Characterization of Carotenoids Content and Composition of Saffron from Different Localities

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Abstract: The most essential carotenoids for humans are found in plants that are normally yellow, orange, and red coloured pigments. They are typically and mostly lipophilic in nature, but some unique plant species may yield water-soluble carotenoids. Saffron or *Crocus sativus* contains hydrophilic carotenoids named crocin. Thus, this paper will describe the extraction and characterization of hydrophilic and lipophilic carotenoids (colour properties) obtained from saffrons of different geographical origins. They are specifically the Iranian, Turkish, and Kashmiri saffron respectively. Maceration techniques have been employed to extract the targeted compounds, whereas the characterization of the compounds has been analysed using HPLC. The extraction and characterization of carotenoids in saffron from different geographical origins found that the amount of crocin content was substantially higher in Iranian saffron, which was 11414.67 ± 516.34 µg/g DW followed by Turkish and Kashmiri saffron. Lipohilic carotenoids (i.e. crocetin, β-carotene, and zeaxanthin) were detectable in Iranian and Turkish saffron but absent in Kashmiri saffron. Similarly, the highest amount of crocetin content was found in Iranian saffron at 1054.73 ± 50.31 µg/g DW, while the highest amount of β-carotene and zeaxanthin was found in Turkish saffron at 512.92 ± 79.98 µg/g DW and 252.04 ± 60.34 µg/g DW, respectively. There was a marked difference in carotenoid composition sourced from different localities. Various environmental factors like climatic conditions, agricultural practices, stigma separation, and storing and drying processes may play an important role to explain such difference.

Keywords: Active pharmaceutical ingredients, carotenoid, Saffron, natural pigment.

INTRODUCTION

Carotenoids, in particular, have been discovered in various natural sources like vegetables, fruits, algae, and crustaceans. Approximately 700 carotenoids have been found, contributing to the different colours of yellow, orange, and red [1-4]. The carotenoids are typically categorized according to their functional groups, specifically carotenes and xanthophylls. Carotenes like β-carotene, α-carotene, and lycopene consist of hydrocarbon, which is made up of only carbon and hydrogen, whereas xanthophylls contain oxygenated functional groups like lutein, zeaxanthin, β-cryptoxanthin and astaxanthin [4-6].

β-carotene is one of the most well-known food carotenoids and is sometimes found together with α-carotene in certain foods. It can be detected in carrot, mango, and apricots, whereas α-carotene is typically found in carrot and pumpkin. Lutein, the dihydroxy derivative of β –carotene is commonly detected in yellow or orange fruits and flowers, as well as green vegetables. Meanwhile, lycopene is a typical food carotenoid found in many red fruits and vegetables, such as watermelon, pink guava, grape, and tomato [4, 6]. In contrast, astaxanthin is the major form of carotenoid detected in marine animals like salmon, shrimp, lobster and crab, as well as other microorganisms [7]. Unique carotenoids like bixin may be sourced in annatto, while crocin is present in saffron [6]. The red pigment from bixin is widely and typically used in the food, pharmaceutical, cosmetic and textile industries accordingly [8]. However, the primary carotenoids in a type of food may differ depending on the genetics, locality, seasonality and handling techniques [4, 9-11].

According to the BCC Research Report [12], the global market value for carotenoids fetched up to $1.5 billion in 2017 and is expected to reach $2.0 billion by 2022, at a compound annual growth rate (CAGR) of 5.7% for the period of 2017-2022. The geriatric population growth concerning health care and disease prevention is a major factor that boosts the demand for carotenoids. Moreover, the demand for natural

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colorants is another factor fuelling market growth. Research and Development (R&D) for the invention of high-value natural carotenoids becomes a prospect during the forecast period [12].

The most essential carotenoids for humans are found in plants that are normally yellow, orange, and red coloured pigments. They are typically and mostly lipophilic in nature, but some unique plant species may yield water-soluble carotenoids. Saffron or *Crocus sativus* contains hydrophilic carotenoids named crocin ($C_{44}H_{64}O_{24}$). The utilization of saffron as a colouring agent was started during the ancient eras specifically for textiles [13]. The growing demand for saffron in Malaysia has inspired the researchers in cultivating this 'red gold' spice via *in vivo* and *in vitro* conditions. The results have successfully shown that saffron cultivation is promising for mass propagation, plant conservation as well as commercialization in Malaysia [14].

