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Original article

Valorisation of *Crocus sativus* flower parts for herbal infusions: impact of brewing conditions on phenolic profiling, antioxidant capacity and sensory traits

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ABSTRACT Saffron production from *Crocus sativus* flowers produces large amounts of by-products that may represent an excellent source of polyphenols. The aim of this work was to evaluate infusions originating from different brewing processes and from different saffron flower portions, in terms of both functional and sensory traits. For this aim, total polyphenols and total flavonoids, *in vitro* antioxidant assays and an untargeted phenolic profiling were applied. In general, tepals showed higher polyphenol and flavonoid content than stamen infusions, and their bioactive content depended more on brewing temperature than brewing time. These findings were consistent with both antioxidant capacity and phenolic profiling. Multivariate statistics highlighted polyphenols discriminating 'boiled' vs. 'cold' infusions, being mainly flavonoids, phenolic acids and the alkylphenol 5-pentadecylresorcinol (showing a strong down-accumulation at the higher brewing temperatures). Positive correlations could be highlighted between anthocyanins, flavones, flavonols and lignans, and the *in vitro* antioxidant assays. In general, cold brewing was successful in extracting phenolic compounds and provided better sensory properties, thus indicating that this may represent a valuable strategy to develop saffron-based functional beverages with better consumers' acceptability.

Keywords Antioxidant capacity, circular economy, floral bioresidues: valorisation of by-products, functional beverages, herbal infusions, metabolomics, polyphenols, saffron.

Introduction

Saffron, the orange-red dried stigma of the *Crocus sativus* flower, is the most expensive spices by weight in the world due to its production costs. The spice is produced from flower stems and stigmas, and up to 68 kg of flowers (~230 000 flowers) are required to produce 1 kg of saffron. However, during its processing, up to 63 kg of floral bioresidues, including tepals and stamens are generated (Serrano-Díaz *et al.*, 2012). Recent studies have reported that both these floral parts may represent a good source of bioactive molecules, mostly polyphenols (Montoro *et al.*, 2012; Cusano *et al.*, 2018; Senizza *et al.*, 2019). Tepals and stamens have been reported to be rich in anthocyanins such as cyanidin, delphinidin 3-*O*-glucoside, malvidin 3-*O*-glucoside. Additionally, *C. sativus* tepals are a valuable source of kaempferol and its glycosides (Tuberoso *et al.*, 2016; Menghini *et al.*, 2018; Xu *et al.*, 2019).

Consistently, tepal extracts have been shown to possess several biological activities (Moratalla-Lopez *et al.*, 2019) comprising *in vitro* antioxidant activity (Termentzi & Kokkalou, 2008; Montoro *et al.*, 2012; Sánchez-Vioque *et al.*, 2012; Serrano-Díaz *et al.*, 2013) and antidiabetic properties (Menghini *et al.*, 2018; Wali *et al.*, 2020). *In vivo* studies have reported that tepals administered by oral gavage (20 mg/kg body weight for 6 days) are hepatoprotective in rats (Omidi *et al.*, 2014), whereas in humans an antidepressant activity of tepals (30 mg/day) was observed (Mottaghipisheh *et al.*, 2020). Important antifungal, cytotoxic and antioxidant activities have also been demonstrated for stamens and perianth (Zheng *et al.*, 2011).

Herbal infusions have long since been used as therapeutic vehicles and could provide the ideal medium to

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deliver water-soluble phytochemicals compared to the dried herb (Poswal *et al.*, 2019).

Herbal teas include aqueous infusions in hot or cold water for an unspecified amount of time, to extract the phytochemical constituents of plant materials. Tea, brewed from the leaves of the plant *Camellia sinensis*, is the most consumed beverage in different parts of the world. However, infusions are also prepared from roots (i.e. ginger, liquorice), leaves (nettle, *Urtica dioica L.*; Spearmint), flowers (lavender, chamomile, hibiscus), seeds (fenugreek, *Trigonella foenum-graecum L.*) and other organs belonging to different plant species (Poswal *et al.*, 2019). Recent studies have demonstrated that herbal teas represent an excellent food source of bioactive compounds or phytochemicals such as phenols and flavonoids (Pyrzynska & Sentkowska, 2019). Indeed, *in vitro* and *in vivo* studies have explored a wide range of herbal teas revealing antioxidant properties and potential clinical benefits in chronic conditions, including diabetes, high blood pressure, obesity (Chandrasekara & Shahidi, 2018; Poswal *et al.*, 2019) and cancer (Talib *et al.*, 2020). However, the composition of these infusions varies not only according to the plant species but also according to the brewing conditions (time, temperature) adopted (Venditti *et al.*, 2010; Castiglioni *et al.*, 2015; Damiani *et al.*, 2019). In previous studies, we demonstrated that the brewing conditions of infusions from *C. sinensis* (common tea) and *Aspalathus linearis* (rooibos tea) influence the polyphenol content and *in vitro* antioxidant capacity, and that cold infusions showed similar or higher polyphenol content and antioxidant capacity than the traditionally prepared hot beverages (Venditti *et al.*, 2010; Damiani *et al.*, 2019).

Starting from these background conditions, the aim of the present study was to characterise the functional and sensory traits of infusions prepared from tepals and stamens of *C. sativus* flowers as a function of the brewing method used. To this object, an untargeted metabolomics approach was chosen to comprehensively screen the phenolic compounds released from the flower parts of *C. sativus* during brewing, and the phenolic profile of the tested samples was then correlated to their *in vitro* antioxidant capacity. A sensory analysis was also done to establish a preference rating for the infusions flavour, taste, colour and overall acceptability. On the whole, our work has a twofold interest: (i) the possible use of tepals and stamens in the framework of valorising saffron by-products; and (ii) the development of functional beverages where the potential health benefits of saffron by-products are optimised.

Materials and methods

Reagents and equipment

All chemical reagents were obtained from Sigma-Aldrich (Milan, Italy) and used as received. *Crocus sativus* flowers were kindly donated by Azienda Agraria Lorenzini (Ancona, Italy), black tea was purchased from a local retail shop, while red rooibos was obtained from Bokkeveld rooibos (Nieuwoudtville, Northern Cape, South Africa). The polyphenol standard compounds reported in Untargeted UHPLC-ESI-QTOF-MS phenolic profiling (>98% purity) were purchased from Extrasynthese (Genay, France). The same bottled mineral water purchased from local retail shops was used to prepare the herbal infusions. For analytics, ultrapure water was used throughout and obtained from a Milli-Q Reference A+ system from Millipore (Merck, Darmstadt, Germany). All spectrophotometric measurements were recorded on a microplate reader (Synergy-HT, BioTek, Winooski, VT, USA).

