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HPLC quantification of major active components from 11 different saffron (*Crocus sativus* L.) sources

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Abstract

Eleven certified saffron samples (*Crocus sativus* L.), one each from Azerbaijan, China, Greece, France, India, Iran, Italy, New Zealand, Spain, Turkey and the Sigma Chemical Company, were analyzed by using an HPLC photodiode array detection method. This analysis quantified the 10 major saffron compounds in each sample and their concentration was analyzed at three different wavelengths. Results indicated that the Greek, Indian, New Zealand, and Spanish saffron extracts possessed the highest concentrations of water-soluble glycosidic carotenoids ($\geqslant$8.0%) suggesting that they could be a good source of this type of metabolites for further biological evaluation.

Keywords: Saffron; *Crocus sativus* L.; Quantitative analysis; HPLC; Glycosidic carotenoids; Crocins

1. Introduction

Saffron is the dried stigmas of a flower scientifically identified as *Crocus sativus* L. Although the source of saffron is unknown, it apparently originated in the area of Iran, Turkey and Greece, but now it is also successfully cultivated in such European countries as Spain, Italy, France, and Switzerland, as well as in Morocco, Egypt, Israel, Azerbaijan, Pakistan, India, New Zealand, Australia and Japan. While the world's total annual saffron production is estimated to be 190 tons, Iran produces about 90% of the total (Fernandez, 2004; Negbi, 1999; Xuabin, 1992).

Since ancient times, saffron has been used as a medicinal plant and a culinary spice (Basker & Negbi, 1983; Rios, Recio, Giner, & Mañez, 1996; Zhou, Sun, & Zhang, 1978). The stigmas contain carbohydrates, minerals, vitamins and such pigments as carotenes, and flavonoids (Abdullaev, 1993; Winterhalter & Straubinger, 2000). The bitter-taste is produced by the picrocrocin ($C_{16}H_{26}O_7$), a monoterpene glycoside precursor of safranal ($C_{10}H_{14}O$), the main volatile oil responsible for the aroma. β-Glucosidase action on picrocrocin liberates the aglycone, 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC, $C_{10}H_{16}O_2$; MW 168), which is then transformed to safranal by dehydration during the drying process of the plant material (Lozano, Delgado, Gómez, Rubio, & Iborra, 2000; Sujata, Ravishankar, & Venkataraman, 1992).

The colour of saffron comes from the water-soluble glycosidic *cis*- and *trans*-carotenoids crocins, glucosyl esters of crocetin (8,8′-diapocarotene-8,8′-dioic acid; $C_{20}H_{24}O_4$). For commercial purposes the quality of the colouring power of a saffron sample depends on the quantification of the crocin analogues by colorimetric (Li, Lin, Kwan, & Min, 1999) and chromatographic techniques, such as TLC, GC-MS, and HPLC (Zareena, Variyar, Gholap, & Bongirwar, 2001).

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Several methodologies have been described using different analytical tools to establish purification protocols for the bioactive constituents of saffron, i.e. picrocrocin, safranal and crocins (Castellar, Montijano, Manjón, & Iborra, 1993; Iborra, Castellar, Canovas, & Manjón, 1992; Sujata et al., 1992; Tarantilis, Polissiou, & Manfait, 1994; Tarantilis, Tsoupras, & Polissiou, 1995). The chemical composition of saffron samples from Spain (Iborra et al., 1992; Lozano, Castellar, Simancas, & Iborra, 1999; Lozano et al., 2000), Greece (Kanakis, Daferera, Tarantilis, & Polissiou, 2004; Tarantilis et al., 1994; Tarantilis & Polissiou, 1997; Tarantilis et al., 1995), China (Li & Wu, 2002; Li et al., 1999), India (Sujata et al., 1992; Zareena et al., 2001), Germany (type *Electus pulvis*) (Straubinger, Bau, Eckstein, Fink, & Winterhalter, 1998; Straubinger, Jezussek, Waibel, & Winterhalter, 1997), and Iran (Bolhasani, Bathaie, Yavari, Moosavi-Movahedi, & Ghaffari, 2005) indicated that the estimated amounts of constituents strongly depended on the methods employed for drying, extraction, separation and quantification.

