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# A Successful Talmudic-Flavored High-Performance Liquid Chromatographic Analysis of Carthamin from Red Safflower Dyeings

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### Introduction

The international flavor of this research is evident from the historical documentation and archaeological record pertaining to the usage of this plant in numerous geographical locations throughout various archaeological periods. It is believed that safflower originated in western Asia<sup>1</sup> or southern Asia,<sup>2</sup> and that it was known to the ancient Egyptians.<sup>3</sup> Brunello writes that safflower seeds, named *nas* in the texts of the VI Dynasty, have been found in the tombs of the Theban Period of 2000 BC.<sup>4</sup> It has been reported that the culture of safflower gradually extended from Egypt to the Mediterranean area much before the Roman era.<sup>5</sup> This plant was also well known to the ancient Greeks, who used its seeds for a purgative and adopted safflower as their ancient official color.<sup>6</sup> Safflower is mentioned in the writings of Aristotle, Theophrastus, and Galenus,<sup>7</sup> and in the *Talmud*. In antiquity, it was also cultivated in the Levant, Persia, India and China, and, according to Leggett, its earliest use was for food and medicine.<sup>8</sup> Japanese books on natural dyeing concur that safflower entered China about 2,000 years ago along the Silk Route and then made its way to Japan in about the 5th to 6th century.<sup>9</sup>

This annual thistle of the *Compositae* family grows to a height of about 1 m and was – and still is – valued for its seeds and for its petals.

Two colorants can be produced from the petals: a water-soluble yellow and an alkali-soluble pinkish red.<sup>10</sup> The names of these respective dyes are safflor yellow A (or safflomin-A) and carthamin,<sup>11</sup> also known as carthamic acid.<sup>12</sup> Both dyes are used for textile dyeing,<sup>13</sup> as food colorants and, especially carthamin, in the cosmetic industry.<sup>14</sup> The petals of this plant are sold today in Arab markets, and a common use of the ground florets by the local population is to sprinkle them as seasoning and colorant over freshly cooked rice. Throughout history, it was known that the seeds produce safflower oil. So popular has safflower become that a computer search on the internet yields thousands of citations for ‘safflower’. These web sites are mostly from various companies that deal with herbal or alternative medicine, and from a perusal of their information pages, it seems that safflower is a ‘panacea’ for everything that ails you or might ail you.

Although analyses of ancient textiles from the Middle East have not, as of now, detected the presence of the yellow or the red dye component, nevertheless the use of the red dyestuff is well known in the Far East. The oldest chemically confirmed presence of red safflower in a dyeing is in the Chinese Kesi silk panels, dating from the 10th to the 12th centuries.<sup>15</sup>

This paper reports on the first successful development and use of a high-performance liq-

uid chromatographic (HPLC) method for the separation and detection of carthamin and other red dyes extracted from red safflower dyeings. Previous investigators have detected the major red dye carthamin, also known as carthamic acid, extracted from red safflower dyeings by means of visible spectrophotometry<sup>16</sup> and by chemical tests.<sup>17</sup> HPLC was utilized in the past to analyze the main red colorant extracted from cultured cells from the tissues of flower buds before and after blossoming. In the current study, various solvent systems were tested to determine the most efficient one for stripping the red dyes from red cotton and silk dyeings from the Middle East and the Far East.

### What's in a name?

The etymology of the various appellations for safflower is important for tracing its movement from one geographical area into another throughout history. For researchers of everyday life, living habits, and crafts in ancient Israel and the surrounding regions during the Roman and Byzantine periods, the *Talmud* is an indispensable historical source of information. The *Talmud* is a multi-volume work, often difficult to understand, that discusses religious and civil laws relating to the Jewish population living in ancient Israel and also to those dispersed in Babylonia. It dates to the first five centuries of this era and was written mostly in Aramaic, using Hebrew characters, and in Hebrew. Thus it is necessary to combine this historical record, together with the chemical analyses of dyes from the Middle East, in order to get a more complete picture of the ancient technology associated with textile dyeing in that part of the world.<sup>18</sup>

The *Talmud* mentions safflower as a dye-plant in a number of different etymological forms, but there is no mention in the *Talmud* and related ancient Jewish writings as to whether the plant was used as a yellow or a red dyestuff, or as both. The thistle (*qôtzäh*) was described together with woad – Hebrew *isatis* (pronounced 'ee-sä-tees') or *istis* ('ees-tees') – and madder (*pooah*) in connection with textile

dyeing. The following citation refers to quantities of dyestuffs that should not be carried into the public domain – a work activity that should not be performed on the Shabbat, the day of rest. Hence, the *Talmud* states:<sup>19</sup>

(The prohibited quantities are for) *isatis*, *qotzah*, and *pooah*, enough to dye a small garment. And how much is a 'small garment'? – such as a hair-net.