Infusion preparation

The *Crocus sativus* flowers were manually picked and then hand processed in the laboratory to separate tepals (T) and stamens (S) from the flowers. The separated parts were immediately left to dry in a thermostatic oven for 5 h at 40 °C. Four different infusions were prepared from T and S as depicted in Fig. 1, using in each case 0.25 g of plant material and 25 mL of water in a glass cup with a lid. These proportions were chosen as they simulate a real cup of herbal tea preparation (2.5 g in 250 mL). The hot infusion (H) was prepared by pouring boiling water (100 °C) over the plant material and then left to stand for 5 min at room temperature (±21 °C) with occasional manual stirring. For the boiled infusions (B), boiling water was poured on each sample, and then left to boil continuously for 5 min in a thermostatic bath at 100 °C with occasional stirring. The room temperature infusions (RT) were prepared by pouring room temperature water on each sample, which was then continuously stirred for 2 h at room temperature (±21 °C) on a Biosan orbital shaker at 140 rpm. The cold infusions (C) were prepared in the same way as the RT ones, but they were kept under continuous stirring at 4 °C overnight. These infusion conditions were chosen based on those previously reported in the literature and which reflect standard brewing conditions for the preparation of a cup of tea (Venditti *et al.*, 2010; Castiglioni *et al.*, 2015; Damiani *et al.*, 2019). The rooibos (R), stigmas (St) and black (Bt) teas were prepared using the hot (H) infusion method. Once prepared, each infusion was filtered through a fine mesh strainer, centrifuged at 1000 ***g*** for 10 min, filtered through 12 μm Albet filter paper and stored at −20 °C until use.

Phytochemical analysis

Total polyphenols content

Total polyphenols (TPC) were evaluated using the Folin–Ciocalteu method as previously described in (Bacchetti *et al.*, 2020) using 200 μL of each infusion.

Gallic acid was used to create the standard curve and the results are expressed as mg gallic acid equivalents (GAE) per litre (mg GAE/L).

Total flavonoids content

Flavonoids (TFC) were quantified using the colorimetric aluminium chloride assay as previously described (Bacchetti *et al.*, 2020), using 50 µL of infusions. Catechin was used to develop the standard curve and the results are expressed as mg of catechin equivalents per litre (mg CE/L).

In-vitro antioxidant capacity assays

The antioxidant capacity of T and S infusions was determined using the oxygen radical absorbance capacity (ORAC) assay on 25 µL of diluted samples (1:100) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay on 12 µL of the undiluted sample, as previously described (Bacchetti *et al.*, 2020). The final ORAC values were calculated using the net area under the fluorescence decay curves (AUC) and the data are expressed as mM of Trolox Equivalents (mM TE) obtained from the Trolox standard curve, while the capability to scavenge the DPPH radical was calculated using the following equation:

$$\%\text{Inhibition} = \frac{\text{Abs control} - \text{Abs sample} \times 100}{\text{Abs control}}$$

The ferric ion-reducing antioxidant power (FRAP) assay was also used following the method described by Tomasina *et al.* (2012) on 10 µL of infusions. Trolox was used for calibrations, and the results are expressed as mM Trolox equivalents (mM TE).

Untargeted UHPLC-ESI-QTOF-MS phenolic profiling

An ultrasonic-assisted extraction was used to prepare aqueous extracts (1:20 w/v) from lyophilised samples.

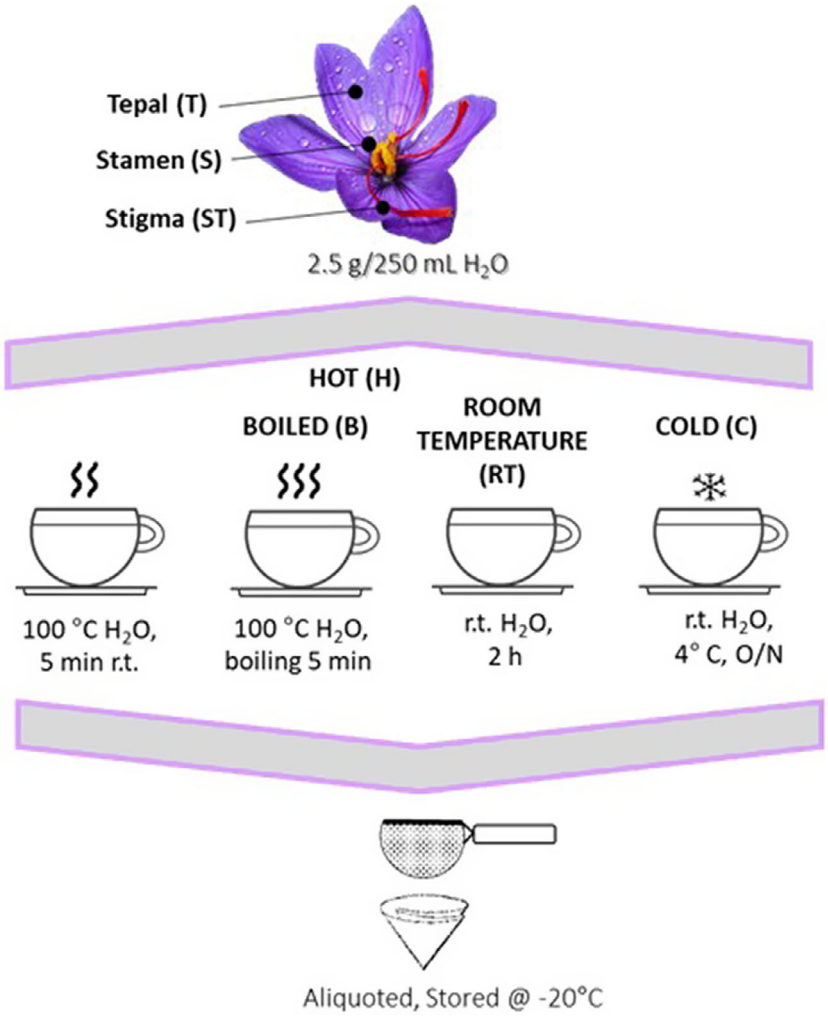

Figure 1 A graphical outline of the different brewing conditions used for the preparation of Crocus sativus tepal (T), stamen (T) and stigma (ST) infusions.

