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Hot and cold drying of edible flowers affect metabolite patterns of extracts and decoctions

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ABSTRACT

Edible flowers are rich in bioactive compounds and pigments that are on increasing demand in nutraceutical, medicinal, food, cosmetic and dyeing industries. This study evaluated the anthocyanins, phenolics and antioxidant activity of eight edible flowers (*Bellis perennis* L., *Centaurea cyanus* L., *Dianthus carthusianorum* L., *Lavandula angustifolia* Mill., *Primula vulgaris* Huds., *Rosa canina* L., *Rosa pendulina* L. and *Viola odorata* L.) after 24 h of hot drying (50 °C; natural convection stove) or cold drying (CD) (22 °C; heat-pump drying system). The hot-dried and cold-dried materials were then used to prepare ultrasound-assisted extracts, which are used as food additives or as a source of natural colourants, or decoctions (DECs), which are used for herbal teas. The edible flower UAEs and DECs had high amounts of anthocyanins (up to 3,284.6 mg C3G · 100 g^{-1}), phenolics (up to 9,034 mg · 100 g^{-1}) and antioxidant activity (866 µmol TE · g^{-1}, 4,901 mmol Fe^{2+} · kg^{-1}), with roses having the highest amount. The anthocyanin content and phenolic profile (flavonols, benzoic acids, cinnamic acids and flavanols) of edible flowers are found to be affected by drying and extraction methods. For decoctions, CD was found to be the most efficient drying method for all the parameters. For UAEs, CD resulted in a higher anthocyanin content, while hot drying resulted in a higher phenolic content, particularly flavanols. Overall, the studied edible flower extracts and decoctions can contribute to healthy and coloured substances, which can be used for designing innovative natural products. *Rosa pendulina* and *R. canina* are the most promising sources.

Keywords: additives, anthocyanins, heat pump drying, HPLC, natural colourants, phenolics

INTRODUCTION

The European diet is continuously changing due to emerging consciousness on the value of different foodstuff, not only from a nutritional point of view but also looking at sustainability and health-promoting ingredients that can improve welfare of consumers (Pires et al., 2017). In this scenario, many studies explored the potential of edible flowers in the human diet as food, supplements or additives and revealed several interesting sources of bioactive compounds (Fernandes et al., 2017; Zhao et al., 2019; Takahashi et al., 2020; Demasi et al., 2021a). Different aspects of edible flowers are currently under investigation, for example quality attributes, traditional consumption, processing, chemical and nutritional characterisation or biological activities (Zhao et al., 2019; Takahashi et al., 2020), but most of the studies focused on the flavonoid content (e.g. anthocyanins and phenolic compounds), a class of low-molecular weight phenolic compounds commonly present in fruits and vegetables. They are fundamental components in nutraceutical, cosmetic, medicinal and pharmaceutical applications. Indeed, flavonoids modulate enzyme functions and exert positive effects on health, such as anti-inflammatory, anti-carcinogenic, antioxidant and anti-mutagenic (Panche et al., 2016).

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Flowers can also be a source of natural colourants for applications in food, cosmetic and dyeing industries, providing a safer option for human health and the environment, counteracting the disadvantages of synthetic dyes (Sivakumar et al., 2011; Bujak et al., 2022).

Fresh flowers have a short shelf-life after harvest (5–15 days depending on the species), and different techniques can be adopted to extend their duration (Fernandes et al., 2019; Zhao et al., 2019; Demasi et al., 2021b). Among these, industry has been using heat treatment techniques for centuries, such as pasteurisation, sterilisation, blanching and drying (Xu et al., 2021). Dehydration is commonly used to prolong the shelf life and preserve quality because it inhibits enzymatic degradation and limits microbial contamination, as well as reduces the weight of the product for more sustainable transport and storage (Fernandes et al., 2019; Zhao et al., 2019; Lu et al., 2020; Qiu et al., 2020). Many different drying methods can be used, such as fluidised bed drying, spray drying, freeze drying, microwave drying, cool wind drying, osmotic drying and combinations of these methods (Fernandes et al., 2019; Stelluti et al., 2021; Xu et al., 2021). However, the application of heat (hot air drying and sun drying) is the most used technique worldwide because of its simple operation, low cost but long drying time, despite some drawbacks, such as undesirable nutritional and biochemical changes that may affect the product quality (Boyar et al., 2017; Fernandes et al., 2019; Qiu et al., 2020). Indeed, chemical composition, aroma component and appearance of herbs and flowers could be altered by the drying process, as well as changes in the tissue anatomy and cellular ultrastructure (Lu et al., 2020; Marchioni et al., 2022; Vallino et al., 2022). Flowers are extremely sensitive to heat compared to other parts of the plant; thus, drying them at low temperature could be a valid alternative to hot drying (HD) because colour, shape and chemical properties could be better preserved. Low-temperature drying, or cold drying (CD), can be achieved through the combination of airflow drying and a heat pump, which works on the principle of refrigeration: It cools an air stream, which renders the air dry and condenses the water contained in it, by recovering the latent heat of evaporation through water vapour removal, which permits air recirculation (Patel and Kar, 2012; Boyar et al., 2017).

Flower extracts may be used as ingredients, particularly for beverages, or additives, or as source of anthocyanins to improve the colour of cosmetics, textiles and food, for example yogurt (Sivakumar et al., 2011; Pires et al., 2018, 2019; Bujak et al., 2022). Ultrasound-assisted extraction (UAE) is an effective method to obtain plant extract faster and more efficiently than the traditionally used maceration. Indeed, ultrasound-assisted extraction causes the instantaneous formation and collapse of cavitation bubbles, which foster cell wall destruction, improving the solvent penetration and the release of phytocompounds (Medina-Torres et al., 2017; Zhao et al., 2019; Stelluti et al., 2021). Edible flowers can be used to prepare wines, functional beverages (e.g. sport drinks, functional bottled water and energy drinks) and herbal teas (Boyar et al., 2017; Pires et al., 2017; Xu et al., 2021), which are defined as beverages made from plants other than *Camellia sinensis* L. and are highly consumed worldwide (Pereira et al., 2017). The demand for these products is estimated to be increasingly popular (Males et al., 2022). Some flower species are used to prepare herbal teas, such as rose, chamomile, primula, poppy, marigold, echinacea, lavender, rosemary, gentian and chrysanthemum (Boyar et al., 2017; Lu et al., 2020; Falla et al., 2022).

The aim of this study was to understand the potential of eight different edible flowers to be used in two different forms – either as ultrasound-assisted extracts (UAEs) or as decoctions (DEC) – for industrial applications. The more suitable drying technique between HD and CD was evaluated for both types of products to achieve better quality in terms of anthocyanin and phenolic contents and antioxidant activity.

MATERIALS AND METHODS

Plant material

The study used eight edible flowers, namely, *Bellis perennis* L., *Centaurea cyanus* L., *Lavandula angustifolia* Mill., *Primula vulgaris* Huds. and *Viola odorata* L., which are known and consumed edible flowers, while *Dianthus carthusianorum* L., *Rosa canina* L. and *Rosa pendulina* L. were selected for their interestingly high levels of phenolic compounds and antioxidant activity. All these species have been recently characterised fresh at harvest and during cold storage (Demasi et al., 2021a, 2021b).

For each species, 200 g of fresh flowers were harvested at their full bloom stage in 2019 in meadows of Turin (Italy), except for *D. carthusianorum* and *R. pendulina*, which were harvested in mountain areas around Turin (45°18′03.6″N 7°13′08.4″E and 45°18′03.6″N 7°11′31.2″E, respectively). Flowers were placed in sealed polyethylene bags, stored at 4 °C in a portable refrigerator and transported to the facilities of the Department of Agricultural, Forest and Food Sciences (Grugliasco, Italy, 45°03′58.5″N; 7°35′29.1″E) to be immediately dried.