There are however, no reports analysing and comparing the chemical composition of four or more saffron samples originating from different locations (Li et al., 1999; Lozano et al., 1999; Zalacaín, Díaz-Plaza, Blázquez, Carmona, & Alonso, 2004). Therefore, the aim of the present investigation was to develop an HPLC analytical protocol useful for the identification and quantification of the major components in 11 commercial samples of saffron from different countries for quality control comparison. Up-scaling the same protocol permits preparative level isolation of the major compounds for biological activity investigation.

2. Materials and methods

2.1. Plant materials and chemicals

Certified dried saffron stigma samples were obtained from plants harvested in 2003 from each of the following countries: Azerbaijan (Belgya, Apsheron), China (Tibetan saffron), France (Poitou), Greece (Krokos Kozanis), India (Baby Brand), Iran (Abbaszadeh, Reg. No. 70070), Italy (Cooperativa Altopiano di Navelli), New Zealand (Eight Moon Saffron, Rangiora), Spain (La Mancha) and Turkey (No brand name). All samples were directly provided by local expert saffron growers in order to avoid any adulteration with foreign plant material. In addition, a commercial sample was purchased from Sigma (Product S8381, Sigma–Aldrich Corp., St. Louis, MO, USA). The material was stored in our laboratory in the dark at 4 °C until processed. 2-Nitroaniline (Sigma) was used as the internal standard for HPLC analysis. Methanol and acetonitrile were of HPLC grade (Merck KGaA, Darmstadt, Germany). Water was distilled, deionized and further purified through an ultrafiltration system (Easypure RF, Barnstead Thermolyne, Dubuque, IA, USA).

2.2. Extraction

Saffron stigmas (50 mg) were suspended in 10 ml of methanol–water (50:50, v/v) and magnetically stirred during 24 h at 4 °C in the dark. After extraction, samples were centrifuged at 30,000*g* for 20 min to eliminate plant residues and then the supernatant was collected and filtered through a nylon membrane (Acrodisc 13, 0.45 μm pore size × 13 mm diameter, *Waters*, Milford, MA, USA) (Lozano et al., 1999). Before quantitative chromatographic analysis, 1 ml of 2-nitroaniline (0.5 mg/ml) was added as an internal standard to 1 ml of each tested sample (Caballero-Ortega et al., 2004). For preparative HPLC, 20 mg of the Greek sample were pulverized and suspended in 1 ml of methanol–water (50:50, v/v) and extracted as described above.

2.3. HPLC equipment

HPLC analysis was performed on a multisolvent delivery system (*Waters*, Milford, MA, USA) equipped with a pump (Model 600E), a multiple UV wavelength photo-diode array detector (Model 996), linked to a computer system (Optiflex GX280, *Dell Computer Corp.*). Empower Software 2002 (Waters) was used for equipment control, data acquisition, and processing of the chromatographic information.

2.4. Quantitative determination

A Spherisorb RP C_{18} column (*Waters*, Milford, MA, USA, 25 cm length, 4.6 mm internal diameter, 10 μm particle size with a pore diameter of 80 Å) was used for all analyses. A linear gradient of methanol (10–100%) in water (15% of acetonitrile) was used as a mobile phase with a flow-rate of 1.0 ml/min for a maximum elution time of 60 min at room temperature. The sample size was 50 μl of the test solution (Abdullaev et al., 2002). The analyses were triplicated for each sample. Picrocrocin, HTCC and kaempferol were detected at 250 nm, safranal at 310 nm and all crocins at 440 nm, whereas the internal standard was detected at the above three mentioned wavelengths (Lozano et al., 1999). For the internal standard, a calibration curve was prepared using final concentrations of 0.015, 0.031, 0.062, 0.125, 0.25, 0.5, and 1.0 mg/ml in triplicate (Lindsay, 1992). Quantitative determinations were made taking into account the molecular coefficient absorbance of each peak obtained at the wavelength of maximum absorbance of the respective ingredient as previously reported (Lozano et al., 1999) and they are expressed in milligrams per gram of saffron stigmas. The R^2 range was from 0.9833 to 0.9990 and the slope R.S.D. values between injected samples were lower than 5%.

