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RESEARCH NOTE

Colour stability of carthamin under alkaline conditions

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Carthamin was dissolved in alkaline solutions and the colour stability examined by the spectrophotometric-assay technique. The experimental results suggest that the red colour of carthamin can be retained for 24 h or so when treated pertinently, even under alkaline conditions. The practical importance of these findings is mainly in regard to carthamin extraction from raw starting materials with alkalies.

INTRODUCTION

Carthamin is a well-known and useful red dyestuff, recently attracting attention as one of many harmless bio-colorants for processed foods. Its coloration is unique; however, it is very unstable in solutions, particularly if alkaline. Carthamin is usually extracted from processed florets of dyer's saffron with relatively high concentrations of K_2CO_3 (Saito *et al.*, 1992). It is therefore obvious that the yield of carthamin dye is greatly affected by the amount of K_2CO_3, the extraction intervals, the environmental conditions, and the skill of the colorant engineers. On standing in alkaline solutions, the red colour fades progressively to reddish orange, orange–yellow, yellow, and light yellow. The original fine colour can never be recovered from the off-colour solutions: the colour change is irreversible. To overcome this disadvantage and increase the yield of carthamin, it seems necessary to study the effect of alkalies on the red coloration systematically. However, as far as we are aware, no work on this topic has ever been presented in the literature.

The following experiments have been conducted to see if alkaline treatment influences the carthamin red coloration substantially. Initial studies of this type should allow suggestions to be made on how to extract alkali labile carthamin more effectively.

MATERIALS AND METHODS

Materials

Carthamin was prepared from processed florets of dyer's saffron according to the method of Saito *et al.* (1992). NaOH, K_2CO_3, glycine, sodium citrate, citric acid, and acetone were supplied by Wako Pure Chemical (Osaka, Japan). Water used in this study was deionized/distilled carefully before experimental use.

Preparation of carthamin solution

Prior to the experiments, carthamin (2 mg) was dissolved, if not stated otherwise, in 50mM citric acid/sodium citrate buffer, pH 5·0 (10 ml). Carthamin solution (0·2 mg carthamin/ml) buffered with 50mM glycine/NaOH, at pH 10·0, was also prepared. Solutions were stored at 3–4°C in the dark. The residual solutions kept for over 24 h were discarded, and fresh ones were prepared.

Test for stability of carthamin

Carthamin in 72·4mM K_2CO_3 (0·2 mg/ml) was left at 27°C for given intervals in the dark. After the preincubation, the solution was acidified to become pH 5·0 by the addition of citric acid and immediately measured at 521 nm or 300–600 nm in a Hitachi model U-3210 spectrophotometer. Blank runs were carried out in parallel with the above experiments in two different buffers, citric acid/sodium citrate (50mM, pH 5·0) and glycine/NaOH (50mM, pH 10·0); UV/VIS absorbances were then assayed spectrophotometrically. At least three replications were used for all experiments. The spectra thus recorded were compared closely with each other.

RESULTS AND DISCUSSION

Flavonoids, inclusive of carthamin, are known to be usually very unstable in alkaline media, and their

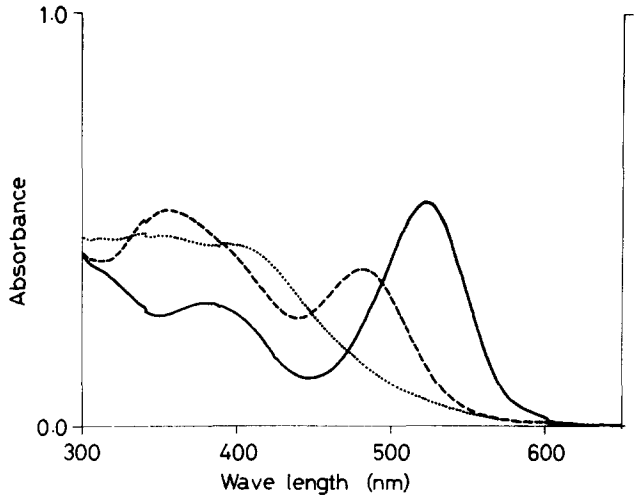

Fig. 1. UV/visible spectra of carthamin in acidic or basic solution. A carthamin sample (2 mg) was dissolved in 50mM citric acid/sodium citrate buffer, pH 5·0 (10 ml), 50mM glycine/NaOH, pH 10·0, or in 72·4mM K_2CO_3, pH 11·4 (10 ml) and each UV/visible spectrum was recorded immediately. ———, 50mM Citric acid/sodium citrate; · · · · · · ·, glycine/NaOH; – – – –, K_2CO_3.

