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Preliminary studies on edible saffron bio-residues during different post-harvest storages

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Huge quantities of floral bio-residues (tepals, stamens, and styles) are produced annually after the removal of stigmas from *Crocus sativus* flowers. This material is rich in polyphenols and completely edible. In this work, some preliminary analyses were performed on saffron bio-residues kept in different post-harvest storage conditions (dried at 34 °C or frozen at -20 °C, respect to fresh flowers stored at 4°C as control) in order to select which treatment was able to preserve more quantities of bioactive compounds (polyphenols and carotenoids) in these floral tissues. Analysis of volatiles highlighted a significant presence of monoterpenes and non-terpenoid substances in control flowers, while dried flowers were characterised by a high percentage of phenylpropanoids. Analyses carried out on dried or frozen anthers and tepals provided good amount of compounds with antioxidant activity (polyphenols, anthocyanins and flavonoids in tepals and carotenoids in anthers).

Keywords: *Crocus sativus* L., edible flowers, volatile organic compounds, secondary metabolites, flowers conservation

INTRODUCTION

Edible flowers are inflorescences good to eat, and used in human diet in various parts of the world. In both traditional and innovative cuisine, edible flowers are used as dishes decoration or real ingredient in many recipes. Their increasing success relies in their colours, beauty, interesting flavour and tastes, along with important nutritional, antioxidant and antimicrobial properties [1]. However, edible flowers are very delicate and their shelf life is limited after harvesting; only few studies have been performed on this field until now.

Saffron (*Crocus sativus* L.) is a plant belonging to Iridaceae family, and flowers are composed of six purple tepals, three yellow stamens and a white style that culminates in a brilliant red stigma, divided into three threads (Fig.1). Stigmas represent the main reason for which saffron is cultivated: once removed and dried, stigmas become one of the most expensive spice of the world (saffron spice). However, stigma represents only 7.4% (w/w) of the total flower weight [2]. Since 68 kg of saffron flowers are required to produce 1 kg of spice, huge quantities of floral bio-residues (tepals, stamens and styles) are produced [2]. In addition, since the world annual saffron production is estimated around 300 tons per year (Iran produces 76% of total) [3] an exorbitant amount of saffron bio-residues are generated.

Focusing on Italy, the hectares cultivated with saffron are around 50, the kilograms of saffron spice produced per year are 450-600 kg (*https://www.zafferanoitaliano.it/lo-zafferano-in-italia/osservatorio-economico.html*), and the quantity of floral bio-residues produced per year is around 28350-37800 kg.

In general, saffron bio-residues are discarded as waste, added to compost, and hence used as fertilizer [4] or valorised as natural textile dye [5]. To date, the use of saffron bio-residues as food is less widespread and maybe less known too.

Saffron flowers are completely edible, characterised by floral aroma with notes of honey [6], which remain after harvesting and stigmas removal. On the palate, these flowers have a pleasant bitter aftertaste; this characteristic makes them suitable to be included in vegan and vegetarian recipes, as well as in meat and fish preparations. In addition, dry flowers are able to enhance the saffron bitter note in dishes that already contain this spice. From a healthy point of view, some authors have identified in saffron floral bio-residues an interesting source of polyphenols (included flavonoid and anthocyanins) and other antioxidant molecules, quantified in dried flower tissues by chromatographic techniques combined with mass spectrometry [4,7-9]. These compounds have multiple biological effects as shown in Hosseini *et al.* [10] that reviewed saffron petal pharmacological properties. In addition, some contributions to saffron floral bio-residues nutritional value are reported [11, 12].

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Fig. 1. Whole fresh flower (without stamen) of *Crocus sativus* L.:(A), frozen (B) and dried (C) saffron bio-products.

Currently, dry saffron tepals can be purchased for human consumption; in addition, it is not possible to exclude their use as drinks decoration in ice-cubes, as suggested in different websites. Post-harvest storage condition affects nutritional and antioxidant components that, generally, decrease after limited periods. To ensure a healthy product to consumers, it is essential to find out the best way to preserve these molecules and hence conserve the flowers. Serrano-Díaz *et al.* [13] compared different dehydration treatments (temperatures vs speed of airflow) to determine which combination would have allowed to maintain the highest levels of polyphenols and anthocyanins in dried saffron bio-residues.