2.5. Preparative analysis and spectrometric identification of pure compounds

A Spherisorb RP C_{18} column (Waters, 25 cm length, 20 mm internal diameter, 10 μm particle size with a pore

diameter of 80 Å) was used for this analysis. The above-mentioned linear gradient was used with a flow-rate of 10 ml/min during an elution time of 60 min. The amount of sample injected was 500 μl of the test solution. Eluates across the peaks with t_R of 8.3 (picrocrocin), 16.0 (HTCC), 17.8 (3-gentiobiosyl-kaempferol), 25.4 (*trans*-crocin 4), 28.7 (*trans*-crocin 3), 31.9 (*trans*-crocin 2′), 35.0 (*cis*-crocin 4), 35.9 (safranal), 38.1 (*trans*-crocin 2), and 44.4 (*cis*-crocin 2) were collected by the technique of heart cutting (Hostettmann, Marston, & Hostettmann, 1998). Each collected peak was rapidly evaporated under vacuum using a centrifugal solvent evaporator system (CentriVap, Labconco, MO, USA) and further submitted to low resolution fast atom bombardment mass spectrometry (LRFAB-MS) analysis. Positive-ion LRFAB-MS was registered using a glycerol matrix on a SX102A spectrometer (JEOL, Tokyo, Japan). Xenon was used as collision gas. The accelerating voltage was 10 keV, and the resolving power was 3000. Mass spectra were determined in duplicate and the pseudomolecular ion $[M + Na]^+$ was detected for each peak. The observed fragmentation pattern was used for crocin identification by comparison with previously reported spectra (Carmona et al., 2005).

3. Results and discussion

3.1. Qualitative analysis

The chemical composition of saffron samples from 11 different sources was determined using reverse phase C_{18} HPLC. The chromatographic conditions employed allowed identification of 10 major components in each sample and a well-resolved baseline separation was obtained. Each component was identified by comparison of its retention time as previously described in the literature (Li et al., 1999; Lozano et al., 1999; Tarantilis et al., 1995) as well as by LRFAB-MS analysis through the detection (m/z) of its corresponding pseudomolecular ion $(M + H)^+$ (Carmona et al., 2005; Tarantilis et al., 1995). Fig. 1 shows three representative chromatograms, one with the highest concentration of analysed glycosidic carotenoids (1A), the second as an example for the Sigma product (1B) and a third one showing the profile for the Tibetan sample (1C). The peak identification is as follows: number 1, 2 and 3 detected at 250 nm were picrocrocin, HTCC and 3-gentiobiosyl-kaempferol, respectively; peak number 7 (310 nm) was safranal and peak numbers 4 to 6, and 8 to 10 (440 nm) were *trans*-crocin 4, *trans*-crocin 3, *trans*-crocin 2′, *cis*-crocin 4, *tran*-crocin 2, and *cis*-crocin 2, respectively. According to our analysis different saffron samples did not differ in their chemical composition, but did differ in the concentration of each component.