A more piquant Talmudic account of the wondrous powers of this plant – with its different Aramaic names (shown in bold type below) – can be found within the discussion regarding various ailments, maladies and other 'indispositions', either real or allegorical, and their herbal remedies. The relevant citation follows:<sup>20</sup>

The Rabbis taught: If one 'uses his bed' [has sexual relations] while standing, then he will be seized with spasms. If, while sitting, then he will be seized with *däläriä* [diarrhoea or shortness of breath or impotence]. If she is on top and he is on the bottom, then he will (also) be seized with *dalaria*. ... Rabbi Joshua ben Levi said: The medication for dalaria is *därdärä*. What is *dardara*? Abbaye said, *môriqä* [yellow/green] of the thorns. Rabbi Pappa chewed and swallowed it (while) Rabbi Pappi chewed and spit it out.

Abbaye said: Whoever is not fit for the 'way of the world' [sexual relations], let him bring three *k'fiza* [small measure] of *kôrtem* of the thorns, and he should grind them and cook them in wine and drink it. Rabbi Yohanan said: (Indeed) it is they that returned me to my youth(ful sexual prowess).

According to the last-mentioned Talmudic discussion from nearly two millennia ago, the safflower plant was an important ingredient in that aphrodisiac potion, which may have served as the first completely Kosher Jewish 'Viagra'.

The Middle Eastern origin of this plant undoubtedly gave birth to its various etymological variants. Table 20.1 shows the similar names of this plant in different languages. The word

‘safflower’ is composed of two parts: ‘saf’ + ‘flower’. The second half of the word is obvious, and the first half (‘saf’) is borrowed from the Arabic word for the plant, ‘*usfur* (also ‘*asfar*’), which includes the first two consonants of ‘saf’. ‘Safflower’, whose beginning syllable is identical to ‘saffron’ (*Crocus sativus* L.), has often been confused with the latter. This confusion is not only etymological in origin, but also deliberate, as the safflower petals have been used as a cheap substitute and as a diluent for the much more expensive, and similar in appearance, saffron pistils. Both produce a yellow dye, which has been (and still is) used as a food color and as a seasoning. Hence, the use of the additional names ‘false saffron’ or ‘bastard saffron’ in various cultures for this ‘culprit’. Etymological ambiguities in differentiating between safflower and saffron also exist with the variant Aramaic and Hebraic names of these plants. For instance, the Aramaic *moriqa* has often been interpreted both as saffron and as safflower. However, in the author’s belief, the use of the word *moriqa* by itself refers to saffron, whereas the use of that word in conjunction with ‘thistle’ indicates safflower, as in the above Talmudic reference.

The Latin name (*Carthamus*) is derived from the Semitic form of that name (*qurtema* and similar variants), as seen in Table 20.1. In fact, the Semitic root *qrtn* means ‘cut’, ‘nip’, ‘truncate’, as, for example, in the act of collecting these thistle flowers.

### Dyeing with red safflower

In order to determine the solvent system of choice for the extraction of the red dye from textile dyeings, cotton and silk were dyed according to the recipe published in Schwebbe’s manual.<sup>21</sup> For each 100 g of textile (cotton or silk) to be dyed, 200 g of safflower florets were used with 3 L of water containing 50 g of soda. The entire procedure, from the yellow and red extractions to the dyeing, was performed at room temperature. The petals for this experiment were obtained in an Israeli-Arab market at Kafr ‘Anbata, and the yellow and red components were selectively extracted from the yellow-red florets according to the following scheme.

#### *Extraction of the yellow component from the petals.*

First the yellow colorant was removed from the florets by simple repeated overnight washings with fresh samples of water (about three to four), until the solution was no longer yellow in appearance.

