Canadian Chemical Transactions Year 2016 | Volume 4 | Issue 3 | Page 389-397

Research Article

DOI:10.13179/canchemtrans.2016.03.0329

Gas Chromatographic Determination of Phytosterols and Fatty Acids Profile in Saffron Petals

Javad Feizy[1] and Nastaran Reyhani[2]

[1]*Food Chemistry Department, Research Institute of Food Science and Technology, P.O.Box 91735-147, Mashhad, Iran*
[2]*Testa Quality Control Laboratory, Mashhad, Iran.*

**Corresponding Author, Email: j.feizy@rifst.ac.ir; feizy.j@gmail.com*

Received: *July 26, 2016* ***Revised:*** *September 15, 2016* ***Accepted:*** *September 18, 2016* ***Published:*** *October 2, 2016*

Abstract: Saffron petals are one of the main by-products in saffron processing that is producing in large amounts annually. The purpose of this study was to determine phytosterols and fatty acids of the saffron petals using gas chromatographic technique. The saffron petal samples were obtained from the flowers of Torbateheydariyeh farms. The main fatty acids in the oils of saffron petals were linoleic acid (28.48 g/100g), linolenic acid (21.06 g/100g) and palmitic acid (16.21 g/100g). The major sterols were fucosterol (47.65 g/100g), stigmasterol (32.69 g/100g), sitosterol (10.17 g/100g) and fagarasterol (9.48 g/100g). In this work, we reported fatty acids composition and phytosterols content of the saffron petals, for the first time. As a conclusion, the saffron petals for nutrition value could be used in food and pharmaceutical applications

Keywords: Phytosterol; Fatty acid; Saffron petal; GC

1. INTRODUCTION

Sterols are substances that derived from hydroxylated polycyclic isopentenoids having a 1, 2-cyclopentanophenthrene structure. These compounds have a total of 27-30 carbon atoms (the number of carbon atoms in the biosynthetic precursor squalene oxide) in which a side chain with carbon atoms more than or equal of seven, is attached at the carbon 17 position (C-17). Their structures are closely related and varied depending on the extent of modifications of the ring system and side chain variations [1]. The main biological functions of phytosterols are their cholesterol-reducing properties and anticancer effects [2, 3].

The main phytosterols found commonly in plants [4] include sitosterol, stigmasterol and campesterol. They have much stand out due to their nutritional value as natural components of regular diet [5, 6]. The most common phytosterols and phytostanols are sitosterol (3β-stigmast-5-en-3ol), sitostanol (3β,5α-stigmastan-3-ol), campesterol (3β-ergost-5-en-3-ol), campestanol (3β,5α-ergostan-3-ol), stigmasterol (3β-stigmasta-5,22-dien-3-ol) and brassicasterol (3β-ergosta-5,22-dien-3-ol). The chemical structure of reported pytosterols in saffron petals are shown in Fig. 1.

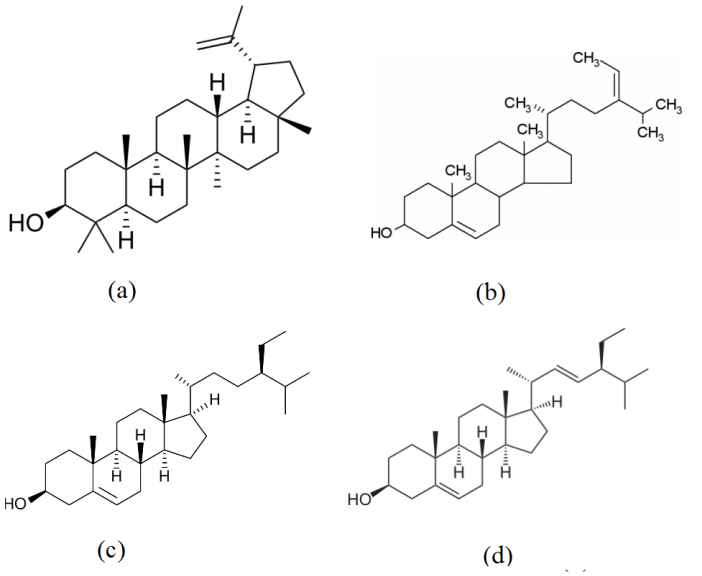

Figure 1. Chemical structure of Fagarasterol (a), Fucosterol (b), Sitosterol (c) and Stigmasterol (d)