3.2. Quantitative analysis

Table 1 shows the concentration of each detected compound in the 11 tested samples. The results indicate

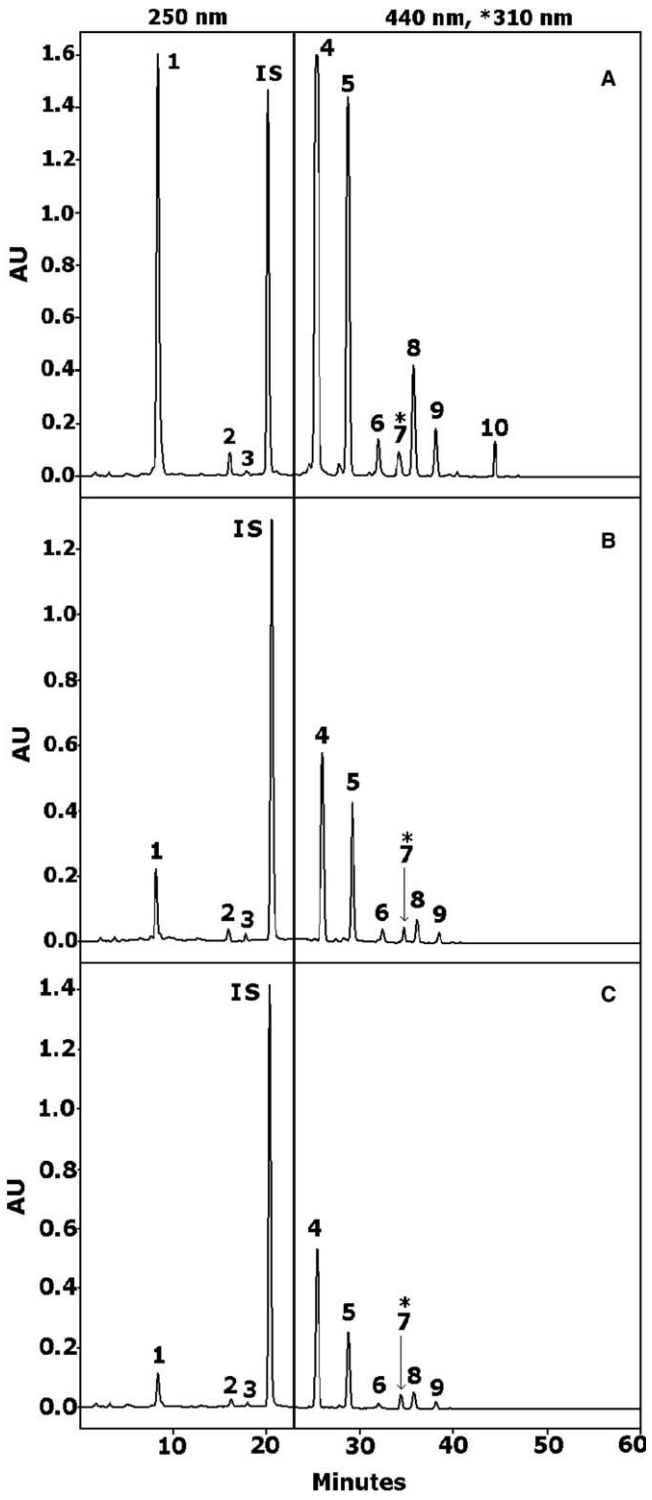

Fig. 1. HPLC fingerprint chromatograms of saffron samples from Greece (cultivated sample) [A], Sigma Product S8381 [B], and China (non-cultivated sample) [C], recorded at three wavelengths. A Waters Spherisorb C_{18} column, a linear gradient of methanol (10–100%) in water (15% of acetonitrile), and a flow rate of 1.0 ml/min were used for quantitative determinations. Saffron components: picrocrocin (peak 1); HTCC (peak 2); 3-gentiobiosyl-kaempferol (peak 3); *trans*-crocin 4 (peak 4); *trans*-crocin 3 (peak 5); *trans*-crocin 2′ (peak 6); safranal (peak 7); *cis*-crocin 4 (peak 8); *trans*-crocin 2 (peak 9); *cis*-crocin 2 (peak 10). IS: internal standard, 2-nitroaniline.

