Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(1):111-115

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Aroma of Kashmir saffron (*Crocus sativus* L.) and variation of safranal content by different drying methods

***Syed Muzaffar[1], Khaliquz Zaman Khan[1], Mohd Riaz[3], Javid Ahmad Mir[2] and Ajaz Ahmed[1,3]**

[1]Department of Chemistry, University of Kashmir, Srinagar, Jammu and Kashmir, India
[2]Central Institute of Temperate Horticulture (CITH), Srinagar. Jammu and Kashmir, India
[3]Govt. P.G., Degree College, Rajouri, Jammu and Kashmir, India

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ABSTRACT

Saffron is known from ancient times, and not only as a spice used in food but as dyeing, beauty product and especially for its pharmacological properties. Actually, its main use is in food as,it is one of the few spices that is capable of transmitting color, aroma, and flavor. Commercial saffron enterprises demand aroma evaluation more and more insistently through simple and reproducible techniques. Consumer knowledge of the spice has improved and they begin to demand saffron that possesses quality in a much wider sense. This study reports the effects of drying methods (Shade-drying and microwave drying) on Safranal content of saffron (Crocus sativus L.) and simultaneous method validation is presented by Several parameters: RSD, LOD, LOQ, Spike amount, % Recovery.. Samples of saffron were analysed by High Performance liquid chromatography. Result suggested that microwave drying retain maximum concentration of safranal (0.072± 0.003 mg/100mg of stigma) as compared with sample dried under shade, safranal ranged from (0.045± 0.004 mg/100mg of stigma). It is concluded that Significant increase in the relative safranal contents at high temperatures are most likely due to direct thermal conversion of picrocrocin at these high temperatures. So, microwave drying could be the best drying method for saffron stigmas in order to retain its aroma.

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

INTRODUCTION

Saffron (*Crocus sativus*L.) is known as one of the earliest cultivated plants [1]. 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 [2]. 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.

Picrocrocin(C16H26O7) is considered to be the main bitter principle of saffron. It is a monoterpene glycoside precursor of safranal (C10H14O). b-Glucosidase action on picrocrocin liberates the aglycone, 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC, C10H16O2) which is transformed to safranal by dehydration during the drying process of the plant material [15,16]. Lower moisture about 12% according to ISO 3632-2 (2003) preserves quality characteristics of product during longer storage periods [17]. Here method validation and effects of drying methods (Shade-drying and microwave drying) on safranal content of saffron (*Crocus sativus*L.) is presented

EXPERIMENTAL SECTION

Plant materials and chemicals

Saffron stigma sample collected from Pampore of pulwama region, Kashmir, India. About 60–70 mg of sample was weighed than dried under two different conditions i.e. shade drying and microwave drying. For Shade drying about 30 mg of fresh stigmas were dehydrated by spreading them on a piece of paper at room temperature for five consecutive days and remaining 30-40 mg were dehydrated under electric microwave oven (1000w, 4 mints) in the Department of Chemistry, University of Kashmir. Both dried samples were extracted with 10 mL of methanol separately afterwards sonicated for 1 hr, than extracted sample was filtered using whatmann filter paper and 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 than subjected to HPLC for simultaneous quantitation of safranal. The experiments were carried out at Central Institute of Temperate Horticulture (CITH), Srinagar.

Chemicals

Standard, safranal were purchased from Sigma–Aldrich. HPLC Methanol and HPLC acetonitrile were purchased from Fisher Chemicals.

System Suitability

System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by injecting 50 µL of mixture of standard solution of assay concentration of safranal six times. The % RSD was found to be 0.55, which was acceptable as it is less than 2%.