The beneficial effects of phytosterols in human health have triggered much study on the development of analytical methods for determination of phytosterols in diet sources. General methods used for the determination of phytosterols include gas liquid chromatography [7] and high performance liquid chromatography [8]. Mass spectrometry methods connected with gas and liquid chromatography have accepted as power tools for the detection of phytosterols in complicated samples [9, 10]. Liquid chromatography in nano scale has been used to analyze phytosterols in plant oils such as olive oil [11].
Isolation and extraction of sterols from plant tissues or oilseeds needs initial solvent extraction, supercritical fluid extraction (SFE), or supercritical fluid fractionation (SFF) followed by various clean-up and chromatographic procedures. For subsequent characterization and quantification of sterol compounds, the crude isolate can be separated by a wide variety of chromatographic techniques including column chromatography (CC), gas chromatography (GC), thin-layer chromatography (TLC), normal phase high-performance liquid chromatography (NP-HPLC), reversed-phase high-performance liquid chromatography (RP-HPLC) and capillary electrochromatography (CEC). The sterols can be detected with flame ionization detection (FID), UV detection (UV), evaporative light scattering detection (ELSD), infrared detection (IR), nuclear magnetic resonance detection (NMR) and mass spectrometry (MS) [1].

Fatty acids are components of foods that serve as a source of energy for man and animals. The type of fatty acids consumed has important implications for human health, especially with respect to concentrations of serum lipids and the risk of coronary heart disease [e.g., 2-5]. C_{20}-polyunsaturated fatty acids such as arachidonic acid, eicosapentaenoic acid or dihomo-γ-linolenic acid located in biological membranes serve as precursors for eicosanoids [12, 13].

GC with flame ionization detector is the common method for fatty acids determination in foods. There are other chromatographic techniques, notably high-performance liquid chromatography (HPLC) [14], where alternative derivatives, such as those with UV chromophores, are better. Picolinyl esters or pyrrolidide derivatives have special properties for GC-mass spectrometry (MS) of fatty acids [15]. Most fatty acids don't absorb UV naturally and don't show fluorescence; therefore there is no method for determination of fatty acids by spectrophotometer and Spectrofluorometer.

Saffron petals are one of the by-products of fields that, so far, no appropriate way found for reusing it. The amount of this by-product is more than 10000 tons each year [16]. Nowadays, the only

application of saffron petals is in dye industry, which is not flourished yet [17]. It has been reported that ethanol extract of saffron petals possesses antidepressant activity [18]. Phenolic compounds are likely to be the biologically active components of the petals [19]. Flavanoids and anthocyanins are among phenolic compounds of this species [20, 21]. Petals of Crocus sativus are rich in flavonoids and anthocyanins. The protective activity of carotenoid against cancer has obtained support from a large number of epidemiological and experimental studies [22-25].

Therefore, in this study, the phytosterols and fatty acids composition of saffron petals were investigated as methyl esters by gas chromatographic technique. This is the first report on fatty acids composition and phytosterols content of saffron petals from Iran.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Stigmasterol (95%), β-sitosterol (95%), fagarasterol (94%), fucosterol (93%), and cholesterol (94%) were purchased from Sigma-Aldrich (MO, USA). All organic solvents used in the study were of HPLC grade purchased from Fisher Scientific (PA, USA). Potassium hydroxide purchased from Merck (Darmstadt, Germany). Ultra-high purity (UHP) water, prepared from tap water, was pre-treated using Elix reverse osmosis cartridges before filtration by a Milli-Q system from Millipore (Bedford, MA, USA) and used throughout the study. All other chemicals used were of analytical grade commercially available, unless otherwise stated.

2.2 Sample Preparation

For fatty acid methyl esters (FAMEs) preparation, appropriate amount of sample was weighed in a beaker and 200 ml hexane added to the sample vessel. After 1 h the solvent was evaporated using a rotary evaporator. The remained extracted oil estrificated by addition of 7 ml of heptane and 2 ml of KOH/MeOH mixture at 40-50 ◦C for 15 min. Then the supernatant was gathered and injected to the gas chromatograph instrument.

For sterols, 50 ml of ethanolic potassium hydroxide 2.32M mixture was added to 2 gr of sample and refluxed for 1 h. The refluxed solution was then cooled to room temperature and transported to a separating funnel and 50 ml distilled water and 100 ml of hexane were added to the funnel. After shaking the funnel for 20 min the supernatant was added to the rotary flask and dried in the room temperature. One ml of chloroform was added to the dried mass and the obtained solution was injected to the gas chromatograph instrument.