Table 1
HPLC quantitative analyzes of 10 saffron metabolites from 11 different saffron sources

Compound	Sample (mg/g of stigmas)										
	Greece	India	New Zealand	Spain	France	Azerbaijan	Italy	Turkey	Iran	Sigma	China
Picrocrocin	5.95 ± 0.053	7.87 ± 0.064	7.90 ± 0.578	8.14 ± 0.407	5.97 ± 0.119	3.34 ± 0.223	5.80 ± 0.311	5.67 ± 0.132	3.69 ± 0.008	0.83 ± 0.068	0.53 ± 0.027
HTCC	0.17 ± 0.100	0.32 ± 0.007	0.28 ± 0.010	0.13 ± 0.010	0.21 ± 0.008	0.13 ± 0.004	0.07 ± 0.003	0.42 ± 0.025	0.20 ± 0.003	0.02 ± 0.004	0.03 ± 0.002
3-Gentiobiosyl-kaempferol	0.14 ± 0.005	0.16 ± 0.006	nd	nd	0.25 ± 0.031	0.04 ± 0.003	0.04 ± 0.004	0.07 ± 0.004	0.19 ± 0.007	0.04 ± 0.003	0.03 ± 0.003
Safranal	1.29 ± 0.051	1.24 ± 0.084	0.47 ± 0.067	0.88 ± 0.125	0.81 ± 0.049	0.98 ± 0.059	0.53 ± 0.049	0.84 ± 0.093	0.65 ± 0.064	0.35 ± 0.054	0.22 ± 0.050
trans-crocin 4	40.77 ± 0.420	37.54 ± 0.001	41.21 ± 0.197	38.41 ± 0.580	38.43 ± 0.188	39.08 ± 1.697	38.25 ± 2.062	36.35 ± 2.160	38.41 ± 0.685	6.53 ± 0.091	6.29 ± 0.001
trans-crocin 3	30.36 ± 0.001	22.13 ± 0.001	31.26 ± 0.457	24.43 ± 0.108	27.74 ± 0.001	27.25 ± 0.001	28.28 ± 0.355	25.32 ± 1.096	23.58 ± 1.371	4.00 ± 0.001	2.44 ± 0.001
trans-crocin 2′	2.16 ± 0.001	1.01 ± 0.001	1.32 ± 0.001	0.92 ± 0.001	1.27 ± 0.001	1.16 ± 0.001	0.69 ± 0.001	0.58 ± 0.001	1.15 ± 0.001	0.11 ± 0.001	>0.0 ± 0.001
cis-crocin 4	10.14 ± 0.120	9.10 ± 0.001	0.31 ± 0.001	5.76 ± 0.001	5.89 ± 0.001	7.49 ± 0.001	2.31 ± 0.001	5.21 ± 0.001	4.73 ± 0.001	0.53 ± 0.001	0.31 ± 0.001
trans-crocin 2	2.84 ± 0.001	2.61 ± 0.001	0.05 ± 0.001	2.12 ± 0.001	1.72 ± 0.001	2.09 ± 0.001	0.64 ± 0.001	1.32 ± 0.001	1.33 ± 0.001	0.03 ± 0.001	>0.0 ± 0.001
cis-crocin 2	0.23 ± 0.001	3.29 ± 0.001	0.46 ± 0.001	2.21 ± 0.001	>0.00 ± 0.001	0.09 ± 0.001	1.85 ± 0.001	0.95 ± 0.001	0.12 ± 0.001	nd	nd
Total	94.06 ± 0.07	85.25 ± 0.02	83.27 ± 0.13	83.02 ± 0.12	82.28 ± 0.04	81.65 ± 0.20	78.45 ± 0.28	76.72 ± 0.35	74.04 ± 0.21	12.43 ± 0.02	9.86 ± 0.01

Instrumental conditions as described in Fig. 1. Results are expressed as means ± standard deviations.
nd: not detected.