The samples were then centrifuged at 6000 ***g*** for 10 min at 4 °C and the supernatant was transferred to an HPLC vial for subsequent analysis. The phenolic profile of each sample was investigated through an untargeted metabolomics approach. In this regard, the equipment consisted of ultra-high-pressure liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UHPLC-QTOF-MS). The instrumental conditions were previously optimised and described (Senizza *et al.*, 2019). The chromatography was based on reverse phase separation, using a C18 column Agilent Zorbax eclipse plus (50 mm × 2.1 mm, 1.8 μm) and following a water-acetonitrile binary gradient (from 6% acetonitrile to 94% acetonitrile in 32 min). Accurate masses in the range of 100–1200 m/z were screened in a positive full-scan mode and at a rate of 0.8 spectra/s. The infusions' extracts were analysed in triplicate, with an injection volume of 6 μL. Also, the injection sequence was randomised, and both blank (extraction solvent only) and pooled quality control samples were injected.

The software Agilent Profinder B.07 was used to putatively annotate the raw mass features, using the 'find-by-formula' algorithm based on the isotopic pattern (i.e. monoisotopic mass, isotopic spacing and isotopic ratio) of each compound and reaching the annotation against the Phenol-Explorer 3.6 database. Therefore, a level 2 confidence in annotation (i.e. putatively annotated compounds with some structural identification, i.e., data-dependent approach) was achieved, considering a typical 5-ppm tolerance for mass accuracy. The raw data set was obtained after post-acquisition filtering (only those compounds identified within 100% of replications within at least one treatment were retained), baselining and normalisation by using the software Agilent Mass Profiler Professional B.12.06. Finally, to provide semi-quantitative data from the annotated mass features, the isobaric compounds were removed from the raw data set and all the polyphenols annotated were classified into classes and sub-classes. Polyphenols were then quantified by using standard solutions of pure (>98%) standard compounds representative of each class/sub-class (i.e. cyanidin for anthocyanins, quercetin for flavonols, luteolin for flavones and other flavonoids, catechin for flavan-3-ols, ferulic acid for phenolic acids, sesamin for lignans, resveratrol for stilbenes, tyrosol for tyrosols and other remaining phenolics). The results are expressed as mg equivalents/g dry matter (DM), considering three replicates ($n = 3$).

Sensory analysis

A sensory evaluation of the tepal infusions was carried out by human volunteers to determine how the different brewing conditions impact the aroma, taste and overall hedonistic value of hot and cold tepal infusions (H and C). The infusions were prepared as reported in Fig. 1 but doubling the volume and quantity of plant material (0.5 g in 50 mL H_2O). A group of 12 healthy volunteers (age 23–50 years) interested in sensory evaluation and familiar with tea drinks were enrolled in the study. These evaluators were asked to avoid smoking, brushing their teeth, using perfume, and eating or drinking anything except water, within 1 h before the tasting session. Before the evaluation, a training session was conducted by a tea master to explain the definitions of the quality attributes of tea concerning sensory analysis. Subsequently, one infusion at a time was offered to each evaluator who was asked to express his/her opinion on a questionnaire that was divided into three parts: (i) Evaluators' identifiers (age, gender, physiological condition that may influence the test such as smoking, allergies, stress, colds, etc.); (ii) test for smell, colour, taste (bitter, astringent, sweet, persistence); (iii) test for aroma using macro and micro descriptors (in brackets): floral, spicy, plant (green grass, cooked greens, herbs), undergrowth (woody, earthy, mineral) and fruity (wild berries, citrus, tropical, dry fruits). The intensity scale was expressed from 0 to 5 corresponding to the perception: 'absent' = 0, 'barely' = 1, 'fairly' = 2, 'rather' = 3, 'highly' = 4 and 'extremely' = 5. These sensory descriptors were chosen based on descriptive terms used to describe the attributes of a certain product (Drake & Civille, 2003). Personal evaluation of the overall liking was expressed on a verbal scale: very good, good, fair and unpleasant. The assessors were asked to rinse their palate with lukewarm water between evaluations.

Statistical analysis

The colorimetric assays were performed on a minimum of triplicate independent replicates, and the results are reported as means ± SD.

The metabolomics data set was aligned and normalised using the Agilent Mass Profiler Professional B.12.06 software, as previously described (Senizza *et al.*, 2019). In particular, the metabolomic data set was exported into SIMCA 13 (Umetrics, Malmo, Sweden) and a supervised orthogonal projection to latent structures discriminant analysis (OPLS-DA) was then carried out. Additionally, Hotelling's T2 and permutation testing were also used to exclude the presence of strong outliers ($P > 0.05$) and overfitting respectively. The variable importance in projection approach (VIP) was then used to depict the most discriminant compounds (VIP score > 1), allowing the discrimination between the different brewing methods. Also, for each VIP marker compound, the Log Fold Change (FC) variations were extrapolated for each possible comparison. Finally, a one-way analysis of variance

(ANOVA) was carried out with GraphPad PRISM 8.2 software. Tukey's multiple comparison test ($P < 0.01$) post hoc analysis was used to compare means. Pearson's correlations ($P = 0.01$, two-tailed) were also calculated using IBM SPSS software (Version 25.0).

Results and discussion

Total polyphenols (TPC) and total flavonoids (TFC) analysis

Phytochemical analysis in terms of spectrophotometric TPC and TFC content released from stamens and tepals according to the different brewing conditions, is reported in Table 1. Concerning TPC, the results clearly show that tepal infusions have a higher content than stamen infusions (186–83 mg GAE/L vs. 105–94 mg GAE/L). Furthermore, the different brewing conditions did not affect TPC in stamen infusions, whereas significantly higher TPC values were noted for tepal infusions prepared using the boiled, room temperature and cold brewing methods with respect to the hot brewing one. A similar trend can also be noted for TFC, where the levels were higher in tepal infusions prepared using the aforementioned brewing methods (91–34 mg CE/L vs. 55–28 CE/L).

