SENSORIAL AND FUNCTIONAL ATTRIBUTES OF HERBAL INFUSIONS CONTAINING SAFFRON

*Anastasia Kyriakoudi, Evangelia Pouliou, Stella A. Ordoudi and Maria Z. Tsimidou**

Aristotle University of Thessaloniki (AUTH), School of Chemistry, Laboratory of Food Chemistry and Technology, Thessaloniki, Greece. Emails: tsimidou@chem.auth.gr, steord@chem.auth.gr, ankyria@chem.auth.gr, evipouliou.chem@gmail.com

Abstract – The present study aimed at examining the sensorial and functional attributes of three herbal infusions containing saffron. Results are discussed with regard to their chemical composition, which is expected to be rather complex taking into consideration the synthesis of the commercial blends. Such studies are of great importance for the development of functional saffron based products that are also acceptable by consumers.

Keywords: saffron, herbal infusions, bitter taste picrocrocin, phenolic compounds

1. INTRODUCTION

Teas (from the dried leaves of *Camellia sinensis*) and herbal teas (from leaves, flowers, roots, stems, etc., of other plant species) are among the most widely consumed beverages worldwide. They are considered as functional beverages and are consumed not only for their sensory attributes but also for the biological activities assigned to certain ingredients present at high concentrations. Currently, a growing body of evidence from epidemiological studies associates the consumption of herbal teas with a variety of health effects such as anticancer, antimutagenic, antioxidant actions, etc. [1].

Saffron, the most expensive spice in the world, derives from the dehydrated red stigmas of the plant *Crocus sativus* L. The spice is highly valued in the food and beverage industry for the (i) bright orange yellow hues that are attributed to a group of water-soluble apocarotenoids, the crocins, which are sugar esters of crocetin (8,8'-diapocarotene-8,8'-dioic acid), (ii) the unique bitter taste that is mainly assigned to the colorless monoterpene glucoside picrocrocin (4-(*β*-D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde) and (iii) the delicate aroma mainly due to the presence of safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) (Fig. 1) [2]. Apart from its applications in the food industry, a lot of pharmacological actions have been attributed to certain of these apocarotenoids. For this reason, in a recent review, Kyriakoudi, Ordoudi, Roldán-Medina and Tsimidou [3] named saffron as a functional spice. Currently, saffron is added to commercial food and beverage products both for its sensorial as well as functional properties. Due to the high content of biologically active hydrosoluble metabolites (~50% of dried stigmas weight) [4] herbal tea blends with saffron do not need very high amounts of the latter. This is of commercial importance considering the huge difference in price between saffron and the other herbs and plant materials commonly used in these teas.

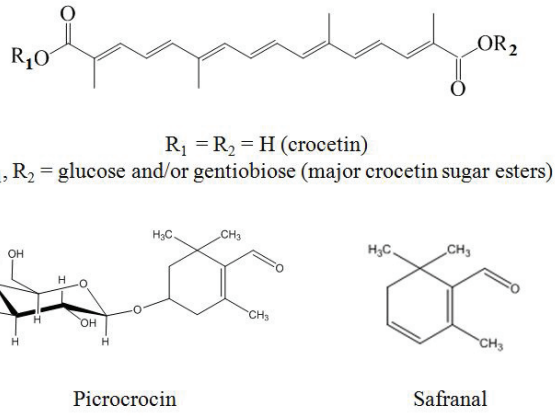

Fig. 1. Chemical structures of the major saffron bioactive compounds.

2. AIM OF THE STUDY

In the present study, the sensorial and functional attributes of three herbal infusions containing saffron were examined and discussed with regard to their chemical composition. Due to the synthesis of the commercial products this is expected to be complex as indicated from the data in Table 1.

Table 1. Ingredients and chemical composition of the examined herbal tea blends with saffron.

Herbal infusion	Major ingredients	Expected major compounds in infusion based on literature	Ref.
 #1	Orange peel, hibiscus, cinnamon, apple, cloves, natural flavours, saffron	Catechin, epicatechin and derivatives, rutin, hydroxycinnamic acid glycosides, cinnamaldehyde, *trans*-4-GG crocetin ester, picrocrocin	[5,6]
 #2	Apple, rosehips, orange leaves, orange peels, natural flavors, lemon peel, sweet blackberry, honey granules, saffron	Proanthocyanidins, catechin, hesperetin, gallic acid, chlorogenic acid, ferulic acid, ascorbic acid, *trans*-4-GG crocetin ester, picrocrocin	[7-9]
 #3	Black tea, spearmint, lemon peel, natural flavors, saffron	Epigallocatechin, gallic acid, chlorogenic acid, rutin, rosmarinic acid, *trans*-4-GG crocetin ester, picrocrocin	[10-14]

3. MATERIALS AND METHODS

3.1. Samples

Commercial herbal tea blends with saffron (1.1% w/w) were purchased from the market (Thessaloniki, Greece).

