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Effects of Storage and Cultivation on Crocin Content of Dried Stigma of Saffron (*Crocus sativus* L.)

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ABSTRACT

Saffron (*Crocus sativus* L.) is known as one of the earliest cultivated plants. This plant is used in food, cosmetics, dying and pharmaceutical uses. Saffron stigma contains rare water-soluble carotenoids including the major one known as crocin. These compounds have a high economical value as a culinary spice and are used for their flavoring and coloring properties. They are subject of ongoing scientific research for their protenoids like effects on human health due to their high antioxidant characteristic. The study aimed to find the effects of cultivation condition and storage on crocin content of dried stigma in saffron. This study was carried out at the Forests and Rangelands Research Institute, Tehran. Samples were collected during November 2006 from two (Tehran and Alborz) provinces. They were dried in the shade. Dried stigma samples of saffron were kept for 20 months under three conditions: 1. under light (at the room temperature), 2. under darkness (at the room temperature) and 3. at 4°C in the refrigerator. Samples were analyzed during November 2008 by High- Performance Liquid Chromatography (HPLC). The results showed that the crocin content from Alborz province in light, dark and refrigerated conditions were 290, 410 and 300 ppm, respectively. Similarly, the samples from Tehran province showed that their crocin content varied showing 300, 300 and 280 ppm crocin under light, dark and refrigerated conditions respectively. It is concluded that the dried saffron stigmas could be best preserved under dark conditions.

Key Words : *Crocus sativus* L ., Crocin, Storage condition, HPLC

Kurutulmuş Safran (*Crocus sativus* L.) Stigmasının Krokin İçeriği Üzerine Depolama ve Yetiştirme Koşullarının Etkisi

ÖZET

Safran (*Crocus sativus* L.) tarımı yapılan ilk bitkilerden biri olarak bilinir. Bu bitki, gıda, kozmetik, boya ve farmasötik alanlarda kullanılır. Safran stigması, suda nadir çözünen karotenoidleri, özellikle de krokin olarak bilinen bileşiği içerir. Bu bileşikler, yemeklerde kullanılan baharat gibi yüksek ekonomik değere sahip olup, koku ve renk verici özellikleri için kullanılır. Yüksek antioksidan özelliği nedeniyle insan sağlığı üzerindeki protenoidler benzeri etkilerinden dolayı bu bileşikler sürekli bilimsel araştırma konusu olmaktadır. Bu çalışma, kurutulmuş safran krokini içeriğine yetiştirme ve depolama koşullarının etkilerini bulmaya yönelik yapılmıştır. Çalışma, Orman ve Meralar Araştırma Enstitüsü, Tahran, İran'da gerçekleştirildi. Numuneler iki (Tahran ve Elburz) ilden Kasım 2006 döneminde toplanmıştır. Örnekler gölgede kurutulmuştur. Kurutulmuş safran stigmaları üç farklı koşulda 20 ay boyunca depolanmıştır: 1. Işık altında (oda sıcaklığında), 2. karanlıkta (oda sıcaklığında) ve 3. buzdolabında 4°C sıcaklıkta. Numuneler Yüksek Performanslı Sıvı Kromatografi (HPLC) ile Kasım 2008 tarihleri analiz edilmiştir. Sonuçlar, ışık altında, karanlıkta ve soğuk koşullarda Elburz ilindeki numunenin krokin içeriği sırasıyla 290, 410 ve 300 ppm olduğunu göstermiştir. Benzer şekilde, Tahran şehrinden elde edilen numunelerde krokin

içeriği sırasıyla, ışık altında, karanlıkta ve soğuk koşullarda 300, 300 ve 280 ppm bulunmuştur. Kurutulmuş safran stigması için en iyi depolamanın karanlık koşullarda olduğu sonucuna varılmıştır.