Phylogenetically, *C. sativus* is classified in the clade Angiosperms, clade Monocots, order Asparagales, family Iridaceae, subfamily Crocoideae and genus Crocus [15]. It is a triploid perennial sterile plant cultured by rhizomes and produces the most expensive saffron spice in the world [16]. This makes up merely 7.4% of its whole plant section [17]. Saffron's flower contains three stigmas that only weigh about 6 mg, hence, to produce 1 kg of the stigmas, more than 150,000 saffron flowers must be harvested, and the stigma will be carefully separated, consequently, the production of this spice produced more than 90 % by-products. Hence, its meticulous process, which requires a long-time procedure becomes one of the factors for its expensiveness [17]. Saffron color, taste, and aroma quality is commonly dependant on various factors, such as soil, climate, agricultural practices that includes harvest time, postharvest treatments, as well as dehydration treatment [16, 18]. The main supplier of saffron globally is Iran, accounting for nearly 94% of the worldwide saffron production, followed by Greece, Kashmir, and Morocco [19]. The increasing demand of this imported spice in Malaysia has initiated the researchers in cultivating this 'red gold' spice under *in vivo* and *in vitro* condition and surprisingly, the results have successfully proven that saffron cultivation is promising for mass propagation, plant conservation as well as commercialization in this tropical country [14].

Since the ancient eras, the dry stigmas of saffron is known as a valuable spice that is widely used in daily life as a dye, cosmetics, in cooking, and for medical purposes [13]. In the biomedical industry, its usage is fronted by its main biological compound boasting various antioxidant properties, as well as perks of anticancer, antimicrobial, and anti-inflammatory benefits. Hence, the compound is credited towards preventing and healing numerous health problems as reported by many researchers [13, 20, 21]. Despite the expensive price, crocin high water solubility and stability at varying pH, light, oxidation and resistance to microbiological attacks have allowed its pigment to be commercially used in the food, bakery and beverage industries, with the FDA recognizing it as a coloring agent [22]. Colorant from saffron is of natural food colorants categorized under 'exempt from certification' of FDA and EU which named CI natural yellow [23, 24]. As a whole, due to its excellent bioactivities and hydrophilic property, crocin is chosen for this study in order to explore its potential as hydrophilic biocolourant to be utilized in various product applications.

Additionally, crocin is the main pigment of saffron that produces a yellowish red color primarily due to glycosylated esters of a dicarboxylic acid, whereas its solubility is due to the saccharide link with either glucose, gentibiose or neapolitanose [25]. It has seven conjugated double bonds at the central unit and consists of four side-chain methyl groups, whereby the end groups are esterified with one, two and three glucose units [26, 27]. It is also a group of hydrophilic glycosyl esters of crocetin (8,8′-diapo-ψ,ψ′-carotenedioic acid) with molecular weight 976.972 g/mol. The mobility of the solution may be enhanced with the presence of a high volume of water [28]. Meanwhile, methanolic, ethanolic or aqueous solutions are commonly used to extract crocin from saffron [29, 30]. Crocin displayed more yellow than bixin (another hydrophilic carotenoid) due to its fewer number of carbon atoms in its conjugated chain, hence, became the best alternative to replace the utilization of tartrazine due to its water solubility and a similar shade of colour [31, 32].

Therefore, this paper will describe the extraction and characterization of hydrophilic and lipophilic carotenoids (color properties) obtained from saffron of different geographical origins. They are specifically the Iranian, Turkish, and Kashmiri saffron respectively. Maceration techniques have been employed to extract the targeted compounds, whereas the characterization of the compounds has been analyzed using HPLC.

MATERIALS AND METHODS

Sample Preparation

Dried saffron stigmas have been sourced from Spice Bazaar, Istanbul, Turkey, which originated from

Iran, Turkey, and Kashmir respectively. The samples have been stored in the freezer at -20°C prior to further processing.

