Research Article

Effect of Drying Methods on the Colour of Kashmir Saffron (*Crocus sativus* L.) and Simultaneous Method Validation

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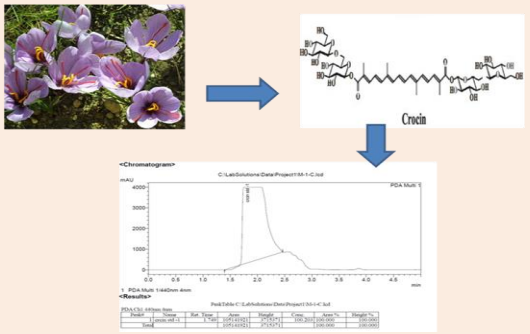
Abstract

This study reports the effects of drying methods (Shade-drying and microwave drying) on crocin content of saffron (*Crocus sativus* L.) and simultaneously method validation is presented. Various samples of saffron were analysed seven each from shade drying and microwave drying by HPLC. Several parameters have been taken into account and evaluated for the validation of method, namely: RSD, LOD, LOQ, Spike amount, % Recovery. Result suggested that microwave drying retain maximum concentration of crocin (4.13± 0.103 mg/100mg of stigma) as compared with sample dried under shade, where crocin level was (3.7± 0.298 mg/100mg of stigma). Further there is significant ($P<0.05$) decrease in its quantity in case of shade drying. It is concluded that microwave drying could be the best drying method for saffron stigmas in order to retain its color.



Keywords: *Crocus sativus* L, Crocin, HPLC Quantification

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Introduction

Saffron spice is made up of dried stigmas of Crocus sativus L. Currently, it is being 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 [1,2]. In recent decades, biological and medical properties of this spice and its constituents have again much scientific attention. It has been proposed that saffron is effectual against arteriosclerosis, while reducing cholesterol levels in the blood [3, 4]. Many in vivo tests on tumors in rats, as well as in vitro trials on established cellular lines, have been carried out [5, 6, 7, 8, 9]. It is highly valued as a culinary spice for its flavouring and colouring properties [10].

Interest in the impact of saffron carotenoids on human health is growing due to their high antioxidant capacity [11, 12, 13]. The major components of saffron are crocins, picrocrocin and safranal. Crocin is responsible for the color of saffron, whereas picrocrocin and safranal are responsible for its bitter taste and aroma [14]. In other words Saffron's quality depends on its three major metabolites providing the unique colour and flavour to the stigmas. 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 [15]. Saffron odour is obtained during drying process by hydrolysis of picrocrocin to volatile safranal. By the way, coloring strength of saffron increases when reduction of moisture content during drying results in hydrolysis of crocin pigment [16]. Lower moisture about 12% according to ISO 3632-2(2003) preserves quality characteristics of product during longer storage periods[17]. Therefore, this study was conducted to determine the effects of drying methods (Shade-drying and microwave drying) on crocin content of saffron (*Crocus sativus* L.) and simultaneously

method validation was performed by taken into an account of several parameters, namely: SD, RSD, LOD, LOQ, Spike amount, % Recovery etc.

Material and Methods

Plant materials and chemicals

Saffron stigma samples collected from pulwama region of Kashmir, Jammu and Kashmir, India
Full- bloomed flowers on each experimental plot were picked by hand at approximately from 6 to 8 am of the same day. The flowers were transported and kept in cool condition (4 °C) before treatment. Stigmas for the experiments were separated by hand from flowers at 24 °C indoor. After weighing, the samples were dried under two different conditions; shade drying (5 days) and microwave (1000w, 4 mints) drying in the Department of Chemistry, University of Kashmir. About 30 g of fresh stigmas were dehydrated by spreading them on a piece of paper at room temperature for five consecutive days and 30 g were dehydrated under electric microwave oven (1000w, 4 mints). Moisture content was calculated. Afterward, Methanol extract of stigmas were analyzed by HPLC in order to investigate the effects of drying method on saffron quality. All experiments were repeated seven times for method validation. The experiments were carried out at Central Institute of Temperate Horticulture (CITH), Srinagar.

