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Spectrophotometric Measurement of Valuable Pigments from Petals of Safflower (*Carthamus tinctorius* L.) and their Identification by TLC Method

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Abstract: Safflower (*Carthamus tinctorius* L.) is a composite plant. It's flowers are an essential dye-stuff for preparing edible carthamin and safflower yellow dyes, which has been increasingly applied as a colour additive for processed foods as well as cosmetic rouges and medicinal tablets. A technique for the analysis of carthamin and Carthamus yellow is described. The teqnique involves the following 3 steps: Extraction, measurement of visible absorption spectrum of the color and thin-layer chromatography. Dried safflower powder was used for extraction of water soluble yellow and the water insoluble carthamin was obtainable through alkaline extraction, acidification and cellulose adsorption. The pigment extracts were identified by using thin-layer chromatography and spectrophotometery. Spectrophotometeric absorption spectra in invisible wavelength showed the absorption maxima for carthamin red at 520 nm in acetone extraction. Similarly water soluble safflower yellow showed 405 nm absorption maxima. The R_f values were measured by thin-Layer chromatography. Because these dyes are natural and have clinical effects, they are potentially useful for dying different food products.

Key words: Carthamin, safflower yellow, spectrophotometric, thin-layer chromatography

INTRODUCTION

The flower of safflower, *Carthamus tinctorius*, is utilized for producing medicines, as food colorants and natural red dye. Many investigations have disclosed the chemical structures of the yellow and red pigments contained in the flower petals (Kuroda 1930, Obara and Onodera 1979, Takahashi *et al*., 1982). In recent years, public concern over the use of synthetic dyes in foods has grown rapidly and the general public prefer the use of natural dyes in foods (Goda *et al*., 1997; Hirokado *et al*., 1999). Water soluble yellow and water insoluble red carthamin pigments can be extracted from safflower florets. These pigments have some medicinalvalue such as curative effect on coronary heart diseases, myocardial infection, cerebral thrombosis and some gynaecological uses (Kulkarni *et al*., 1997). In the future, natural food dyes will continue to be widely acceptable in food products due to their non-allergic and non-carcinogenic properties (Rudimetova *et al*., 2001). In Iran, safflower is cultivated mainly as an oilseed crop. Seeds are harvested but valuable florets go to waste. Hence, it was thought worthwhile to explore these florets for extraction of colorants that can be used in different food products. In this study, we decided to extract and identify the important pigments from safflower florets.

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), Kiselgel 60 F_{254} (1005554 Merck) Silica gel G (type 60) for thin layer chromatography (from Merk). The spectrophotometer model was Biowave s2100 UV/VIS Diode Array spectrophotometer.

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 (1gr) was suspended in 20 mL of 0.5% w v^{-1} sodium carbonateand 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

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ottain 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 andcentrifuged. 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, then centrifuged for 5 min at 3500 rpm. The acetone layer was filtered and used for Spectrophotometeric measurment.

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.

Spectrophotometric measurement: The spectrophotometric measurement of carthamin (acetone washing of reddishcellulose) and yellow pigment (water extract) was followed from 380-620 nm for carthamin and from 385-500 nm for safflower yellow.

Thin-layer chromatography: Thin-layer chromatographic identification was employed as reported by Rudometova *et al*. (2001). The R_f values of yellow pigment and the red carthamin were examined on silica gel. Two kinds of thin layer plates were used namely silica gel G and Kiselgel 60 F_{254}.The chromatographic solution consisted of distilled water: isobutanol: etanol: formic acid (4:7:4:4).

RESULTS AND DISCUSSION

The spectrum of extracts of samples have distinctive maximum peak oflight absorbing at 520 nm (Fig. 1). This peak is typical for carthamin extract (Saito and Takahashi 1985). For safflower yellow, maximum peak at 405 nm was obtained (Fig. 2). Similar type of observations have also been reported by Wu and Fu (1993). However, they found that optical density for yellow pigment is at 400 nm. On the other hand, Kulkarni *et al*. (1997) found that yellow pigment have maximum optical density at480 nm. This discrepancy may be related to varietal differences. For the aim of confirmation, the results of the spectrophotometeric analysis of extracts went through chromatic division on silica gel plates for thin-

