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Effect of solvent extraction on qualitative parameters of saffron edible extract

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ABSTRACT

Saffron, known as expensive spice on the world and distinguished by its delicate flavor and attractive yellow color, is basically cultivated in Iran, Spain, and India. Besides to its use as spice, saffron is also esteemed as medical plant and since short time, has been investigated as a new safe source of natural antioxidants for the food industry. Saffron stigma includes crocin, picrocrocin, safranal. The objective of this study was to find a suitable solvent with optimized extraction temperature and time to produce saffron extract with improved quality factors. For this purpose, the active compounds of saffron were extracted using different solvent (water, aqueous ethanoland aqueous methanol) and different solvent concentration (30%, 50% and 80%) in different times (1, 5 and 72 h). The effect of time and solvent concentration on the extraction yield of three major constituents of saffron was investigated at 25°C, 40°C and 60°C. Results showed that the optimal parameters to extract the target compounds from saffron were as follows: extraction solvent: 50% aqueous ethanol; extraction condition 5h at 25 °C. The crocin, picrocrocin and safranal content of the extract produced were respectively 423.9, 49.51 and 133.1 compared to 229.7, 27.92 and 75.17 for the control.

KEYWORDS

Color;
Saffron stigma;
Hydro alcoholic;
Ethanol;
Methanol.

INTRODUCTION

Nowadays there is a wide range of materials in the word, which are used for different purpose in food processing. So because of this reason the place of worldwide usage of natural, healthy products is considerable these days. Saffron is one of the most important export products and plays a significant role in income and employment of saffron producers. It is a major product in Khorasan, Fars and Kerman provinces in Iran[1,2]. Iran, Greece, India, Morocco, Kashmir, Spain and Italy are among main countries dealing with Saffron production. The chemistry of saffron has been investigated in detail[3]. The major pigment, a water-soluble carotenoid giving saffron its value as a dye, is crocin, a yellow-red pigment found at levels of up to 2%. Picrocrocin (<4%) is a bitter-tasting principle that hydrolyses to glucose and safranal (<4%), on drying.

Stigma which is the basic part of commercial saf-

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fron has special color, odor andflavor. Crocin (C_{44} H_{64} O_{24}) is the most influential chemical in the coloring power of saffron. It is a rare carotenoid found in nature which can easily dissolve in water. In comparison to other carotenoids, crocin has a wider application as a colorant in food and medicine, mainly because of its high solubility (Figure 1)[2,4-6].

A glucose known as picrocrocin (C_{16} H_{26} O_7) is the major factor for the bitter taste of saffron. This bitter substance can undergo crystallization, through acid hydrolysis, producing safranal (a glucose and aldehyde) (Figure 2)[2,3,7]. Safranal is the compound mainly responsible for the aroma of saffron spice and can be used as a measure of saffron quality (Figure 3)[8].