#### *Extraction of the red component from the petals*

The florets were removed from the water extractions, squeezed, and placed in the propor-

**Table 20.1** Historical and popular names of safflower, *Carthamus tinctorius* L., in various languages.

<i>Scientific language</i>	
Colour Index <sup>a</sup>	Natural Red 26 & Natural Yellow 5
<i>Semitic and Arabic languages</i>	
Aramaic/Hebrew	Qurtama, Qurtema, Qurtam (in modern Hebrew); Dardara & Qotzah (thorn, thistle); Kharia; Moriqa (of the ‘thistle’)
Arabic	‘Usfur, ‘Asfar; Qurtum, Qorton; Khiri
Latin	Carthamus
English	Safflower, Dyer’s thistle, False saffron, Bastard saffron, Dyer’s saffron
<i>European languages<sup>b</sup></i>	
Italian	Cartamo, Zaffrone, Zaffranone, Zafferano bastardo, Asfore, Grogio
French	Carthame officinal, Faux safran, Graine de perroquet, Safran Bâtard, Safran d’Allemagne, Vermillon de Provence
German	Borstenkraut, Deutscher Saflor, Falscher Saffran, Färber-Saflor, Wilder Saflor, Türkische Saflor
Spanish	Cartamo, Azafaran bastardo, Alazor, Azafran romi

<sup>a</sup> *The Colour Index* 1971 (see note 12), p. 3227 for Yellow and p. 3240 for Red.

<sup>b</sup> Brunello 1973 (see note 3).

tionate quantities of water containing the sodium carbonate (which yielded a solution of pH 10.5 – 11.0) for one hour. The color of the florets turned light yellow and the resulting orange solution was filtered from the florets by means of a kitchen sieve. A sufficient amount of 10% citric acid was added until the pH of the resulting solution was between 4.0 and 5.0, at which point the solution's color changed to pink.

### *Dyeing of cotton and silk with the red colorant*

The previously obtained dye solution was then divided in order to dye cotton threads and a silk fabric by leaving each textile overnight in its solution while stirring the textile a number of times. The dyeing was then repeatedly rinsed the next day with cold water. The final rinsing was conducted with a little acetic acid and cream of tartar and the dyeing was then hung to dry.

### Extraction of the red component from a dyeing

Various solvents were tested at various temperatures for their ability to extract the red colorant from red safflower dyeings. In each case, 150  $\mu\text{L}$  of the solvent was used on a 1 cm cotton thread that was dyed in this study, and each sample was

treated to the solvent investigated for 10 minutes. Room-temperature extractions were performed by placing the thread in the solvent in an ultrasonic bath. For temperatures up to 100  $^{\circ}\text{C}$ , the sample was placed in a plastic, 1.5-mL Eppendorf vial and was secured in a boiling water bath. The test with N,N'-dimethylformamide (DMF) at 150  $^{\circ}\text{C}$  was performed with the dyed sample placed in a glass vial that was then inserted into an aluminum heating block. The efficiency of extraction was determined by a visual inspection of the color of the textile sample before and after extraction and of the color and depth of color of the resulting dye solution. These qualitative observations were sufficient for the determination of an efficient solvent system for the extraction of the red colorant from red safflower dyeings. This set of experiments is summarized in Figure 20.1.

The typical solvent system that has often been used for the extraction of red anthraquinonoid and yellow flavonoid dyes, hydrochloric acid (3M) and methanol, was also utilized in the current study on red safflower dyeings. However, the use of this solvent system resulted in a pale yellow solution, and left the dyed sample nearly the same color as before the attempted extraction. Thus, this acidic methanolic solvent system can only extract the yellow colorants still present in red safflower dyeings, but not the red carthamin colorant itself.

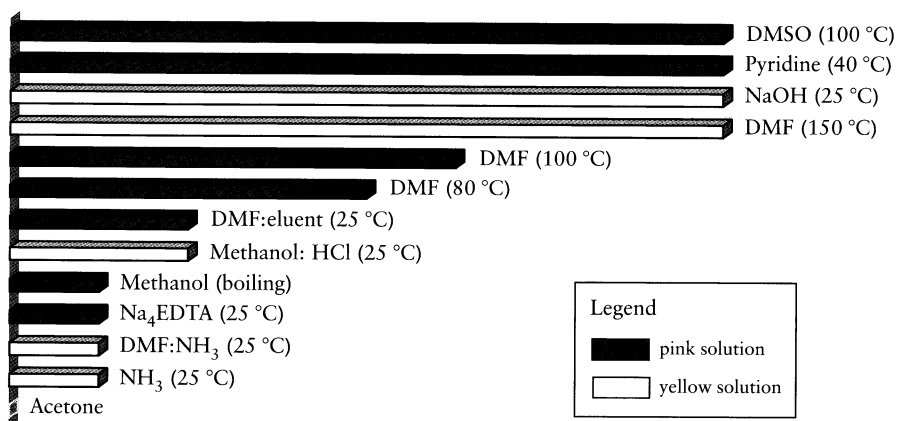


Figure 20.1 Comparison of the carthamin-extraction abilities of various solvents from a red safflower dyeing.