These results indicate that the bioactive compound contents from tepal infusions are more dependent on brewing temperature than brewing time. Indeed, for the hot brewing methods (H and B), microwaving the brew for 5 min to maintain a constant boiling brew, compared to simply pouring boiling water on tepals and then leaving the brew to stand at r.t. for 5 min where the temperature gradually drops, impacts the degree of phytochemical extraction. Compared to conventional heating, microwave dielectric heating induces increased molecular motions and accelerates energy transfer, resulting in greater penetration of solvent across the sample matrix thus translating into a higher extraction efficiency (Duarte *et al.*, 2014). This confirms a previous study where two hot preparation methods for rooibos (*Aspalathus linearis*) herbal teas were compared (Damiani *et al.*, 2019), which showed that TFC content was highest by 5 min constant boiling in a microwave. In general, for the same brewing time, a higher water temperature (e.g. 85–90 °C vs. 70–75 °C) increases the extraction of bioactive compounds from tea infusions, further supporting our data (Vuong *et al.*, 2011; Castiglioni *et al.*, 2015; Santos *et al.*, 2016).

Regarding the cold infusions, the extraction efficiency appears similar whether infusions are carried out for 2 h at r.t. or overnight at 4 °C. Therefore, increasing infusion time but at a lower temperature (4 °C vs. r.t.) does not impact the extraction of phytocompounds from tepals. A 2 h infusion was chosen since we previously demonstrated that at this time point, extraction efficiency was maximum compared to lower steeping times for white and green teas (*C. sinensis*) (Castiglioni *et al.*, 2015). It is interesting to note that both cold-brewing methods are as efficient as microwave-assisted boiling, possibly because, despite the lower temperatures of the former, tepals are infused for a longer time, leading to comparable phytocompound extraction efficiency.

Table 1 Total polyphenol and flavonoid contents in stamen and tepal infusions under different brewing conditions as reported in Fig. 1: hot (H), boiled (B), room temperature (RT) and cold (C)

		Total polyphenols mg GAE/L	Flavonoids mg CE/L
Tepals	H	83 ± 22[a]	34 ± 17[a]
	B	159 ± 12[b]	74 ± 22[b]
	RT	186 ± 55[b]	89 ± 17[b]
	C	186 ± 36[b]	91 ± 17[b]
Stamens	H	105 ± 20[a]	28 ± 17[a]
	B	80 ± 15[a]	55 ± 16[a]
	RT	94 ± 29[a]	45 ± 25[a]
	C	99 ± 24[a]	46 ± 24[a]

Data are expressed as mean ± SD, $n = 6$. Different letters indicate statistical differences between samples in each column (Tukey's post hoc multiple comparison test $P < 0.05$).
CE, catechin equivalents; GAE, gallic acid equivalents.

Impact of different brewing conditions on antioxidant capacity of stamen and tepal infusions

Because of the complexity of oxidation processes, three different *in vitro* assays were chosen to study the antioxidant capacity of stamen and tepal infusions, which differ in their determination principles, and which together give a more comprehensive picture than relying on a single assay (Pulido *et al.*, 2003; Prior *et al.*, 2005). The results reported in Fig. 2 distinctly show that tepal infusions have a greater antioxidant capacity than infusions prepared with stamens. Regardless of the assay used, this can be observed for all the brewing methods employed except the hot infusion, where there are no statistical differences between the hot tepal infusions and most of the stamen infusions prepared using the four different methods. Interestingly, especially for the tepal infusions, it can be noted that brewing in cold or room temperature water leads to the same antioxidant capacity as the boiled infusions, likely ascribable to the reasons mentioned in the previous section.

Some differences in the results can be observed among the assays employed; for example, the FRAP assay shows that the hot infusions are always

statistically less potent in terms of antioxidant capacity than those prepared with the other three brewing conditions, for both stamen and tepals; whereas with the DPPH assay, the stamen infusions prepared with room temperature water had the lowest antioxidant capacity. The DPPH assay has previously been used on both methanolic and acid hydrolysed extracts of saffron powders (Urbani *et al*., 2016) where antioxidant capacity was reported to be higher for the hydrolysed extracts (between 20 and 40% inhibition). This inhibition range is comparable to that reported here on our stamen water infusions, but lower than those found for the tepals infusions. With the ORAC assay, all the stamen infusions had a comparable antioxidant capacity, regardless of the preparation method. Notwithstanding these small differences, the results obtained using the three different assays all correlate well among each other, with Pearson's coefficient values exceeding 0.95 in every case (Table S1). In addition, the antioxidant capacity determined with all three assays, strongly correlates with TPC and TFC (Table S1) where Pearson coefficient values are in the range 0.895–0.978. These data confirm that the higher *in vitro* antioxidant capacity observed in infusions obtained with tepals than stamens is likely due to the higher polyphenol content.

This is the first study looking at infusions prepared with whole, isolated saffron flower parts aimed at human consumption; hence comparison of antioxidant capacity, TPC and TFC with other studies is not possible, especially since most studies are performed on freeze-dried samples, using different hydro-alcoholic mixtures and at higher concentrations compared to those used for drinking infusions. Despite this limitation, our results are in accordance with data from (Serrano-Díaz *et al*., 2012) who showed that TPC, TFC and anthocyanins were more abundant in tepals than stamens of freeze-dried *C. sativus* isolated flower parts, although they found no differences in antioxidant capacity measured with the ABTS assay among these flower parts (Serrano-Díaz *et al*., 2012). However, differences in antioxidant capacity were detected in a study by Menghini *et al*., who compared water and olive oil extracts of stigma (CST) and by-products of the saffron industry (tepals + antlers = CTA) (Menghini *et al*., 2018). They found that both TPC and TFC were higher in water CTA than in water CST extracts, and that these correlated well with the antioxidant activity, in accordance with our observations. Previously, Tuberoso *et al*. (2016) analysed water extracts obtained from soaking dried, ground and liquid nitrogen-frozen tepals in room temperature water under stirring in the dark for 30 min. In contrast to our results, they found no correlations between antioxidant activity (FRAP, ABTS assays) of these extracts with TPC and total anthocyanin content. Most likely, the infusion methods in our study are determinants for extraction efficiency and thus also for the subsequent parameters analysed.

Since a recurrent trend can be observed in Fig. 2, showing that the boiling (B) and cold (C) brewing methods appear to lead to a higher antioxidant capacity, these two conditions were chosen for fingerprinting the phenolic profiles of stamen and tepal infusions using an untargeted metabolomics approach. The results obtained lead to gaining further insights into the impact these two brewing methods have on *C. sativus* phenolic profile and hence antioxidant activity.

