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Diurnal variability of stigma compounds of saffron (*Crocus sativus* L.) at different harvest times

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ABSTRACT

Saffron (Crocus sativus L.) is the most valuable spice in the world that widely used for food industries and pharmaceutical preparations. In this study, we examined and evaluated variation of stigma composition of saffron due to harvest at different hours of day in Gonabad climatic conditions. The flowers of saffron were collected at different times (06:00, 12:00, 18:00 and 6:00 h of after flower emergence day) and their stigmas were separated from flowers. After drying in shade, stigma composition analyzed using a GC-MS system. The main identified compounds were safranal, picrocrocine, crocin, cic-crocin and crocetin. Other components included 1,8-cineol, α-pinen, oleoanolic acid, kaempferol, 3-gentiobiosyl-kaempferol, cineole, and echinocystic acid. Our results revealed that different harvesting times significantly influenced the compounds percentage of saffron. The high content of crocin and picrocrocin resulted in harvest at last harvest time (6:00 h of after flower emergence day). Although, safranal content decreased due to delay of harvest and highest value was obtained from first harvest time (06:00 h).

Key words: Diurnal variation, Saffron (*Crocus sativus* L.), GC-MS, Stigma quality.

INTRODUCTION

Saffron (*Crocus sativus* L.) is a perennial plant from the family *Iridaceae* with great economic value that widespread throughout the temperate and sub-tropical regions originally grew from the Western Mediterranean to Iran, India (Kashmir), China and Japan [1-4]. In Iran, saffron is grown in the eastern region of the country, primarily in Khorasan province. The dried stigmas of saffron are a very expensive species that is mainly used as an herbal medicine or food coloring and flavoring agent in different countries [5-6]. So, its high value has made saffron the object of

frequent adulteration, and also being the object of intense chemical and biotechnological research [7]. The amount of the volatile component of the essential oil in dried stigma tissues is the most important indicator of the quality of the saffron. Various techniques have been used to extract and identify the stigma composition of saffron. The best technique for analyzing the volatile components of saffron is GC-MS. The high sensitivity, the low limit of detection, the possibility of analyzing a great number of analytes and of rapidly identifying them by mass spectra have made GC-MS one of the most widespread analytical techniques for scientific researches [8].

Fernandez [9] reported that the main constituents of saffron to be carotenoids, glycosides, monoterpenes, aldehydes, picrocrocin and antocyanins, flavonoids, vitamins, amino acids, proteins, starch and minerals. The essential oil (maximum 1%) contains several terpenes (pinene, cineole) and carbonyl compounds. Its most abundant constituent is safranal ($C_{10}H_{14}O$). Safranal is one of the compounds considered to be responsible for the aroma of saffron spice and measurement of its levels, has been used in the past as a measure of saffron quality [10]. Picrocrocin ($C_{16}H_{26}O_7$) is considered to be the main bitter principle of saffron. b-Glucosidase action on picrocrocin liberates the aglycone, 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC, $C_{10}H_{16}O_2$) which is transformed to safranal by dehydration during the drying process of the plant material [11-12]. The intense color is due to cartotenoid type pigments. Although saffron contains some conventional carotenoids (α- and β-carotene, lycopene and zeaxanthin), its pronounced staining capability is mostly caused by crocetin esters. crocetin is a dicarboxylic acid with a carotenoid-like C_{18} backbone which is formed from carotenoid precursors. Crocin, a diester of crocin with gentobiose, is the single most important saffron pigment. Crocins dissolve easily in water to provide an orange-red solution. This is the reason for its application as a food colorant [13-14].

Although, synthesis of volatile compounds in medicinal plants controlled by genetic processes, but those composition and concentration obviously influenced by environmental factors and also by agronomic conditions, harvesting time and the type of processing [10], so that these facrors cause changes in growth of medicinal plants, quantity and quality of their volatile compound. The environmental factors as light (quality, intensity and duration), temperature, altitude, humidity and soli properties alone or in combination have significant influence on chemical composition and secondary metabolite situation of medicinal and aromatic plants. The temperature in day length usually is variable from morning to night. It is found that temperature has more effect on essential oil of medicinal plants [17-18].

DeVasconcelos *et al*. [19] reported variation during the daytime in the chemical constituents of the essential oil of *Ocimum gratissimum* leaves. They showed a considerable variation in the yield of eugenol, i.e. 98% at 12.00 to 11% at 17.00. Studies made on the effects of ontogenetic and diurnal variations in the essential oil composition of *O. onites* [20-21] and *Rosmarinus officinalis* L. [18] showed that the variations depending on different times of the day and development period can influence the composition and the herbal productivity of these plants.