Acetone did not produce any visible extraction. Very weak extractions were obtained with the following systems: boiling methanol, an aqueous solution of the tetrasodium salt of ethylenediaminetetraacetic acid (EDTA), each of which produced very pale pink solutions, ammonia (1%), and an equivolume solution of DMF and ammonia (1%), the latter two producing very faint yellow solutions. An equivolume solution of DMF and the initial HPLC eluent composition, which consisted of methanol, water, and 5% phosphoric acid in the ratio 30:60:10, respectively, produced a faint pink solution.

More promising results were obtained with DMF at 80 °C and even better results at 100 °C. Using DMF near its boiling point of 153 °C stripped the red dye nearly completely from the textile fibers; however, during the course of the extraction process, the solution turned from pink to yellow. This indicated that the high temperature used decomposed the red dye to one or more yellow colorants. Sodium hydroxide (0.2M) at room temperature also stripped the red dye from the fibers, but produced a yellow solution. However, this solvent was not deemed a suitable candidate as its pH is too high for the subsequent HPLC analysis, and, in addition, it is more 'esthetically pleasing' to produce a red-colored solution from the extracted red dye, rather than the yellow solution actually obtained.

Warm pyridine is an excellent solvent for the extraction of the red dye. A room-temperature equivolume mixture of pyridine and water was used by Taylor.<sup>22</sup> However, pyridine's noxious and nauseating odor, which is detectable even with the use of a laboratory hood, presents problems for its use. Of all the solvents investigated in this study, it was found that the best system for the extraction of carthamin and its relatives was found to be dimethylsulfoxide (DMSO) at 100 °C. This is a low volatility liquid (high boiling point) and, with appropriate common-sense laboratory precautions, is safe to use. This solvent nearly completely stripped the red dye from the fibers, even with only one extraction, and produced a beautiful pink-red dye solution. Chemistry and esthetics were in harmony.

## HPLC analyses of the stripped red dye

In order to test the HPLC analysis method developed in this study for the detection of red safflower in a dyeing, three geographically different red safflower dyeings were analyzed by extracting the red dye with hot DMSO and injecting the filtered, extracted dye solution into the chromatograph. The samples investigated included the following:

1. A pink cotton thread, dyed in this study using local Israeli-Arab grown safflower.
2. A pink cotton thread (Leeds Collection No. A383), dyed using Chinese safflower by Su Grierson of Perth, Scotland.
3. A red silk fabric, dyed using Chinese safflower by Sachio Yoshioka and provided by Professor Masako Saito of Kyoritsu Women's University, Tokyo, Japan.

The Waters chromatographic system used in this study consisted of a 600E Controller Pump and a 996 Photodiode Array (PDA) detector, with a C18, 4  $\mu$ m, 3.9  $\times$  150 mm Nova-Pak column and a 10  $\mu$ L sample loop, with an eluent flow rate of 1 mL/minute. The HPLC gradient elution method used is the one used by the author for the detection of other red dyes from vegetal and insect sources.<sup>23</sup> This method consists of the following elution scheme:

Time (minutes)	Methanol %	Water %	H <sub>3</sub> PO <sub>4</sub> (5% w/v) %
0 $\rightarrow$ 1	30	60	10
1 $\rightarrow$ 3	30 $\rightarrow$ 60	60 $\rightarrow$ 30	10
3 $\rightarrow$ 15	60 $\rightarrow$ 90	30 $\rightarrow$ 0	10
15 $\rightarrow$	90	0	10