To the best of our knowledge, few studies are performed on *C. sativus* floral volatile bio-residues until now. Volatile organic compounds are identified and quantified in defatted hydro-alcoholic extract [9, 14], in essential oil and concretes [6, 15]. In addition, Bergoin [6] determined the volatile fraction of fresh cut flowers.

In this work, total content of some antioxidant and healthy molecules (polyphenols, flavonoids, anthocyanins and carotenoids) were determined, both in dried and frozen saffron flowers without stigmas. On the other hand, volatile analyses were performed in order to give, for the first time, some information on how flowers' aroma change in different post-harvest condition, as integration of secondary metabolites data.

.MATERIALS AND METHODS

Plant material

Crocus sativus flowers, deprived of stigmas, were collected at the end of October 2017 in a small local production site (Agriturismo "Il Poggio di Marò") located in Badalucco (Imperia, Liguria, Italy) (43.922647N, 7.846080E), in the north-west of Italy. Fresh flowers were analyzed after storage at 4 °C for 5 hours (control), or kept at -20 °C. Dry flowers were obtained by drying the fresh flowers at 34 °C for 5 days; this material was kept at room temperature until usage.

Volatiles analysis. HS-SPME analysis

Flowers were analysed for their spontaneous volatile emission by HS-SPME. For each storage condition, three biological replicas were performed.

Each sample was introduced into a 25-mL glass conical flask closed with aluminium foil and allowed to equilibrate for 30 min. After the equilibration time, a Supelco SPME device (Supelco analytical, Bellefonte, PA, USA) coated with polydimethylsiloxane (PDMS, 100 □m) was inserted through the septum and then the fibre was exposed to the headspace of the vial for 30 min at room temperature. Once sampling was finished, the fibre was withdrawn into the needle, thus transferred to the injection port of the GC and GC–MS system. All the SPME sampling and desorption condition were identical for all the samples.

GC-MS analysis

GC-MS analysis was performed with a Varian CP-3800 apparatus equipped with a DB-5 capillary column (30m x 0.25 µm i.d. film thickness 0.25 µm) and Varian Saturn 2000 ion-trap mass detector. The oven temperature was programmed rising from 60 °C up to 240 °C at 3 °C/min; injector temperature 220 °C; transfer-line temperature 240 °C; carrier gas He (1 mL/min). The identification of the constituents was based on the comparison of the retention times (Rt) with those of pure reference samples and their linear retention indices (LRIs) determined relatively to a series of *n*-alkanes. The mass spectra were compared with those listed in the known commercial libraries NIST 2014 and Adams 2001 [16,17] and in a home-made mass spectra library built up from pure substances and components of known essential oils and MS literature data [16,17].

Biochemical analysis

Flowers dried at 34 °C for 5 days or frozen at -20 °C were used for biochemical analyses. The flowers

were separated in different tissue extracts: 1) only anthers (A), 2) only tepals (T), 3) pool of anthers and tepals (A+T), equally represented. For each analysis, three biological and two technical replicates were performed.

Total carotenoid content

100 mg of frozen flower tissues and 40 mg of dried material were used to extract carotenoid in 5 mL of 100 % (v/v) methanol. All samples were left at 4 °C overnight before reading the absorbance at 665 nm, 652 nm and 470 nm in a SHIMADZU UV-1800 spectrophotometer. Total carotenoid content was determined using the proper formulas reported in Lichtenthaler [18].

Total anthocyanin content

Anthocyanins were extracted from 100 mg of frozen flower tissues and 40 mg of dried material in 500 µL of ethanol/HCl (v/v 99/1%), according to Bretzel *et al.* [19] with slight modification. In brief, after 30 min of incubation at 4°C, 250 □L of distilled water and 750 µL of $CHCl_3$ were added to the samples, and centrifuged at maximum speed for 5 min. The supernatant was used to read the absorbance at 535 nm. The total anthocyanin content was expressed as milligrams of malvin-choride equivalents (ME) per gram of dry or fresh weight.

Total phenolic content

200 mg of frozen flower tissues and 40 mg of dried material were extracted in 2 mL of 70 % (v/v) methanol. After 30 min of incubation at 4 °C, the extract was centrifuged at maximum speed for 10 min and the supernatant was used also for the determination of the antioxidant activity (DPPH assay) and the total flavonoid content.

Total phenolic content was performed by following the Folin – Ciocalteu method [20], with some modification according to Bretzel *et al.* [19]. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry or fresh weight.

