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Comparing Stability of Carthamin and Safflower Yellow Pigments at PH, Temperature and Light, from Safflower (*Carthamus tinctorius* L.) Florets

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Abstract: Safflower (*Carthamus tinctorius* L.) belongs to composite family. Its florets contain edible carthamin and safflower yellow dyes. The pigments are very useful for dying foods and cosmetics. The natural pigments also have medicinal properties. In this research, external factors suspecting to influence the chemical nature of carthamin and safflower yellow in aqueous media were studied. Dried florets were powdered and used for the extraction of carthamin which was obtainable through alkaline extraction, acidification and cellulose adsorption. Also, dried safflower powder was used for extraction of water soluble yellow pigment. Aqueous solutions of the pigments were exposed to some external factors such as temperature (10, 30, 50 and 70°C), pH (below 2 and above 7), light (400 Lux and UV). The results showed at higher temperature carthamin was more readily decomposed, but safflower yellow isn't affected so much by the temperature. Occurring above and below the pH range in which carthamin is most stable (pH 3-5.5), increased degradation of the pigment. The pigments in aqueous solution were exposed to dark, visible and ultraviolet light. Loss of safflower yellow coloration increased. These results were studied spectrophotometerically to check patterns of stability of the pigments. Comparing the pigments indicates safflower yellow is more stable than carthamin in temperature and pH treatment, but carthamin is more stable than safflower yellow in light treatment. Most synthetic pigments have carcinogenic properties, whereas natural pigments have biological value and belong to natural components of food products.

Key words: Carthamin, safflower yellow, external factors, spectrophotometeric, *Carthamus tinctorius*

INTRODUCTION

The dried petals of safflower (*Carthamus tinctorius* L., compositae) contained red and yellow pigments and have been used traditionally for dyeing fabrics, as food colorings and cosmetics and in painting s in Korea and other countries (Henry and Francis, 1996; Watanabe *et al*., 1997; Cho *et al*., 2000). The structures of safflower red and yellow pigments are reported as having unique structures of C-glycosyl quinochalcone moieties that exist only in *Carthamus tinctorius* (Meshly *et al*., 1993; Kazuma *et al*., 2000).

Natural colorants often are very sensitive to external factors and sooner or later, deteriorate from their original coloration. Temperature, UV-light, pH, gas phase, metal ions and certain chemicals are known to all be decisive instigators for facilitating colour bleaching (Kanehira *et al*., 1990).

Deterioration of colorants is induced especially when colorants are exposed to light, in which UV-light plays a leading role (Saito and Utsumi, 1996; Kanehira *et al*., 1990). Carthamin is very unstable in solution, especially when left at high temperature in day light (Kanehira *et al*., 1990). The sensitivity of carthamus pigments to atmospheric oxygen and UV light in air and/or to pH in aqueous solutions has been observed frequently during the course of extraction and purification of the colouring matter. Carthamin, especially in the Free State, gradually faded from normal red to reddish orange, orange yellow and finally to pale yellow in aqueous media below pH 6.5 above pH 7.0, it is readily dicoloured to a brownish yellow. Safflor yellows are changed to brownish-yellow substances under alkaline conditions (Takahashi *et al*., 1984).

However, because of an increasing use of petals of *C. tinctorius* for pharmaceuticals and food pigments the research of the external factors on the stability of the pigments is necessary. In Iran, safflower is cultivated mainly as an oilseed but valuable florets go to waste. In this study, we decided to determine the effects of some external factors on the preservation of chemical nature for the pigments.

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MATERIALS AND METHODS

Dry florets were purchased from a local market (in Iran). The chemicals used in the present study were obtained from the following sources: Cellulose microcrystalline Avicel® (2330). The spectrophotometer model was Biowave s2100 UV/VIS Diode Array spectrophotometer. The pHmeter model was Meltler delta 340. For UV irradiation, OMRONH 3CR was used.

Dye extraction: Extraction of water insoluble carthamin and yellow water-soluble pigment from safflower florets were essentially carried out as described previously (Kulkarni *et al*., 1997) but with some modifications as follows:

Extraction of carthamin: Fine dry floret powder (1 g) was suspended in 20 mL of 0.5 % w v^{-1} sodium carbonate and stirred at room temperature for 30 min. The floating pieces were removed by centrifugation at 3500 rpm for 15 min and the supernatant was retained at 5±1 °C. The resulting suspension was added to fresh 20 mL 0.5% sodium carbonate and stirred for further 30 min and centrifuged and this process was repeated for one more time. The cooled extracts were mixed together and was acidified to attain a pH of with 0.5% citric acid and used for adsorption of carthamin. Adsorption of carthamin from acid extract was performed using a modified method (Kulkarni *et al*., 1997). Cellulose powder (0.5 g) was suspended in acid solution, stirred with a magnetic stirring apparatus for 30 min at room temperature and centrifuged at 3500 rpm for 15 min. Supernatant was discarded. The pellet was resuspended in distilled water and centrifuged. The washing was repeated 5-6 times under the same conditions until a colorless supernatant was obtained. The pellet was suspended in 10 mL of acetone, intermixed for 5 min and then centrifuged for 5 min at 3500 rpm. The acetone layer was filtered and used for Spectrophotometeric measurement.