Crocin and picrocrocin compounds degrade naturally in the cells of stigmas during drying, storage, and extraction. The degree of degradation depends on temperature, humidity, light

irradiation and other compounds in the environment. Alonso *et al.* [22] noted that increase of temperature caused to oxidation of crocin and decomposition of picrocrocin of saffron stigma.

In the present study, we investigated the change of chemicals attributing spice properties of saffron stigma at different times of harvest in Gonabad region.

MATERIALS AND METHODS

This study was conducted on an experimental field in Gonabad, state of Khorasan Razavi, Iran (34°21' N, 58°42' E; 1150 m above sea level) in autumn 2011. The design of experiment was in completely randomized design (CRD) manner with three replications. In each replication, the flowers were picked by hand in four times (06:00, 12:00, 18:00 and 6:00 h after day of flower emergence) and stigmas were removed from the flowers and then, air-drying of stigma was performed in a shady place at room temperature for 10 days. Then they were used for the analysis of essential oil composition.

Saffron composition analyzed by Hewlett-Packard GC-MS (model 5890 series II) with a HP-5 capillary column (30 m length, 0.25 mm of inside diameter, and film thickness 0.25 μm). The transfer line and detector temperatures were set at 220 and 290 °C, respectively. Helium was used as carrier gas at a flow rate 1 mL min^{-1}. Ionisation of the sample components was performed in electron impact mode (EI, 70 eV). The chromatograms of each sample were compared to the standard injections. The target peaks were confirmed by retention time and mass spectra data. Confirmed integrated peaks were then used to determine the analyte percentage.

Finally, data for essential oil compositions were subjected to analysis of variance (ANOVA) using statistical analysis using SAS statistical software package. Differences between treatments were assessed using the F-test, and Duncan's Multiple Range Test (DMRT) was calculated at the 0.05 probability level.

RESULTS AND DISCUSSION

Sixteen components were found in the stigma constitute and 12 were identified. The major components were safranal, picrocrocine, crocin, cic-crocin and crocetin. Other components included 1,8-cineol, α-pinen, oleoanolic acid, kaempferol, 3-gentiobiosyl-kaempferol, cineole, and echinocystic acid. ANOVA summary for the effect of harvesting hours on stigma composition were reported in Table 1. Our results showed that various hours of harvest have a significant influence on the percentage of stigma components.

Quality of saffron stigma depends on the concentration of its three major metabolites (crocin, picrocrocin, and safranal) providing especial color and flavor of stigmas [14]. Comparison of means for crocin percentage (Figure 1) reveals that the highest content of crocin (18.20%) was obtained in harvest at the last time of harvest (06:00 h after day) and stigmas obtained from flowers harvested at first time (06:00) had the lowest crocin content (14.50%). Crocin is water soluble compound in saffron stigma and is the reason for application of saffron as a food colorant. The color strength changes more noticeably than the aroma and bitterness, it is believed

that the shelf life of saffron is related mainly to the fate of its pigment and consequently the decrease of color strength [23].

As shown it Figure 2, in the case of picrocrocine, saffron flowers harvested at second (12:00) and last (06:00 h after day of emergence) harvest time showed the highest amounts (7.66 and 7.40%, respectively) and those harvested at first (06:00) and third (16:00) harvest time achieved the lowest values (6.96 and 6.63%, respectively). Safranal content also decreased as harvest delayed. The maximum Safranal content in the essential oil (1.36%) was recorded at the Ist harvesting time i.e. at 06:00 and the minimum value (0.80%) was observed at the 4th harvest i.e. at 06:00 after day of flower emergence, however, the difference between it and 3rd harvest treatment (16:00) was not statically significant (Figure 3). In terms of crocetin, the high content (5.53%) was obtained from harvest at 06:00 h, while, delay in harvest caused to decrease of crocetin content so that the lowest content (3.73%) was obtained from harvest at 6:00 h after day of flower emergence (Figure 4). Results of mean comparison illustrated that other components identified in saffron stigma in this trial changed due to harvest time treatments (Table 2).

Table 1. Analysis of variance of stigma composition as affected by different harvest hours

		Mean of Squares											
S.O.V	Df	α-pinen	Echinocystic acid	Cineole	1,8-cineol	Picro-crocine	3-gentiobiosyl-kaempferol	Kaempferol	Corcetin	Oleoanolic acid	Cic-crocin	Crocin	Safranal
Treatment	3	1.85**	5.03**	1.61**	3.43**	0.62**	3.97**	1.34**	1.83**	3.20**	4.95**	7.56**	0.18**
Error	8	0.046	0.021	0.030	0.025	0.052	0.026	0.035	0.017	0.021	0.020	0.148	0.007
CV (%)		3.82	2.47	3.72	3.76	3.19	2.65	5.17	2.96	2.48	1.79	2.32	8.11

*** = Significant at 1% probability*.

Table 2. Day time fluctuations in stigma composition of saffron during four harvesting hours.