2.3 Sterol analysis

GC analysis was carried out by Agilent 7890A coupled with flame ionization detector. The HP-5 capillary column with dimensions of 30 m ×0.32 mm and 0.25 µm film thickness was used for the analysis. One µL of sample was injected with split mode (1:10). Helium used as a carrier gas at flow rate of 1 ml min^{-1} and the total run time was 25 min and the equilibration time was 0.5 min. The oven temperature was 260 ◦C. The temperature of the injection port and detector was 300 ◦C and 310◦C, respectively. .

The compounds were analyzed by GC–MS (Agilent 5977 MSD). MS data were acquired with full scan (50–500 m/z) mode for identification with 4 min of solvent delay.

Fatty acid analysis

Chromatographic analysis was carried out on a Agilent apparatus, model 7890A, equipped with a flame ionization detector, split/splitless injector, and a fused silica capillary column BPX-70 (120 m, 0.25 mm i.d. and 0.25 µm stationary phase). The operation parameters were: column temperature of 198 ºC (Isothermal). The injector and detector temperatures were kept at 250 and 300 ºC, respectively. The gas flow rates used were 1 mL min^{-1} carrier gas (N2), 15 mL min^{-1} make-up gas (N2), and 30 mL min^{-1} and 300 mL min^{-1} flame gas, H_2 and zero air, respectively. The sample split mode was 1:100. The injections were carried out in duplicate and the injection volume was 1 µL. The Chemstation software was used for data collection, and calculation of all parameters.

3. RESULTS AND DISCUSSION

Up to now, there is no information about fatty acids composition and phytosterols of saffron petals, but Arapcheska et al [26] investigated fatty acids composition of saffron (Crocus sativus L.) from different origins. In their studied three different saffrons from Hungary, Spain and Greek were studied. The obtained results showed that the Hungarian saffron sample contained lauric acid (C12:0) 7.675; myristic acid (C14:0) 3.343%; palmitic acid (C16:0) 37.101%; heptadecenoic acid (C17:1) 0.273%; stearic acid (C18:0) 9.939%; oleic acid (C18:1) 3.102%; linoleic acid (C18:2) 24.966% and linolenic acid (C18:3) 14.209%. Fatty acids composition of the Spanish saffron samples was pentadecanoic acid (C15:0) 6.094%; palmitic acid (C16:0) 21.434%; stearic acid (C18:0) 1.683%; oleic acid (C18:1) 10.135%; linoleic acid (C18:2) 52.684% and linolenic acid (C18:3) 7.971%. The most abundant fatty acids in the Greek saffron samples was linoleic acid (C18:2) 40.104%; palmitic acid (C16:0) 33.910%, oleic acid (C18:1) 10.397%; linolenic acid (C18:3) 10.206%; lauric acid (C12:0) 4.229% and pentadecanoic acid (C15:0) 1.153%.

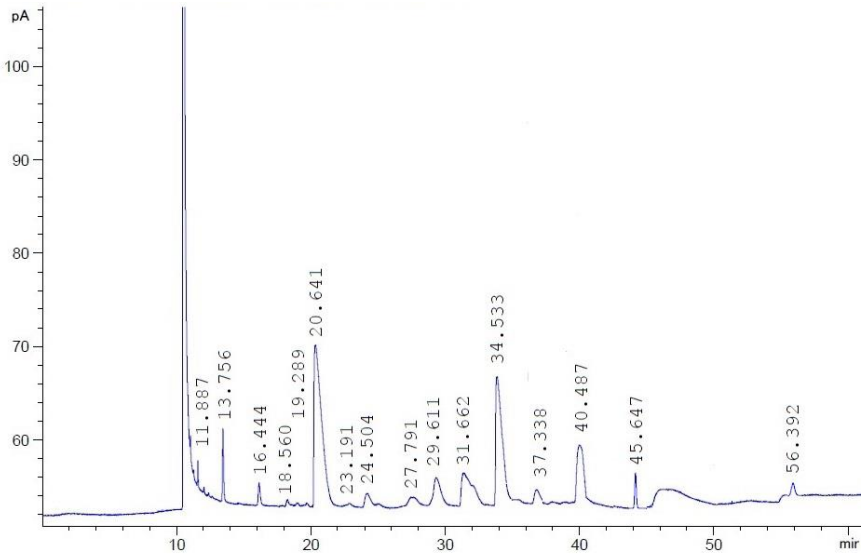

Figure 2. Capillary gas chromatographic profiles for fatty acid methy esters (FAMEs) from saffron petals.