Drying

Flowers uniform in shape and colour were selected, and half of harvested material was placed in aluminium trays for preparing at least seven replicates and then inserted in a natural convection stove (DRY-Line, DL 115. VWR, Leuven, Belgium) operating at 50 ± 2 °C for 24 h. Afterwards, the flower samples were cooled down at room temperature. The other part of the harvested material was placed in perforated plastic trays (at least three replicates) and placed in a heat pump drying system (NWT-5, North West Technology, Boves – CN, Italy) operating at low temperature (22 ± 2 °C) for 24 h.

Samples derived from stove drying were labelled HD in the text, while samples from heat pump drying were

labelled CD. The samples were weighted before and after drying to assess the percentage of water extracted in each method within 24 h. Dry flowers were pictured to assess their visual appearance and then grinded in a mortar to obtain a powder to be extracted and analysed. All the results of the analyses were expressed on a dry weight basis.

Extraction

Both hot-dried and cold-dried materials of each species were extracted through two different procedures, namely, UAE and DEC. In UAE, a dry flower powder (0.5 g) was mixed with 25 mL of deionised water and extracted at room temperature with ultrasound equipment (Reus S.à r.l., Drap, France) at 23 kHz for 15 min (Demasi et al., 2021a, 2021b; Stelluti et al., 2021). The solution was filtered with one layer of filter paper (Whatman No. 1, Maidstone, UK) and centrifuged at 3,000 rpm for 10 min, and the supernatant was filtered through a 0.45-mm PVDF syringe filter (CPS Analitica, Milano, Italy).

In DEC, a dry flower powder (1 g) was mixed with 200 mL of deionised water, warmed up to the boiling point, boiled for 5 min and then cooled down for 20 min (Pereira et al., 2017). Similarly to UAE, the solution was filtered, centrifuged and filtered through a syringe filter. All the extractions were performed in triplicate, and the extracts were stored at −20 °C for the following phytochemical analyses.

Total anthocyanin content

The total anthocyanin content (TAC) was estimated using the pH differential method as previously used in the same edible flower species (Demasi et al., 2021b; Stelluti et al., 2021). Each sample was diluted in two 10-mL volumetric flasks (1 mL of sample for each flask) containing two different buffer solutions: one at pH 1.0 (KCl and HCl – 25 mM) and the other at pH 4.5 ($C_2H_3NaO_2$ and $C_2H_4O_2$ – 0.4 M). The absorbances of the flasks were read at 510 nm and 700 nm by using a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, CA, USA) after 20 min in the dark at room temperature. The results were expressed in milligrams of cyanidin-3-*O*-glucoside per 100 g (mg C3G · 100 g^{-1}) of the sample.

Total phenolic content

The total phenolic content (TPC) was analysed using the Folin–Ciocalteu method as previously used in the same edible flower species (Demasi et al., 2021a, 2021b; Stelluti et al., 2021). An measure of 200 µL of the flower extract was mixed with 1,000 µL of diluted (1:10) Folin–Ciocalteu reagent. The samples were left in the dark at room temperature for 10 min, and then 800 µL of Na_2CO_3 (7.5%) was added. After 30 min in the dark at room temperature, absorbance was read at 765 nm, and the results were expressed in mg of gallic acid equivalents (GAEs) per 100 g (mg GAE · 100 g^{-1}) of the sample.

Antioxidant activity

Three methods were used to evaluate the antioxidant activity of edible flower extracts (Demasi et al., 2021a; Stelluti et al., 2021): 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging methods, and the ferric reducing antioxidant power (FRAP) assay.

In the DPPH assay, the DPPH radical solution (DPPH, 100 µM) was prepared by the reaction of 2 mg of DPPH with 50 mL of MeOH up to the absorbance of 1.00 (± 0.05) at 515 nm. A measure of 3 mL of DPPH was mixed with 40 µL of the flower extract. The absorbance of the solution was read at 515 nm after 30 min in the dark at room temperature.

In the ABTS assay, the ABTS radical cation solution ($ABTS^+$) was obtained by the reaction of 2.45 mM $K_2S_2O_8$ and 7.0 mM ABTS and incubated for 12–16 h in the dark at room temperature. The $ABTS^+$ was then diluted with deionised water to reach an absorbance of 0.70 (± 0.02) at 734 nm. Then, 2 mL of diluted $ABTS^+$ was mixed with 30 µL of the flower extract. The absorbance of the solution was read at 734 nm after 10 min in the dark at room temperature.

The percentage of both DPPH and ABTS inhibition was calculated as $[(Abs_0 - Abs_1/Abs_0) \cdot 100]$, where Abs_0 is the absorbance of the control and Abs_1 is the absorbance of the sample. This inhibition capacity was plotted against Trolox calibration curves, and the results were expressed as micromole of Trolox equivalents per gram (µmol TE · g^{-1}).

In the FRAP assay, 900 µL of a solution containing 2,4,6-tripyridyltriazine (TPTZ, 10 mM in HCl 40 mM), $FeCl_3 \cdot 6H_2O$ (20 mM) and a buffer solution at pH 3.6 ($C_2H_3NaO_2$ + $C_2H_4O_2$ in water) was mixed with 30 µL of the flower extract and 90 µL of deionised water. The mixture was placed at 37 °C for 30 min, and its absorbance was then read at 595 nm, the results of which were expressed as millimoles of ferrous iron (Fe^{2+}) equivalents per kilogram (mmol Fe^{2+} · kg^{-1}).

Phenolic profile

The phenolic profiles of all the extracts were assessed using high-performance liquid chromatography (HPLC) with diode array detection (DAD) (Agilent 1200, Agilent Technologies, Santa Clara, CA, USA), provided with a Kinetex C18 separation column (4.6 · 150 mm, 5 mm, Phenomenex, Torrance, CA, USA). Thirteen phenolic compounds were investigated, namely flavonols (hyperoside, isoquercitrin, quercetin, quercitrin and rutin), flavanols (catechin and epicatechin) and phenolic acids (cinnamic acids: caffeic, chlorogenic, coumaric and ferulic acids; benzoic acids: ellagic and gallic acids). The separation of compounds was achieved using different mobile phases, according to previous validated methodology on edible flowers (Demasi et al., 2021a; Stelluti et al., 2021). The compounds were identified by comparison with retention times and UV spectra of analytical standards (purity >95%; Sigma Aldrich, St.

Louis, MO, USA), and their quantification was carried out using calibration curves at the same chromatographic conditions, and the results were expressed as mg · 100 g^{-1}.

Statistical analyses

The effects of different drying methods, HD and CD, on the edible flower quality have been evaluated using the analysis of different parameters, considering two types of extracts: UAE, which is suitable for beverages or food additives, and DEC, which is suitable for hot beverages (tisane and infusion). Data were analysed accordingly, maintaining separation between UAE and DEC.

Differences between HD and CD of the same species concerning the water content, TAC, TPC, ABTS and classes of phenolic compounds were evaluated by mean comparison. The effects of drying (HD and CD) and extraction (UAE and DEC) methods, species and their interactions on phytochemical data (TAC, TPC, FRAP, DPPH and ABTS) and on classes of phenolic compounds were computed using generalised linear models (GLMs) with gamma distribution. Raw data of FRAP, DPPH and ABTS were transformed into standard scores and averaged to obtain the Relative Antioxidant Capacity Index (RACI) (Sun and Tanumihardjo, 2007) and the Global Antioxidant Score (GAS) (Leeuw et al., 2014). The nonparametric Kruskal–Wallis test by stepwise comparison was performed on TAC, TPC, ABTS, RACI and GAS to evaluate differences between species that were subjected to the same drying method.

