safflower petals or seeds are still being used as traditional medicine for the treatment of cardiovascular and hematological disease such as angina pectoris, cerebral hemorrhage, cerebral arterioscrelosis, rheumatalgia, amenorrhea, and menorrhalgia (Tang and Eisenbrand, 1992).

Although the natural red color from safflower (Obara and Onodera, 1979) has a great possibility to be applied more sophisticately to a wide range of industry, it appears to have a problem that the red color fades gradually when it is in aqueous solution (Kanehira *et al.*, 1990). To overcome this off-colored problem, it is necessary to study the detailed discoloration kinetics of the red pigment, carthamin (Scheme 1), in aqueous solution. In connection of previous work (Kim *et al.*, 1996) where the red pigment, carthamin, was efficiently isolated and identified from *Carthamus tinctorius*, physical stability of carthamin in aqueous solution was determined at various ranges of pH and temperature conditions.



Scheme 1. Structure of Carthamin.

MATERIALS AND METHODS

Materials

Dried safflower (*Carthamus tinctorius*) was purchased from local market and stored at 4°C. Cellulose and dimethyl-d_6 sulfoxide were obtained from Sigma Chemical Co. (St. Louis, U.S.A.). Sephadex LH-20 was pur-

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chased from Pharmacia Fine Chemicals (Uppsala, Sweden). Other chemicals including methanol, acetone and pyridine of first grades were bought from Hayman Ltd. (Witham Essex, UK).

Purification of carthamin

Isolation procedures of the red pigment were described previously (Kim *et al.*, 1996). Briefly, after washing dried petals of safflower with water and then methanol to remove yellow pigments, the red pigment was extracted with 0.5 M Na_2CO_3 and then acidified with 0.5 M citrate. The red pigment extracts were further chromatographed on cellulose and Sephadex LH-20 columns. The collected fractions were essentially homogeneous as determined by TLC (n-BuOH/AcOH/H_2O=4:1:5) and NMR measurements (Oxford FT-NMR). ^{1}H NMR (400 MHz, DMSO-d_6): 19.02 (1H×2, br s, enolized β-triketone), 8.34 (1H, s, methine), 7.56 (1H×2, d, J=16.0 Hz, 3'-H), 7.55 (2H×2, d, J=8.4 Hz, 2''-H, 6''-H), 7.40 (1H×2, d, J=16.0 Hz, 2',-H), 6.84 (2H×2, d, J=8.4 Hz, 3''-H, 5''-H), 3.84 (1H×2, d, J=8.0 Hz, 1'''-H), 3.82 (1H×2, d, J=9.4 Hz, 6'''-H), 3.56 (1H×2, d, J=9.4 Hz, 6'''-H), 3.38 (1H×2, br s, 2'''-H), 3.13 (1H×2, br s, 3'''-H), 3.11 (1H×2, m, 4'''-H), 2.92 (1H×2, br s, 5'''-H).

Procedure for kinetic runs

Thermal degradation reactions were carried out in acidic (0.1 M acetate buffer, pH 5.0), neutral (0.1 M phosphate buffer, pH 7.0) and alkaline (0.1 M phosphate buffer, pH 12.0) conditions. After the buffer solutions (0.9 ml) in sample cuvette reached to desired temperature (25, 40, 50, 60, 70, 80°C), the reaction was initiated by injecting 100 μl of carthamin stock solution (3.5 mg carthamin/1 ml methanol). Degree of degradation was measured by absorbance changes at 520 nm (pH 5.0), 505 nm (pH 7.0), and 484 nm (pH 12.0) with UV/VIS spectrophotometer (Milton Roy Spectronic 3000).

RESULTS AND DISCUSSION

Although flavonoids in general, including carthamin, are known to be usually unstable in alkaline media (Kanehira *et al.*, 1990), Saito and Mori (1994) reported that the stabilities of carthamin in alkaline media (72.4 mM potassium carbonate, pH 11.4) or in acidic buffer (50 mM citrate, pH 5.0) were similar. However, no critical data including decomposition reaction rate constants or thermodynamic parameters have been emerged in their experiments. To solve these problem, thermal decomposition kinetic studies were conducted and compared the stabilities of carthamin at acidic, neutral and alkaline pH conditions. Carthamin showed characteristic UV/Vis spectra at different pH values (Fig. 1), indicating that carthamin possesses different structures at various pHs. The color of carthamin at acidic (pH 5.0) or neutral (pH 7.0) buffers was pink-red with maximum absorption at 520 and 505 nm, respectively. (Fig. 1 A, B) However, when carthamin was dissolved in alkaline (pH 12.0) buffer, the color was changed to orange-yellow with λ_{max} of 485 nm. (Fig. 1 C).