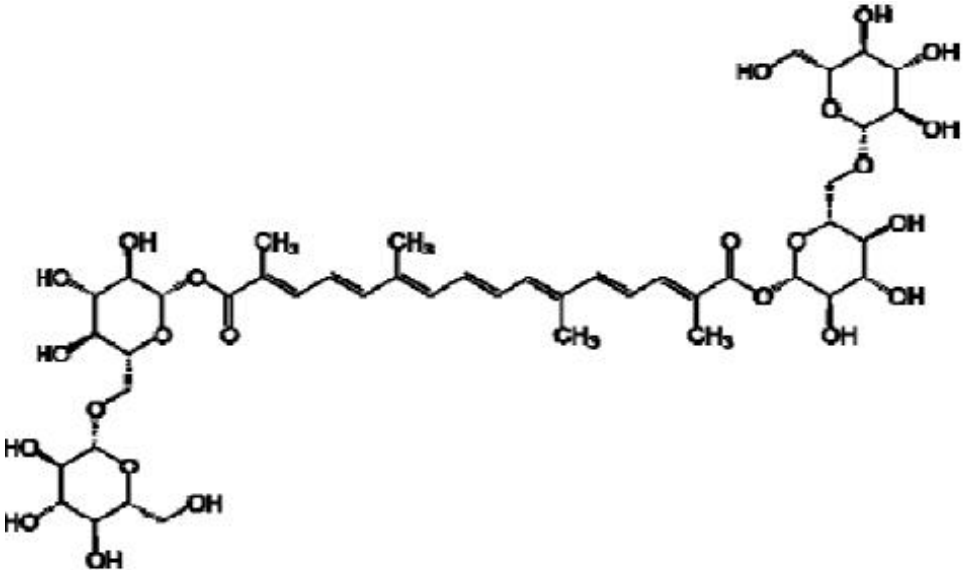

Figure 1 : Crocin structure

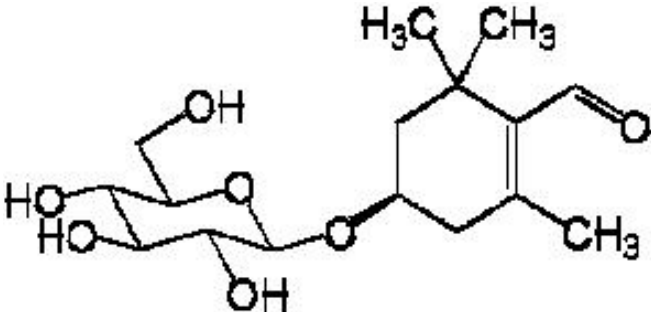

Figure 2 : Picrocrocin structure

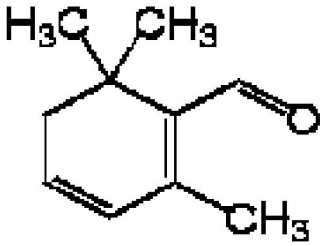

Figure 3 : Safranal structure

Saffron extract is a liquidextract of saffron which is treated to enhance the natural coloring and flavoring agents, without losing any of its natural properties[9]. In production of saffron extract, water or a mixture of water and another solvent is used to extract the color and flavor of saffron. This product is ready to use, and can be directly incorporated intoany food system[6,10].

There are many extraction techniques, such as maceration, digestion, infusion, decoction, percolation, hot continuous extraction, counter-current extraction, supercritical fluid extraction, microwave-assisted extraction, phytonic processes, or ultrasound-assisted extraction. The maindifferences among these techniques are related to the design of the reactors, the solvents used, the time and temperature of the processes or the use of new technologies (microwave, ultrasound, supercritical fluids, and enzymes)[1,11,12]. Among the mentioned techniques, solvent extraction using cheap solvents is the most economical one. As no research had been done on saffron extract production, this study conducted to produce an edible saffron extract using 3 polar solvents and to compare the quality parameters of extracts in order to establish an economical extraction method in which the ratio of solvents, time and temperature of maceration are optimized.

MATERIAL AND METHODS

Plant material

The whole plants of Crocus sativus were col-

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lected from Bahraman saffron company in Khorasan province inIran. Samples were dried in environmental temperature then pulverized into powder by a disintegrator and sieved with stainless steel sieves to classify the particle size. The powdered samples were kept in a dry and dark place until use.

Reagents

Solvents used in the extraction, such as methanol, ethanol, and were of analytical food grade and purchased from Merck (Germany) and SiminTak factory (Iran).

Saffron extractions

Dried and pulverized stigmas of C. sativus L, (10g) were extracted with three different solvent (aqueous ethanol (50% v/v),aqueous methanol (50% v/v), and distillated water)and three different distilled aqueous ethanol concentration (30%, 50% and 80%), in three different temperature (25°C, 40°C and 60°C) separately for 1, 5 and 72h and centrifuged. Then the aqueous extract from each step, was evaporated (Heidolph, model: Rota vacvario power unit No: 11-300-004-33-3, Germany) on the 40°C to separate alcoholic solvent. The filtrate and alcohol-free extract was then analyzed by UV spectrophotometer. The parameters investigated include extraction solvent, such as crocin, picrocrocin and safranal. All experiments wereprepared in triplicate. Dried stigmas of C. sativus L was analyzed as the control treatment at 25°C.

Coloring test

For measuring saffron quality factors (crocin, safranal and picrocrocin) international standard ISO 3632-2 was used. The absorbance readings of the extract were measured on an UV vis recording spectrophotometer, Cecill, Japan (Model CE 1021) at 257, 330 and 440 nm representing λ_{max} for picrocrocine, safranal, and crocin, respectively. Distilled water was used as blank. The results were expressed as $E^{1\%}_{\gamma MAX}$

$$E^{1\%}_{\gamma MAX} = \frac{A_{\gamma MAX} \times 10000}{(100 - H)}$$

Where A is the absorbance at λ_{max}, m the mass of saffron sample (g), and H is the mass fraction of moisture and volatile content of the sample. H was determined to be 7 % for the samples used in this study[12].