	α-pinen	Echinocystic acid	Cineole	3-gentiobiosyl-kaempferol	Kaempferol	Oleoanolic acid	1,8-cineol	Cic-crocin
Harvest time				(%)				
06:00	5.63b	4.33d	3.83c	6.96b	4.46a	4.60d	5.63a	9.46a
12:00	4.90c	6.60b	5.33a	7.66a	3.83b	5.83c	4.53b	8.53b
18:00	5.30bc	7.30a	4.23b	6.63b	3.46c	6.16b	3.73c	7.73c
06:00	6.73a	5.53d	5.20a	7.40a	2.86d	7.10a	3.16d	6.43d

Mean followed by similar letters superscripted in each column, are not significantly different at the 5% level

One more important factor that influences the oil composition of medicinal plants is the time of harvesting. In the literature there is limited data available for the role of day time harvest on yield and chemical composition of essential oil. Environmental factors affect saffron's quality [14]. Bouvier *et al.* [24] noted that carotenoid content is influenced by different environmental conditions such as temperature. Figueiredo *et al.* [25] reported that essential oil diurnal fluctuations may be related with the activity of the plant pollinator. In other cases, the variations in the composition of the oils were correlated with weather conditions (day length, temperature and humidity) and to the attack of fungal pathogens [26]. Air temperatures measured at four harvest times were 6, 12, 15 and 10 °C, respectively. It is reported that fluctuation of daily

temperature is the most important environmental factor in flower initiation of saffron [27]. It seems that in our experiment, variation of weather parameters (mainly sunlight and temperature) during day time is the most important factor that affected the percentage of stigma constitutes of saffron.