The HPLC chromatograms of these three samples are given in Figures 20.2, 20.3, and 20.4, respectively, and the ultraviolet (UV)-visible (UV-Vis) spectrum of the main red colorant, carthamin, obtained from the photodiode array (PDA) detector in each run is given in Figure 20.5. The retention times ( $t_R$ ), the relative retention times ( $RRT = t_R/t_{R,carthamin}$ ), and the visible  $\lambda_{max}$  data obtained from these chromatograms are summarized in Table 20.2. From the

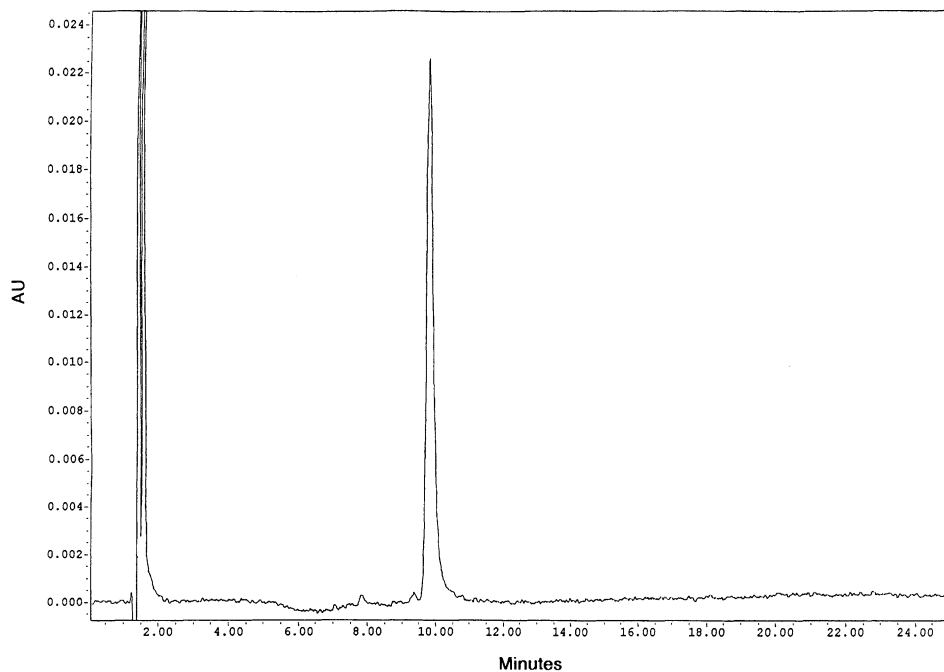


Figure 20.2 HPLC chromatogram (at 520 nm) of the hot DMSO extraction from an Israeli-Arabic red safflower dyeing.

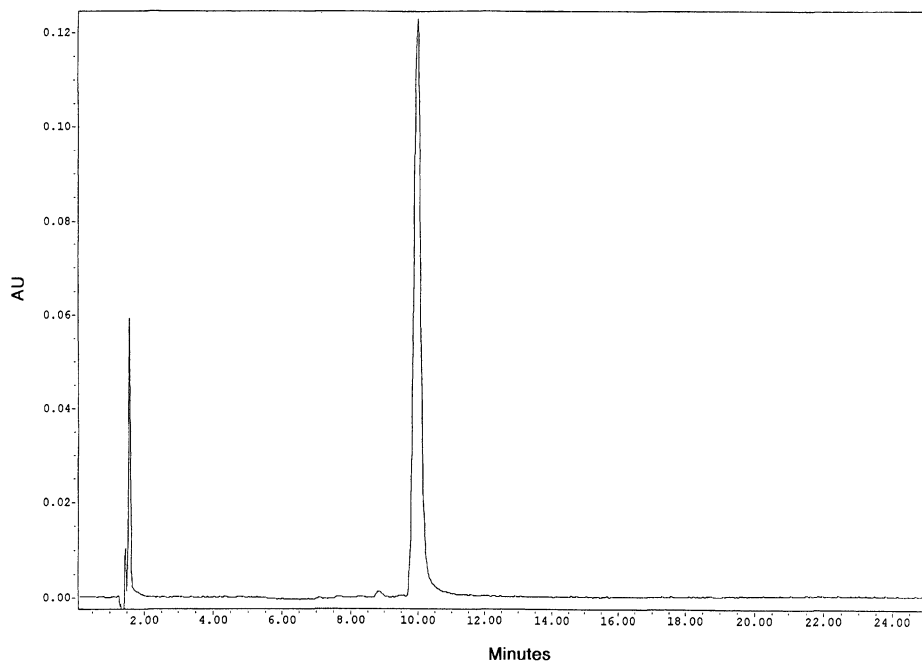


Figure 20.3 HPLC chromatogram (at 520 nm) of the hot DMSO extraction from a Scottish-Chinese red safflower dyeing.