RESULT AND DISCUSSION

Effect of solvent

The selection of the most appropriate solvent for extracting the compounds of interest from the sample is an essential step for developing any extraction method. In this study, water, methanol and ethanol were tested to extract crocin, picrocrocin, and safranal from saffron (crocus sativus). Figure 4 shows the effects of different solvents on the extraction yield of the target compounds. Three different solvents exhibited different effects on the extraction yield under same extraction conditions. Water is not suitable solvent for extracting the target compounds. Because crocin, picrocrocin, and safranal are polar compounds, solvents with high polarities such as methanol and ethanol are better for their extraction. Methanol, with lower polarity than ethanol, exhibited a lower extraction yield. This result indicates that solvents show different extraction results on the objective constituents due to polarity and viscosity differences between them.

TABLE 1 : Comparison of $E^{1\%}_{\gamma MAX}$ values obtained using different solvent

Type of solvent	$E^{1\%}_{440}$ Crocin	$E^{1\%}_{330}$ Safranal	$E^{1\%}_{257}$ Picrocrocin
Control (X ± SD)	229.7±0.68^{c}	27.92±0.4^{c}	75.17±0.33^{c}
Water (X ± SD)	125.4±0.11^{d}	24.99±0.33^{d}	67.18±0.13^{d}
Water/Methanol (X ±SD)	273.3±0.46^{b}	31.32±0.25^{b}	84.78±0.74^{b}
Water/Ethanol (X ± SD)	423.9±0.23^{a}	49.51±0.09^{a}	133.1±0.33^{a}

The extraction time was 5h, extraction temperature was 25 °C, solvent concentration 50%v/v and the ratio of solvent to material was 20:1

Figure 4 shows the saffron extraction results carried out under different type of solvent with fixed conditions of other factors, such as extraction solvent concentration (50 %v/v), extraction temperature (25 °C).

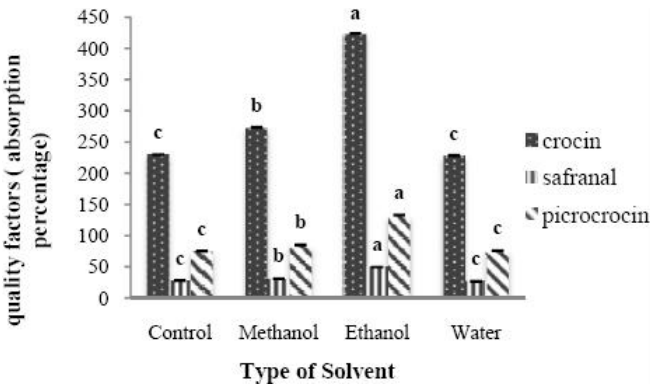

Figure 4 : Effects of solvent on the quality factors of saffron extract

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Effect of aqueous ethanol solvent concentration

TABLE 2 shows that the extraction of the target compounds in 50 % aqueous ethanol are better than in 30% and 80%aqueous ethanol, respectively. Although the use of 50 % aqueous ethanol as the extracting solvent produced higher yields of crocin, picrocrocin and safrasnalthan80 % aqueous ethanol, and its disadvantage is, over 50 % aqueous ethanol is a toxic solvent, which makes it more harmful to health. It has been observed that sometimes the addition of small percentage of water to the extraction solvent helps to increase the extraction yield of the target compounds from the sample (TABLE 1).

TABLE 2 : Comparison of $E^{1\%}_{\gamma MAX}$ values obtained using differentaqueous ethanol solvent concentration

Aqueous EthanolSolvent Concentration	$E^{1\%}_{440}$ Crocin	$E^{1\%}_{330}$ Safranal	$E^{1\%}_{257}$ Picrocrocin
Control (X ± SD)	229.7±0.68^{c}	27.92±0.4^{c}	75.17±0.33^{d}
30% (X ± SD)	297.9±0.37^{b}	38.22±0.49^{b}	100.60±0.44^{c}
50% (X ± SD)	423.9±0.23^{a}	49.51±0.09^{a}	133.1±0.33^{b}
80% (X ± SD)	420.7±3.92^{a}	49.57±0.18^{a}	135.7±0.90^{a}

The extraction time was 5h, extraction temperature was 25 °C, extraction solvent was water/ethanol and the ratio of solvent to material was 20:1