Extraction Procedure

Hydrophilic carotenoid (crocin) extraction followed the method described by [33] with a slight modification. 1 g of saffron stigma was added into a volumetric flask containing 1 liter of distilled water (Water Purification Systems, Millipore S.A.S) before the mixture is placed on the platform shaker (Unimax 1010 DT, Heidolph, Germany). It was then agitated for 30 minutes in ambient temperature prior to being heated in the oven (UF55, Memmert) for another 30 minutes at a temperature of 60°C. Next, the saffron mixture was kept in the dark for 24 hours at room temperature (approximately 25°C), followed by a filtration process before the resulting pigment was transferred into a conical flask. Then, 1 liter of distilled water was added again into the sample to extract the remaining pigment. The maceration process was repeated until the liquid becomes colorless. Then, the combined crocin pigment was concentrated using a rotary evaporator (Hei-VAP Precision, Heidolph, Germany) equipped with a water bath at 45 °C and operated at 75 to100 rpm speed at 45 mbar vacuum condition. The concentrated extract with a final volume of 10 ml was then freeze-dried (Alpha 1-4 LD Plus, Martin Christ) for 3 days until completion. Finally, a good-quality of extract was maintained by storing the powdered crocin pigment in a sealed amber bottle in a freezer at -20 °C prior to further analysis.

In contrast, the procedure for lipophilic carotenoid extraction followed the method used by [10]. 1 g of dried saffron stigma was added into 50 ml tubes and rehydrated via the addition of 1 ml distilled water. Then, 5 ml of different solvents (i.e. acetone: methanol mixture (9:1, v/v), acetone, methanol, ethanol) prepared with 0.1 % butylated hydroxytoluene (BHT) was added into each tube. BHT is used to avoid any oxidation and isomerization from occurring during the extraction [9]. The samples were then stored overnight in a dark condition at room temperature, whereby each had been prepared in triplicate. The next day, the samples were vortexed and centrifuged for 2 minutes at 13 500 g (NU-C200R-E, Nuaire, USA), with the resulting supernatant being transferred into 50 ml graduated polypropylene centrifuge tubes. This step was then repeated by adding 5 ml of the same solvent until the supernatant of the sample becomes colourless (normally two or three times). Then, an equal volume of hexane and distilled water (5 ml) was added to extract the carotenoid from the combined supernatants. The solution was then allowed to separate, with the upper hexane layer containing the carotenoids being collected. This procedure was repeated with hexane alone until it becomes colourless. After that, the combined upper layer was dried to completion under a gentle stream of oxygen-free nitrogen. Tubes were then capped and sealed with parafilm to exclude oxygen and immediately stored at -20 °C prior to further analysis.

Analysis of Carotenoids Content by High–Performance Liquid Chromatography (HPLC) Analysis

1 mg of dried crocin and 1 mg of dried lipophilic carotenoids extract respectively were dissolved in 1 ml methanol and 1ml ethyl acetate accordingly. 450 µl of sample was then added into Whatman Mini-UniPrep Syringeless Filters vial. Triplicate samples was prepared, with three injections being performed for each vial. The HPLC analysis had subsequently followed the procedure described by previous authors [10, 34] with the HPLC Agilent 1200 series (Agilent Technologies, USA) being used to analyze the carotenoid content extracted from *C. sativus* stigma. It is equipped with a binary pump with autosampler injector, micro vacuum degassers, and thermostatted column compartment, whereas the reverse phase column is a ZORBAX Eclipse XDB-C_{18} end-capped (5 µm), sized at 4.6 x 150 mm. An HPLC grade of acetonitrile: water (9:1 v/v) had been prepared as eluent A, whereas HPLC grade of ethyl acetate was designated as eluent B. Meanwhile, Ultrapure water 18.0 MΩ was prepared using a Millipore S.A.S water purification system.

The solvent gradient used had been established as follows: 0-40% solvent B (0-20 min), 40-60% solvent B (20-25 min), 60-100% solvent B (25- 25.1 min), 100% solvent B (25.1-35 min) and 100-0% solvent B (35-35.1 min). The flow rate was set at 1.0 ml min^{-1} and the injection volume of 10 µl. The column has been thermostated at 25 °C, with carotenoid peak identification performed at the range of 350 to 550 nm. The stop time was set at 40 minutes, with individual carotenoid detection done at maximum absorption wavelength according to the carotenoids: crocin and crocetin (440 nm), zeaxanthin (452 nm) and β-carotene (454 nm). The concentrations of the carotenoids were determined using their standard curve with regards to their structures and properties. The chromatographic peak was then identified by comparing the retention

time and spectra against the carotenoid's standard. The carotenoids concentration was quantified in terms of a microgram per 1.0 g dry weight of the sample (µg/g DW).