Table 1. Fatty acid compositions of saffron petals

Fatty acid	Common name	Fatty acid content ± SD (g per 100 g)
C8:0	Caprylic acid	0.12 ± 0.03
C10:0	Capric acid	0.28 ± 0.07
C12:0	Lauric acid	1.16 ± 0.11
C14:0	Myristic acid	0.66 ± 0.08
C14:1	Myristoleic acid	0.17 ± 0.07
C15:0	Pentadecanoic acid	0.13 ± 0.02
C15:1	---	0.10 ± 0.02
C16:0	Palmitic acid	16.21 ± 2.84
C16:1	Palmitoleic acid	0.55 ± 0.40
C17:0	Margaric acid	1.10 ± 0.26
C17:1	Heptadecenoic acid	0.61 ± 0.55
C18:0	Stearic acid	4.01 ± 1.61
C18:1 cis	Oleic acid	6.13 ± 1.29
C18:2 cis	Linoleic acid	28.48 ± 2.47
C20:0	Arachidic acid	0.36 ± 0.21
C18:3	Linolenic acid	21.06 ± 2.53
C20:1	Gadeloic acid	9.18 ± 1.56
C20:2	---	1.10 ± 1.06
C20:3	Elaeostearic acid	2.52 ± 1.41
C20:4	Arachidonic acid	1.56 ± 0.29
C22:0	Behenic acid	2.19 ± 0.64
Saturated fatty acid	----	26.22
Unsaturated fatty acid	---	71.46

Results of this study illustrated the nutritional potential of the sterols and fatty acids profiles of saffron petals. The fatty acid composition of saffron petals are shown in Table 1. The most abundant fatty acids were linoleic, linolenic and palmetic acid. Linoleic acid (C18:2) was the most abundant polyunsaturated fatty acids in saffron petals. Results showed that saffron petals had 28.48±2.47 (g/100g) linoleic acid (C18:2). Another quantitatively high unsaturated fatty acid in the saffron petals was linolenic acid (C18:3) with the mean value of 21.06±2.53 (g/100g). Palmitic acid (C16:0), which was the most abundant saturated fatty acid, amounted to 16.21±2.84 (g/100g). Stearic acid (C18:0) was found in small amount with mean value of 4.01±1.61 (g/100g). The other unsaturated fatty acids were paulinic (C20:1) and oleic acid (C18:1) with mean value of 9.18±1.56 and 6.13±1.29 g/100g, respectively (Fig. 2). Other fatty acids in lower scale are shown in Table 1.

Phytosterols are plant-derived compounds that are similar in structure and function to cholesterol. Table 2 presents the content and the composition of sterols in the saffron petals. The major sterols in saffron petals were fucosterol, stigmasterol, sitosterol and fagarasterol. The mean value concentrations of fucosterol, stigmasterol, sitosterol and fagarasterol were 47.65±0.2, 32.69±0.27, 10.17±0.10 and 9.48±0.21 g/100g, respectively (Fig. 3).

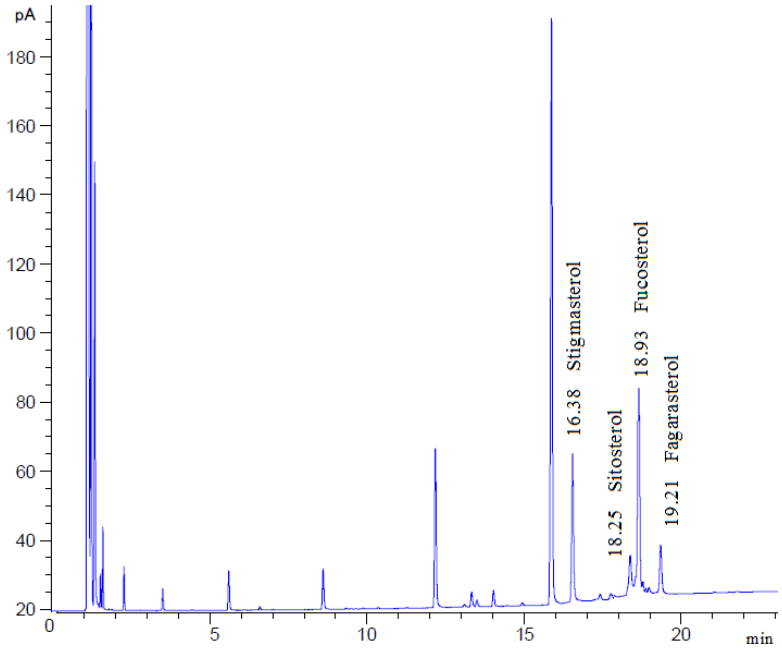

Figure 3. Gas chromatographic analysis of phytosterol in saffron petals.