Anahtar Kelimeler: *Crocus sativus* L ., Krokin, Depolama koşulları, HPLC

INTRODUCTION

Saffron (*Crocus sativus* L.) is known as one of the earliest cultivated plants [5]. This plant is an important crop cultivated as the source of its spice for at least 3,500 years. Saffron is a perennial spice and has been spread out in Mediterranean and west Asia [10]. Currently, it has been cultivated more or less intensively in Iran, India, Pakistan, Greece, Spain, Italy, Turkey, France, Switzerland, Israel, Pakistan, Azerbaijan, China, Egypt, United Arab Emirates, Japan, Afghanistan, Iraq and recently Australia (Tasmania) [12]. Saffron is classified into Magnoliophyta division, class Liliopsida and order Asparagales. It is a member of Iridaceae family and *Crocus* L. genus [4]. It is highly valued as a culinary spice for its flavouring and colouring properties [14], and is the subject of ongoing scientific research for its potential medicinal properties. Interest in the impact of saffron carotenoids on human health is growing due to their high antioxidant capacity [1, 13, 17].

The major components of saffron are crocins, picrocrocin and safranal. Crocins is responsible for the color of saffron, whereas picrocrocin and safranal are responsible for its bitter taste and aroma [3]; in other word Saffron quality depends on its three major metabolites providing the unique colour and flavour to the stigmas. Picrocrocin ($C_{16}H_{26}O_7$) is considered to be the main bitter principle of saffron. It is a monoterpene glycoside precursor of safranal ($C_{10}H_{14}O$). 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, 18]. The aroma of saffron comes from an essential oil, which is primarily composed of the terpene aldehyde, safranal, being the most abundant volatile component in the stigmas of saffron (>60% of essential oil) [15, 16]. The absorbance maximum for safranal is 330 nm. The dye substances collectively referred to as the crocins, come from the water-soluble glycosidic cis- and trans-carotenoid crocin, glucosyl esters of crocetin. Crocins dissolve easily in water to provide an orange-red solution. This is the reason for its application as a food colorant. The absorbance maxima of crocins are at about 440 nm in distilled water [ISO/TS 3632, 2003]. The study aimed to find the effects of cultivation condition and storage on crocin content of dried stigma in Iranian saffron.

MATERIALS and METHODS

The trial was carried out at the experimental fields in Tehran and Alborz provinces and chemical laboratory of the Research Institute of Forests and Rangelands, Agriculture Ministry, Karaj, Iran, during 2006-2008. Saffron corms obtained from the Research Institute were used as the study material. Corm planting was done on 30th September 2010. Planting was performed as 20 x 10 cm of plant spacing. No fertilization and 1 time irrigation were applied to the experiment. Saffron flowers were collected during November 2006 from experimental fields at two locations. They were dried in the shade. Dried stigma of saffron were kept for 20 months under three conditions: 1. light (at the room temperature), 2. darkness (at the room temperature) and 3. at 4°C in the refrigerator. Samples were analyzed during November 2008 by High- Performance Liquid Chromatography (HPLC).

Analyzing of Samples

Extraction Combinations of Saffron

For the determination of crocins in saffron, samples were extracted with 6 mL of degassed methanol and sonicated for 1 h and then stored overnight. The whole process was carried out in darkness at room temperature, samples were removed from darkness, sonicated for one more hour, and brought to volume with previously degassed methanol. Each extracted sample was filtered using a 1 mL tuberculin syringe and a 0.20 µL sample was then injected into an HPLC.

HPLC System Conditions

The HPLC system used for sample harvested during 2006 was Knauer, Model Well Chrom 2000, Maxi-star K-1000 pump and detector spectrophotometer K-2500 with Erospher 100 C_{18} column using 250 mm x 4 mm, 5 µm column cm in length and diameter of 4 mm and the mobile phase ethyl acetate, isopropanol and water (56: 34: 10) with a flow rate 0/6 mL per minute, the amount 0.20 µL of sample was injected into the device for 40 minutes.

Preparation of Standard Solution

Composition for preparing a standard curve crocin ($C_{44}H_{64}O_{24}$ Mr 976.98) was purchased from Fluka Company. Three different dilutions of standard samples with methanol (0.024; 0.048 and 0.072 mg/ml) was prepared.