RESULTS AND DISCUSSION

Analysis of Carotenoids Contents by HPLC Analysis

The HPLC chromatograms of crocin have been detected at 2.5 to 2.6 minutes of retention time, as shown in Figures **1**, **2**, and **3**. Meanwhile, the peaks of lipophilic carotenoids, which are specifically the crocetin, zeaxanthin and β-carotene of Iranian and Turkish saffron have been detected at the retention time of 1.7, 9.9, and 27.9 minutes respectively, as shown in Figures **4** and **5**. The result of carotenoid content is as presented in Table **1**, whereby the highest amount of crocin and crocetin content is found in Iranian saffron at 11414.67± 516.34 µg/g DW and 1054.73 ± 50.31 µg/g DW, respectively. Meanwhile, the highest amount of β-carotene and zeaxanthin are found in Turkish saffron, whereas Kashmiri saffron only shows the presence of crocin at the lowest amount compared to Turkish and Iranian saffron.

The highest amount of crocin and crocetin in Iranian saffron, as detected in this study, is supported by previous reports, whereby Iranian saffron is recognized as the greatest saffron producer in terms of quality and quantity [36]. Furthermore, Iranian climates of arid and semi-arid have rendered it a special factor for the

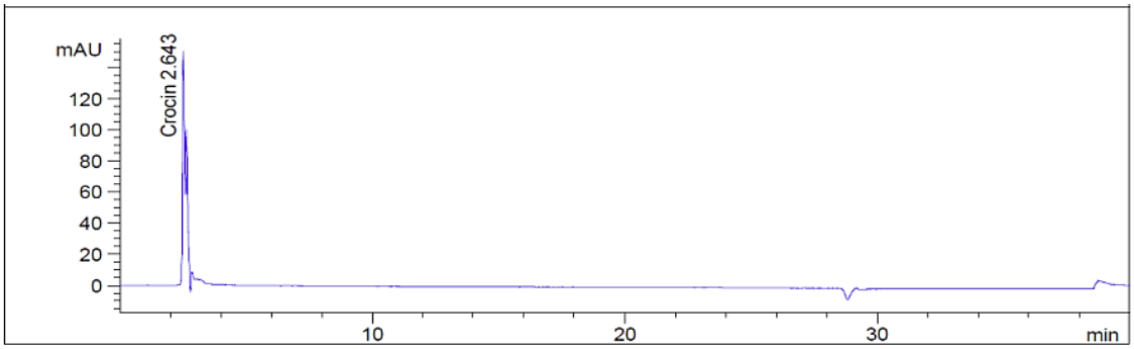

Figure 1: HPLC chromatogram of crocin in Kashmiri saffron sample.

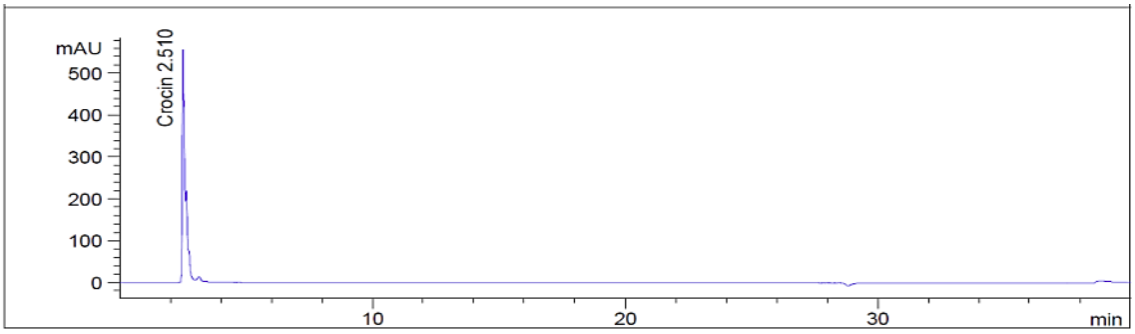

Figure 2: HPLC chromatogram of crocin in Turkish saffron sample.

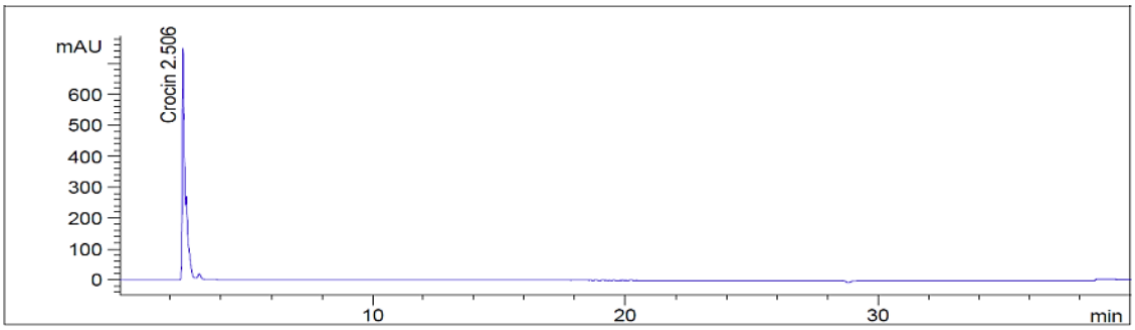

Figure 3: HPLC chromatogram of crocin in Iranian saffron sample.