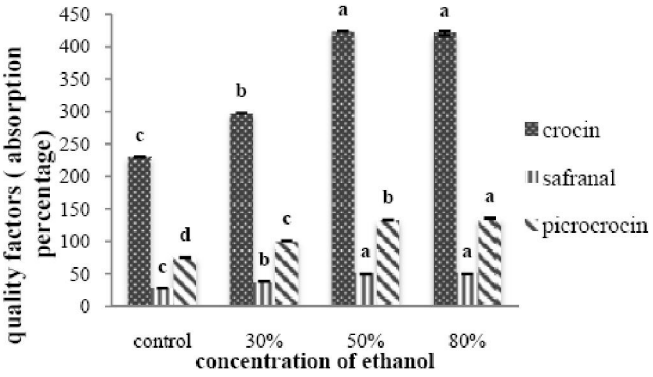

Figure 5 : Effects ofaqueous ethanolsolvent concentration on the quality factors of saffron extract

Figure 5 shows the effects of different concentrations of aqueous ethanol on the extraction yield of crocin, picrocrocin and safrasnalfrom crocus sativus. Other extraction conditions were fixed at: extraction time: 5h, extraction temperature: 25 °C, and ratio of solvent to material 20:1. A 50 % aqueous ethanol solution showed the highest extraction efficiency and was chosen as the optimal solvent for the following extraction experiments.

Effect of temperature

The influence of the temperature on theextraction yields of crocin, picrocrocin, and safranal from saffron (crocus sativus) was evaluated (TABLE 3). The extractions were performed with 50%aqueous ethanol at three different temperatures (25°c,40°c and 60°c) respectively. The extraction time was 5 h. The data shown in Figure 6 indicated no significantdifferences of extraction yields of the objective constituents whenthe temperature was increased from 25 to 40 °c. Ahigher temperature will lead to excess work in theextraction process, causing the decreasing in amount of saffron quality factors specially safranal. For commercial application, a temperature 25^{0}C (environmental temperature) should be optimum to avoid waste of quality factors such as safranal (saffron odor) and without any needs to heating equipment.

TABLE 3 : Comparison of $E^{1\%}_{\gamma MAX}$ values obtained using different temperature

Temperature	$E^{1\%}_{440}$ Crocin	$E^{1\%}_{330}$ Safranal	$E^{1\%}_{257}$ Picrocrocin
Control (X± SD)	229.7±0.68^{c}	27.92±0.4^{c}	75.17±0.33^{c}
25°c (X ± SD)	423.9±0.23^{a}	49.51±0.09^{a}	133.1±0.33^{a}
40°c (X ± SD)	344.6±0.23^{b}	35.38±0.09^{b}	113.7±0.22^{b}
60°c (X ± SD)	324.4±0.11^{c}	30.36±0.33^{c}	103.7±0.3^{c}

The extraction time was 5h, extraction solvent was water/ethanol, solvent concentration 50%v/v and the ratio of solvent to material was 20:1

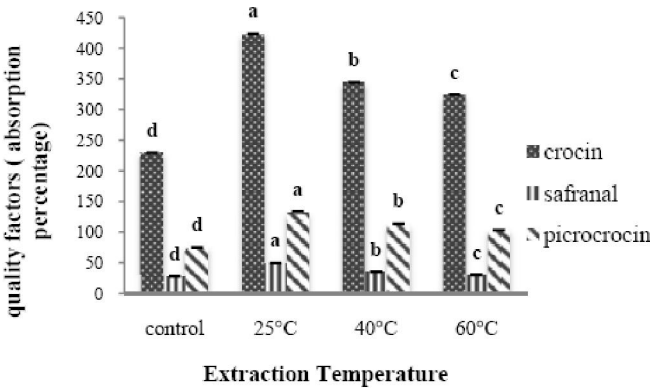

Figure 6 : Effects of extraction temperature on the quality factors of saffron extract

Effects of extraction time

TABLE 4 shows the effect of maceration time under same conditions (5 hours at 25 °C; solvent concentration 50%v/v (ethanol/water); ratio of solvent to material: 20:1) on quality factors of saffron extract. The results indicated that when extraction time increased from 1 to 5h, the extraction yields of crocin, picrocrocin, and safranal increased from 268.9 to 423.9, 42.63 to 49.51, and 92.34 to 133.1, respectively. After 5h, the extrac-

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tion yield is stable. These results can be explained as the effects of mixing and rupture of plant cells, which caused the intensification of mass transfer and thus closed interaction between the solvent and the plant tissues. Along with the increase of extraction time, all the plant cells will be completely cracked by mixing effect, and the extraction yield will increase within certain time duration. As the plant cells rupture, impurities such as insoluble substances, as well as lipids suspend in the extraction liquid, resulting in the lower permeability of the solvent. Dissolved constituents will also reabsorb on the smashed plant particles due to their relatively large specific surface areas lowering yields of recovered compounds. Hence, 5h is suitable time duration for the extraction of crocin, picrocrocin, and safranal (Figure 7).