Chemicals

Seven saffron samples were analyzed for quality. Standard, crocins were purchased from Sigma–Aldrich. HPLC Methanol and HPLC acetonitrile were purchased from Fisher Chemicals.

Sample preparation

Dried and accurate weighted Saffron stigma were cut using a razor blade to ‘‘grind’’ the saffron as evenly as possible. Approximately 60–70 mg of each sample was weighed in a 25 mL volumetric flask for extraction. Samples were extracted with 10 mL of methanol and sonicated for 1 hand then stored overnight. The whole process is carried out in darkness and at room temperature. Samples were removed from darkness. Each extracted sample was filtered using whatmann filter paper and than reduced under pressure using rotary evaporator. Obtained extracts were stored. Finally extracted samples were dissolved in HPLC grade methanol at a final concentration of 100 mg/ml. samples were filtered through 0.2 µm syringe filters. A 20µl filtered sample was than injected into an HPLC coupled to a PDA detector.

HPLC analysis

The analysis was carried out in a Shimadzu HPLC (Kyoto, Japan) equipped with quaternary pumps, degasser coupled to a photo-diode-array detector and injection value with a 20 µl loop. Separation was carried out with an injection volume of 20 µl, a flow rate of 1ml min-1 with 35-40 minutes of run time. The analysis was replicated for each sample. Crocin was detected at 440nm, whereas the standard was detected at the above mentioned wave lengths (Lozano *et al.* 1999). Chromatographic separations were performed on C18 (250 mm × 4.6 mm), 5 µm column using a solvent system consisting of 75% acetonotrile and 25% methanol in an isocratic mode. The mobile phase was filtered through a 0.45 µm membrane filter (millifore, Bedford, MA, USA) before analysis. Class WP software (version 6.1) from Shimadzu was used for instrument control, data acquisition and data processing. Quantitative determinations were made by taking into account the respective peak areas of standards at particular retention time versus concentration and expressed in milligrams per gram of saffron stigmas.

Statistical analysis

Results were presented as mean ± standard deviation (S.D). Data were statistically analyzed using one-way ANOVA, compare means - one sample T-Test, paired sample T-Test, test for linearity and ANOVA table was used to determine the differences between shade drying and microwave drying. Statistical significance was considered at $P \leq 0.05$.

Results and discussion

Variability in quality of saffron dried under shade and microwave condition are presented in table 1. Both the drying treatments produced saffron with final moisture content at or below the recommended maximum (12 %) required by the ISO-3632 standard.

Our result suggested that microwave drying retain maximum concentration of crocin (4.13± 0.103 mg/100mg of stigma) as compared with sample dried under shade, where crocin level was (3.7± 0.298 mg/100mg of stigma). Further there is significant ($P<0.05$) decrease in their quantity in case of shade drying.

A broad range of values is reported for these saffron components and the amount varies greatly from country to country. Reported values for crocins vary from 0.85% to 32.4% dry weight [18]. Other values reported vary between 2.9 mg% (29 mg/g) [19] and 4.6 mg% (45.99 mg/g) [20] for Iranian saffron and 6.7 mg% (67.3 mg/g) for Indian saffron. Safranal levels reported by some researcher are around 0.80 mg% (8 mg/g) [21]. Our results showed the total crocin content increased, when saffron sample dehydrated at moderate temperature in microwave oven. The loss of this quality compound under shade drying treatment might be due to enzymatic degradation. Moreover, the developed HPLC method was used for simultaneous determination of crocin from dried stigma of saffron. Several parameters have been taken into account and evaluated for the validation of method, namely: RSD, LOD, LOQ, Spike amount, % Recovery. The retention time of crocin in sample solution was 1.749 **(Figure 1, Figure 2).**