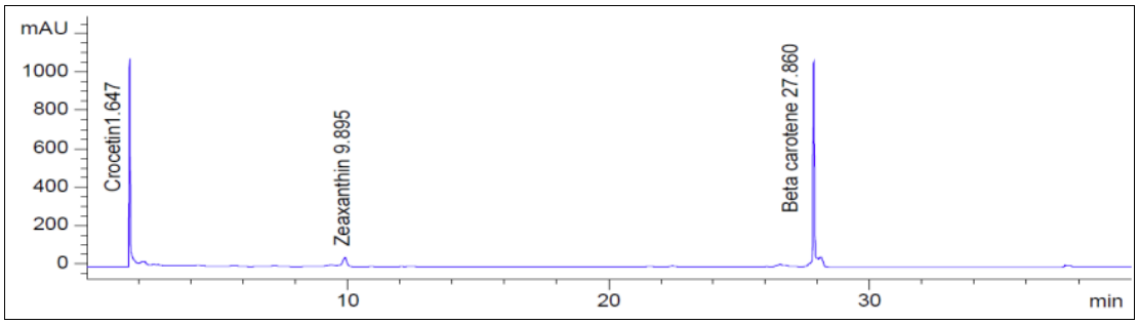

Figure 4: HPLC chromatogram of crocetin, zeaxanthin, and β-carotene in Turkish saffron sample.

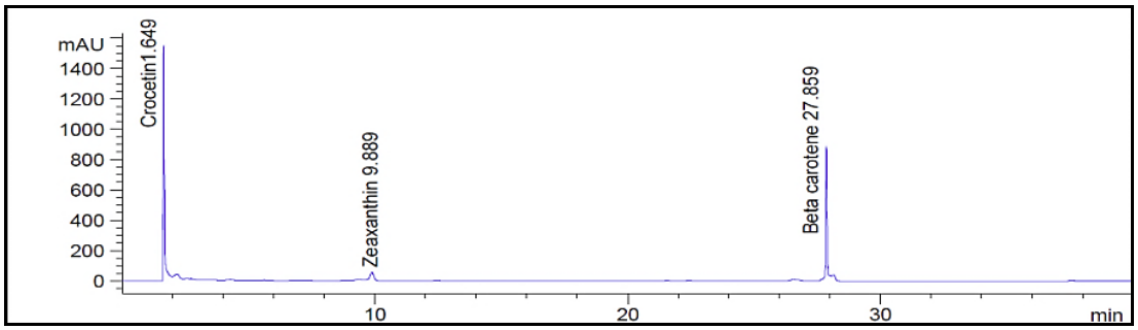

Figure 5: HPLC chromatogram of crocetin, zeaxanthin and β-carotene in Iranian saffron sample.

Table 1: Individual Carotenoids Content in Saffron from Different Localities

Carotenoids	Saffron origin	Amount (µg/g DW)
Crocin	Kashmir	311.63 ± 38.79
	Turkey	8201.70 ± 154.59
	Iran	11414.67± 516.34
Crocetin	Kashmir	ND
	Turkey	186.64 ± 15.43
	Iran	1054.73 ± 50.31
β-carotene	Kashmir	ND
	Turkey	512.92 ± 79.98
	Iran	343.46 ± 87.06
Zeaxanthin	Kashmir	ND
	Turkey	252.04 ± 60.34
	Iran	61.23 ± 9.58

*Results in mean ± standard deviation; DW- dry weight; ND-Not determined.

cultivation of quality saffron, especially in North Khorasan, Fars, Kerman and Yazd provinces. Globally, nearly 95 % of saffron in 2005 was produced by Iran, with 82 % of it being exported [19]. Meanwhile, other countries that also record saffron production are Greece, followed by Morocco, Kashmir, Turkey, Spain, and Italy. Furthermore, the results of the recent study are also in agreement with previous studies that detected other carotenoids like β-carotene and zeaxanthin in saffron at a lower concentration [30, 37-39]. In addition, the carotenoid biosynthesis involving a specific chromoplast pathway during the development of the saffron is responsible for the high concentration of apocarotenoid in stigmas [15].