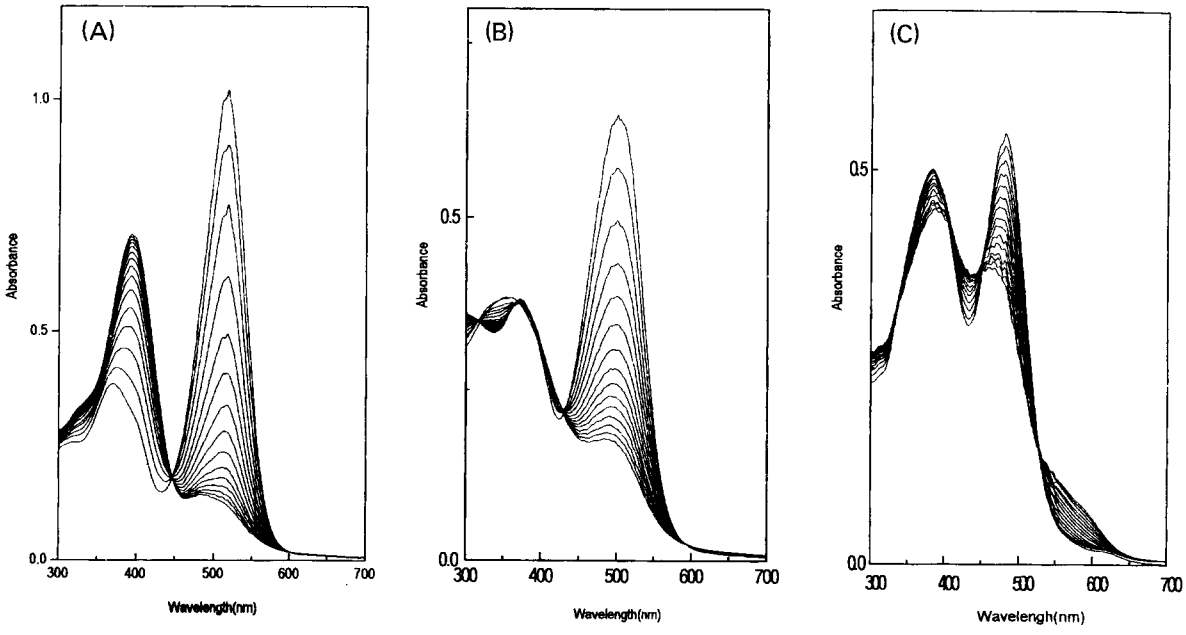

Fig. 1. Thermal degradation of carthamin in different pHs at 25°C. Spectra were scanned with 1 hr interval. Buffer systems used were 0.1 M acetate, pH 5.0 (A); 0.1 M phosphate, pH 7.0 (B); 0.1 M phosphate, pH 12.0 (C).

Table I. Rate constants (k) and half life ($T_{1/2}$) values of thermal degradation reactions of carthamin at acidic (pH 5.0, 0.1 M acetate buffer), neutral (pH 7.0, 0.1 M phosphate buffer), and alkaline (pH 7.0, 0.1 M phosphate buffer) conditions at different temperatures

Temperature (°C)	Rate constant (s^{-1})$\times 10^4$			Half-life (h)		
	pH 5.0	pH 7.0	pH 12.0	pH 5.0	pH 7.0	pH 12.0
25	0.481	0.377	0.154	4.00	5.10	12.50
40	1.38	0.987	0.481	1.39	1.95	4.00
50	2.96	2.46	1.48	0.65	0.78	1.30
60	6.79	5.50	2.57	0.28	0.35	0.75
70	13.9	8.88	6.42	0.14	0.22	0.30
80	27.5	18.6	–	0.07	0.10	–