Table 1. Main volatile compounds (%) and classes of compounds (%) in fresh flowers analysed after 5 hours post-harvest (C), frozen flowers after storage at -20°C (F), dried flowers (D); n=3. The total refers to all the compounds detected, even those not shown in the table. Abbreviations: mh - monoterpene hydrocarbons; om – oxygenated monoterpene; sh - sesquiterpene hydrocarbons; os - oxygenated sesquiterpenes; nt – non-terpenoid substances; ac – apocarotenoids; pp – phenylpropanoids; LRI - Linear retention index. Compounds are listed according to their elution on the DB-5 column.

Class	Compounds	LRI	Relative content %		
			C	F	D
nt	2(5H)-Furanone	923	-	-	11.94
mh	α-Pinene	940	13.03	1.92	0.26
mh	□-Pinene	980	3.55		
mh	p-Cymene	1026	3.73	3.64	-
mh	Limonene	1031	8.27	43.71	1.37
om	1,8-Cineole	1034	20.61	-	-
nt	Phenyl acetaldehyde	1045	8.27	19.8	1.08
mh	γ-Terpinene	1062	2.54	2.4	tr
nt	Nonanal	1102	-	-	2.86
nt	Phenyl ethyl alcohol	1112	24.88	8.22	3.45
om	Camphor	1145	2.61	3.81	0.37
ac	Safranal	1201	-	-	3.13
om	(E)-Geranyl acetate	1381	-	-	1.77
nt	Dodecanal	1408	-	-	2.54
pp	(E)-Methyl isoeugenol	1495	-	-	56.99
	TOTAL (%)		**98.65**	**97,81**	**93.62**
mh	monoterpene hydrocarbons		37.64	57.05	1.54
om	oxygenated monoterpenes		23.99	9.86	4.01
	Total monoterpenes		61.63	66.91	5.55
sh	sesquiterpenes hydrocarbons		1.46	0.17	1.30
os	oxygenated sesquiterpenes		-	-	0.68
	Total sesquiterpenes		1.46	0.17	1.98
nt	not terpenoids substances		35.24	29.45	27.02
ac	apocarotenoids		-	0.98	2.09
pp	phenylpropanoids		0.33	0.30	56.99

Total flavonoid content

The flavonoid content was determined according to Kim *et al.* [21] with some modification [22]. The absorbance was read at 510 nm and the total flavonoid content was expressed as milligrams of

(+)- catechin equivalents (CE) per gram of dry or fresh weight.

DPPH Radical-Scavenging Activity Assay

The antioxidant activity of different flower's tissues was determined by using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging method [23]. Different aliquots of the methanolic extract (2, 5, 10, 15 µL) were added to 0.25 mM (w/v) DPPH methanol solution to reach a final volume of 1 mL. After 30 min of incubation at room temperature in the dark, the bleaching of DPPH was measured at 517 nm. Ascorbic acid was used as control (1 mg/mL). The DPPH scavenging effect (%) was calculated as [(Abs0 − Abs1/Abs0) × 100], where Abs0 is the absorbance of the control and Abs1 is the absorbance of the sample. Results were reported as IC_{50} (mg/mL), namely the extract concentration required to obtain 50% of antioxidant activities.

Statistical analysis

Data were statistically analysed by ANOVA followed by Fisher's probable least-squares difference test with cut-off significance at $p \leq 0.05$ (lowercase letters) or p $p \leq 0.001$ (capital letters).

RESULTS AND DISCUSSION

Volatiles emitted by C. sativus floral bio-residues after different post-harvest storages

Volatiles identified and quantified in saffron flowers (without stigmas) stored at 4 °C for 5 hours (control), frozen at -20 °C or dried at 34 °C for 5 days are reported in Table 1. Control flowers were characterised by high levels of monoterpenes (61.63 %) and not- terpenoid substances (35.24 %). More in detail, phenyl ethyl alcohol (24.88 %), 1,8-cineole (20.61 %), and α-pinene (13.03 %) were the most abundant compounds in fresh flowers after 5 hours of post-harvest (Table 1). The "floral", "sweet honey", and "fruity" notes were attributed to phenyl ethyl alcohol [6]; since this compound was detected here as the most abundant, our results are consistent with the aroma perceived by smelling fresh cut saffron flowers, even deprived of stigma [6]. On the other hand, 1,8-cineole has a camphor-like odour, and it could help to give pungent and spice aftertaste to saffron tepals.