Due to the high-cost production of saffron, various fraudulent methods have been applied to manipulate its properties. This includes the addition of artificial or

natural colorants among them to substitute or enhance the colors [40]. For instance, the content of crocetin ester in *Gardenia jasminoides* fruit has allowed the producers some option in substituting saffron. Therefore, several simple procedures may be executed to ensure the saffron authentication, as described by [40]. Identification measurement is also regulated by international trade to ensure that exported saffron meets the requirements and specifications of the ISO/TS 3632 standard.

In terms of quality control in consideration of the economical aspect, ultraviolet-visible spectro-photometer (UV-Vis) analysis is a common procedure that is employed instead of the analytical procedure using high-performance liquid chromatography (HPLC) or gas chromatography (GC) [37, 40]. The most important element that contributes to the color, taste, and aroma of the saffron is due to a group of carotenoids and crocetin esters (carotenoid derivatives), picrocrocin (a monoterpene glucoside), and volatile safranal (a monoterpene) respectively [41, 42]. Therefore, the quality in terms of the color commonly analyzed at maximum absorbance, 440 nm (coloring strength) has been valued and utilized as a coloring agent in the food and beverage industry.

CONCLUSION

The outcomes of the extraction and characterization of carotenoids in the saffron extract from different geographical origins may be summarized as follows:

1. The amount of crocin content was found to be substantially higher in Iranian saffron, which is 11414.67± 516.34 µg/g DW followed by Turkish and Kashmiri saffron.

2. Lipohilic carotenoids (i.e. crocetin, β-carotene, and zeaxanthin) were detectable in Iranian and Turkish saffron but absent in Kashmiri saffron. Similarly, the highest amount of crocetin content was found in Iranian saffron at 1054.73 ± 50.31 µg/g DW, while the highest amount of β-carotene and zeaxanthin were found in Turkish saffron at 512.92 ± 79.98 µg/g DW and 252.04 ± 60.34 µg/g DW respectively.

3. There was a marked difference in carotenoid composition sourced from different localities. Various environmental factors like climatic conditions, agricultural practices, stigma separation, and storing and drying processes may have played an important role to explain such differences.

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REFERENCES

[1] Mezzomo N, Ferreira SRS. Carotenoids functionality, sources, and processing by supercritical technology : A review. J Chem 2016; 1-17. https://doi.org/10.1155/2016/3164312

[2] Polyakov NE, Leshina TV, Meteleva ES, Dushkin AV, Konovalova TA, Kispert LD. Water-soluble complexes of carotenoids with Arabinogalactan. J Phys Chem B 2009; 113: 275-282. https://doi.org/10.1021/jp805531q

[3] Rodriguez-Amaya DB, Rodriguez EB, Amaya-Farfan J. Advances in food carotenoid research: chemical and technological aspects, implications in human health. Ma1 J Nutr 2006; 12(1): 101-121.

[4] Fortes C. Carotenoids in cancer prevention. In: Baer-Dubowska W, Bartoszek A, Malejka-Giganti D, editors. Carcinogenic and anticarcinogenic food components. United States of America: CRC Press 2006; p. 283-302.

[5] Rivera S, Vilaró F, Canela R. Determination of carotenoids by liquid chromatography/mass spectrometry: Effect of several dopants. Anal Bioanal Chem 2011; 400(5): 1339-1346. https://doi.org/10.1007/s00216-011-4825-6

[6] Rodriguez-Amaya DB. A guide to carotenoid analysis in foods. Washington, DC, USA: OMNI Research; 2001.

[7] Ushakumari UN, Ramanujan R. Astaxanthin from shrimp shell waste. Int J Pharm Chem Res 2012; 1(3): 1-6.

[8] Santos DT, Albuquerque CLC, Meireles MAA. Antioxidant dye and pigment extraction using a homemade pressurized solvent extraction system. Procedia Food Sci 2011; 1: 1581-1588. https://doi.org/10.1016/j.profoo.2011.09.234

[9] Arvayo-Enríquez H, Mondaca-Fernández I, Gortáres-Moroyoqui P, Lopez-Cervantes J, Rodríguez-Ramírez R. Carotenoids extraction and quantification: a review. Anal Methods 2013; 5(12): 2916-2924. https://doi.org/10.1039/c3ay26295b

[10] Othman R. Biochemistry and genetics of carotenoid composition in potato tubers. Lincoln University; 2009.