Untargeted phenolic profile and discrimination of the different brewing methods

The phenolic profile of both stamen (S) and tepal (T) infusions prepared using the boiled (B) and cold (C) brewing methods was evaluated through an untargeted metabolomic approach followed by multivariate

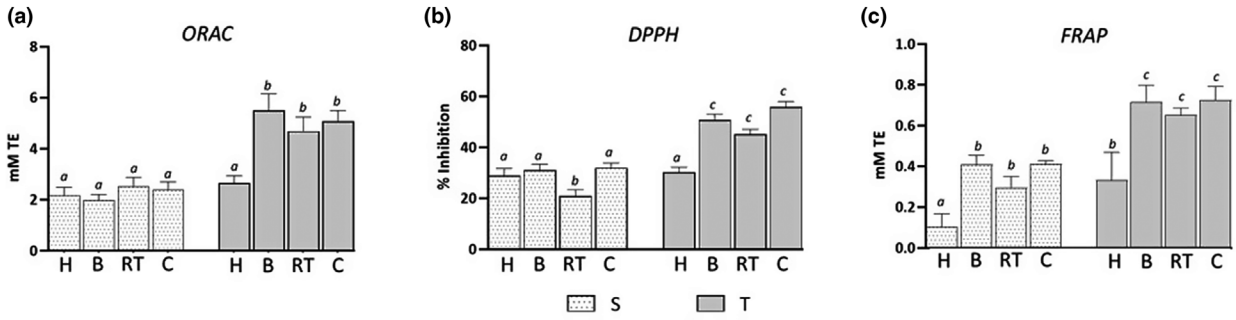

Figure 2 Effect of different brewing conditions on the antioxidant capacity measured using the ORAC assay (a), DPPH assay (b) and FRAP assay (c) of Stamen (S) or Tepal (T) infusions. The brewing conditions are as reported in Fig. 1: hot (H), boiled (B), room temperature (RT) and cold (C). Error bars represent ± SD, $n = 6$. Different letters indicate statistical differences between samples (Tukey's post hoc multiple comparison test, $P < 0.05$). TE, Trolox equivalents.

Table 2 Quantification per classes/sub-classes of phenolic compounds identified from UHPLC-QTOF-MS data for the different tepal (T) and stamen (S) infusions prepared as reported in Fig. 1: cold (C), boiled (B)

Class equivalent (mg/g DM)	TC	TB	SC	SB	Significance
Anthocyanins	13.03 ± 0.43[a]	9.47 ± 3.1[a]	2.61 ± 0.44[b]	3.20 ± 0.55[b]	**
Flavones	5.79 ± 0.80[a]	8.72 ± 2.14[a]	2.00 ± 0.23[b]	2.62 ± 0.62[b]	**
Flavonols	6.14 ± 0.57[a]	8.78 ± 3.09[a]	2.70 ± 0.06[b]	2.40 ± 0.31[b]	**
Flavan-3-ols	0.90 ± 0.28	0.63 ± 0.08	0.60 ± 0.16	0.87 ± 0.06	ns
Phenolic acids	3.14 ± 0.46	2.62 ± 0.32	2.21 ± 0.37	2.50 ± 0.22	ns
Lignans	15.58 ± 1.37[a]	11.30 ± 2.71[b]	5.06 ± 0.55[c]	10.14 ± 1.11[b]	*
Other phenolics (LMW)	16.04 ± 1.68[a]	14.01 ± 1.28[a]	21.97 ± 1.82[b]	30.27 ± 2.37[c]	**
Stilbenes	1.26 ± 0.24	2.21 ± 0.11	1.47 ± 0.42	1.99 ± 0.41	ns
Total phenolics	61.9	57.7	38.6	54.0	

Results are expressed as mean values (mg/g DM) ± SD, $n = 12$. Different letters indicate statistical differences between the different infusions for each class (Tukey's post hoc multiple comparison test. *$P < 0.05$, **$P < 0.0001$).
DM, dry matter; LMW, lower molecular weight; ns, not significant.

statistics. A total of 464 phenolic compounds were putatively annotated, to include 257 flavonoids (i.e. 69 anthocyanins, 66 flavonols, 29 flavan-3-ols and 93 other flavonoids), 95 phenolic acids (of which 67 were hydroxycinnamic acids), 76 low-molecular weight (LMW) phenolics, 29 lignans and 7 stilbenes. The detailed list containing all the phenolic compounds annotated is reported in Appendix S1, together with their abundance and composite mass spectra. Semi-quantitative results are presented in Table 2. The cumulative phenolic contents ranged from 38.6 (for the stamen cold infusions) up to 61.9 (for the tepal cold infusions) mg Eq./g DM. When considering both brewing methods, the tepal infusions exhibited higher phenolic content than the stamen infusions. The phenolic profile of the tepals infusions was mainly represented by flavonoids, with anthocyanins being the most abundant sub-class (i.e. 13.03 and 9.47 mg Eq./g DM for TC and TB respectively). Notably, anthocyanins, flavonols and other flavonoids were significantly ($P < 0.0001$) higher in the tepal infusions than in the stamen infusions, with no difference among cold and boiled brews. On the contrary, low-molecular weight phenolics were significantly ($P < 0.0001$) higher in the stamen infusions, being the most abundant class, with the boiled stamen infusions exhibiting the highest value (30.27 mg Eq./g DM). Considering that the tepal infusions showed the highest phenolic contents, only slight differences were detected by the semi-quantitative analysis between the cold and boiled brewing methods (i.e. lignans significantly higher in TC than in TB; $P < 0.05$). In addition, the stamen infusions prepared using the boiled method resulted in higher cumulative phenolic content than the cold stamen infusions, with significantly higher amounts of LMW phenolics and lignans (30.27 and 10.14 mg Eq./g DM respectively).