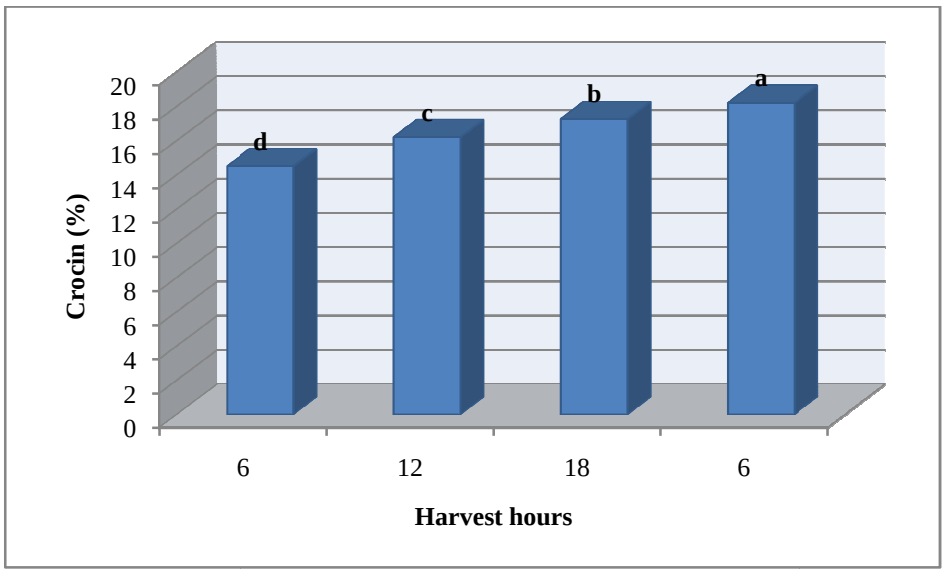

Figure 1. Effect of harvest hours on crocin content of saffron stigma

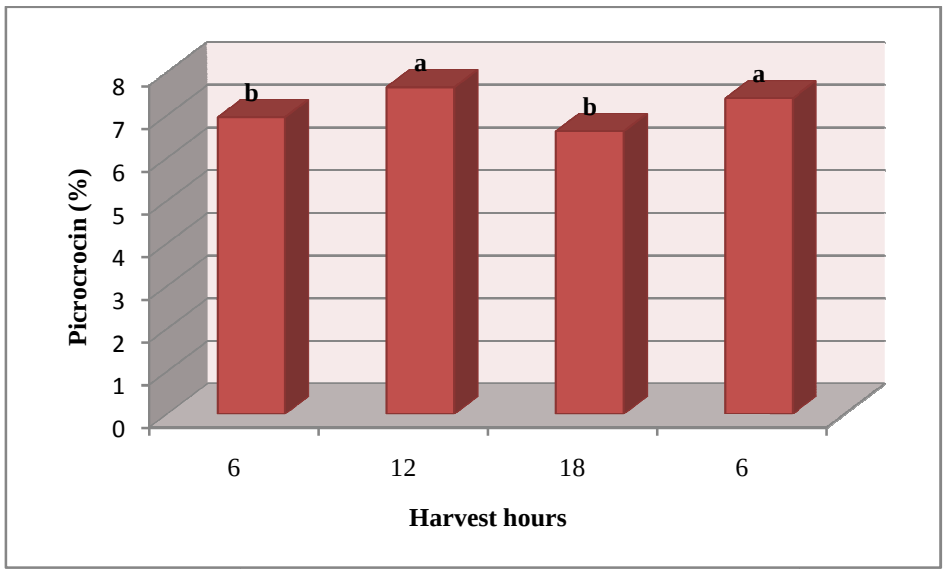

Figure 2. Effect of harvest hours on picrocrocin content of saffron stigma

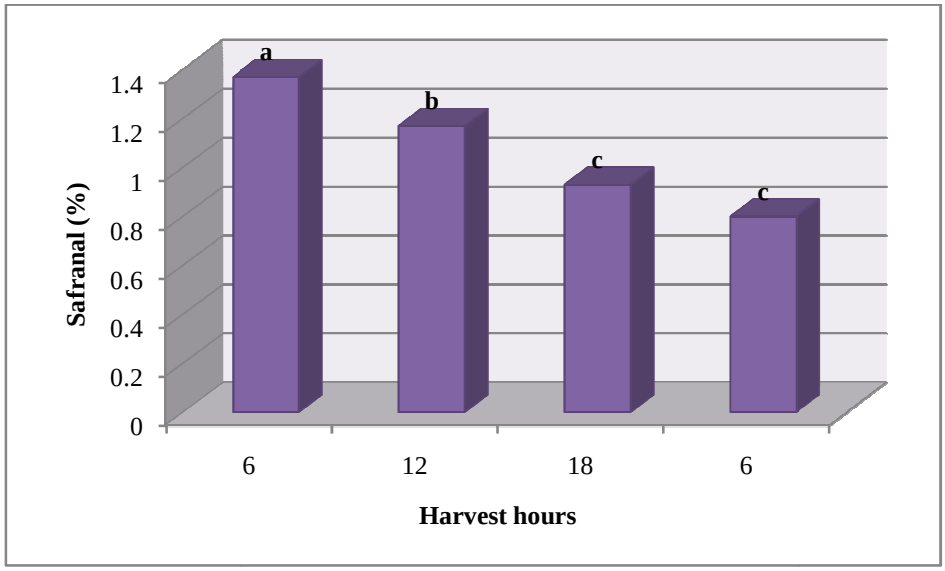

Figure 3. Effect of harvest hours on safranal content of saffron stigma

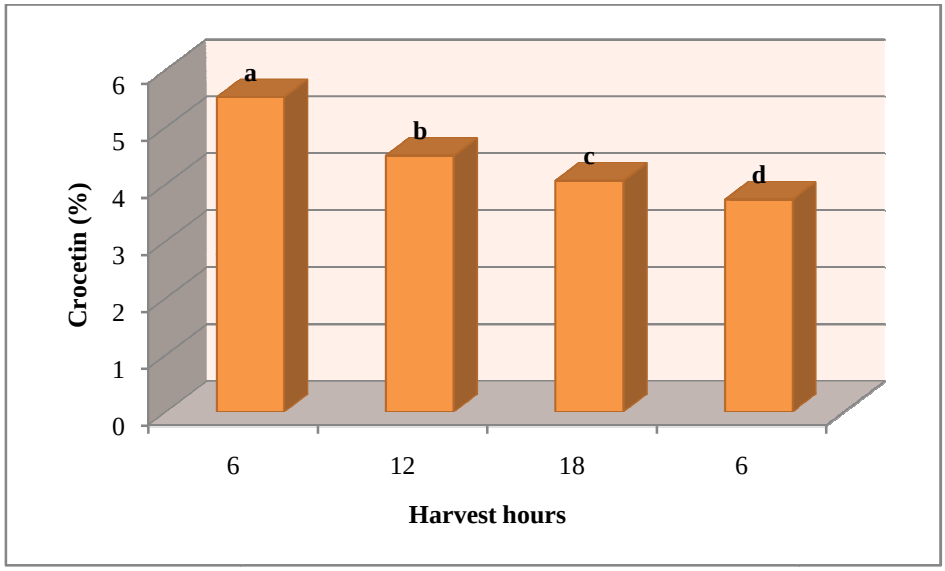

Figure 4. Effect of harvest hours on crocetin content of saffron stigma

REFERENCES

[1] M. Negbi, B. Dagan, A. Dror, D. Basker. *Isr. J. Bot*,. **1989**, *38*, 95-113.
[2] J.L. Rios, M.C. Recio, R.M. Giner, S. Manez. Phytotherap. Res., **1996**, 10, 189-193.
[3] N. Vurdu, S. Allahverdiev, H. Vurdu. *Kastamonu. Ed. J*., **1997**, 4, 85-89.
[4] M.J. Sheykhdavodi, M.A. Ebrahimi Nik, M. Pourreza Bilondi, M. Atashi, M. Seyedian. *Australian J. Crop. Sci*., **2010**, 4(5), 295-300.
[5] D. Basker, M. Negbi. *Econ. Bot*., **1983**, 37, 228-236.
[6] J. Escribano, M.J.M. Diaz-Guerra, H.H. Riesse, A. Alvarez, R. Proenza, J.A. Fernandez. *Planta Med*,. **2000**, *66*, 157-162.

[7] J.A. Fernandez. *Recent Res. Devel. Plant Sci*., **2004**, 2, 127-159.
[8] L.H. Jia, Y. Liu, Y.Z. Li. *J. Chinese Pharma. Sci*., **2011**, 20, 404-409.
[9] J.A. Fernandez. *Adv. Phytomed*., **2006**, 313-330.
[10] F. Hadizadeh, M. Mahdavi, S.A. Emami, Z. Khashayarmanesh, M. Hassanzadeh, J. Asili, M. Seifi, H. Nassirli, A. Shariatimoghadam, R. Noorbakhsh. *Acta Hort*., **2007**, 739, 405-409.
[11] O. Plessner, M. Negbi, D. Basker. *Isr. J. Bot.*, **1989**, *38*, 1-7.