LOD and LOQ

LOD and LOQ of marker was determined based on signal-to-noise (S/N) ratio by following ICH guidelines. For LOD determination, the known concentration of serially diluted standard solutions (10–30 µg/ml) were analyzed along with blank MeOH sample (six times). S/N ratio (3:1) was performed standard by comparing measured signals from samples with known low concentration of analyte with those of blank samples to establish the minimum concentration at which the analyte can be reliably detected. LOQ determination was also carried out by the same process to establish the minimum concentration at which the analyte can be reliably quantified by keeping typical signal to noise ratio 10:1

Linearity

A good linearity was achieved in the concentration ranges of 10 µg /mL – 30 µg/mL for safranal. The regression equations and correlation coefficient for the reference were y = 0.043+0.931x, R2 = 0.995. The experiment was performed six times and the mean was used for the calculations. The data was analyzed by linear regression least squares fitting. The statistical data obtained is given in Table 1

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. Safranal was detected at 310nm, whereas the standard was detected at the above mentioned wave lengths [18]. Chromatographic separation 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, to determine the differences between shade drying and microwave drying. Statistical significance was considered at P≤ 0.05.

RESULTS AND DISCUSSION

Assay

The developed HPLC method was used for determination of safranal from dried stigma of saffron. The sample working solution (50 µL) was injected and the area of safranal peak was measured. From the calibration curve, the amount of safranal in dry stigma of *saffron* was calculated. The retention time of safranal in sample solution was 6.804. Both the drying treatments produced saffron with final moisture content at or below the recommended maximum (12 %) required by the ISO-3632 standard. From microwave drying mean assay value of safranal was found to be 0.072 mg per 100 mg of extract with SD as ±0.003 and from shade drying mean assay value of safranal was found to be 0.045 mg per 100 mg of extract with SD as ±0.004 **fig 1,2**. Further there is significant (P<0.05) decrease in its quantity in case of shade drying.

System Suitability and Accuracy

The signal-to-noise ratio of 3:1 and 10:1 was used to establish LOD and LOQ, respectively, the LOD and LOQ of safranal was 2.5µg/ml and 7.5µg/ml respectively and the % RSD 3.81 was found. Accuracy of the method was studied using the method of standard addition. Standard safranal solutions were added to the extract of the dried stigma of saffron and the percent recovery was determined at three different levels, than safranal content was determined and the percent recovery was calculated. The results of recovery analysis are shown in Table 2 for both drying methods. A broad range of values is reported for this saffron components and the amount varies greatly from country to country. Safranal levels reported by some researcher are around 0.80 mg% (8 mg/g) [19]. Other values reported for safranal vary between a minimum of 0.06 mg/g and a maximum of 0.29 mg/g [20].

Fig 1: HPLC chromatogram shows total content of safranal from saffron stigma after microwave drying