RESULTS

Water content

The percentage of water extracted from edible flowers after 24 h of HD and CD is reported in Figure 1. The water obtained through HD ranged from 60.4% (*L. angustifolia*) to 87.8% (*P. vulgaris*) and that through CD ranged from 58.4% (*C. cyanus*) to 88.1% (*P. vulgaris*). Significant differences between the two drying methods have been highlighted in three species; HD was more effective in *C. cyanus* and *V. odorata*, while CD was more effective in *D. carthusianorum*.

Phytochemical characteristics

The overall effect of drying and extraction methods, species and their interactions on the phytochemical characteristics evaluated (TAC, TPC and antioxidant activity) was significant, except for the drying method on DPPH and the interaction of drying–extraction–species on FRAP assay (Table 1).

Specifically, data on the effects of the two drying methods on plant extracts and DEC of the different species are shown in Figures 2–6.

Total anthocyanin content

Drying methods affected the TAC of the various edible flowers in both extracts and DECs (Figure 2).

Considering UAE (Figure 2, left), the TAC was influenced by the drying method in three of eight species. Specifically, HD led to a higher TAC in *C. cyanus* (1,101.3 mg C3G · 100 g^{-1}), while CD led to a higher TAC in *R. pendulina* (1,750.3 mg C3G · 100 g^{-1}) and *V. odorata* (3,284.6 mg C3G · 100 g^{-1}). The values in HD extracts ranged from 74.3 (*L. angustifolia*) to 1,101.3 (*C. cyanus*) mg C3G · 100 g^{-1}, while in CD extracts, they ranged from 120.6 (*L. angustifolia*) to 3,284.6 (*V. odorata*) mg C3G · 100 g^{-1}.

Considering DEC (Figure 2, right), the TAC was influenced by the drying method in all the species, except for *L. angustifolia*. In particular, HD led to a higher TAC in *B. perennis* (42.6 mg C3G · 100 g^{-1}), *C. cyanus* (181.1 mg C3G · 100 g^{-1}) and *R. canina* (54.3 mg C3G · 100 g^{-1}),

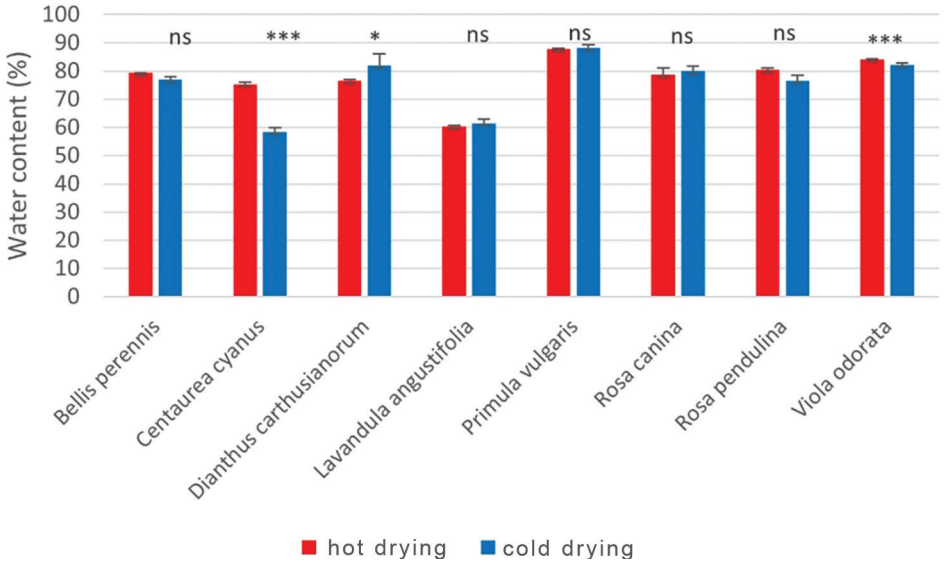

Figure 1. Percentage of water (%) extracted from edible flowers after 24 h of HD and CD. Significance of the Kruskal–Wallis post hoc test is provided. * = $p < 0.05$, *** = $p < 0.001$, CD, cold drying; ns, not significant.

Table 1. Effects of different drying and extraction methods, species and their interactions on TAC, TPC and antioxidant activity of edible flowers according to GLM.

	TAC	TPC	Antioxidant activity (DPPH)	Antioxidant activity (ABTS)	Antioxidant activity (FRAP)
Drying	***	***	ns	***	*
Extraction	***	***	***	***	***
Species	***	***	***	***	***
Drying · Species	***	***	***	***	**
Extraction · Species	***	***	***	***	***
Drying · Extraction	***	***	***	***	*
Drying · Extraction · Species	***	***	***	***	ns

* = $p < 0.05$.
*** = $p < 0.001$.
ABTS, 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; GLMs, generalised linear models; ns, not significant, TAC, total anthocyanin content; TPC, total phenolic content.

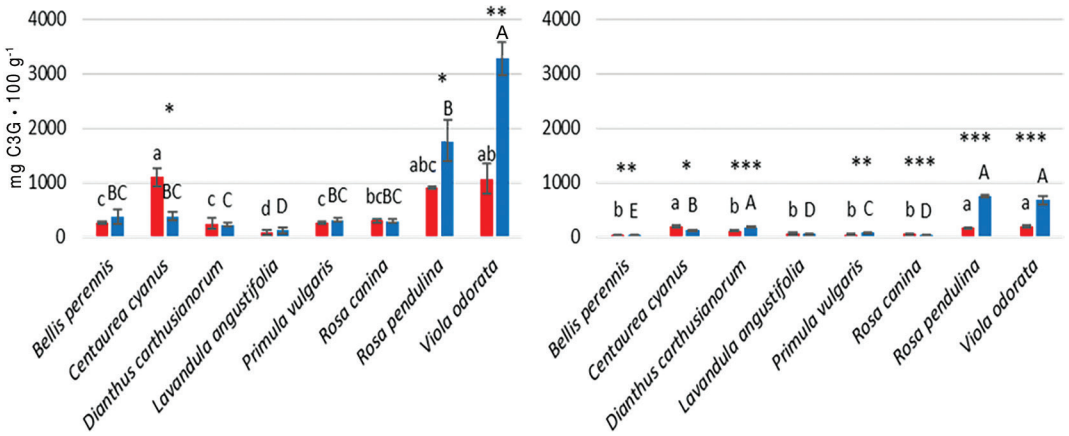

Figure 2. TAC of edible flower extracts (UAE, left) and DEC (right) after HD (red bars) and CD (blue bars). Small case letters indicate significant differences between species along HD, while capital letters between species along CD. Asterisks indicate significant differences between HD and CD considering the single species. *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$. CD, cold drying; DEC, decoctions; HD, hot drying; TAC, total anthocyanin content; UAE, ultrasound-assisted extract.