3.2. Standards, reagents and solvents

Trans-crocetin di-(*β*-D-gentiobiosyl) ester (*trans*-4-GG crocetin ester) was laboratory isolated by semi-preparative reversed-phase high performance liquid chromatography (RP-HPLC) according to Kyriakoudi and Tsimidou [15]. In brief, the system consisted of two Marathon IV series HPLC pumps (Rigas Labs, Thessaloniki, Greece), a Rheodyne injection valve (model 7125) with a 250 μL fixed loop (Rheodyne, Cotati, CA) and a diode array linear UVIS-206 multiple wavelength detector (Linear Instruments, Fermont, CA). Separation was carried out on a Nucleosil 100 C18 (250 x 10 mm i.d.; 7 μm) chromatographic column (Macherey-Nagel, Düren, Germany). The solvents used were water (A) and methanol (B). The gradient was 0 min, 30% (B), 0-10 min, 45% (B), 10-20 min, 70% (B), 20-30 min, 100% (B), 30-40 min, 100% (B), 40-50 min, 30% (B) and the flow rate 3.0 mL/min. Monitoring was at 440 nm. Purity of isolated *trans*-4-GG crocetin ester (97%) was checked (a) chromatographically by RP-HPLC-DAD in the range of 200 – 550 nm and calculated as the percentage of the total peak area at 440 nm and (b) by Nuclear Magnetic Resonance (NMR) spectroscopy, recording the 1H 1D spectra at 300 MHz on a Brucker 300AM spectrometer (Rheinstetten, Germany). All standards, reagents and solvents were of the highest purity required.

3.3. Preparation of infusions

The preparation of infusions was carried out by immersing one tea bag in 400 mL of boiling water (90 °C) for 5 min according to the instructions of the manufacturer. For each type of infusions 9 bags from 9 different packages belonging to different lots were used. Aliquots of 100 mL per infusion were combined to form a representative sample (900 mL) that was used in all further analyses.

3.4. Panel selection and training

Thirty three healthy subjects, aged between 21 and 57 years old, with no history of taste disorders were recruited from the staff and students at AUTH (School of Chemistry). Their training was accomplished in three phases: (A) taste sensitivity investigation according to ISO 3972 [16], (B) familiarization with bitter taste recognition and detection thresholds according to the same standard and (C) training on Ascending Forced Choice (AFC) of Limits according to the ASTM E-679 standard [17] as previously described in detail by Chrysanthou,

Pouliou, Kyriakoudi and Tsimidou [18]. Seven trained assessors participated in the evaluation of the sensorial attributes of the herbal infusions. Assessors were asked to avoid eating, smoking, or drinking coffee and tea 1 hour prior testing. The infusions were served in triads, hot (50 °C) and cold (10 °C), in special coded cups. The assessors were requested to answer (i) whether they could recognize the bitter taste in each infusion and to describe it and (ii) whether they could recognize or not the characteristic taste of saffron. All of the sensory evaluations were conducted at individual booths in the sensory laboratory at the School of Chemistry (AUTH).

3.5. Total phenol content estimation using the Folin-Ciocalteu assay

The total phenol content of the infusions was determined spectrophotometrically by the Folin-Ciocalteu (F-C) assay according to Kyriakoudi, Tsimidou, O'Callaghan, Galvin and O'Brien [19]. In brief, in a 10 mL volumetric flask, 5 mL of water, 0.4 mL of each representative sample and 0.5 mL F-C reagent were mixed. After exactly 3 min, 1.0 mL of saturated sodium carbonate solution (37%, w/v) was added and the mixture was agitated. The volume was adjusted with water and the flask left in the dark for 1 h at room temperature. The absorbance was measured at 750 nm (U-2000 Hitachi UV-Vis spectrophotometer, Tokyo, Japan) against a blank prepared in the same way with deionized water in the place of the infusion aliquot. Caffeic acid (CA) was used as a reference standard, and results were expressed as mg caffeic acid/L herbal infusion using a proper calibration curve. All measurements were carried out in triplicate and the results were expressed as the mean value of three independent measurements.

3.6. DPPH radical scavenging activity

The DPPH radical scavenging activity of the infusions was assessed according to the procedure presented by Nenadis and Tsimidou [20]. Briefly, samples (0.05 mL) were added to 2.9 mL of a 0.1 mM methanolic solution of $DPPH^{\bullet}$. The absorbance at 515 nm was recorded at the start and after 30 min incubation. All measurements were performed in triplicate and the results were expressed as the mean value of three independent measurements.

3.7. Liquid chromatographic analysis

The crocetin sugar esters (CRTSEs) present in the examined infusions were identified and quantified at 440 nm by high performance liquid chromatography (HPLC) as previously described by Kyriakoudi, Chrysanthou, Mantzouridou and Tsimidou [4]. In brief, the HPLC system consisted of a pump, model P4000 (Thermo Separation Products, San Jose, CA, USA), a Midas autosampler (Spark, Emmen, The Netherlands) and a UV 6000 LP diode array detector (DAD) (Thermo Separation Products, San Jose, CA, USA). Separation was carried out on a Discovery HS C18 (250 x 4.6 mm i.d.; 5 μm) column (Supelco, Bellefonte, USA). The solvents used were a mixture of water:acetic acid (1%, v/v) (A) and acetonitrile (B). The linear gradient was 20 to 100% B in 20 min. The flow rate was 0.8 mL/min. The injection volume was 20 μL. The analytical samples were prepared after proper dilution with deionized water (1:2, v/v) and filtration through a 0.45 μm membrane filter. Chromatographic data were processed using the ChromQuest Version 3.0 software (Thermo Separation Products, San Jose, CA, USA). Monitoring was in the range of 200-550 nm. Quantification of the total CRTSEs content was accomplished with the aid of a proper calibration curve of *trans*-4-GG crocetin ester. Picrocrocin concentration was calculated from the established ratio pricrocrocin/crocetin sugar esters content (0.5) due to peak overlapping at 250 nm (λ_{max} of picrocrocin) [18].