that the differences might be due to the origin of the sample, to the dissimilar drying processes possibly involving varied time periods, as well as to storage conditions. Greek saffron had the highest total concentration of components (94.06 mg/g of stigmas) followed in order by the Indian, New Zealand, Spanish, French, Azerbaijanian, Italian, Turkish, and Iranian saffron extracts, while the sample purchased from Sigma (12.43 mg/g of stigmas) as well as the Tibetan variety from China (9.86 mg/g of stigmas) showed the lowest. Although, Sigma described their product as authentic Spanish saffron, it had low concentration of components in comparison to the sample analysed from La Mancha, Spain. Greek saffron had the highest concentrations of *trans*-crocin 2′, 2 and *cis*-crocin 4; New Zealand saffron had the highest concentration of *trans*-crocin 4 and 3; *cis*-crocin 2 was present in large amounts in the Indian sample. This variation could be the result of different drying processes used, or the time and conditions under which the plant product was packed and stored in each country, all of which could affect the concentration of glycosidic carotenoids as they are thermally labile and photochemically sensitive components (Tarantilis et al., 1995). In case of Tibetan saffron, the lower quantities of components could be due to the shorter and non-protruding stigmas found in this non-cultivated sample collected in the field (Tibetan Medicine Encyclopaedia).

Table 1 also shows that picrocrocin, as precursor of the saffron aroma components (safranal and HTCC), was present in large amounts in both the Spanish and New Zealand samples (8.14 and 7.9 mg/g of stigmas, respectively), while the highest concentration of HTCC was found in the Turkish sample (0.42 mg/g of stigmas). Previously obtained results from Spanish saffron also reported a similar picrocrocin and HTCC concentration (Iborra et al., 1992). 3-Gentiobiosyl-kaempferol was not detected in either the Spanish or the New Zealand sample.

To obtain and quantify HTCC and safranal, different strategies as microsimultaneous hydrodistillation-extraction (MSDE), ultrasound-assisted extraction (USE), steam distillation (SD), vaccum head space method (VHS), gas chromatography (GC) (Kanakis et al., 2004) and high-performance liquid chromatography (HPLC) have been employed (Iborra et al., 1992; Lozano et al., 1999; Tarantilis et al., 1994, 1995,1997). These components are present in very small amounts in the fresh stigma and its degree of concentration strongly depends on drying and storage conditions (Lozano et al., 2000). The high concentration of safranal in the tested Greek sample agrees with a previous report describing it as a spice rich in safranal. Kanakis and collaborators (2004) reported that the amount of safranal obtained by MSDE and USE were in the range of 288.1–687.9 and 40.7–647.7 mg/100 g, respectively. Our results (Table 1) on 100 g basis for each tested samples, show similar data.

Quality control for crocins has been obtained by HPLC assays (Li et al., 1999; Tarantilis et al., 1995). Our results in Table 1 show that *trans*-crocin 4 is the

most abundant ingredient in all saffron samples followed by *trans*-crocin 3 and *cis*-crocin 4, although present in all of the cultivated samples, *trans*-crocin 2, 2′, and *cis*-crocin 2 were produced in lower concentrations. Crocins, as other carotenoids, are stable only in their natural form and are subject to considerable degradation when isolated (Tarantilis et al., 1994), perhaps explaining the different component ratios found in the tested samples. It is evident that a relationship between the country of origin and the composition based only on our results is not possible since environmental, genetic (e.g. varieties) and cultivation practices affect the chemical composition of saffron. Investigations demonstrated that crocins inhibit different types of tumor cell growth (Abdullaev, 2002; Abdullaev et al., 2002, 2003, 2004), which could suggest that saffron with high carotenoid concentration may be a source for antitumor agents. New biotechnological techniques and approaches should be used to produce high quality saffron in large quantities at reduced production cost. Finally, the simple and specific HPLC method which this study developed can be used for the quality control that allows for quantification of the major biologically active glycosidic carotenoids in different saffron samples.

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References

Abdullaev, F. I. (1993). Biological effects of saffron. *BioFactors, 4*, 83–86.