TABLE 4 : Comparison of $E^{1\%}_{\gamma MAX}$ values obtained using different extraction times

Extraction Times	$E^{1\%}_{440}$ Crocin	$E^{1\%}_{330}$ Safranal	$E^{1\%}_{257}$ Picrocrocin
Control (X ± SD)	229.7±0.68^{c}	27.92±0.4^{c}	75.17±0.33^{c}
1h (X ± SD)	268.9±0.23^{b}	42.63±0.31^{b}	92.34±0.45^{b}
5h (X ± SD)	423.9±0.23^{a}	49.51±0.09^{a}	133.1±0.33^{a}
72h (X ± SD)	424.2±0.42^{a}	49.34±0.22^{a}	134.0±0.29^{a}

The extraction solvent was water/ethanol, extraction temperature was 25 °C, solvent concentration 50%v/v and the ratio of solvent to material was 20:1

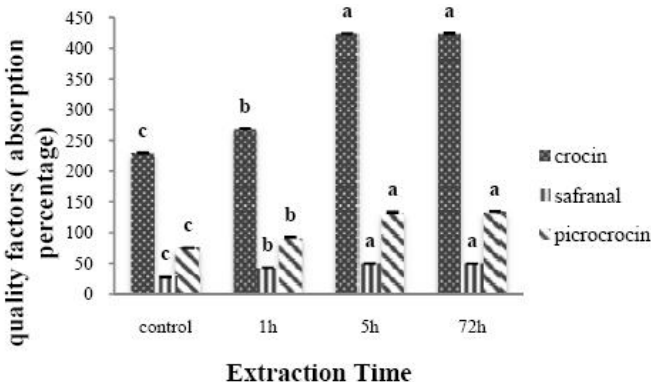

Figure 7 : Effects of extraction time on the quality factors of saffron extract

CONCLUSION

Saffron has a food and medicinal history usage in the world and is widely used as a food additive. This plant is the most precious and expensive spice in the world. The saffron filaments, or threads, are actually the dried stig-mas of the saffron flower and have red colors. These components are often dried and used in cooking as a sea-soning and coloring agent and it is most expensive. There are several reports showing that saffron has phar-macological effects including anti-tumor, antidepressant, neuro protective and anti-oxidative effects[12, 13].

This study investigated the use of classic extraction of three Carotenoid pigment, including crocin, picrocrocin and safranal from crocus sativus (saffron). The experiments suggest that the optimal parameters to extract the target compounds are as follows: extraction solvent: 50% water/ethanol; extraction time: 5h; extraction temperature: 25°C. This conditions lead to rise up saffron liquid extract quality compared to dried stigma.

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REFERENCES

[1] S.G.Agrawal, et al; US Patent, Patent no, US 7070823 **(2006)**.
[2] M.S.Moghaddasi; Journal of Medicinal Plants Research **4(6)**, 427-430 **(2010)**.
[3] P.Winterhalter, M.Straubinger; Food Rev.Intl., **16**, 39-59 **(2000)**.
[4] M.Bolandi, H.B.Ghoddusi; Flavor Science, Recent Advances and Trends, **43**, 323-326 **(2006)**.
[5] M.Carmona, A.M.Zalacain, A.Sanchez, J.Novella, G.L.Alonso; Agric.Food Chem., **54**, 973-979 **(2006)**.
[6] F.Hadizadeh, S.A.Mohajeri, M.Seifi; Pakistan Journal of Biological Sciences, **13**, 691-698 **(2010)**.
[7] H.H.Abd El-Baky, F.K.El Ba, G.S.El-Baroty; Am-Euras.J.Agric., Environ.Sci., **2(6)**, 792-800 **(2007)**.
[8] M.Bolandi, F.Shahidi, N.Sedaghat, R.Farhoush, H.Mousavi-Nik; **1(1)**, 51-55 **(2010)**.
[9] A.V.Loskutov, C.W.Beninger, G.L.Hosfield, C.K.Sunk; Food Chemistry, **69**, 87-95 **(2000)**.
[10] G.Fernandez; US Patent, Patent no,6458399, **(2002)**.
[11] H.Hosseinzadeh, M.Modaghegh, Z.Saffari; Ecam, **6(3)**, 343-350 **(2007)**.
[12] H.Hosseinzadeh, A.Sadegi, T.Ziaee; International Journal of Phototherapy & Psychopharmacology, ISSN: 0944-7113 **(2008)**.
[13] H.Hosseinzadeh, F.Shamsaie, S.Mehri; Phcog.Mag., **5**, 419-24 **(2009)**.