For identification of the obtained sterols, the total ion chromatogram of the saffron petals solution were acquired in scan mode, peaks were identified by their mass spectra. The main compounds were identified by comparing their mass spectra with a GC–MS spectral library (Wiley and NIST). A representative chromatogram is illustrated in Fig. 4.

Table 2. Composition of sterols in saffron petals (n=3)

Sterol	Retention time (min)	Mean ± SD (g per 100 g)
Stigmasterol	16.38	32.69 ± 0.27
Sitosterol	18.25	10.17 ± 0.10
Fucosterol	18.93	47.65 ± 0.20
Fagarasterol	19.21	9.48 ± 0.21

Han Jia et al [27] determined the chemical constituents of volatile components in saffron from the Tibet and compared the chemical composition differences in the saffron. The total volatile components were extracted by ultrasonic assisted solvent extraction (USE), using five different solvents: diethyl ether, ethanol, ethyl acetate dichloromethane and acetone. The results obtained by gas chromatography-mass spectrometry (GCMS) showed that the saffron extracted with diethyl ether contained stigmasterol and sitosterol. The concentrations of stigmasterol and sitosterol were 11.80 and 4.82 g/100g, respectively. The concentrations of these sterols in saffron petals were more than those of Han Jia et al study in saffron. According to these finding, it can be concluded that saffron petals represents a very important dietary source of phytosterols.

Figure 4. TIC and MS spectra of studied phytosterol.

4. CONCLUSION

An analytical method was applied to determine phytosterols and fatty acids to improve the knowledge of these types of compounds (sterols and fatty acids) and their distributions in saffron petals. Due to high mass production of saffron petals which are considered as agricultural waste annually, extraction of such compounds worthy.

The saffron petals are a good source of phytosterols. It was demonstrated that it is possible to extract a considerable quantity of phytosterols from saffron petals. Nowadays, phytosterols are prepared from expensive sources. Given that each year, several tons of saffron petals are produced which are discarded and considered as agricultural waste. So a rich source of phytosterols could be very beneficial in food and pharmaceutical industries.

REFERENCES

[1] Abidi, S.L. Chromatographic analysis of plant sterols In foods and vegetable oils, *J Chromatogr A* **2001**, *935*, 173-201.

[2] Awd, A.B.; Fink, C.S. Phytosterols as anticancer dietary components: Evidence and mechanism of action. *J Nutr* **2000**, *130*, 2127-2130.

[3] Andersson, S.W.; Skinnerm J.; Ellegard, L.; Welch, A.A.; Bingham, S.; Mulligan, A.; Andersson, H.; Khaw, K.T. Intake of dietary plant sterols in inversely related to serum cholesterol concentration in men and women in the EPIC Norfolk populations: A crosssectional study. *Eur J Clin Nutr*, **2004**, *58*, 1378-1385.

[4] Akihisa, T.; Kokke, W.; Tamura, T. Naturally occurring sterols and related compounds from plants in Physiology and Biochemistry of sterols. Ed by Patterson G W and Nes W D. Am. Oil. Chem. Soc. Champaign. IL. **1991**. 172-228

[5] Eussen, S.R B.M,; Feenstra, T.L.; Toxopeus, I.B.; Hoekstra, J.; Klungel, O.H.; Verhagen, H.; VanKaren, H.J.; Rompelberg, C.J.M. Costs and health effects of adding functional foods containing physterols/stanols to statin therapy in the prevention of cardiovascular disease. *Eur J Pharmacol*, **2011**, *668*, S91-S100.

[6] Jones, P.J.; Abumweis, S.S. Phytosterols as functional food ingredients: Linkages to cardiovascular disease and cancer. *Curr Opin Clin Nutr Metab Care*, **2009**, *12*, 147-151.