Extraction of safflower yellow: One gram of fine floret powder was suspended in 15 mL distilled water and stirred for 30 min. Floating pieces were removed by centrifugation and the supernatant was retained at 5±1 °C. The resultant suspension in distilled water was stirred for further 30 min and centrifuged. The supernatant was then filtered to separate suspended particles of floret powder.

Effect of the external factors

Temperature: Colour changes in carthamin and safflower yellow solutions were examined at 4 different temperature (10, 30, 50 and 70°C) by mixing 1 mL carthamin solution with 1 mL 50 mmol citrate/phosphate buffer pH 7.0 and 1 mL safflower yellow solution with 10 mL 50 mmol citrate/phosphate buffer pH 5.0 incubating for various times. Changes in absorbance were followed with a Biowave S2100 UV/VIS Diode Array Spectrophotometer at 520 nm for carthamin and 450 nm for safflower yellow.

pH values: The stability of the red and yellow coloration was observed in 1-70% (by vol.) formic acid and 0.001-25% Na_2CO_3 (or 50 mmol Na_2CO_3/$NaHCO_3$ buffer) at pH ranges below 2 and above 7. The incubation was started immediately after addition of 1 mL carthamin solution or safflower solution to 1 mL test medium for 30 min at 30°C and then used for spectrophotometer measurement at 520 nm for carthamin and 450 for safflower yellow.

Light irradiation: About 1 mL carthamin solution in 1 mL 50 mmol citrate/phosphate buffer pH 7.0 or 1 mL safflower yellow solution in 10 mL 50 mmol citrate/phosphate buffer pH 5.0 were irradiated with white (400 Lux) and UV light. And changes in absorbance at 0-300 min were measured by Biowave S2100 UV/VIS spectrophotometer.

Data analysis: The data were analyzed using Analysis of Variance (ANOVA). Means were separated by using the Tukey-MRT adjustment at $p = 0.05$. Linear lines were drawn to define all time-absorbance relation for each temperature. Values are the mean±SE of 3 replicates.

RESULTS AND DISCUSSION

Temperature is an external factor that influences on the stability of carthamin and safflower yellow (Fig. 1 and 2). The ultraviolet/visible spectrophotometric measurements are shown that the absorption decreases, suggesting that the destruction of carthamin is time-dependent. Carthamin losses its normal red color to orange-yellow at high temperature by increasing the time. But various temperatures hadn't considerably effect on safflower yellow degradation. Similar types of observation have also been reported by Kanehira *et al*. (1990), they found that the normal red coloration of carthamin solution preserved at less temperature and dark. Saito and Murata (1994) found that safflower yellow B is sensitive to temperature and easily decomposed at higher temperature (and pressure). This discrepancy may be related to lack of purification.

In Fig. 3, the expriments showed that acidic pH was more effectible on carthamin degradation and basic pH was less effectible. But at Fig. 4, the experiment showed that safflower yellow was more stable at acidic pH. Saito and Mori (1994) reported that carthamin is very stable at sodium carbonate solution. Yoon *et al*. (2003) found that safflower yellow is relatively stable to

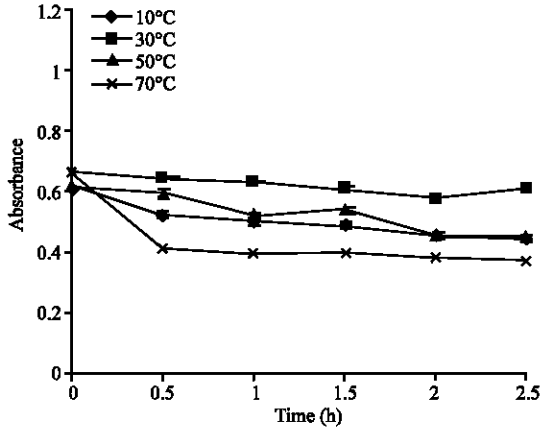

Fig. 1: Effect of various temperatures on carthamin stability in aqueous medium

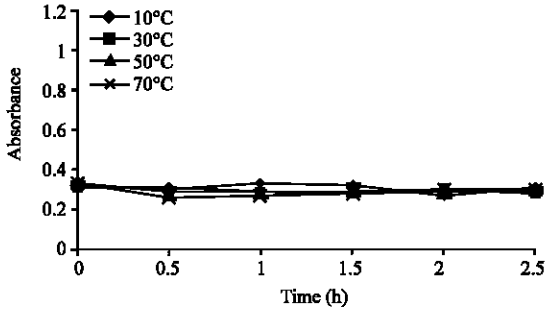

Fig. 2: Effect of various temperatures on safflower yellow stability in aqueous medium