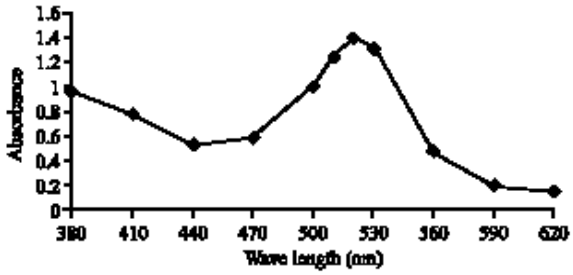

Fig. 1: Optical density of acetone washing of reddish cellulose

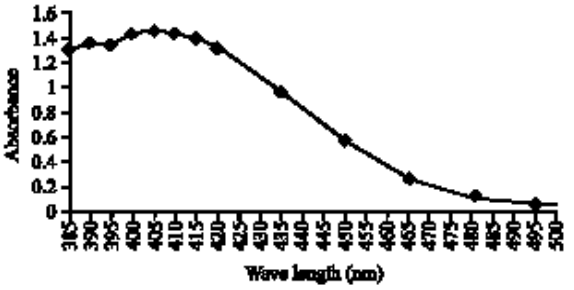

Fig. 2: Optical density of water soluble yellow pigment of safflower

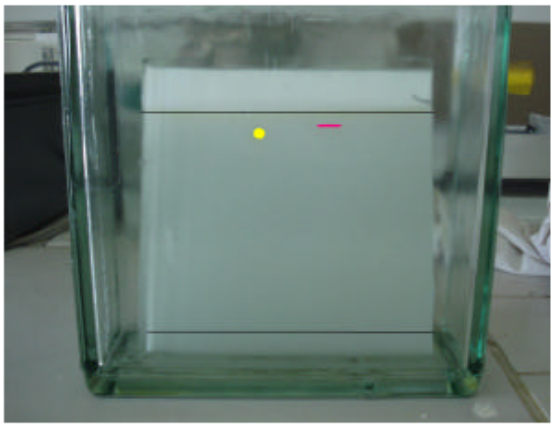

Fig. 3: Colorific pigments from safflower flowers in Thin-Layer Chromatogram using Silica gel type G

layer chromatography. Two different silica gels were examined:1) Silica gel G (type 60), 2) Kiselgel 60 F_{254}(1005554 Merck). The chromatogram of the extracts is presented in Fig. 3 and 4. In both types of gels, carthamin ascended in the form of a red horizontal line. But safflower yellow ascended in the form of a circular yellow spot for silica gel G and tailed spot for silicagel Kiselgel 60 F_{254} (1005554 Merck). The R_f values are given in Table 1. For different silica gels, R_f values were different. This can be connected with the sort of silica gel plates. These results are comparable with those obtained

Table 1: The results of chromatographic and Spectrophotometric research of carthamin and safflower yellow

Sample	Color	Maximum absorbance nm	Silica gele type	R_f values
Acetone washing of redish cellulose (carthamin)	Red	520	Silica gel G	0.93
			Kiselgel 60 F_{254}(1005554 Merck)	0.88
Water-soluble yellow pigment	Yellow	405	Silica gel G	0.85
			Kiselgel 60 F_{254}(1005554 Merck)	0.78

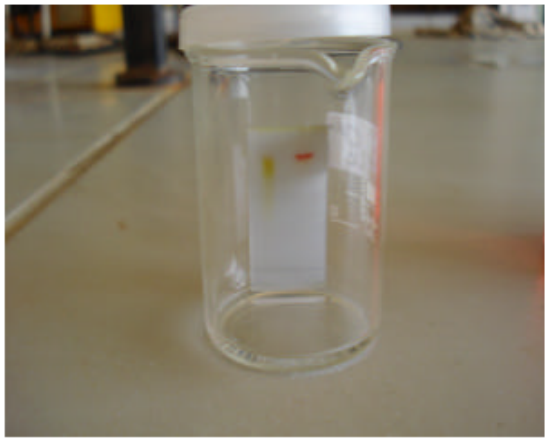

Fig. 4: Colorific pigments from safflower flowers in Thin-Layer Chromatogram using Kiselgel 60 F_{254}

by Rudometova *et al*. (2001).The experiments showed that some additional operations were needed to obtain carthamin in the concentration and purity needed for analysis. These were increasing the number of times for adding sodium carbonate to florets, increasing theamount of sorbent-cellulose powder to 1.0 g, increasing the number of repeatedelutions. Thus it is shown that the potential exist for carthamin and yellow pigmentto be extracted from safflower grown in Iran. These pigments are valuable as food dyes.

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