instabilities are pronounced under various external conditions. Carthamin in K_2CO_3 and glycine/NaOH solutions remains orange–yellow or yellow for several hours when left at room temperature in the dark (Fig. 1). Without any foundation, the unfamiliar tincture of these solutions is often mistaken for unknown substances. However, certain flavonoids are known to remain intact in mild alkaline solutions. In the case of carthamin containing K_2CO_3 solution, this also holds true: the red colour is not changed much, even after being kept in the dark for 2 h at 27°C (Fig. 2). The orange–yellow colour shifts to the original visible-absorbance region (521 nm) and, at the same time, the red colour is

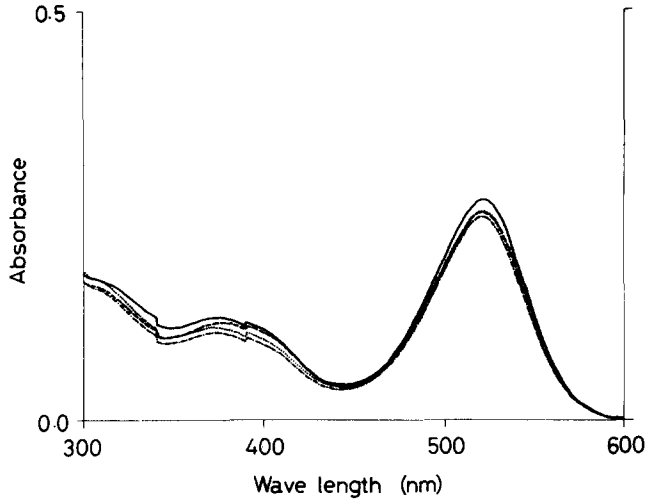

Fig. 2. UV/visible pattern of carthamin solution after being treated with or without K_2CO_3. Carthamin (0·2 mg/ml) in 1% (w/v) K_2CO_3 was left for given intervals at 27°C. At the end of each pre-incubation period, the solution was acidified to pH 5·0 with citric acid and the UV/visible spectrum obtained as shown in the figure. ———, Control; – – – –, 0 min; · · · · · · ·, 30 min; – · – · –, 60 min and 120 min.

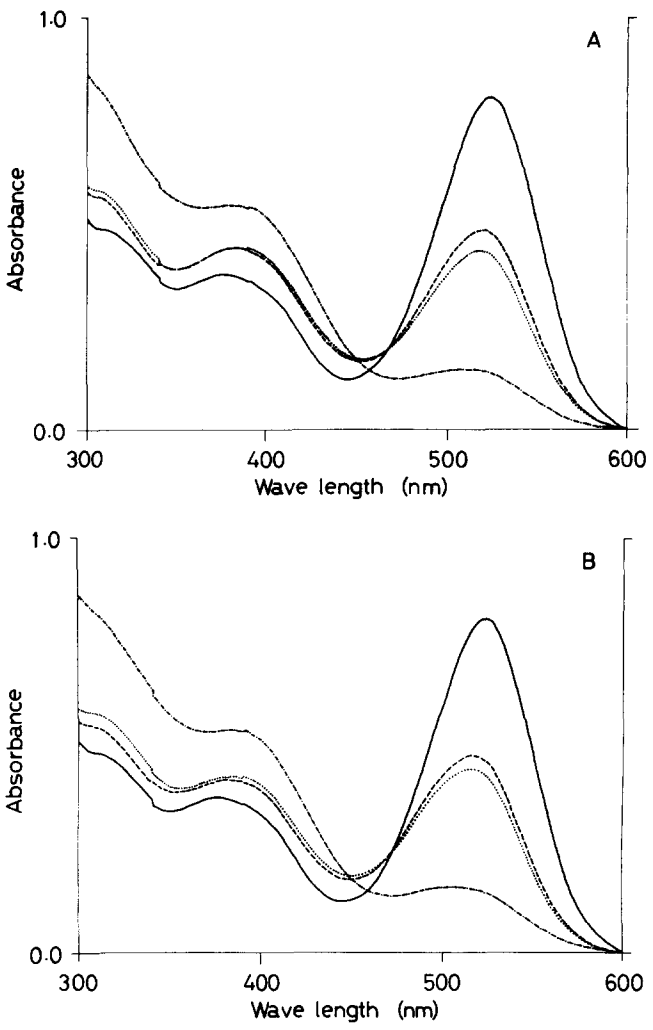

Fig. 3. Comparison of UV/visible absorption pattern of carthamin treated with or without K_2CO_3. (A) Carthamin incubated in 50mM citric acid/sodium citrate buffer at pH 5·0. (B) Carthamin incubated in 72·4mM K_2CO_3 at pH 11·4 and acidified with citric acid to pH 5·0 just before UV/visible measurement. ———, 0 Time; – – – –, 2·5 h; · · · · · · ·, 4·0 h; – · – · –, 20 h.