4. RESULTS AND DISCUSSION

The manufacturer's instructions were adopted in all experiments in order the results for the sensory and functional properties of all infusions to have a common basis for comparison [21]. Crocetin sugar esters and picrocrocin concentrations of the examined infusions due to the presence of saffron in the herbal blends are shown in Table 2. As shown, the total CRTSEs and picrocrocin content were found to be similar in all of the examined infusions. Differences among the infusions were observed regarding their total phenol content as determined by the F-C assay (Table 2). In particular, the highest total phenol content was observed for infusion #3 which is derived from a blend rich in black tea (> 50% w/w). Black tea is a well-established rich source of bioactive polyphenols. Specifically, *Camelia sinensis*

leaves (tea) contain high amounts of flavan-3-ols such as catechin and epicatechin derivatives [22]. Lower total phenol content was observed for infusions #1 and #2 that derived from blends that are labeled to contain orange peel and rose hips (> 50% w/w) and apple and hibiscus (> 50% w/w), respectively, as the major ingredients. In those cases compounds such as proanthocyanidins (i.e. derivatives of epicatechin), anthocyanins, ascorbic acid and flavonoids (e.g. hesperetin, rutin) are expected to prevail.

The DPPH radical scavenging activity of the studied infusions (Table 2) showed a similar trend to that evidenced for the total phenol content. Infusion 3# was found to exert the highest antiradical activity followed by infusions #2 and #1. The observed differences in activity can be enhanced by differences in the antioxidant potency of the individual antiradical compounds present in the extracts. Considering that aqueous extracts of saffron are not expected to contain appreciable amounts of phenolic compounds or to exhibit *in vitro* radical scavenging activity [19,23], the obtained values of radical scavenging activity were attributed to the presence of phenolic compounds from the other plant materials. However, saffron CRTSEs are expected to exhibit *in vivo* antiradical activity as previously shown by Ordoudi, Befani, Nenadis, Koliakos and Tsimidou [23].

The herbal infusions were then used in order to verify if the taste of picrocrocin was perceivable in them. It is worth mentioning that the concentration of picrocrocin 2.5-3.3 mg/L was ~half of the detection and recognition threshold values estimated by Chrysanthou, Pouliou, Kyriakoudi and Tsimidou [18] but were lied within the confidence interval values (i.e. 1.86 and 16.22 mg/L, 95% confidence level). The assessors judged as bitter all of the three herbal infusions. This result can be attributed not only to picrocrocin but also to the presence of phenolic compounds known for their bitter taste. The perception of the bitter taste was found to be more intense when infusions were served cold. This observation is in line with literature [24]. On the contrary, the unique taste of saffron was clearly recognized in infusions #2 and #3 when they were served hot (50 ^{o}C) probably due to the distinctive aroma of its volatiles. Thus, in infusion #1 that contains cinnamon and cloves, the taste of saffron was not recognized, which partially can be due to masking effects from the presence of their volatiles.

Table 2. Total crocetin sugar esters and picrocrocin content, total phenol content and % radical scavenging activity of the examined herbal infusions containing saffron.

Herbal infusion	Total CRTSEs content[a,b]	Calculated picrocrocin content[c]	Total phenol content[a]	Radical scavenging activity[a]
	mg/L herbal infusion		mg/L herbal infusion	%RSA
#1	4.9 ± 0.1[a]	2.5 ± 0.0	46.8 ± 1.8	19.3 ± 2.6
#2	6.7 ± 0.6[b]	3.3 ± 0.3	60.3 ± 4.9	28.9 ± 0.9
#3	5.8 ± 0.6[a,b]	2.9 ± 0.3	143.5 ± 3.6	72.4 ± 2.8

[a]Mean value of three independent measurements ± sd. [b]Different lowercase letters within the same column indicate significant differences among infusions according to Duncan's test ($p < 0.05$). [c]Crocetin esters content HPLC x 0.5 = picrocrocin content calculated.

5. CONCLUSIONS

Herbal infusions can constitute a very good source of functional ingredients for consumers, who can consume a number of cups on a daily basis. Taking into account that till now the only means to intake functional ingredients of saffron is to season food with it the examined products provide a way for daily consumption of crocins. The concomitant presence of other valuable phenolic compounds from different sources increase the interest of consumers in these new products, which were also acceptable from the sensorial point of view.

ACKNOWLEDGMENTS

A.M. is acknowledged for partial funding of this work. The authors are grateful to all panellists for availability and participation in the sensory tests.

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