Furthermore, differences and similarities between tepal and stamen infusions were investigated through supervised modelling based on multivariate orthogonal projection to latent structures discriminant analysis (OPLS-DA). The model obtained was characterised by fully acceptable parameters, being goodness-of-fit = 1 (R^2Y) and goodness-of-prediction = 0.88 (Q^2Y). The OPLS-DA score plot (Fig. 3) showed clear differences between tepal and stamen infusions, regardless of the brewing method used. Also, this model allowed the discrimination between boiled and cold brewing methods, and this was more evident for tepal infusions (on the left side of the score plot). The Variable Importance in Projection (VIP) approach was used to depict the most discriminant compounds (VIP score > 1) according to the comparison 'boiled' vs. 'cold' brewing for both stamen and tepal infusions. These marker compounds are reported in Table 3 with their respective VIP scores and LogFC values. In detail, 33 polyphenols were identified as the most discriminant compounds, being mainly flavonoids and phenolic acids. Notwithstanding, 5-pentadecylresorcinol, an alkylphenol, exhibited the highest VIP score (1.367) by showing a strong down-accumulation for the comparison 'boiled' vs. 'cold' for both stamen (LogFC = −19.79) and tepal (LogFC = −17.71). A slight down-accumulation was also recorded for other discriminant compounds with a VIP score > 1.3, namely p-HPEA-EDA, syringic acid/gallic acid ethyl ester and the lignan cyclolariciresinol/lariciresinol. Gallic acid 4-*O*-glucoside, chrysoeriol 7-*O*-(6''-malonyl-glucoside) and (+)-gallocatechin 3-*O*-gallate were strong up-accumulated in the comparison SB vs. SC, while 24-methyllathosterol ferulate and gallic acid 3-*O*-gallate were significantly down-accumulated. When

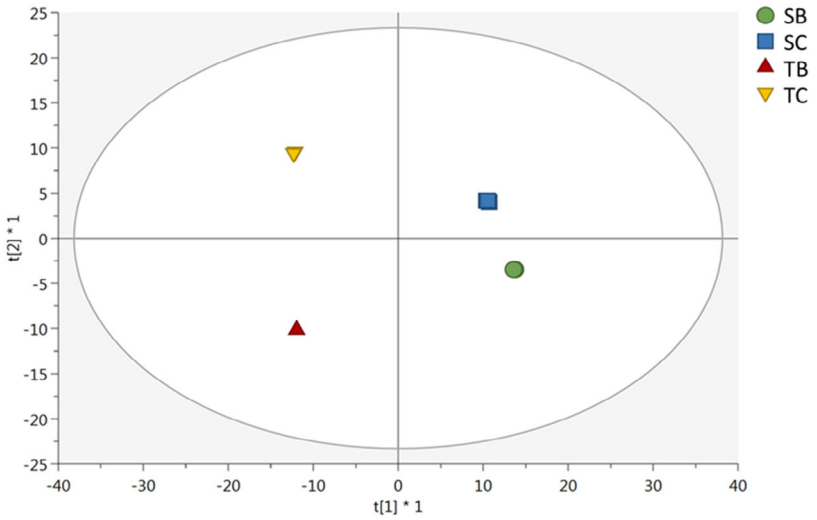

Figure 3 OPLS-DA score scatter plot obtained considering different brewing conditions, cold (C) and boiled (B) of stamen (S) and tepal (T) infusions.

considering the comparison TB vs. TC, the most up-accumulated compound was 5-pentacosenylresorcinol (LogFC = 17.65), while petunidin 3-*O*-(6''-acetyl-galactoside) was the most down-accumulated (LogFC = −18.28).

Finally, the possible correlations between the phenolic profile of the infusions and the *in vitro* antioxidant assays were evaluated through a two-tailed Pearson analysis (Table S1). The Pearson coefficients revealed significant ($P < 0.05$; $P < 0.01$) and positive correlation between some classes/sub-classes of compounds, namely anthocyanins, flavones (other flavonoids), flavonols and lignans, and the *in vitro* antioxidant assays (i.e. ORAC, DPPH, FRAP), thus revealing their contribution to the antioxidant capacity of the samples. The highest correlation coefficients were recorded for anthocyanins, which strongly correlated with ORAC (0.896; $P < 0.01$), DPPH (0.929; $P < 0.01$) and particularly with FRAP (0.936; $P < 0.01$) assays. Notwithstanding, a negative correlation was pointed out between LMW phenolics and ORAC (−0.867; $P < 0.01$), DPPH (−0.839; $P < 0.01$) and FRAP (−0.864; $P < 0.01$) assays.

Comparative total polyphenols content (TPC) and antioxidant capacity in hot infusions of *Crocus sativus* (all flower parts), rooibos and black tea

The TPC and antioxidant capacity of all the flower parts of *C. sativus* (tepals, stigmas, stamens) were compared with those of two popular beverages, namely rooibos (from *A. linearis*) and black tea (from *C. sinensis*) prepared using the same hot brewing method. The infusions were prepared by pouring boiling water over the plant parts and infusing for 5 min at room temperature before filtering, to reflect the most popular method of preparing a hot tea infusion. The results reported in Fig. 4a show that the infusion prepared with *C. sativus* stigma has the lowest TPC, while the infusions prepared with the other flower parts, stamen and tepals, were similar to those of rooibos and black tea. A similar trend was observed regarding the antioxidant capacity determined by the ORAC assay (Fig. 4b), where *C. sativus* infusions from tepals and stamens showed higher values than rooibos and black tea infusions.

Our findings indicate that the different flower parts from *C. sativus* (except for stigmas) could be potentially beneficial for human consumption, such as more popular beverages (e.g. black tea and rooibos) at least in terms of *in vitro* antioxidant capacity. The poor performance of stigmas compared to stamen and tepal infusions is associated with their total phenolic content, and this finding agrees with other literature reports showing that *C. sativus* stigmas are lower in TPC and hence in antioxidant activity compared to other floral parts (Menghini *et al.*, 2018; Xu *et al.*, 2019).

Sensory evaluation

The two spider plots of Fig. 5 show the results of the sensory analysis carried out by volunteers on hot and cold infusions of *C. sativus* tepals; smell, visual and taste (bitter, astringent, sweet and persistent) attributes are reported in Fig. 5a and aroma attributes in Fig. 5b (macro descriptors: floral, spicy, plant, undergrowth, fruits; and micro descriptors: green grass, cooked greens, herbs, woody, earthy, mineral, wild berries, citrus, tropical fruits, dry fruits).