Another compound present in our analyses is limonene (8,27%) (Table1), responsible for the "lemon" and "fresh" notes. Bergoin [6] reported different volatile composition emitted in fresh cut *C. sativus* flowers, with 2-ethylhexan-1-ol and limonene as the principal components. However, in her work the methodology used for the determination by SPME is different to that used in this work.

Freezing process increases limonene and phenyl acetaldehyde levels; conversely phenyl ethyl alcohol decreases. In addition, 1,8-cineole was completely absent. These results show that "floral", "honey" and "lemon" notes could be maintained in home-made ice cubes to make cocktails more colourful and catchy.

Dried flowers were characterised by low level of monoterpenes (5.55%) and high levels of phenylpropanoids (56.99%) and non-terpenoids (27.02%) (Table 1). The most abundant compound was (E)-methyl isoeugenol (56.99%). Compared with frozen flowers, 2(5H)-furanone (11.94%), nonanal (2.84%), dodecanal (2.54%), and (E)-geranyl acetate (1.77%) were detected only in dried flowers (Table 1).

Finally, the analysis of dried flowers' volatiles revealed 3.13% of safranal. The presence of this compound could be due to a careless stigma removal. Indeed, Bergoin [6] who quantified 1.6% of safranal in saffron tepals essential oil, provided the same explanation.

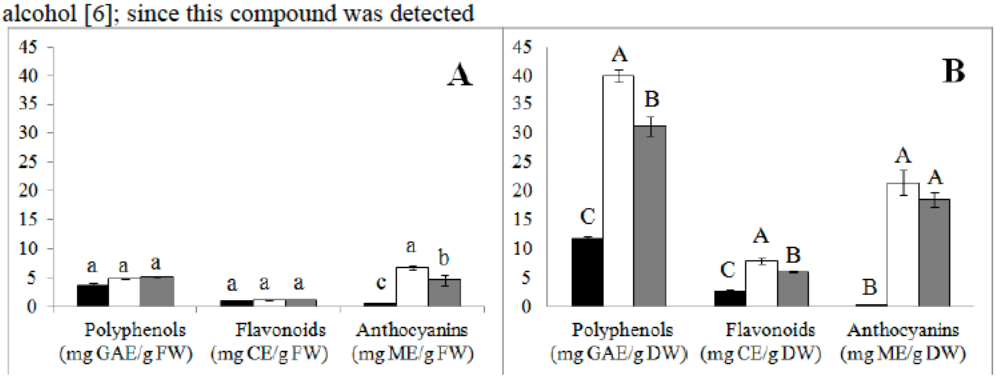

Fig. 2. Total content of polyphenols, flavonoids and anthocyanins in frozen (A) and dried (B) flower organs detected in different flower's organs: anthers (black), tepals (white) and pool of anthers and tepals (grey). Data are shown as mean ± SE, n=3. P < 0.001 (capital letters) or p ≤ 0.05 (lowercase letters).

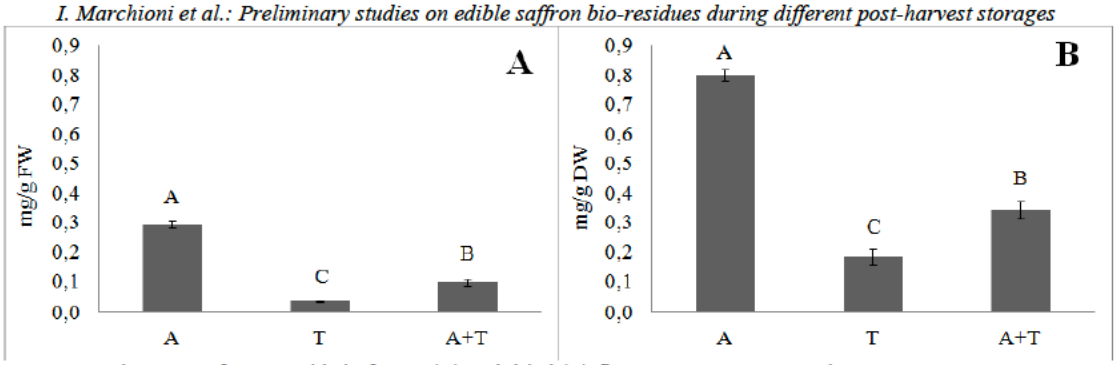

Fig. 3. Total content of carotenoids in frozen (A) and dried (B) flower organs. Data are shown as mean ± SE, n=3. P < 0.001. Abbreviation: A – anthers; T – tepals; A+T – pool of anthers and tepals.