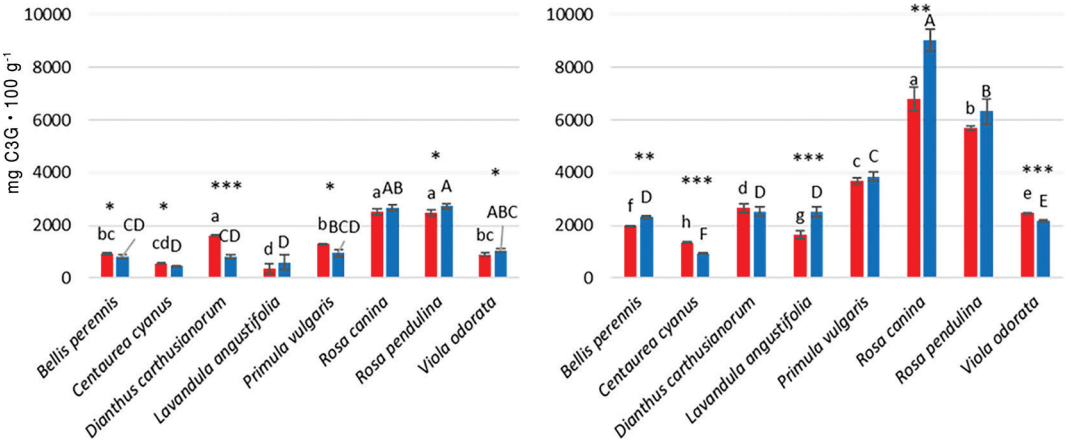

Figure 3. TPC of edible flower extracts (UAE, left) and DEC (right) after HD (red bars) and CD (blue bars). Small case letters indicate significant differences between species along HD, while capital letters between species along CD. Asterisks indicate significant differences between HD and CD considering the single species. *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$. CD, cold drying; DEC, decoctions; HD, hot drying; TPC, total phenolic content.

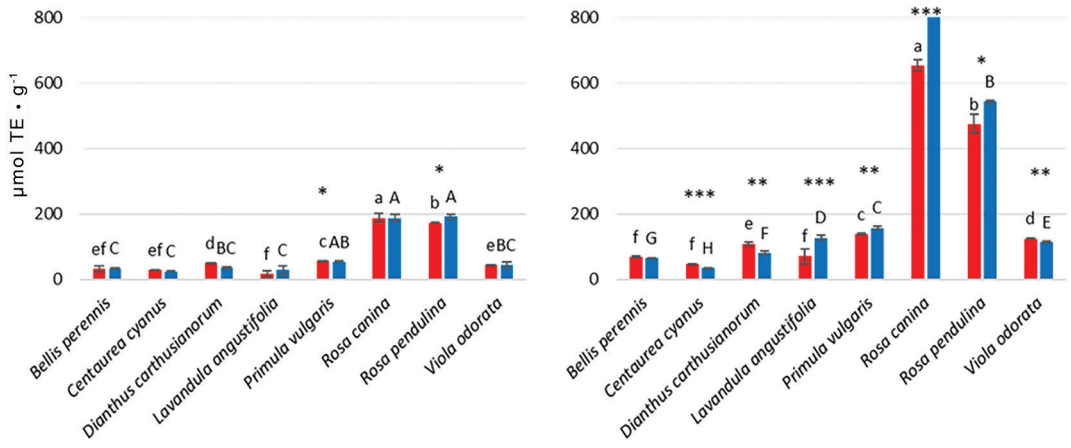

Figure 4. Antioxidant activity evaluated through the ABTS assay of edible flower extracts (UAE, left) and DEC (right) after HD (red bars) and CD (blue bars). Small case letters indicate significant differences between species along HD, while capital letters between species along CD. Asterisks indicate significant differences between HD and CD considering the single species. *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$. ABTS, 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid; CD, cold drying; DEC, decoctions; HD, hot drying; UAE, ultrasound-assisted extract.

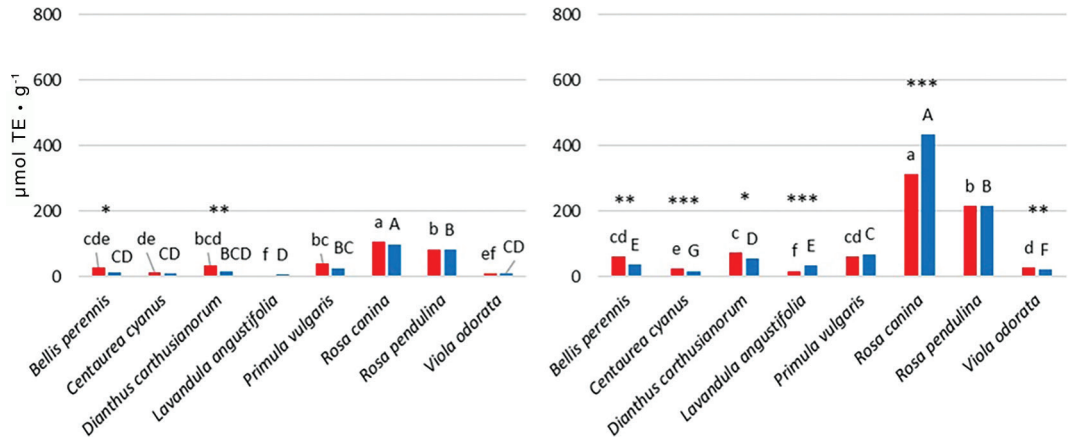

Figure 5. Antioxidant activity evaluated through the DPPH assay of edible flower extracts (UAE, left) and DEC (right) after HD (red bars) and CD (blue bars). Small case letters indicate significant differences between species along HD, while capital letters between species along CD. Asterisks indicate significant differences between HD and CD considering the single species. *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$. CD, cold drying; DEC, decoction; DPPH, 2,2-diphenyl-1-picrylhydrazyl; HD, hot drying; UAE, ultrasound-assisted extract.

while CD values were higher in *D. carthusianorum* (175.4 mg C3G · 100 g^{-1}), *P. vulgaris* (69.8 mg C3G · 100 g^{-1}), *R. pendulina* (740.9 mg C3G · 100 g^{-1}) and *V. odorata* (677.1 mg C3G · 100 g^{-1}). The values in HD ranged from 41.9 (*P. vulgaris*) to 195.4 (*V. odorata*) mg C3G · 100 g^{-1}, while in CD, they ranged from 29.1 (*B. perennis*) to 740.9 (*R. penduline*) mg C3G · 100 g^{-1}.

Total phenolic content

Drying methods affected the TPC of the various edible flowers in both extracts and DECs (Figure 3).

Considering UAE (Figure 3, left), the TPC was influenced by the drying method in six of eight species. HD led to a higher TPC in all the species (*B. perennis* – 934.0, *C. cyanus* – 559.0, *D. carthusianorum* – 1615.7 and *P. vulgaris* – 1,285.8 mg GAE · 100 g^{-1}), except for *R. pendulina* and *V. odorata* where CD was higher (2,730.2 and 1,050.0 mg GAE · 100 g^{-1}, respectively). No significant differences were observed in *L. angustifolia* and *R. canina*.

The range of values in HD was 357.1–2,506.8 mg GAE · 100 g^{-1} in *L. angustifolia* and *R. canina*, respectively, while in CD, the range was 455.5–2,730.2 mg GAE · 100 g^{-1} in *C. cyanus* and *R. pendulina*.

Considering DEC (Figure 3, right), the TPC was influenced by the drying method in five of eight species. HD led to higher values in *C. cyanus* (1,357.9 mg GAE · 100 g^{-1}) and *V. odorata* (2,474.8 mg GAE · 100 g^{-1}), while with CD, values were higher in *B. perennis* (2,308.1 mg GAE · 100 g^{-1}), *L. angustifolia* (2,520.8 mg GAE · 100 g^{-1}) and *R. canina* (9,034.9 mg GAE · 100 g^{-1}). The values in HD ranged from 1,357.9 (*C. cyanus*) to 6799.8 (*R. canina*) mg GAE · 100 g^{-1}, while in CD, they ranged from 932.5 to 9,034.9 mg GAE · 100 g^{-1} in the same species.