regained by acidifying with citric acid, though the visible-absorption maximum is evidently reduced during the pre-incubation. The reduction in the maximal visible peak, however, does not arise from any alkaline effect but results mainly from the lapse of incubation time. Affirmative evidence is shown in the following figures. Figure 3(A) shows the colour change of carthamin plotted against incubation time. The visible-absorption maximum at 521 nm decreases progressively during the prolonged incubation. Figure 3(B) shows similar spectra, registered just after acidification of carthamin/K_2CO_3 at the end of each incubation period. All spectra accord with each other in their finer details, as is also supported by the extracted data for Figs 4(A)–(C). Carthamin in K_2CO_3 solution is far more stable than is usually supposed. From the UV/visible spectra, no sign of any structural change can be perceived. The red pigment possibly keeps its original bichalcone structure, at least in acidic solution, although it may exist as a pseudo-base form at alkaline pH through

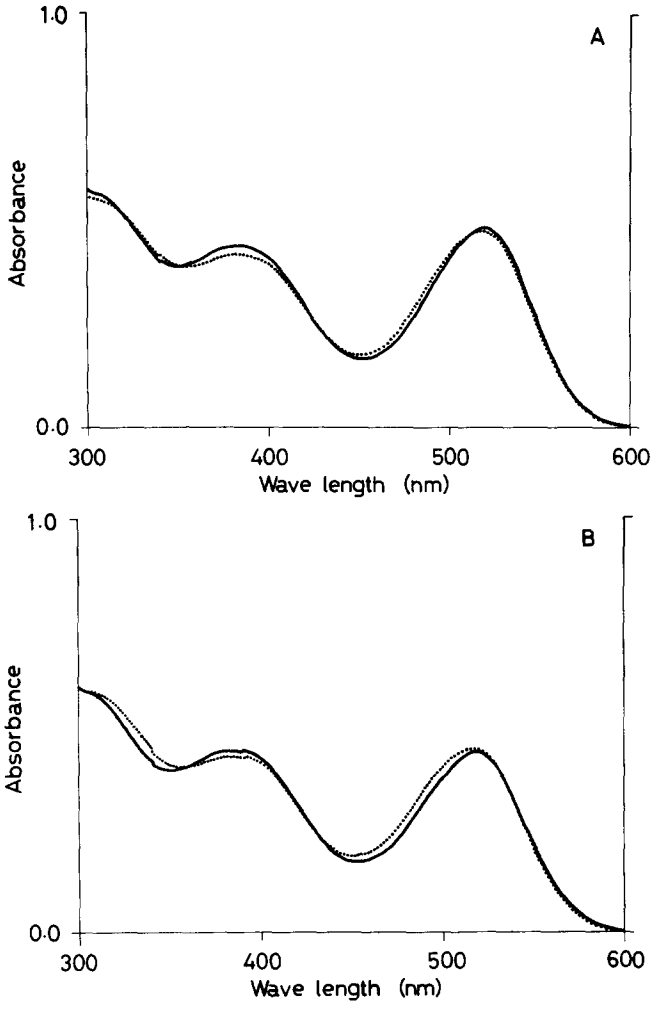

Fig. 4. Comparison of UV/visible absorption profile and tendency to colour-off of carthamin solutions. UV/visible spectra registered at the same incubation times were extracted from Fig. 3(A) and (B) and their spectral patterns compared. (A) 2·5 h; (B) 4·0 h; (C) 20 h. ·····, Carthamin incubated in 50mM citric acid/sodium citrate buffer, pH 5·0. ———, Carthamin incubated in 72·4mM K_2CO_3, pH 11·4 and acidified with citric acid to pH 5·0 just before the UV/visible measurement.

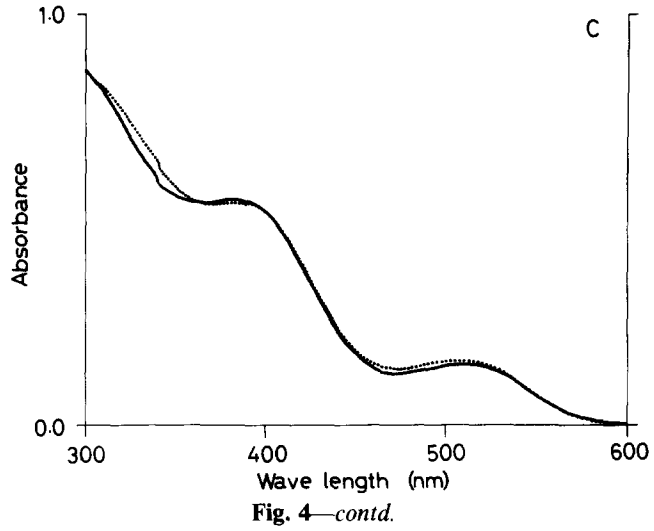

Fig. 4—*contd.*

similar mechanisms to those of other flavonoids (Harlíková & Míková, 1987). Similar patterns could be observed in the UV/visible spectra of a carthamin sample incubated in glycine/NaOH buffer (data are not shown).

The present evidence will remove previous misunderstanding that carthamin is very labile in solutions at alkaline pH. It is therefore proposed that the *Carthamus* dye can be safely kept in alkaline solutions if treated appropriately at low temperature in the dark.

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