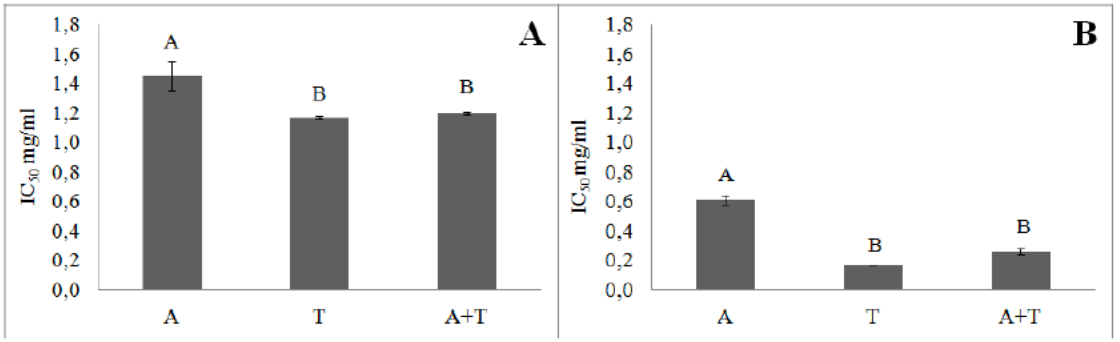

Fig. 4. Antioxidant activity of frozen (A) and dried (B) flower organs. Data are shown as mean ± SE, n=3. P < 0.001. Abbreviation: A – anthers; T – tepals; A+T – pool of anthers and tepals.

Secondary metabolites and antioxidant activity in frozen and dried saffron bio-residues

Total content of polyphenols, flavonoid and anthocyanin in frozen and dry flowers are reported in Figure 2. Anthers and tepals were analysed separately or mixed in pool. These results show that, in general, dried flowers had more secondary metabolites than frozen flowers. In dry flowers (Fig. 2B), tepals show higher total polyphenolic (39.95 mg GAE/g DW), flavonoid (7.82 mg CE/g DW), and anthocyanin (21.49 mg ME/g DW) content than in the other analysed samples; indeed, the same classes of metabolites were less abundant in the mixture of anthers and tepals, followed by anthers. On the other hand, polyphenols, flavonoid and anthocyanins in frozen flowers were lower than 7 mg/g DW (Fig. 2A). Regarding to the dried flowers, our quantifications of these metabolites were lower than that reported in the literature. In fact, Jadouali *et al.* [24] quantified the polyphenol content in tepals and stamens as 65.34 and 35.69 mg GAE/ g powder crude extract respectively. Higher total polyphenolic content was detected in tepals (94 mg GAE/ g dry weight) by Lahmass et al. [25]. The difference between our quantification and that reported in literature could be due to the different cultivation and environmental conditions, along with different extraction methods. Indeed, Jadouali *et al.* [24] performed a maceration in 80% (v/v) methanol, for 24 h at 35°C, and then sample concentration before further analyses. On the other hand, Lahmass *et al.* [25] extracted polyphenols by a maceration of 5 hours in 50% (v/v) methanol before concentration.

Total content of carotenoids in dried and frozen samples are reported in Fig. 3. Dried anthers had the highest carotenoids content (0.8 mg/g DW), while frozen tepals had the lowest (0.037 mg/g FW). Both the floral organs could be used in cuisine, to increase the human diet with different classes of molecules.

Finally, frozen and dried flowers' antioxidant activity (IC_{50} mg/mL) is reported in Figure 4. Based on these results, in general dried flowers showed a higher antioxidant activity than fresh flowers; in addiction, dried tepals had the lowest IC_{50} value (0.17 mg/mL), while fresh anthers the highest (1.45 mg/mL) (Fig. 4). These results are consistent with the previous ones: the higher is the polyphenols, the higher is the antioxidant activity. However, all flowers showed an antioxidant activity lower than the ascorbic acid (IC_{50} 0.01 mg/mL) (data not shown) used as control.

CONCLUSION

Crocus sativus flowers have nutraceutical properties that are maintained during different storage conditions, although their aroma profile

changes during the storage process. Thus, the use of these floral bio-residues can be considered as new source of bioactive and healthy compounds in food or dietary supplements and could be considered as by-product in circular economy parameters.