[11] Manzi F, Flood V, Webb K, Mitchell P. The intake of carotenoids in an older Australian population: the Blue Mountains Eye Study, Public Health Nutr 2002; 5: 347. https://doi.org/10.1079/PHN2002258

[12] McWilliams A. BCC research report overview the: The global market carotenoids. 2018.

[13] Licón C, Carmona M, Llorens S, Berruga MI, Alonso GL. Potential healthy effects of saffron spice (*Crocus sativus* L . stigmas) consumption. Funct Plant Sci Biotechnol 2010; 4 (Special Issue 2): 64-73.

[14] Taha RM, Anuar N, Khan A, Nowrouzi B, Abdullah S, Mohajer S. First novel attempt of saffron (Crocus Sativus L.) cultivation (*in vivo* and *in vitro*) in Malaysia for mass propagation. In: ISER 21st International Conference; Taipei, Taiwan; 2016: p. 17.

[15] Sánchez AM, Winterhalter P. Carotenoid cleavage products in saffron (Crocus sativus L). In: ACS Symposium Series vol. 1134; Washington, DC, USA: American Chemical Society; 2013: p. 45-63. https://doi.org/10.1021/bk-2013-1134.ch005

[16] Nordiyanah A, Taha RM, Noraini M, Sadegh M, Musa SANC, Zul Hazrin ZA. Correlation of colour, antioxidant capacity and phytochemical diversity of imported by principal components analysis. Pigm Resin Technol 2017; 46(2): 107-114. https://doi.org/10.1108/PRT-09-2015-0091

[17] Jinous A, Elahe D-M, Arash M, Rezvan M, Mojdeh H. In-vitro evaluation of *Crocus sativus* L . petals and stamens as natural antibacterial agents against food-borne bacterial strains. Iran J Pharm Sci 2013; 9(4): 69-82.

[18] Carmona M, Zalacain A, Pardo JE, López E, Alvarruiz A, Alonso GL. Influence of different drying and aging conditions on saffron constituents. J Agric Food Chem 2005; 53(10): 3974-3979. https://doi.org/10.1021/jf0404748

[19] Ghorbani M. The Efficiency of saffron's marketing channel in Iran. World Appl Sci J 2008; 4(4): 523-527.

[20] Hosseinzadeh H, Modaghegh M. Saffari Z. *Crocus sativus* L. (saffron) extract and its active constituents (crocin and safranal) on ischemia-reperfusion in rat skeletal muscle. Evid Based Complement Alternat Med; 2007; 9(3): 343-350. https://doi.org/10.1093/ecam/nem125

[21] Urrutia EC, Riverón-Negrete L, Abdullaev F, Del-Angel DS, Martínez NLH, Cruz MEG, Cruz VDP, Silvia-Adaya D, González-Cortés C, Santamaría A. Saffron extract ameliorates oxidative damage and mitochondrial dysfunction in the rat brain. Acta Hortic 2007; 739: 359-366. https://doi.org/10.17660/ActaHortic.2007.739.47

[22] Delgado-Vargas F, Paredes-López O. Natural colorants for food and nutraceutical uses. Boca Raton, Florida: CRC Press; 2003. https://doi.org/10.1201/9781420031713

[23] Aberoumand A. A review article on edible pigments properties and sources as natural biocolorants in foodstuff and food industry. World J Dairy Food Sci 2011; 6(1): 71-78.

[24] Ahmad WA, Wan Ahmad WY, Zakaria ZA. Yusof NZ. Application of bacterial pigments as colorant: The Malaysian perspective, Malaysia: Springer; 2012. https://doi.org/10.1007/978-3-642-24520-6

[25] Licón CC, Carmona M, Rubio R, Molina A, Berruga MI. Preliminary study of saffron (*Crocus sativus* L. stigmas) color extraction in a dairy matrix. Dyes Pigm 2012; 92(3): 1355-1360. https://doi.org/10.1016/j.dyepig.2011.09.022

[26] Liakopoulou-Kyriakides M, Tsatsaroni E, Laderos P, Georgiadou K. Dyeing of cotton and wool fibres with pigments from *Crocus sativus*-Effect of enzymatic treatment. Dyes Pigm 1998; 36(3): 215-221. https://doi.org/10.1016/S0143-7208(97)00013-2