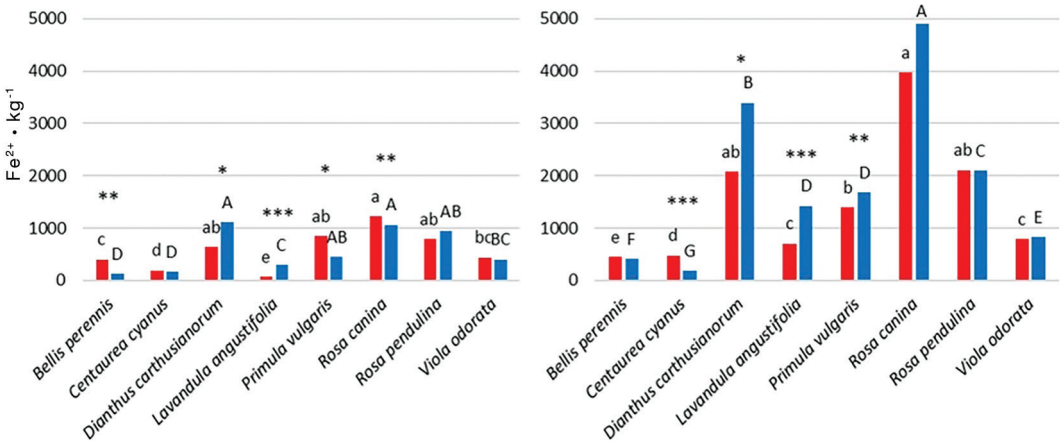

Figure 6. Antioxidant activity evaluated through the FRAP assay of edible flower extracts (UAE, left) and DEC (right) after HD (red bars) and CD (blue bars). Small case letters indicate significant differences between species along HD, while capital letters between species along CD. Asterisks indicate significant differences between HD and CD considering the single species. *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$. CD, cold drying; DEC, decoctions; FRAP, ferric reducing antioxidant power; HD, hot drying; UAE, ultrasound-assisted extract.

Antioxidant activity

The drying methods affected the antioxidant activity measured using ABTS assay in both extracts and DECs (Figure 4).

Considering UAE (Figure 4, left), ABTS was influenced by the drying method only in two species, namely, *P. vulgaris*, which showed higher values with HD (56.9 µmol TE · g^{-1}), and *R. pendulina*, which conversely showed higher values with CD (191.9 µmol TE · g^{-1}). The values in HD ranged from 17.9 (*L. angustifolia*) to 188.1 (*R. canina*) µmol TE · g^{-1}, while in CD, they ranged from 25.1 (*C. cyanus*) to 191.9 (*R. pendulina*) µmol TE · g^{-1}.

Considering DEC (Figure 4, right), ABTS was influenced by the drying method in all, but one, species (*B. perennis*). HD led to a higher antioxidant activity in *C. cyanus* (47.0 µmol TE · g^{-1}), *D. carthusianorum* (109.3 µmol TE · g^{-1}) and *V. odorata* (124.5 µmol TE · g^{-1}), while CD led to higher values in the other four species: *L. angustifolia* (127.6 µmol TE · g^{-1}), *P. vulgaris* (158.0 µmol TE · g^{-1}), *R. canina* (865.7 µmol TE · g^{-1}) and *R. pendulina* (543.1 µmol TE · g^{-1}). The values in HD ranged from 47.0 (*C. cyanus*) to 654.2 (*R. canina*) µmol TE · g^{-1}, while in CD, they ranged from 34.9 to 865.7 µmol TE · g^{-1} in the same species.

DPPH and FRAP assays were also performed to provide a more comprehensive view of the antioxidant activity (Figures 5 and 6, respectively). With DPPH assay, the pattern observed was similar to that of ABTS, with two species (*B. perennis* and *D. carthusianorum*) influenced by the drying method when considering UAE, while six species when considering DEC (*B. perennis*, *C. cyanus*, *D. carthusianorum*, *L. angustifolia*, *R. canina* and *V. odorata*); the range of values was instead lower by half comparing to that of ABTS, but *R. canina* and *R. pendulina* showed the highest antioxidant activity.

The FRAP assay highlighted more differences due to the drying method in UAE (*B. perennis*, *D. carthusianorum*, *L. angustifolia*, *P. vulgaris* and *R. canina*) than in DEC (*C. cyanus*, *D. carthusianorum*, *L. angustifolia* and *P. vulgaris*). In UAE, the highest antioxidant activity was detected in both roses, while it was higher in *R. canina* and *D. carthusianorum* in DEC.

The combination of results obtained with FRAP, DPPH and ABTS assays enabled calculating the RACI and the GAS. RACI (Figure 7) shows that the UAE of both roses have a higher antioxidant activity than the other species, regardless of the drying method used. HD and CD of the other species did not show significant differences but were clearly separated from roses. The RACI of DEC varied to a wider extent, but the trend is similar to that of UAE, with roses at the top of the ranking and *B. perennis*, *C. cyanus* and *L. angustifolia* at the bottom. The same order was obtained with GAS (Figure 8). In UAE, GAS values ranged from 0.007 (HD, *L. angustifolia*) to 0.207 (HD, *R. canina*), while in DEC, they ranged from 0.028 (CD, *C. cyanus*) to 0.898 (CD, *R. canina*).

Phenolic profiles

Overall, the drying method quantitatively affected the phenolic profile of the eight edible flowers but only pertaining to the flavanol content in UAE and DEC, with higher values in HD (393.2 mg · 100 g^{-1} and 971.0 mg · 100 g^{-1}, respectively), while flavonols were higher with CD in DEC (2,093.2 mg · 100 g^{-1}). The amount of all the phenolic classes was significantly influenced by the extraction method, except for benzoic acids, with higher values recorded with DEC (Tables 2 and 3).

However, evaluating each species separately, several different patterns emerged in the phenolic classes' composition in UAE (Figure 9) and DEC (Figure 10) derived from HD and CD. Considering UAE (Figure 7), the total amount of phenolic compounds extracted ranged from 190.3 (*D. carthusianorum*) to 1,869.8 mg · 100 g^{-1}

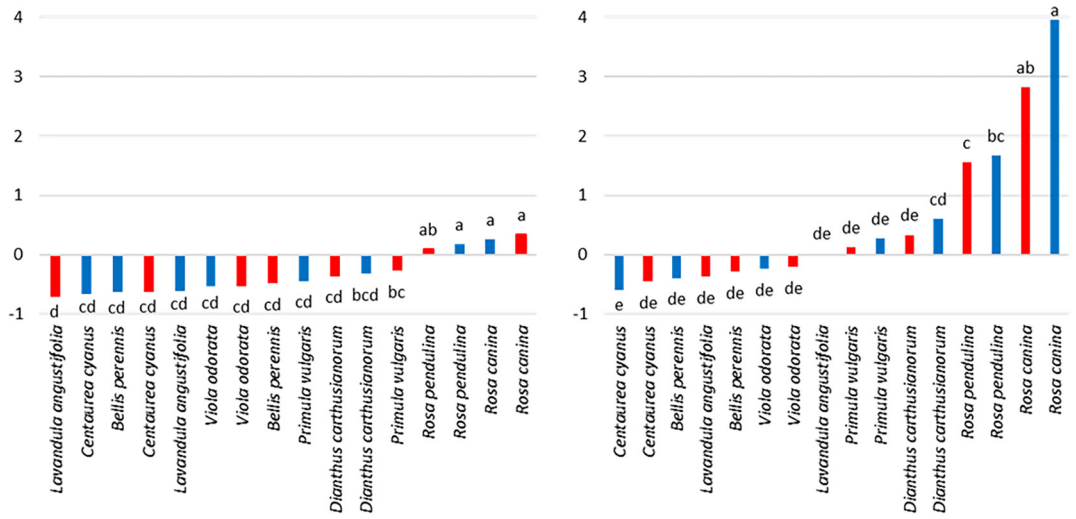

Figure 7. RACI of edible flower extracts (UAE, left) and DEC (right) after HD (red bars) and CD (blue bars). Small case letters indicate significant differences between species, according to the nonparametric Kruskal–Wallis test ($p < 0.05$). CD, cold drying; DEC, decoctions; HD, hot drying; RACI, relative antioxidant capacity index; UAE, ultrasound-assisted extract.