REFERENCES

1. L. Fernandes, S. Casal, J. A. Pereira, J. A. Saraiva, E. Ramalhosa, *J. Food Compost. Anal.*, **60**, 38 (2017)
2. J. Serrano-Díaz, A. M. Sánchez, L. Maggi, M. Martínez-Tomé, L. García-Diz, M. A. Murcia, G. L. Alonso, *J. Food Sci*., 77, C1162 (2012)
3. A. R. Gohari, S. Saeidnia, M. K. Mohmoodabadi, *Pharmacogn Rev*., 7, 61 (2013)
4. K. Zeka, K. C. Ruparella, M. A. Continenza, D. Stagos, F. Vegliò, R. R. J. Aroo, *Fitoterapia*, 107, 128 (2015)
5. S. M. Mortazavi, M. Kamali Moghaddam, S. Safi, R. Salehi, *Prog. Color Colorants Coat,* **5**, 75 (2012)
6. M. Bergoin, PhD Thesis, Institut National Polytechnique de Toulouse, Toulouse, 2005
7. R. Sánchez-Vioque, O. Santana-Méridas, M. Polissiou, J. Vioque, K. Astraka, M. Alaiz, D. Herraiz-Peñalver, P. A. Tarantilis, J. Girón-Calle, *J Funct Foods*, **24**, 18 (2016)
8. J. Serrano-Díaz, A. M. Sánchez, M. Martínez-Tomé, P. Winterhalter, G. L. Alonso, *J. Agric. Food Chem.*, **62**, 3125 (2014)
9. S. Argento, F. Branca, L. Siracusa, T. Strano, E. M. Napoli, G. Ruberto. *Acta Hort*, **850**, 251 (2010)
10. A. Hosseini, B. M. Razavi, H. Hosseinzadeh, *Iran J Basic Med Sci*, **21**, 1091 (2018)
11. K. Mahdavee Khazaei, S. M. Jafari, M. Ghorbani, A. Hemmati Kakhki, M. Sarfarazi, *Food Anal. Methods*, **9**, 1993 (2016)
12. J. Serrano-Díaz, A. M. Sánchez, M. Martínez-Tomé, P. Winterhalter, G. L. Alonso, *J. Food Compost Anal*, **31**, 101 (2013)
13. J. Serrano-Díaz, A. M. Sánchez, A. Alvarruiz , G. L. Alonso, *Food Chem*, **141**, 1536 (2013)
14. B. Tirillini, R. Pagiotti, L. Menghini, E. Miniati, *J. Essent. Oil Res*., **18**, 298 (2006)
15. M. Bergoin, C. Raynaud, G. Vilarem, J. Bessière, T. Talou, *Acta Hort*, **650**, 355 (2004)
16. R. P. Adams, Identification of essential oil components by gas chromatography /quadrupole mass spectrometry, Allured Publishing Corporation, Carol, 2001
17. NIST 2014. National Institute of Standards and Technology. PC Version 1.7 of the NIST/EPA/NIH Mass Spectral Library; Perkin Elmer, Norwalk, CT, 2014.
18. H. K. Lichtenthaler, *Methods Enzymol.,* **148**, 350 (1987)
19. F. Bretzel, S. Benvenuti, L. Pistelli, *Environ Sci Pollut Res*, **21**, 2325 (2013)
20. V.L. Singleton, J.A., Rossi, *Am. J. Enol. Viticult*., **16**, 144 (1965)
21. D. Kim, O. Chun, Y. Kim, H. Moon and C. Lee, *J. Agric. Food Chem*., **51**, 6509 (2003)
22. M. Caser, F. D'Angiolillo, W. Chitarra, C. Lovisolo, B. Ruffoni, Lu. Pistelli, La. Pistelli, V. Scariot, *Plant Growth Regul*., **84**, 383 (2018)
23. W. Brand-Williams, M. E. Cuvelier, C. Berset, *Lebensm.-Wiss. u.-Technol*., **28**, 25 (1995)
24. S. M. Jadouali, Z. Bouzouba□ K. Majourhat, R. Mamouni, S. Gharby, H. Atifi, *Acta Hortic*. **1184**, 301 (2017)
25 I. Lahmass, T. Lamkami, C. Delporte, S. Sikdar, P. Van Antwerpen, E. Saalaoui, V. Megalizzi, *J Funct Foods,* **35**, 341 (2017)