[7] Phillips, K.M.; Ruggio, D.M.; Bailey, J.A. Precise quantitative determination of phytosterols, stanols and cholesterol metabolites in human serum by capillary gas-liquid chromatography. *J Chromatogr B* **1999**, *732*, 17-29.

[8] Kasama, T.; Byun, D.S.; Seyama, Y. Quantitative analysis of sterols in serum by high performance liquid chromatography: Application to the biochemical diagnosis of cerebrotendinous xanthomatosis. *J Chromatogr A* **1987**, *400*, 214-246.

[9] Chevy, F.; Humbert, L.; Woilf, C. Sterol profiling of amniotic fluid: A routine method for the detection of distal cholesterol synthesis deficit. *Prenat Diagn* **2005**, *25*, 1000-1006.

[10] Careri, M.; Elviri, L.; Mangia, A. Liquid chromatography-UV determination and liquid chromatography atmospheric pressure chemical ionization mass spectrometric characterization of sitosterol and stigmasterol in soybean oil. *J Chromatogr A* **2001**, *935*, 249-257.

[11] Rocco, A.; Fanali, S. Analysis of phytosterols in extra virgin olive oil by nano liquid chromatography. *J Chromatogr A* **2009**, *1216*, 7173-7178.

[12] Brenner, R.R. Factors influencing Fatty Acid Chain Elongation and Desaturation. In: Vergroesen, A.J.; Crawford, M. editors. The role of fats in human nutrition. London: Academic Press; **1989**. 45
[13] Chow, C.K.; (Ed.) Fatty Acids in Foods and their Health Implications. 2nd ed. Marcel Dekker, Inc., New York, NY; **2000**,109
[14] Christie, W.W. High-Performance Liquid Chromatography and Lipids: A practical guide 1st Edition, Pergamon Press, Oxford, **1987**.
[15] Harvey, D.J. in Advances in Lipid Methodology One, edited by Christie WW, Oily Press, Ayr. **1992**, 19
[16] Kafi, M.; Rashed, M.; Kocheki, A. Saffron, production and processing. Ferdowsi university of mashhad. **2001**
[17] Fahim, N.K.; Janati, S.S.F.; Feizy, J. Chemical composition of agriproduct saffron (*Crocus sativus* L.) petals and its considerations as animal feed. *GIDA* **2012**, *37(4)*, 197-201.
[18] Karimi, G.; Hosseinzadeh, H.; Khaleghpanah, P. Study of antidepressant effect of aqueous and ethanolic extract of Crocus sativus in mice. *Iran J Basic Med Sci*. **2001**, *4*, 11-15.
[19] GarridoPol, J.L.; Bethencourt, C.D.D.; Revilla, E. Flavonoids composition of hydrolyzed petal extracts of Crocus sativus. *Anal Brometal* **1987**, *39*, 69-77.
[20] Isao, K.; Ikuyo, K.H. Flavonols from saffron flowers, tyrosinase activity and inhibition mechanism. *J Agric Food Chem* **1999**, *47*, 4121-4125.
[21] Hadizadeh, F.; Khalili, N..; Hosseinzadeh, H.; Khair-Aldine, R. Kaempferol from Saffron Petals. *Iran J Pharmceutical Res* **2003**, *2(4)*, 251-252.
[22] Gainer, J.L.; Walls, D.A.; Jones, J.R. The effect of crocetin on skin papillomas and Rous sarcoma. *Oncol* **1976**, *33*, 222-224.
[23] Bendich, A.; Olson, J.A. Biological actions of carotenoids. *FASEB J* **1989**, *3*, 1927-1932.
[24] Moon, R.C. Comparative aspects of carotenoids and retinoids as chemopreventive agents for cancer. *J Nutr* **1989**, *119*, 127-134.
[25] Ziegler, R.G. A review of epidemiological evidence that carotenoids reduce the risk of cancer. *J Nutr* **1989**, *119*, 116-122.
[26] Arapceska, M.; Jankuloski, Z.; Hajrulai-Musliu, Zehra.; Uzunov, R. Comparative analysis of fatty acid composition of saffron (crocus sativus L.) from different origins. Conference paper. October 2014. SaffronOmic **2014**; Wageningen.
[27] Ling-Han, J.; Yi, L.; Yu-Zhen, L. Analysis of volatile components in saffron from Tibet and Henan by ultrasonicassisted solvent extraction and GCMS. *J Chin Pharm Sci* **2011**, *20*, 404-409.

The author declares no conflict of interest