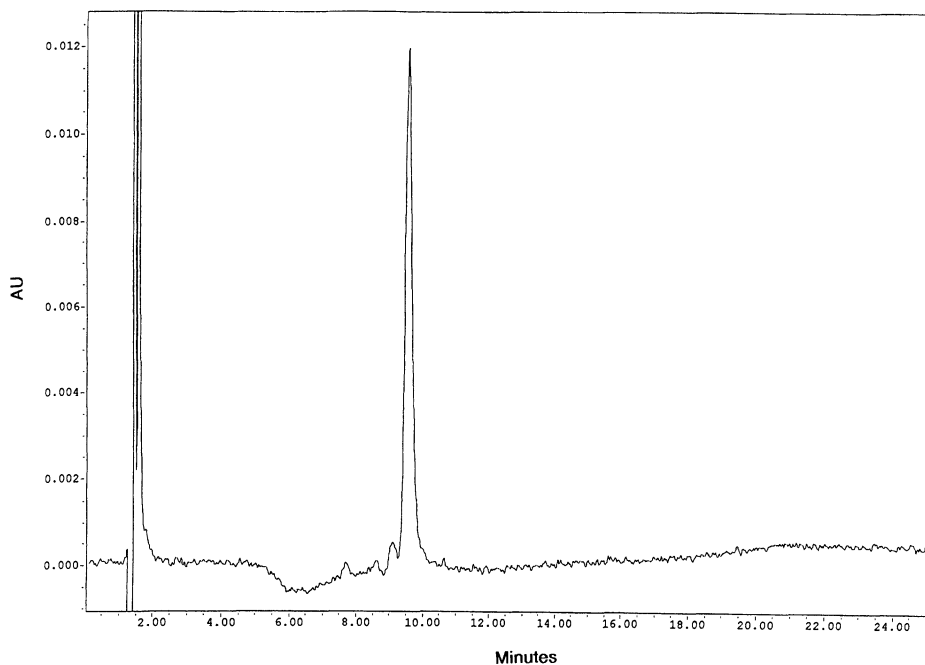


Figure 20.4 HPLC chromatogram (at 520 nm) of the hot DMSO extraction from a Japanese-Chinese red safflower dyeing.

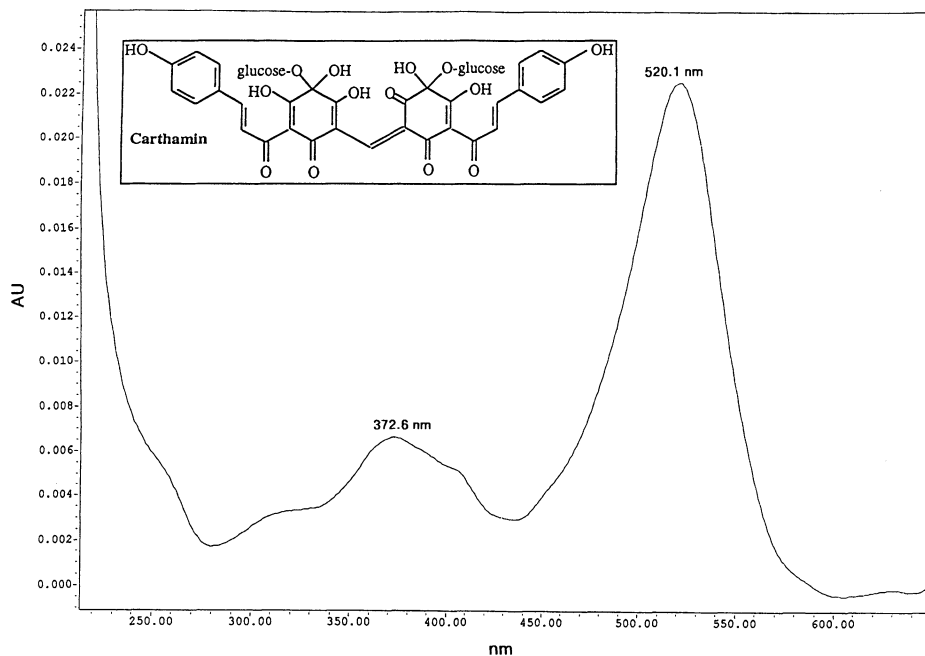
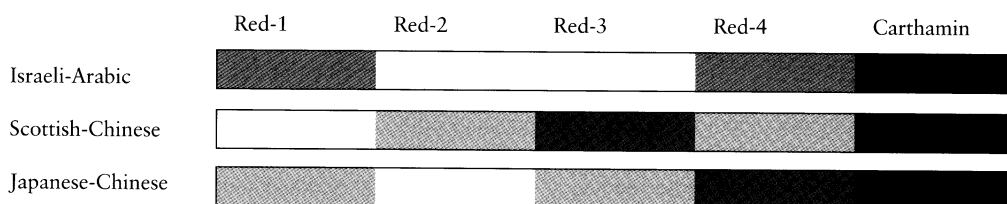