[27] Vickackaite V, Romani A, Pannacci D, Favaro G. Photochemical and thermal degradation of a naturally occurring dye used in artistic painting. A chromatographic, spectrophotometric and fluorimetric study on saffron. Int J Photoenergy 2004; 6: 175-183. https://doi.org/10.1155/S1110662X04000224

[28] Tsimidou M, Biliaderis CG. Kinetic studies of saffron (*Crocus sativus* L.) quality deterioration. J Agric Food Chem 1997; 45(8): 2890-2898. https://doi.org/10.1021/jf970076n

[29] Mohajeri SA, Hosseinzadeh H, Keyhanfar F. Aghamohammadian J. Extraction of crocin from saffron (*Crocus sativus*) using molecularly imprinted polymer solid-phase extraction. J Sep Sci 2010; 33(15): 2302-2309. https://doi.org/10.1002/jssc.201000183

[30] Sánchez AM, Carmona M, Ordoudi SA, Tsimidou MZ, Alonso GL. Kinetics of individual crocetin ester degradation in aqueous extracts of saffron (*Crocus sativus* L.) upon thermal treatment in the dark. J Agric Food Chem 2008; 56(5): 1627-1637. https://doi.org/10.1021/jf0730993

[31] Timberlake CF, Henry BS. Plant pigments as natural food colours. Endeavour 1986; 10(1): 31-36. https://doi.org/10.1016/0160-9327(86)90048-7

[32] National Center for Biotechnology Information [Internet]. PubChem compound database. USA: U.S. National Library of Medicine [cited 2018 Mar 6]: Available from https://pubchem.ncbi.nlm.nih.gov/

[33] Tsatsaroni E, Eleftheriadis I. Comparative study of dyeing properties of two yellow natural pigments-Effect of enzymes and proteins. Dyes Pigm 1998; 37(4): 307-315. https://doi.org/10.1016/S0143-7208(97)00069-7

[34] Radzali SA, Masturah M, Baharin BS, Rashidi O, Rahman RA. Optimisation of supercritical fluid extraction of astaxanthin from *Penaeus monodon* waste using ethanol-modified carbon dioxide. J Eng Sci Technol 2016; 11(5): 722-736.

[35] Butnariu M, Rodino S, Petrache P, Negoescu C, Butu M. Determination and quantification of maize zeaxanthin. Dig J Nanomater Bios 2014; 9(2): 745-755.

[36] Amiri Aghdaie SF. Investigating effective factors on Iran's pistachio exportation. Int J Mark Stud 2009; 1(2): 35-40. https://doi.org/10.5539/ijms.v1n2p35

[37] Lech K, Witowska-jarosz J, Jarosz M. Saffron yellow: characterization of carotenoids by high performance liquid chromatography with electrospray mass spectrometric detection. J Mass Spectrom 2009; 2009(May): 1661-1667. https://doi.org/10.1002/jms.1631

[38] Castillo R, Fernández JA, Gómez-Gómez L. Implications of carotenoid biosynthetic genes in apocarotenoid formation during the stigma development of *Crocus sativus* and its closer relatives. Plant Physiol 2005; 139(2): 674-689. https://doi.org/10.1104/pp.105.067827

[39] Pfander H, Schurtenberger H. Biosynthesis of C20-carotenoids in *Crocus sativus*. Phytochem 1982; 21(5): 1039-1042. https://doi.org/10.1016/S0031-9422(00)82412-7

[40] Sánchez AM, Maggi L, Carmona M, Alonso GL. Authentication of saffron spice (*Crocus sativus* L.). In: ACS Symposium Series vol. 1081; Washington, DC, USA: American Chemical Society; 2011: p. 309-331. https://doi.org/10.1021/bk-2011-1081.ch022

[41] Heidarbeigi K, Mohtasebi SS, Foroughirad A, Ghasemi-Varnamkhasti M, Rafiee S, Rezaei K. Detection of adulteration in saffron samples using electronic nose. Int J Food Prop 2015; 18(7): 1391-1401. https://doi.org/10.1080/10942912.2014.915850

[42] Priscila DCC, Carmona M, Maggi L, Kanakis CD, Anastasaki EG, Tarantilis PA, … Alonso GL. Picrocrocin content and quality categories in different (345) worldwide samples of saffron (*Crocus sativus* L.). J Agric Food Chem 2010; 58(2): 1305-1312. https://doi.org/10.1021/jf903336t

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