Abdullaev, F. I. (2002). Cancer chemopreventive and tumoricidal properties of saffron (*Crocus sativus* L.). *Experimental Biology and Medicine, 227*, 20–25.

Abdullaev, F. I., Caballero-Ortega, H., Riverón-Negrete, L., Pereda-Miranda, R., Rivera-Luna, R., Hernández, J. M., et al. (2002). Evaluación in vitro del potencial quimiopreventivo del azafrán. *Revista de Investigación Clínica, 54*, 430–436.

Abdullaev, F. I., & Espinosa-Aguirre, J. J. (2004). Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials. *Cancer Detection and Prevention, 28*, 426–432.

Abdullaev, F. I., Riverón-Negrete, L., Caballero-Ortega, H., Hernández, J. M., Pérez-López, I., Pereda-Miranda, R., et al. (2003). Use of in vitro assays to assess the potencial antigenotoxic and cytotoxic effects of saffron (*Crocus sativus* L.). *Toxicology In Vitro, 17*, 731–736.

Basker, D., & Negbi, M. (1983). The use of saffron. *Economic Botany, 37*, 228–236.

Bolhasani, A., Bathaie, S. Z., Yavari, I., Moosavi-Movahedi, A. A., & Ghaffari, M. (2005). Separation and purification of some components. *Asian Journal of Chemistry, 17*, 725–729.

Caballero-Ortega, H., Pereda-Miranda, R., Riverón-Negrete, L., Hernández, J. M., Medécigo-Ríos, M., Castillo-Villanueva, A., et al. (2004). Chemical composition of saffron (*Crocus sativus* L.) from four countries. In J. A. Fernández & F. I. Abdullaev (Eds.), *Proceedings of the first international symposium on saffron biology and biotechnology*. *Acta Horticulturae* (Vol. 650, pp. 321–326). Leuven, Spain: International Society for Horticultural Science.

Carmona, M., Zalacain, A., Pardo, J. E., López, E., Alvarruiz, A., & Alonso, G. L. (2005). Influence of different drying and aging conditions on saffron constituents. *Journal of Agricultural and Food Chemistry, 53*, 3974–3979.

Castellar, M. R., Montijano, H., Manjón, A., & Iborra, J. L. (1993). Preparative high-performance liquid chromatography purification of saffron secondary metabolites. *Journal of Chromatography, 648*, 187–190.

Fernandez, J. A. (2004). Biology, biotechnology and biomedicine of saffron. *Recent Research of Development in Plant Science, 2*, 127–159.

Hostettmann, K., Marston, A., & Hostettmann, M. (1998). Preparative chromatography techniques. In *Applications in natural products isolation* (pp. 67–69). Berlin: Springer-Verlag.

Iborra, J. L., Castellar, M. R., Canovas, M., & Manjón, A. (1992). TLC preparative purification of picrocrocin, HTCC and crocin from saffron. *Journal of Food Science, 57*, 714–716.

Kanakis, C. D., Daferera, D. J., Tarantilis, P. A., & Polissiou, M. G. (2004). Qualitative determination of volatile compounds and quantitative evaluation of safranal and 4-hydroxy-2,6,6-thimethyl-1-cyclohexene-1-carboxaldehyde (HTCC) in Greek saffron. *Journal of Agricultural and Food Chemistry, 52*, 4515–4521.

Li, C. Y., & Wu, T. S. (2002). Constituents of the stigmas of *Crocus sativus* and their tyrosinase inhibitory activity. *Journal of Natural Products, 65*, 1452–1456.

Li, N., Lin, G., Kwan, Y. W., & Min, Z. D. (1999). Simultaneous quantification of five major biologically active ingredients of saffron by high-performance liquid chromatography. *Journal of Chromatography A, 849*, 349–355.

Lindsay, S. (1992). High-performance liquid chromatography. In J. Wiley (Ed.), *ACOL series*, 2nd ed. (pp. 229–250). Chichester: John Wiley & Sons.