Among a vareity of factors such as heats, pH, oxygen, metals and light, heat is the most potent factor to affect stability of natural colorants (Kanehira *et al.*, 1990). Thermal degradation reactions of the purified carthamin were carried out in acidic (pH 5.0, 0.1 M acetate), neutral (pH 7.0, 0.1 M phosphate), and alkaline (pH 12.0, 0.1 M phosphate) buffer solutions at different temperature ranges of 25~80°C. Fig. 1 showed the decomposition profiles of carthamin in aqueous buffer solutions at 25°C with 1 h intervals. When carthamin in acidic buffer was incubated at 25°C, the red (λ_{max}=520 nm) color was gradually disappeared, yielding pale-yellow colored solution (λ_{max}=395 nm) with a sharp isosbestic point at 446 nm (Fig. 1A). The carthamin was also gradually degraded in neutral and alkaline buffers yielding yellow colors with isosbestic points at 430, 366 and 317 nm at pH 7.0 (Fig. 1B), and 528, 450, 422, and 331 nm at pH 12.0 (Fig. 1C), respectively. These results suggested that each of carthamin at acidic, neutral, and alkaline pH is degraded to produce a simple degradation product. Degree of thermal degradation was determined by measuring absorbance changes at 520 nm (pH 5.0), 505 nm (pH 7.0), and 485 nm (pH 12.0). When degree of degradation was plotted on a semilogalithmic scale, the plot showed straight lines at each pH condition. Thus the degradation reaction follows simple first-order kinetics. The rate constants of the reaction and half-lives of the carthamin at different pH conditions were summarized in Table I. The thermal degradation rates at pH 12.0 were slower about two to three times than those at pH 5.0 indicating that carthamin is much more stable at alkaline pH than at acidic pH. This result was different from the report of Saito's group (Kanehira *et al.*, 1990) that the red pigment is pH dependent and most stable at pH 1.5~5.5. However, the same group reported later (Saito and Mori, 1994) that carthamin in potassium carbonate solution is not so unstable as usually supposed. The degradation rates at 27°C at pH 5.0 (50 mM citric acid/sodium citrate buffer) and at pH 11.4 (72.4 mM K_2CO_3) were similar and the half-lives of carthamin could be estimated approximatly 3 hours in both cases (Saito and Mori, 1994). No critical data including decomposition rate constants have been reported in their experiments. Present data indicated that the degradation half life values of carthamin at 25°C were 4.0 h in acidic (pH 5.0, 0.1 M acetate) and 12.5 h in alkaline (pH 12.0, 0.1 M phosphate) buffer solutions, respectively (Table I), indicating carthamin is much more stable at alkaline pH than at acidic pH. This discrepency of carthamin stability could be due to differences in experimental conditions such as sample preparation (purity) and buffer compositions. Arrhenius plots of thermal degradation reactions showed activation energies of decomposition of carthamin at pH 5.0, 7.0, and 12.0 were 15.6, 15.7 and 16.8 kcal/mol, respectively, suggesting again that carthamin is more stable at alkaline pH than acidic or neutral conditions. The mechanism of the stability of carthmin at alkaline condition is not clear yet, although phenolate ion of carthamin may do some role. The activation energy for the degradation reaction of betanine (Huang and von Elbe, 1985), the major red pigment in beet powder which is permitted as a food colorant (von Elbe *et al.*, 1974), was reported as 17.6 kcal/mol (pH 5.0, 0.1 M citrate-phosphate buffer) suggesting that carthamin also can be a possible candidate for food, cosmetic, or nutraceutical additives.

In summay, carthamin isolated from flower petals of *Carthamus tinctorius* showed red or orange-yellow color with maximum absorption peak at 520 nm, 505 nm, and 485 nm, in acidic (pH 5.0), neutral (pH 7.0), and alkaline (pH 12.0) buffer solutions, respectively. The pigment was more stable to heat at alkaline (pH 12.0) condition than at acidic (pH 5.0) or neutral (pH 7.0) conditions.

ACKNOWLEDGMENTS

This work was supported by grants from NONDIRECTED RESEARCH FUND, Korea Research Foundation (1995-1998) and Advanced R & D Program, Ministry of Agriculture and Fisheries (1995-1998).

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