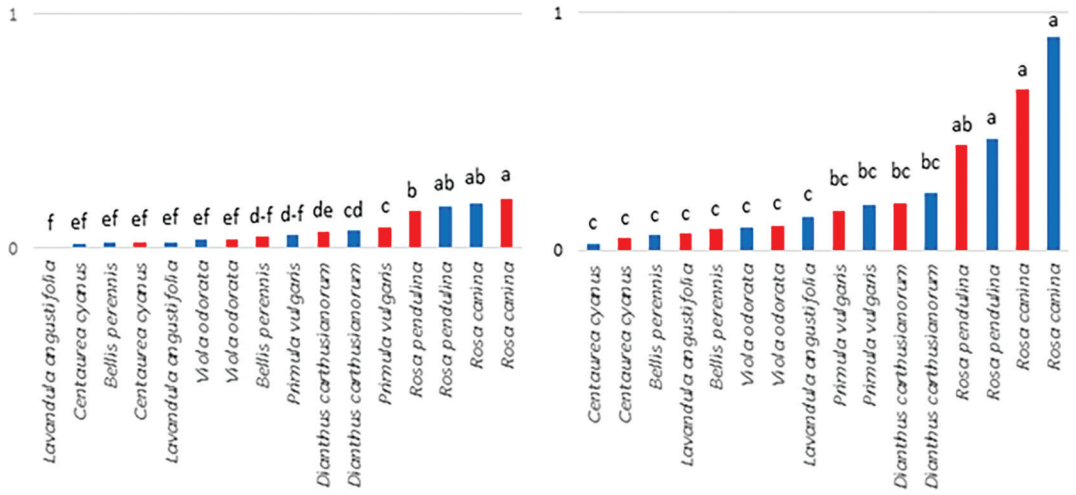

Figure 8. GAS of edible flower extracts (UAE, left) and DEC (right) after HD (red bars) and CD (CD, blue bars). Small case letters indicate significant differences between species, according to the nonparametric Kruskal–Wallis test ($p < 0.05$). CD, cold drying; DEC, decoction; GAS, global antioxidant score; HD, hot drying; UAE, ultrasound-assisted extract.

(*R. pendulina*) with HD, and from 32.3 (*L. angustifolia*) to 2,068.0 mg · 100 g^{-1} (*R. pendulina*) with CD. The four classes were detected in all the species, either in HD, CD, or both, except for benzoic acids that were absent (both in HD and CD) in *L. angustifolia* and *R. pendulina* flowers. The UAE of some species were characterised by a single class of phenolics, namely *B. perennis* and *R. canina* HD, in which only flavonols were identified.

For each species, when a class of phenolics was detected in both HD and CD, a mean comparison between drying methods was performed, and the results are reported in Table 3. In most of the cases, significant differences were recorded, except for flavonols in *L. angustifolia*, *P. vulgaris* and *R. pendulina*. As per cinnamic acids, CD gave a higher number of compounds in two of three species (*P. vulgaris* and *V. odorata*). Flavonols were higher with CD in two species (*C. cyanus* and *D. carthusianorum*) and with HD in the other two species (*R. canina* and *V. odorata*), as also occurred for benzoic acids in *D. carthusianorum*. Finally, flavanols were extracted more with CD in *C. cyanus* and *R. pendulina* and more with HD in *V. odorata*.

Considering DEC (Figure 10), the total amount of phenolic compounds extracted ranged from 150.0 (*D. carthusianorum*) to 3,855.3 mg · 100 g^{-1} (*B. perennis*) with HD, and from 430.3 (*C. cyanus*) to 4,939.6 mg · 100 g^{-1} (*R. canina*) with CD. The four phenolic classes were less frequently detected than UAE, either in HD, or CD, or both, with cinnamic acids being absent (both in HD and CD) in *C. cyanus*, *L. angustifolia* and *R. canina*,

benzoic acids being absent (both in HD and CD) in *B. perennis* and *R. canina*, and flavanols absent (both in HD and CD) in *P. vulgaris*, *R. canina*, *R. pendulina* and *V. odorata*. The DECs of some species were characterised by a single class of phenolics: *D. carthusianorum* HD and *R. canina* HD and CD by flavonols, *L. angustifolia* HD and *C. cyanus* CD by flavanols.

Table 2. Effect of HD and CD on the content (mg · 100 g^{-1}) of different classes of phenolic compounds in edible flower extracts (UAEs) and DECs.

	UAE	DEC	Sign.
Cinnamic acids			
HD	58.5	713.1	***
CD	77.9	505.4	**
Sign.	ns	ns	
Flavonols			
HD	435.3	946.8	*
CD	448.7	2,093.2	***
Sign.	ns	*	
Benzoic acids			
HD	623.7	1,065.8	ns
CD	551.5	844.8	ns
Sign.	ns	ns	
Flavanols			
HD	393.2	971.0	*
CD	181.0	265.5	*
Sign.	*	*	

* = $p < 0.05$.
** = $p < 0.01$.
*** = $p < 0.001$.
CD, cold drying; DEC, decoction; HD, hot drying; ns, not significant, UAE

Again, when a class of phenolics was detected in both HD and CD, a mean comparison between drying methods was performed for each species. The results are reported in Table 4, and significant differences were recorded in all the cases. Considering cinnamic

Table 3. Effects of HD and CD in the yield of phenolic compounds (mg · 100 g^{-1}) in edible flower extracts.

	HD	CD	Sign.
Cinnamic acids			
Lavandula angustifolia	62.4	9.6	*
Primula vulgaris	5.6	66.8	**
Viola odorata	100.8	392.7	***
Flavonols			
Centaurea cyanus	45.5	372.0	***
Dianthus carthusianorum	9.2	28.0	**
Lavandula angustifolia	47.4	22.6	ns
Primula vulgaris	598.9	386.2	ns
Rosa canina	560.8	510.2	*
Rosa pendulina	1,737.5	1,772.8	ns
Viola odorata	286.7	49.2	***
Benzoic acids			
Dianthus carthusianorum	116.1	56.7	***
Flavanols			
Centaurea cyanus	219.3	270.7	**
Rosa pendulina	132.4	288.3	*
Viola odorata	833.8	294.4	***

* = $p < 0.05$.
** = $p < 0.01$.
*** = $p < 0.001$.
CD, cold drying; HD, hot drying; ns, not significant.