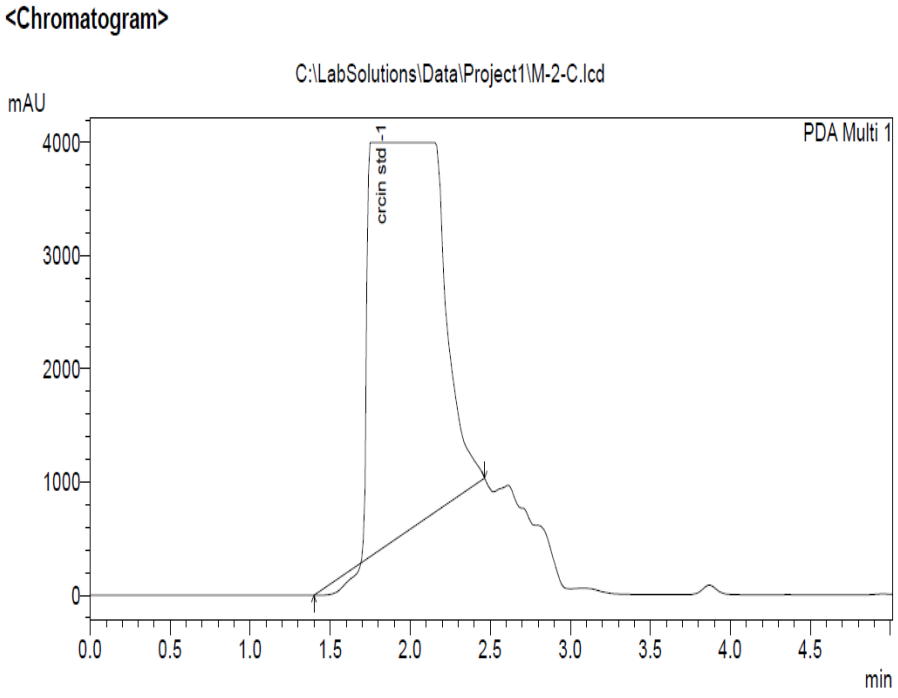

Figure 1 HPLC chromatogram shows total content of crocin from saffron stigma

System suitability tests were used to ensure reproducibility of the equipment. The signal-to-noise ratio of 3:1 and 10:1 was used to establish LOD and LOQ, respectively. From microwave dried samples the LOD and LOQ of crocin was 0.309 mg and 1.03 mg and the % RSD was found to be 2.4% for crocin. Accuracy of the method was studied using the method of standard addition. Standard crocin was added to the extract of the dried stigmas and the percent recovery was determined. The results of crocin along with recovery analysis from both drying treatment are shown in their respective **Table 1.**

Table 1 Differences in crocin content of saffron stigma dried under shade and microwave conditions

Marker Crocin	Evaluated Parameters	Result
Microwave Drying	RT*	1.749
	MEAN CONC.	4.13
	MIN CONC.	4.0081
	MAX CONC.	4.228
N=7	RANGE	0.2201
	VARIANCE	0.011
	SD	±0.103
	S/N RATIO	40.097
	%RSD	2.4%
	LOD	0.309
	LOQ	1.03
	SPIKE AMOUNT*	9.04
	%RECOVERY*	98.19%
Shade Drying	RT	1.749
	MEAN CONC.	3.7
	MIN CONC.	3.4
	MAX CONC.	4.1
	RANGE	0.720
	VARIANC	0.089
	SD	±0.298
	S/N RATIO	12.41
	%RSD	8.05%
	LOD	0.894
	LOQ	2.98
	*SPIKE AMOUNT	8.73
	*%RECOVERY	98.59%

Conclusion

According to findings of current study, microwave drying treatment such as one mentioned here, could be the desired for retaining chemical quality of saffron. Microwave can be used for improving the chemical compound profile of saffron including crocin, which is responsible for color. The method was found to be simple, precise, accurate, specific and sensitive and can be used for routine quality control of herbal raw materials and for the quantification of these compounds in plant materials. It is concluded that microwave drying could be the best drying method for saffron stigmas in order to retain its color.

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