In Fig. 5a, it can be observed that both hot and cold infusions appear to be perceived as somewhat bitter, therefore, barely sweet with almost no

Table 3 Discriminant phenolic compounds according to the comparison 'boiled (B)' vs. 'cold (C)' brewing and considering both stamen (S) and tepal (T) infusions

Discriminant compound	Sub-class	VIP score	Log FC ([SB] vs. [SC])	Log FC ([TB] vs. [TC])
5-Pentadecylresorcinol	Alkylphenols	1.367 ± 0.37	−19.79	−17.71
p-HPEA-EDA	Tyrosols	1.324 ± 0.35	−2.66	−0.75
Syringic acid/Gallic acid ethyl ester	Hydroxybenzoic acids	1.319 ± 0.39	−1.43	−0.23
Cyclolariciresinol/Lariciresinol	Lignans	1.311 ± 0.22	−0.15	−1.40
Isorhamnetin 7-O-rhamnoside/Isorhamnetin 3-O-glucoside	Flavonols	1.289 ± 0.28	−0.30	−2.35
1,2-Disinapoylgentiobiose	Hydroxycinnamic acids	1.283 ± 0.28	−0.27	−4.23
4-Hydroxybenzoic acid 4-O-glucoside	Hydroxybenzoic acids	1.277 ± 0.48	−0.65	0.40
Gallic acid 4-O-glucoside	Hydroxybenzoic acids	1.276 ± 0.66	19.30	1.60
3,4-Dihydroxyphenylglycol	Other polyphenols	1.275 ± 0.49	−1.18	4.46
5-Pentacosenylresorcinol	Alkylphenols	1.261 ± 0.30	−0.31	17.65
Narirutin 4′-O-glucoside	Flavanones	1.256 ± 0.57	−4.73	3.24
Cyanidin 3-O-rutinoside/Petunidin 3-O-rutinoside/Pelargonidin 3-O-sophoroside	Anthocyanins	1.236 ± 0.58	−0.59	1.79
Naringin 6′-malonate	Flavanones	1.215 ± 0.26	−1.13	1.17
Sinapaldehyde/Caffeic acid ethyl ester	Hydroxycinnamics	1.194 ± 0.47	1.75	3.65
Pinoresinol/Matairesinol	Lignans	1.187 ± 0.35	1.43	−0.89
Ligstroside aglycone/p-HPEA-EA	Tyrosols	1.172 ± 0.72	1.89	−0.61
Petunidin 3-O-(6′′-acetyl-galactoside)	Anthocyanins	1.157 ± 0.34	0.32	−18.28
Epirosmanol	Phenolic terpenes	1.142 ± 0.19	1.06	0.10
Cinnamoyl glucose	Hydroxycinnamic acids	1.128 ± 0.99	−1.00	2.09
6′′-O-Malonylglycitin	Isoflavonoids	1.126 ± 0.49	−0.22	0.89
Myricetin 3-O-glucoside	Flavonols	1.101 ± 0.61	−1.67	−0.02
Dehydrodiferulic acid isomers	Hydroxycinnamic acids	1.093 ± 0.33	−0.80	0.59
24-Methyllathosterol ferulate	Hydroxycinnamic acids	1.081 ± 0.19	−16.92	0.59
3/4/5-Feruloylquinic acid	Hydroxycinnamic acids	1.076 ± 0.59	−1.24	1.90
Hydroxycaffeic acid	Hydroxycinnamic acids	1.072 ± 0.24	−2.50	−0.42
Gallic acid 3-O-gallate	Hydroxybenzoic acids	1.072 ± 0.73	−11.13	4.20
Neodiosmin/Diosmin	Flavones	1.061 ± 1.35	−0.35	3.47
Ligstroside	Tyrosols	1.055 ± 0.32	0.20	−0.55
Chrysoeriol 7-O-(6′′-malonyl-glucoside)	Flavones	1.049 ± 0.45	17.33	−0.06
Pigment A/Peonidin 3-O-(6′′-p-coumaroyl-glucoside)	Anthocyanins	1.039 ± 0.79	−1.49	1.16
Prodelphinidin trimer GC-GC-C	Flavanols	1.034 ± 0.27	7.95	−1.07
Quercetin 3-O-glucoside	Flavonols	1.028 ± 0.19	−1.93	−0.19
(+)-Gallocatechin 3-O-gallate/(-)-Epigallocatechin 3-O-gallate	Flavanols	1.015 ± 0.31	17.46	−0.05

Compounds were identified by the VIP (variable importance in projection) approach following OPLS-DA discriminant analysis and are provided together with VIP scores (measure of variable's importance in the OPLS-DA model) and LogFC values (obtained by Fold Change analysis).

astringency. Furthermore, the mouthfeel of cold infusions was considered very persistent, more than the hot ones. Both types of infusions were evaluated as having a rather intense smell, and all volunteers noted the same colour intensity for both infusions. In Fig. 5b, where aroma attributes are reported, the primary attribute perceived by both infusions indicated a grassy hint and a stronger taste of cooked greens coming through for the hot infusion. A taste of wood and undergrowth was also noted for the hot infusion, compared to the cold one. All the other notes were less intense and barely perceived, although the cold infusions were described as rather floral. Regarding overall liking, most of the volunteers rated the cold infusion as being a refreshing and very pleasant drink with floral hints, ideal as a summer drink.

Currently, there are no reported results on the sensory analysis of infusions prepared from *C. sativus* flower parts' by-products, despite them being essential for the promotion of this plant's beneficial properties through herbal infusions, as underlined by Licon *et al.* In their review, which attempts to translate animal doses to human intake of saffron in the diet, a quantity of 30 mg/day for 6–8 weeks, proposed to alleviate depression, could be easily achieved by a cup of 100–150 mL of saffron infusion (Lìcon *et al.*, 2010). The authors conclude their review by recommending taking a saffron infusion as a daily habit to alleviate many diseases. In this context, the results of this present study regarding infusions of *C. sativus* flower parts, aimed at human consumption and where a sensory analysis was carried out, gains significance. An herbal infusion needs to be acceptable and appealing to

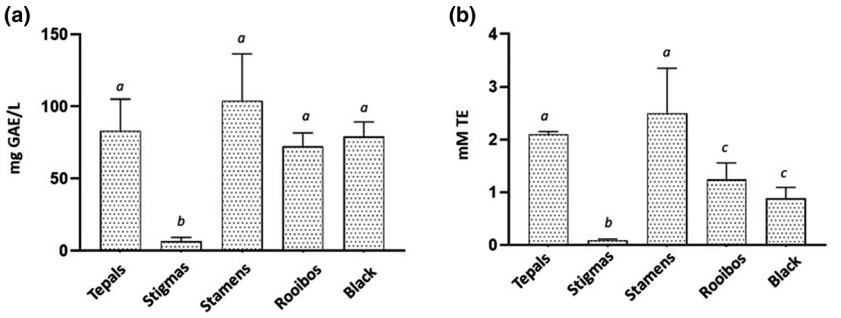

Figure 4 (a) Total polyphenol content, (b) total antioxidant capacity (ORAC assay) of tepal, stigma, stamen, rooibos and black tea infusions prepared using the hot brewing condition (see Fig. 1). Data are expressed as mean ± SD, $n = 6$. Different letters indicate statistical differences between samples (Tukey's post hoc multiple comparison test, $P < 0.05$). GAE, gallic acid equivalents; TE, Trolox equivalents.