Lozano, P., Castellar, M. J., Simancas, M. J., & Iborra, J. L. (1999). Quantitative high-performance liquid chromatography method to analyze commercial saffron (*Crocus sativus* L.) products. *Journal of Chromatography A, 830*, 477–483.

Lozano, P., Delgado, D., Gómez, D., Rubio, M., & Iborra, J. L. (2000). A non-destructive method to determine the safranal content of saffron (*Crocus sativus* L.) by supercritical carbon dioxide extraction combined with high-performance liquid chromatography and gas chromatography. *Journal of Biochemical and Biophysical Methods, 43*, 367–378.

Negbi, M. (1999). Saffron cultivation: Past, present and future prospects. In M. Negbi (Ed.), *Saffron Crocus sativus* L. (pp. 1–17). Amsterdam: Harwood Academic Publishers.

Rios, J. L., Recio, M. C., Giner, R. M., & Mañez, S. (1996). An update review of saffron and its active compounds. *Phytotherapy Research, 10*, 189–193.

Straubinger, M., Bau, B., Eckstein, S., Fink, M., & Winterhalter, P. (1998). Identification of novel glycosidic aroma precursors in saffron (*Crocus sativus* L.). *Journal of Agricultural and Food Chemistry, 46*, 3238–3243.

Straubinger, M., Jezussek, M., Waibel, R., & Winterhalter, P. (1997). Novel glucosidic constituents from saffron. *Journal of Agricultural and Food Chemistry, 45*, 1678–1681.

Sujata, V., Ravishankar, G. A., & Venkataraman, L. V. (1992). Methods for the analysis of the saffron metabolites crocin, crocetin, picrocrocin and safranal for the determination of the quality of spice using thin-layer chromatography, HPLC and GC. *Journal of Chromatography A, 624*, 497–502.

Tarantilis, P. A., Polissiou, M., & Manfait, M. (1994). Separation of picrocrocin, *cis–trans*-crocins and safranal of saffron using high-

performance liquid chromatography with photodiode-array detection. *Journal of Chromatography A, 664*, 55–61.

Tarantilis, P. A., & Polissiou, M. G. (1997). Isolation and identification of the aroma components from saffron (*Crocus sativus* L.). *Journal of Agricultural and Food Chemistry, 45*, 459–462.

Tarantilis, P. A., Tsoupras, G., & Polissiou, M. (1995). Determination of saffron (*Crocus sativus* L.) components in crude plant extract using high-performance liquid chromatography–UV–Vis photodiode-array detection-mass spectrometry. *Journal of Chromatography A, 699*, 107–118.

Tibetan Medicine Encyclopaedia. www.doctorgarma.com/rst/crocus_sativus/.

Winterhalter, P., & Straubinger, M. (2000). Saffron-renewed interest in an ancient spice. *Food Reviews International, 16*, 39–59.

Xuabin, N. (1992). Research progresses on the saffron crocus (*Crocus sativus*). *Zhongcaoyao, 23*, 100–107.

Zalacaín, A., Díaz-Plaza, E.M., Blázquez, I., Carmona, M., Alonso, G.L. FT-NIR spectrometry approach for determining saffron origin. In Fernández, J.A. & Abdullaev F.I. (Eds.), *Proceedings of the first international symposium on saffron biology and biotechnology* (pp. 327–329). Acta Horticulturae: Vol. 650. Leuven, Spain: International society for horticultural science.

Zareena, A. V., Variyar, P. S., Gholap, A. S., & Bongirwar, D. R. (2001). Chemical investigation of gamma-irradiated saffron (*Crocus sativus* L.). *Journal of Agricultural and Food Chemistry, 49*, 687–691.

Zhou, Q., Sun, Y., & Zhang, X. (1978). Saffron, *Crocus sativus* L.. *Journal of the Tradition Chinese Medicine, 28*, 59–61.