Calibration Curve

Standard curve by different concentrations of crocin samples (three samples at concentrations of 0.024; 0.048 and 0.072 mg/mL) and with the injection device is provided. The area under the spectrum of the unknown with its compatibility with the calibration curve, the unknown concentration is obtained (Fig. 1).

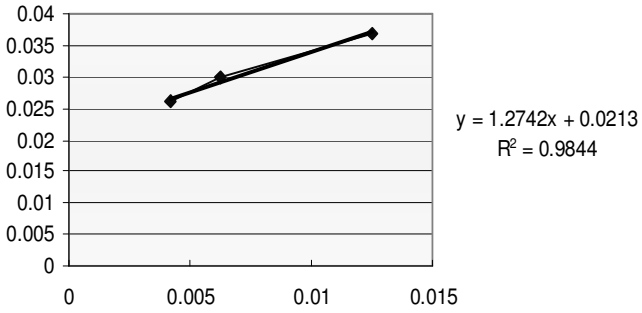

Figure 1. Calibration curve

RESULTS

Crocin content (percentage and ppm.) of dried stigma in two provinces (Tehran and Alborz) and storage conditions (light, dark and at 4℃ in the refrigerator) are shown in Table 1. The results showed that the crocin content of dried stigma from Alborz province in light, dark and refrigerated conditions were 290 ppm, 410 ppm and 300 ppm respectively. Similarly, the samples from Tehran province showed that their crocin content of 300 ppm, 300 ppm and 280 ppm under light, dark and refrigerated conditions respectively. The average of crocin content in Tehran and Alborz locations was 333.33 and 293.33 ppm respectively; and in light, dark and refrigerated conditions were 295 ppm, 355 ppm and 290 ppm, respectively. According to the results in term of crocin content, Alborz province is better compared to Tehran and the best storage conditions for dried stigma is dark environment.

Table 1. The effect of cultivation and storage conditions on the content of crocin in dried stigma

Location	Storage condition	Crocin (%)	Amount (ppm)
Alborz	Exposed to light for 20 months	0.029	290
Alborz	In a dark environment for 20 months	0.041	410
Alborz	Refrigerator for 20 months	0.030	300
Tehran	Exposed to light for 20 months	0.030	300
Tehran	In a dark environment for 20 months	0.030	300
Tehran	Refrigerator for 20 months	0.028	280

DISCUSSION

This study showed that crocin content of samples stored in dark condition from Alborz location was greater compared to other storage conditions but the difference between light and refrigerated conditions was negligible (10 ppm). In Tehran location there was no difference between light and dark storage conditions but under refrigerated conditions crocin content of samples was less than other storage conditions. Jaimand et al. [7] collected Saffron flowers on December 2003 from a field in Torbat-e Heydariyeh, dried and kept samples for 20 months in three conditions (light, dark and in refrigerator). They analyzed Samples by High-Performance Liquid Chromatography (HPLC). The results showed that the crocin content in light, dark and refrigerated conditions were 320 ppm, 380 ppm and 280 ppm, respectively. Raina et al. [19] dried samples in shade environment and at 50℃ and 60℃ temperature condition; they recorded that stigma crocin content by drying at 50℃ and 60℃ was greater compared to drying under shade. Drying samples in shade, take long time. With long drying time, enzymatic and non-enzymatic activities continue and reduce crocin content. The results of Hemmatikakhki [8], Hosseini [9] and Atefi [2] are similar to Raina et al. [19].

CONCLUSION

It is concluded that in terms of crocin content, dried stigma storage under dark condition is better compared to other storage conditions. Since Crocin dissolve easily in water (ISO/TS 3632, 2003), dried stigma under humid conditions is also affected by other factors such as light, oxygen and temperature in agreement with Hemmatikakhki [8]. Therefore it is recommended that the manufacturers, merchants and consumers must store dried stigma under dark, dry condition at room temperature (24-26°C).

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