Figure 20.5 UV-Vis absorption spectrum of red carthamin as obtained by the PDA detector of the HPLC.

**Table 20.2** HPLC analyses of DMSO extractions of red components from red safflower dyeings.

Internal lab code	Source of dyeing	Retention time in minutes (Relative Retention Time), visible $\lambda_{\max}$ in nm				
Safflower locl, #174, S4	Israeli-Arabic (cotton)	7.842 (0.798), 535.9		9.375 (0.954), 522.5	9.824 (1), 521.3	
Safflower (1), #176, S5	Scottish-Chinese (cotton)		8.263 (0.831), 495.8	8.850 (0.890), 511.6	9.473 (0.952), 524.9	9.947 (1), 520.1
Safflower (DMSO), #177, S6	Japanese-Chinese (silk)	7.714 (0.808), 532.2		8.598 (0.901), 520.1	9.115 (0.955), 520.1	9.548 (1), 520.1


**Figure 20.6** Comparison of the presence of various red dyes in safflower dyeings from different geographical regions.

near proportionate identity of the retention times and of the UV-Vis spectra of the main red colorant (carthamin) detected in each run, it is obvious that all three samples analyzed were in fact dyed with red safflower.

This analytical method is very sensitive and can also detect the minor red and yellow components present in each dyeing, as seen from the chromatograms. These minor reds and yellows may be the key to identifying the geographical and botanical provenance of the particular safflower dyeing, although other factors such as soil conditions, dyeing procedure, etc., need to be considered. From Table 20.2 and Figure 20.6, it can be seen that the three different dyeings do have characteristic identifiable components that may help in ascertaining the origin of the dyeing. A total of five different red dyes were detected in this study and their relative retention times are presented in Table 20.2. The correspondence between the geographical origin and the particular red components present is presented in Figure 20.6. It can be seen that all three samples contain the same two dyes, carthamin, and a dye that elutes just before it, Red-4. The Israeli-Arabic dyeing can be identified as it is the only dyeing that is missing two

dyes (Red-2 and Red-3). The Scottish-Chinese dyeing can be characterized as it is deficient in only Red-1, and the Japanese-Chinese is unique in that it is only missing Red-2. Other chromatographic features can be used in addition to the relative retention time parameters, such as the relative absorption areas of the component peaks. These integrations are presented by the darkness of each area in Figure 20.6, i.e., the darker the area the greater its relative composition. Of course, in order to determine unambiguously the geographical and botanical origins of any plant dyestuff, a variety of parameters must be considered, such as soil and climate conditions.

## Conclusion

This investigation has shown that the hot DMSO extraction procedure and the HPLC method described in this paper provide an excellent technique for the clear and efficient analysis of red safflower dyeings. This study is the first successful HPLC analysis of carthamin and other minor red components detected in red safflower dyeings.

### Acknowledgments

I would like to express my deep appreciation to the Dr. Sidney M. Edelstein Foundation for support of this research. The dyed samples provided by Su Grierson and Masako Saito have been invaluable. I would also like to express my gratitude to the assistance that was provided by my student, Mubeen Beidusi, in this research.

### Editor's note

This paper was presented at the 17th Meeting of *Dyes in History and Archaeology*, Greenwich, London, 1998. Address for correspondence: The Edelstein Center, Shenkar College of Engineering and Design, 12 Anna Frank Street, 52526 Ramat-Gan. Paper received 26 March 1999.

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