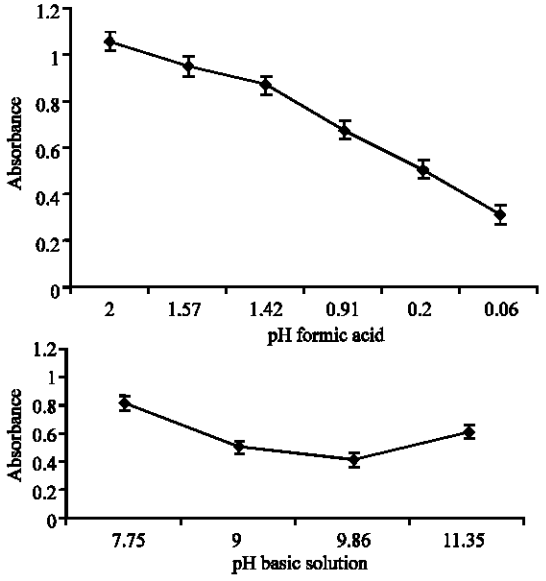

Fig. 3: Effect of pH on carthamin degradation

temperature at acidic pH and preservation of piments on basic pH is less than acidic pH. Kanehira *et al.* (1990) reported that in both of acidic pH and basic pH, carthamin readily lost its normal red coloration.

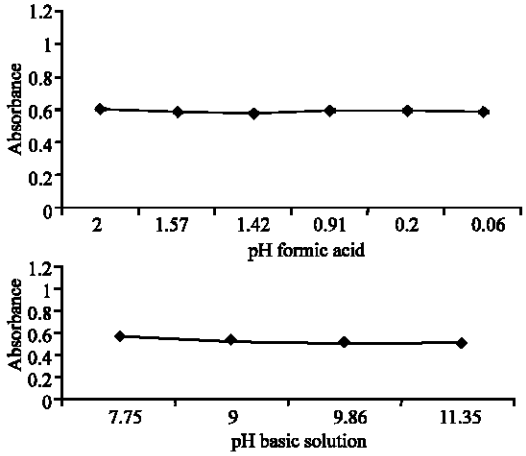

Fig. 4: Effect of pH on safflower yellow degradation

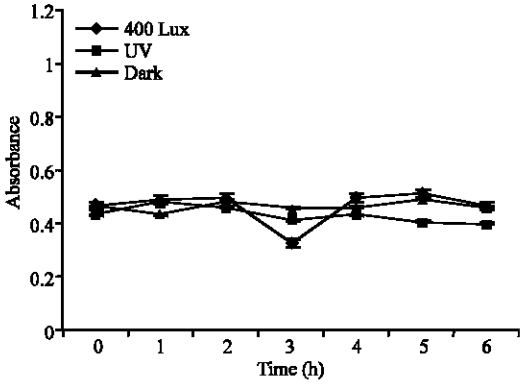

Fig. 5: Effect of light irradiation on carthamin stability

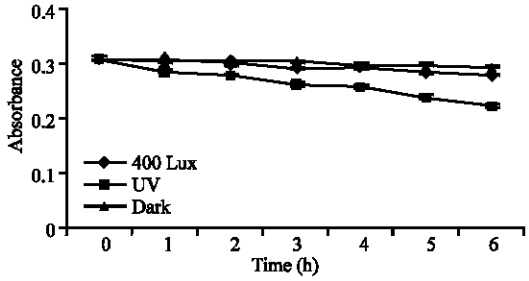

Fig. 6: Effect of light irradiation on safflower yellow stability

The stability of carthamin to dark, white and ultraviolet light was measured at room temperature as shown in Fig. 5. Light has not considerably changes during 6 h on the stability of carthamin. To some extent, carthamin was decreased by ultraviolet light. UV and 400 Lux light had not considerably effect on carthamin but UV light effects on the stability of safflower yellow and decreases it as shown in Fig. 6. Kanehira and Saito found the rate of the degradation depends on light sources. When UV-C light irradiated, the rate of degradation was

more than flourcent bulb. Saito and Murata (1993) reported that finally at higher temperature T UV light and pH are external factors on safflower yellow B degradation.

CONCLUSION

However, comparing of the pigments showed that the temperature effected on carthamin degradation and at high temperature carthamin was more degradated, but temperature hadn't much effect on the safflower yellow decomposition. In acidic pH, carthamin was more decomposed and in basic pH, safflower yellow was more decomposed. Light hadn't considerably effects on carthamin degradation but influenced at safflower degradation. UV light had most effects on safflower yellow decomposition. Comparison of the pigments showed that safflower yellow is more stable than carthamin at temperature and pH. But carthamin is more stable than safflower yellow at light irradiation.

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