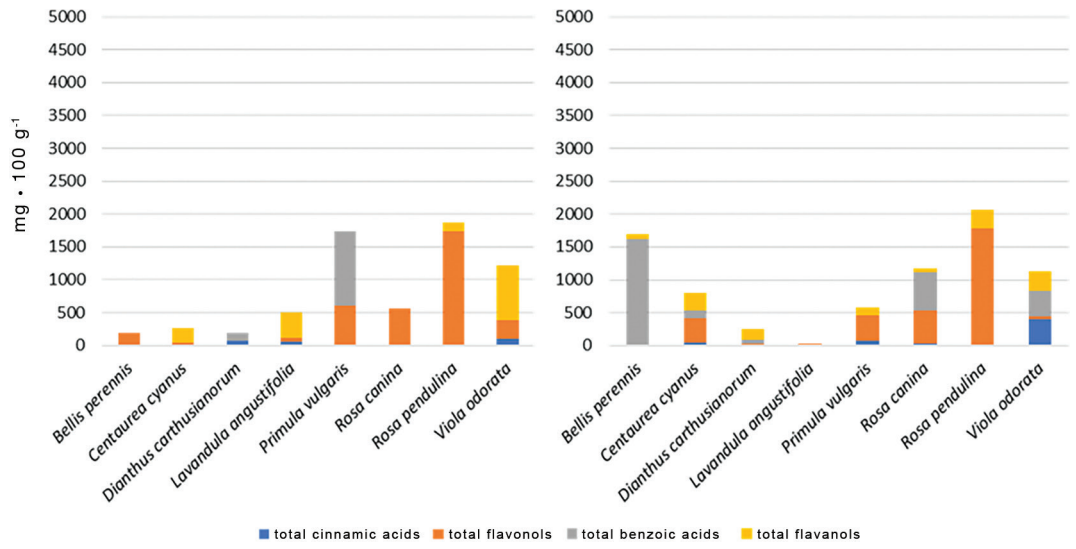

Figure 9. Distribution of different phenolic classes (cinnamic acids, flavonols, benzoic acid, and flavanols) in extracts (UAE) after HD (left) and CD (right). CD, cold drying; HD, hot drying.

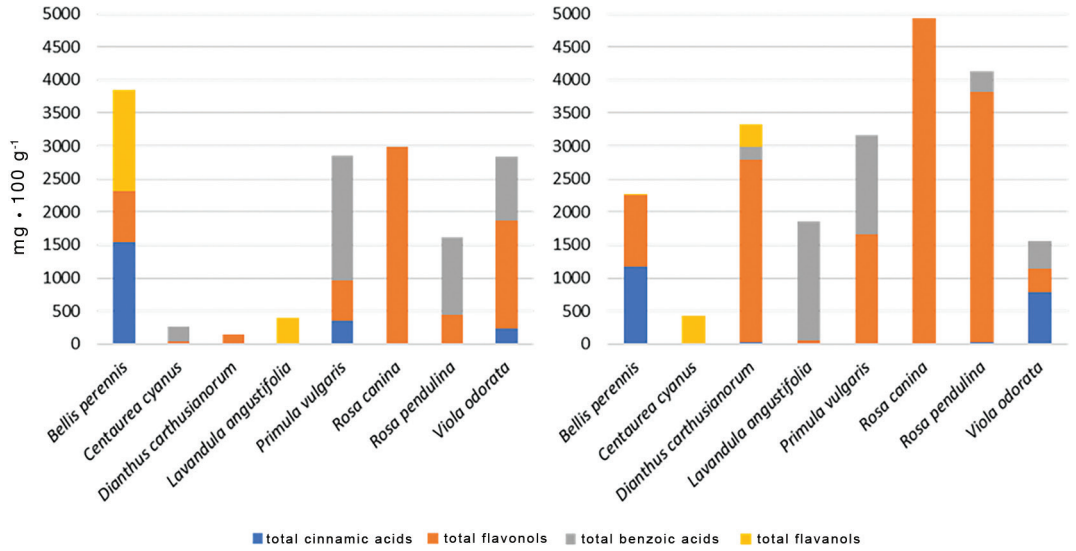

Figure 10. Distribution of different phenolic classes (cinnamic acids, flavonols, benzoic acids and flavanols) in DEC after HD (left), and CD (right). CD, cold drying; DEC, decoctions; HD, hot drying.

Table 4. Effects of HD and CD in the yield of phenolic compounds (mg · 100 g^{-1}) in edible flower DEC.

	HD	CD	Sign.
Cinnamic acids			
Bellis perennis	1,548.8	1,172.9	**
Viola odorata	238.8	790.9	***
Flavonols			
Bellis perennis	768.2	1,081.8	*
Dianthus carthusianorum	150.0	2,762.4	***
Primula vulgaris	609.4	1,663.7	***
Rosa canina	2,987.0	4,939.6	*
Rosa pendulina	447.8	3,793.9	***
Viola odorata	1,627.7	354.1	***
Benzoic acids			
Primula vulgaris	1,895.1	1,501.6	*
Rosa pendulina	1,176.6	314.5	***
Viola odorata	965.4	412.3	***
Flavanols			
Bellis perennis	1,538.2	23.5	***

* = $p < 0.05$.
** = $p < 0.01$.
*** = $p < 0.001$.
CD, cold drying; DEC, decoctions; HD, hot drying; ns, not significant

acids, the two species behaved differently, with higher quantities obtained with HD in *B. perennis* and CD with *V. odorata*. Flavonols were always higher with CD, except for *V. odorata*, where HD was more effective, as occurred also for benzoic acids and flavanols.

Visual appearance

The flowers that underwent HD and CD for 24 h were pictured after drying (Figure 11). Concerning the colour, it is evident that CD retained the green colour of the calyx, when present, while with HD, it changed to a darker, brownish green. Similarly, the colour of petals was more vivid with CD than with HD, where the colours of *L. angustifolia* and *R. canina* almost disappeared, turning to grey and brown, respectively. The shape of flowers responded differently according to the species. In three species (*B. perennis*, *D. carthusianorum* and *L. angustifolia*), the shape was similar in both HD and CD, while in four species (*P. vulgaris*, *R. canina*, *R. pendulina* and *V. odorata*), CD led to curled petals. *C. cyanus* behaved differently as flower appearance seemed to be not affected by CD.

DISCUSSION

Data on extracted water confirmed that generally water represents about 80% of the flower composition (Pires et al., 2017). According to Zhang (2017), the final moisture content of edible flowers should be 10%–12% to better preserve the quality and stability of the product. Our results highlighted that 24 h of HD and CD was sufficient to reach this percentage in *P. vulgaris*, while the other species probably need more time. Particularly, the small percentages of water extracted from *C. cyanus* and generally also from *L. angustifolia* could be due to their high amount of green portion (calix and spike, respectively), which is thicker than petals. However, both drying techniques were sufficient to grind the flowers and obtain the powder to prepare UAE and DEC; moreover, flowers remained unaltered for the following two years when stored in glass jars at room temperature in the dark.

The presence of phenolic compounds, which are non-nutritional plant secondary metabolites, is highly demanded in functional food and beverages to quench reactive oxygen species and reactive nitrogen species in the human body. The control of this oxidative

Figure 11. Appearance of dry flowers of *Bellis perennis*, *Centaurea cyanus*, *Dianthus carthusianorum*, *Lavandula angustifolia, Primula vulgaris*, *Rosa canina*, *Rosa pendulina* and *Viola odorata* after 24 h of HD (left jar) and CD (right jar). CD, cold drying; HD, hot drying.

stress can counteract several age-related diseases, such as inflammations, cancers and cardiovascular, dysmetabolic and neurodegenerative disorders (Del Rio et al., 2013; Zamora-Ros et al., 2013; Aryee et al., 2019; Durazzo et al., 2019; Fraga et al., 2019). The positive properties of phenolics are also exploited in different sectors, from agriculture to food, pharmaceutical and cosmetic industries (Brodowska, 2017). Anthocyanins are natural food additives regardless of the plant source, authorised in Europe with the code E-163 and considered a group of harmless substances (Santos-Buelga and González-Paramás, 2019).