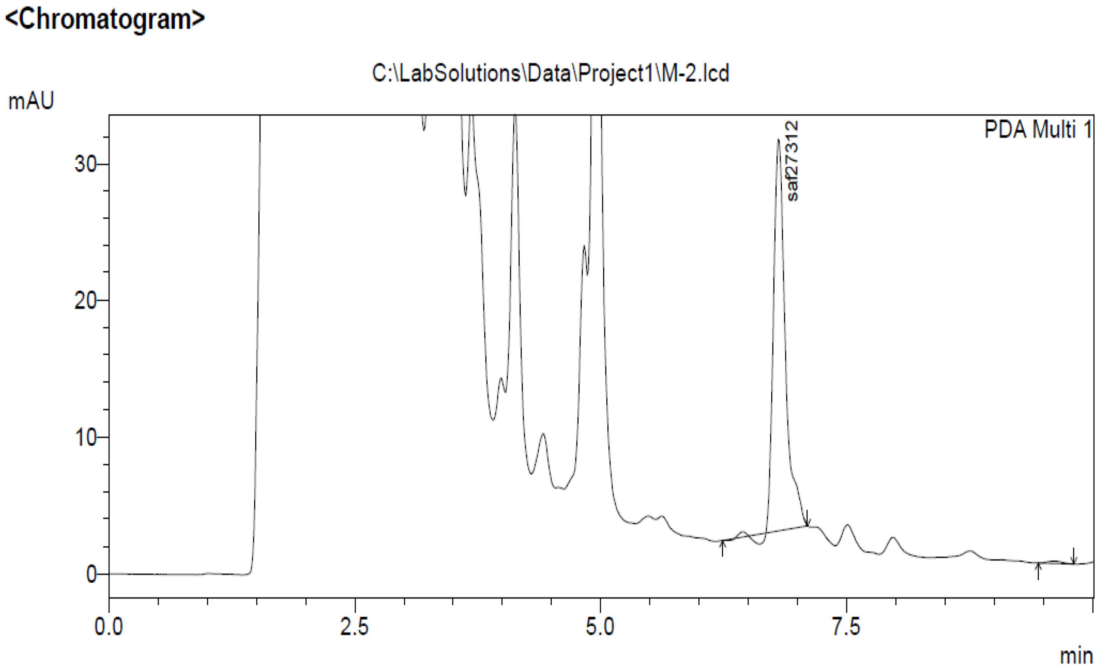

Fig 2 HPLC chromatogram shows total content of safranal from saffron stigma after shade drying

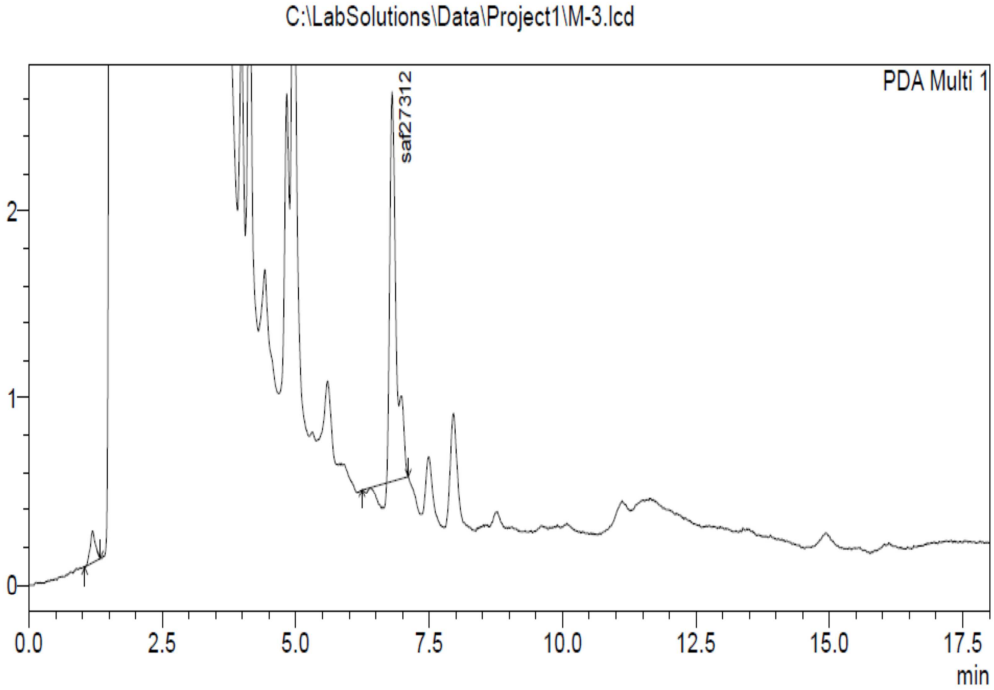

Table: 1 Method validation summary of standard safranal

	Method validation summary	
Marker Saffranal	Linearity range [µg mL-1]	10-30 µg/ml
	Linear equation	y= 0.043+0.931x
	S.D	0.08
	RSD	3.81
	Correlation coefficient (R)	0.995
	LOD	2.5 µg/ml
	LOQ	7.5 µg/ml

	Recovery study for Safranal			
	Amount present in extract(mg/100mg)	Amount added mg	Amount found mg	% Recovery
Microwave	0.075	0.5	0.560	97.39
	0.069 SD=±0.003	1	1.062	99.34
	0.071	1.5	1.548	98.53
Shade drying	0.050	0.5	0.536	97.45
	0.042 SD=±0.004	1	1.031	98.94
	0.044	1.5	1.510	97.79

CONCLUSION

Microwave can be used for improving the chemical profile of saffron in terms of safranal which is responsible for its aroma. Our results showed the total safranal content increased, when saffron sample dehydrated at moderate temperature in microwave oven. Significant increase in the relative safranal contents at are most likely due to direct thermal conversion of picrocrocin in to saffranal .

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