[12] P. Lozano, D. Delgado, D. Gomez, M. Rubio, J.L. Iborra. *J. Biochem. Biophys. Meth*., **2000**, 43, 367-378.
[13] H. Himeno, K. Sano. *Agric. Biol. Chem*., **1987**, 51, 2395-2400.
[14] M. Lage, C. Cantrell. *Scientia Horticulturae*., **2009**, 121, 366-373.
[15] M. Miguel, C. Guerrero, H. Rodrigues, J.C. Brito, F. Duarte, F. Venancio. R. Tavares., **2004**. J. Essent. Oil Res., 16(2), 111-114.
[16] H. Baydar, O. Sagdic, G. Ozkan, T. Karadogan. *Food Control*., **2004**, 15,169-172.
[17] H. Boira, A. Blanquer. *Biochem. Syst. Ecol*., **1998**, 26, 811-822.
[18] S. Ramezani, F. Ramezani, F. Rasouli, M. Ghasemi, M. Fotokian. J. Biol. Sci., 2009, 4(1), 103-106.
[19] S.M.G. DeVasconcelos, A.A. Craveiro, F.J. Abreu Matos, M.I.L. Machado, J.W. Alencar. *Fitoterapia*., **1999**, 70, 32-34.
[20] S. Kizil, A. Ipek, N. Arslan K.M. Khawar. *New Zealand J. Crop. Hort. Sci*., **2008**, 36(1), 71-76.
[21] G. Yaldiz, N. Sekeroglu, M. Ozguven, M. Kirpik. *Grasas y Aceites*., **2005**, 56(4), 254-258.
[22] G.L. Alonso, R. Varon, M.R. Salinas, F. Navaro. *Boll. Chim. Farm*., **1993**, 132, 116-120.
[23] P. Winterhalter, M. Straubinger. *Food. Rev. Intl*., **2000**, 16, 39-59.
[24] F. Bouvier, R. Backhaus, B. Camara. *J. Biol. Chem*., **1998**, 273, 30651-30659.
[25] A.C. Figueiredo, J.G. Barroso, G.L. Pedro, J.C.J. Scheffer. *Flavour Fragr. J*., **2008**, 23, 213-226.
[26] C.N. Hassiotis, D.M. Lazari, K.E. Vlachonasios. *Fresenius. Environ. Bulletin*., **2010**, 19(8), 1491-1498.
[27] R.V. Molina, M. Valero, Y. Navarro, A. Garcia-Luis, J.L. Guardiola. *Scientia Hort*., **2004b**, 103, 361-379.