The lowest values of TAC, TPC and antioxidant activity of dry flowers were mostly recorded in *L. angustifolia* and *C. cyanus* considering both UAE and DEC, except for TAC DEC, where the lowest values were found in *P. vulgaris* and *B. perennis*, subjected to HD and CD, respectively. Overall, UAE was found to extract a higher amount of anthocyanins (Figure 2) than DEC, while DEC allowed to obtain a higher quantity of phenolic compounds (Figures 3 and 6) and a higher antioxidant activity (Figure 4). Considering the drying method, the effect was different according to the product considered: in UAE, CD was more effective on TAC, HD was more effective on TPC, while the drying technique was irrelevant on ABTS; in DEC, CD was found to be the most efficient drying method to get better results for all the parameters.

The values of TAC recorded in edible flowers, up to 3,284.6 mg C3G · 100 g^{-1}, were much higher than that found in commonly consumed vegetables (Li et al., 2012), such as red cabbage (199 mg C3G · 100 g^{-1}) and purple cauliflower (201 mg C3G · 100 g^{-1}), or fruit, up to 414.95 mg C3G · 100 g^{-1} in *Vitis vinifera*, one of the richest sources of anthocyanins (Ribeiro et al., 2015; Solymosi et al., 2015).

The stability of anthocyanins is negatively affected by several factors, such as heat, light and oxygen (Qiu et al., 2020; Cai et al., 2022). Flower pigments are commonly divided into polyene pigments (lutein, carotene) and

phenolic pigments (anthocyanins, flavonoids); unstable glycosides of the latter are easy to hydrolyse and isomerise, resulting in the degradation of the pigments under thermal processing (Xu et al., 2021). In particular, rising the heating temperature during thermal processing has been found to decrease the TAC of edible flowers, causing degradation and discolouration of petals, and our results showed the same behaviour as TAC was much lower in DEC (prepared at boiling temperature, circa 100 °C) than in UAE (prepared at room temperature, circa 23 °C). The higher temperatures were found to cause loss of the anthocyanin content and browning reaction in *Rosa rugosa* flowers (Qiu et al., 2020), confirming what is recorded in this study, where most of the flowers had browning reaction after HD. The main traits that contribute to sales for processed flowers are appearance, aroma and colour, which are determined and affected by several factors, such as hot air during processing (Zhao et al., 2019). The use of low temperature drying and UAE can thus be effective techniques to preserve the visual appearance and TAC of edible flowers, respectively, raising the quality of the dry product. Generally, the effect of the drying method was less evident probably because of the smaller difference in temperature between HD (50 °C) and CD (22 °C). However, heat pump drying is characterised by several positive features, such as obtaining a good visual quality, which is suitable in the sectors where the aesthetic value is essential, like bakeries. Moreover, this equipment has easy-to-control conditions and has high energy efficiency. On the other hand, other characteristics must be evaluated, such as high initial investments, the presence of the refrigerant and the complexity of the structure compared to traditional airflow drying (Lv and Zhang, 2017).

The values of TPC recorded in edible flowers, up to 9,034 mg · 100 g^{-1}, were much higher than those found in commonly consumed vegetables (Cai et al., 2004), such as *Lactuca sativa* (780 mg GAE · 100 g^{-1}) and *Spinacia oleracea* (900 mg GAE · 100 g^{-1}), and infusions of rose, jasmine and osmanthus flowers (375–433 mg GAE · 100 g^{-1} (Hussain et al., 2019). Teas of dry rose petals can reach very high values of TPC and antioxidant activity, as found in this study, representing one of the most promising healthy beverages (Vinokur et al., 2006). Results on dry lavender macerates (Duda et al., 2015) or UAE with different solvents (Dorozko et al., 2019) showed a higher content of TPC (1,183–1,816 mg GAE · 100 g^{-1}) than those found in this study, but we obtained double values when performing DEC on the same species, similar to those obtained with freeze-dried flower DEC (Dobros et al., 2022). DEC was highlighted as the most performant method to extract antioxidant compounds also in aromatic plants *Achillea millefolium* (Dias et al., 2013) and *Melissa officinalis* (Sentkowska et al., 2016).

Concerning TPC, Lu et al. (2020) suggested that the increase in phenolic compounds during the heating process might be due to different causes. The high temperature breaks plant tissue, leading to an easier release of phenolics during the extraction process; moreover, heat can inactivate the enzyme polyphenol oxidase, which usually degrades phenolic compounds in fresh tissues, thus leading to a higher content of TPC; finally, fresh flowers can sense water loss during drying, synthesising phenolic compounds as a defence mechanism.

Concerning the phenolic profiles detected by HPLC, UAE allows obtaining more compounds than DEC in terms of frequency, although their concentrations were much higher in DEC. The effect of drying varied mostly depending on the species, and a general effect was observed only for flavanols, where HD was more effective in both UAE and DEC products.

Phenolic compounds are divided into phenolic acids and flavonoids (Pires et al., 2019). The former are the main compounds produced by plants, including benzoic and cinnamic acids and are used to improve the nutritional, organoleptic and antioxidant properties of food, besides being used in pharmaceutical and cosmetic industries (Kumar and Goel, 2019). The latter include anthocyanins, flavonols and flavanols, all water-soluble pigments that confer red, pink-orange and blue colours to fruits and flowers (Brodowska, 2017; Pires et al., 2019).

In this study, however, a different pattern was observed since benzoic and cinnamic acids were not detected in several species, flavonols and flavanols instead being the most frequent and abundant classes of compounds, confirming previous studies on the same species of fresh (Demasi et al., 2021a) and dry dahlia, rose, calendula and centaurea (Pires et al., 2017). Nevertheless, the pattern of phenolic classes identified in the UAE of the same species of fresh and dry flowers cannot be overlapped as some classes increased after drying and some decreased, depending also on the species. This can be due to the different composition of each extract, in which every compound follows its own process according to conditions.

CONCLUSIONS

Flower species vary widely in terms of phytochemical characteristics and composition; therefore, testing different processing and extraction methods is highly important to improve the knowledge on the more suitable technique to gain the highest amount of bioactive compounds and pigments for each species. This study explored HD and CD applied to eight edible flowers, followed by ultrasound-assisted extraction or decoction preparation. The phenolic profiles detected by HPLC analysis showed peculiar patterns for each species, and both drying and extraction methods differently affected the yield of phytochemicals. Concerning the extraction method, it was seen that a higher amount of TAC can be obtained with UAE, while DEC gained a higher amount of phenolic compounds and greater

antioxidant activity. For DEC, CD was found to be the most efficient drying method for all the parameters. For UAE, CD gave a higher quantity of anthocyanins, while HD a higher quantity of phenolics, particularly flavanols. Overall, the studied flower extracts and DEC can contribute to produce healthy food and beverages and coloured produce. *R. pendulina* and *R. canina* are the most promising sources for designing innovative natural products.

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AUTHOR CONTRIBUTIONS

S.D. – conceptualisation, conceptualisation, methodology, formal analysis, investigation and writing – original draft. M.C. – investigation and writing – review and editing. V.S. – conceptualisation, resources, writing – review and editing, and supervision.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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