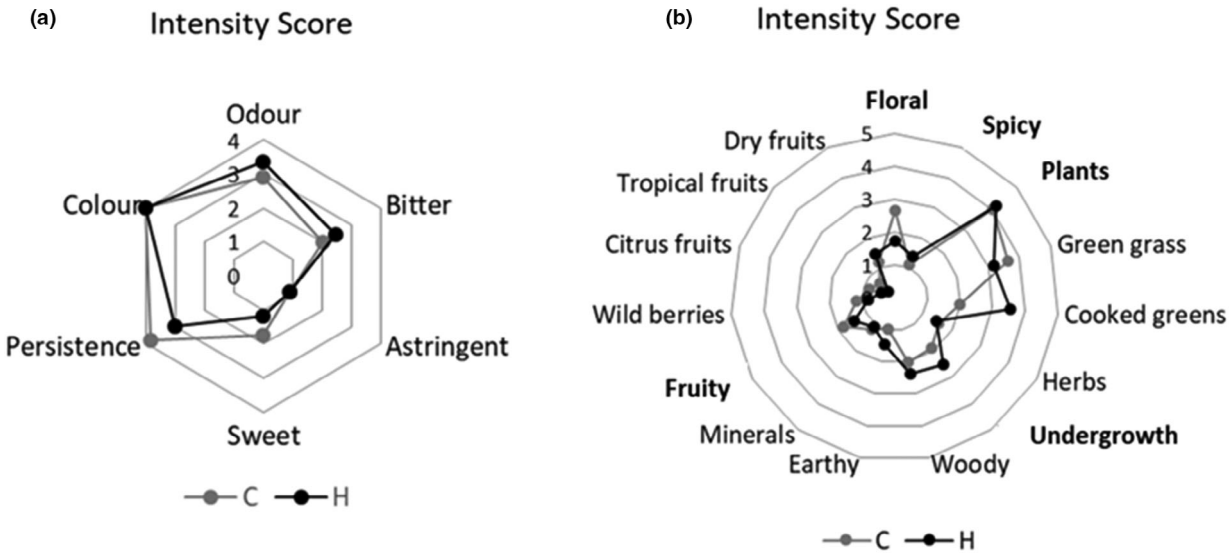

Figure 5 Sensory analysis. Spider plots depicting sensory profiles of the hot (H) and cold (C) tepal infusions prepared as described in Fig. 1. (a). Smell, taste (bitter, astringent, sweet, persistence) and visual attributes. (b) Aroma attributes: macro descriptors are in bold typeface; micro descriptors are in plain typeface.

consumers from the point of view of smell, taste and colour if it has to succeed in the functional food beverage market. To the best of our knowledge, the only literature report on the sensory and functional attributes of saffron was a study on blended herbal teas containing saffron (1.1% w/w) where both a hot (50 °C) and a cold (10 °C) infusion were investigated. These blended teas containing saffron were perceived as bitter, especially when served cold, while the distinctive taste of saffron was clearly recognisable when served hot. Overall, the authors concluded that the blended teas containing saffron were acceptable from the sensory point of view (Kyriakoudi *et al.*, 2016) and that herbal infusions provide a means for the daily intake of functional phytocompounds present in saffron.

Conclusions

Saffron is a worldwide appreciated spice of significant economic and nutritional importance. However, its processing generates high amounts of waste. Hence, the valorisation of saffron by-products through the development of innovative and sustainable high added-value food ingredients could significantly impact agriculture, the nutraceutical and food industry, and ultimately human health. The exploitation of *C. sativus* could, therefore, be extended to include saffron by-products for the preparation of herbal teas, especially considering the rising trend worldwide on consumer demands for functional drinks with positive health attributes (https://www.futuremarketinsights.com/reports/herbal-tea-market, 2022). Saffron tea is a well-known herbal drink, especially in the Middle East, prepared from steeping a few strands of saffron in boiling water, either alone or to flavour tea from *Camellia sinensis*, and other herbal teas and drinks. The present study demonstrates that infusions obtained using tepals and stamen from *C. sativus* are also endowed with high amounts of bioactive compounds representing an excellent source that could

benefit human health. Noteworthy, the flower portion and the brewing method affected the polyphenolic composition of these infusions, with the cold and boiled infusions showing higher polyphenol content and antioxidant capacity than the traditionally prepared hot beverage. The sensory analysis revealed an overall liking for the tepal infusions, especially when served cold. Therefore, the optimisation of saffron-derived infusions plays a pivotal role in determining both functional and sensory traits of the final product. Since the development of sustainable solutions for the management of by-products and food waste is among societal main challenges, the use of saffron flower parts regarded as by-products to prepare functional beverages could also represent an excellent opportunity in the framework of the circular economy.

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Author contributions

Luisa Bellachioma: Formal analysis (equal); investigation (equal); methodology (equal); validation (equal). **Gabriele Rocchetti:** Formal analysis (equal); investigation (equal); methodology (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal). **Camilla Morresi:** Formal analysis (equal); investigation (equal); methodology (equal); validation (equal). **Erika Martinelli:** Formal analysis (equal); visualization (equal); writing – original draft (supporting). **Luigi Lucini:** Supervision (equal); writing – review and editing (equal). **Gianna Ferretti:** Resources (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal). **Elisabetta Damiani:** Conceptualization (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal). **Tiziana Bacchetti:** Resources (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal).

Declarations of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.15713.

Data availability statement

The data that support the findings of this study can be found in Appendix S1 of this article and from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Pearson's correlations coefficient between polyphenols (TPC: Total polyphenols) content) and flavonoids (TFC: Total flavonoids content) and antioxidant capacity assays (ORAC, DPPH, FRAP). $*P < 0.05$, $**P < 0.01$.

Appendix S1. Data set of Saffron infusions, semi-quantitative phenolics, HCA-PCA, LogFC values and OPL-DA.