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Compositional Characteristics and Antioxidant Activity of Edible Rose Flowers and Their Effect on Phenolic Urinary Excretion

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Petals of edible flowers (EF) are rich in biologically active compounds with many proven benefits for human health. However, studies on the effects of EF in humans after consumption are lacking. This pilot explorative study evaluated the changes in urinary phenolic excretion in healthy volunteers to whom different doses of phenolics from edible roses (Gourmet Roses™) have been added to a meal. Rose petals were picked fresh once a week for three weeks, showing significantly increasing values of total phenolic content, total anthocyanin content, and antioxidant activity (measured as ferric reducing antioxidant power (FRAP) and as DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging activities) from the first to the third week. After the meal, direct associations between urinary phenolics and both the EF phenolic content and the antioxidant activity were found in a multiple regression model. These new insights on EF consumption, to be confirmed by larger trials, suggest that the urinary phenolic excretion of healthy volunteers increases with increasing rose phenolic content.

INTRODUCTION

Edible flowers (EF) have been used in human nutrition for hundreds of years and are popular in the European, Middle-East, Chinese, and Indian cultures [Lim, 2014a,b; Pires *et al*., 2019; Scariot *et al*., 2018], thanks to their taste, beauty, and aromas [Takahashi *et al*., 2020]. EF are consumed either fresh or minimally processed or in the form of different preparations [Fernandes *et al*., 2020; Takahashi *et al*., 2020]. Since the late 1980's, studies revealing the EF chemical composition and properties linked to the presence of several bioactive compounds arose [Demasi *et al*., 2020; Falla *et al*., 2020; Fernandes *et al*., 2020; Grzeszczuk *et al*., 2016; Rop *et al*., 2012; Scariot *et al*., 2018], together with an increased awareness of consumers towards the consumption of natural sources of bioactive compounds [Fernandes *et al*., 2020; Rop *et al*., 2012; Takahashi *et al*., 2020]. Petals of fresh EF are rich in vitamins, minerals, and phenolics, a class of biologically active compounds with many proven benefits [Liu, 2003; Loizzo *et al*., 2016; Navarro-González *et al*., 2015; Takahashi *et al*., 2020]. Adequate intake of phenolics could confer benefits for human health, by reducing the risk of cardiovascular, dysmetabolic, and neurodegenerative diseases, and cancer (in particular gastrointestinal neoplasms), by eliciting anti-inflammatory effects, and by favorably modulating the gut microbiota composition [Fraga *et al*., 2019; Zamora-Ros *et al*., 2013]. Furthermore, phenolics have been reported to be inversely associated with all-cause mortality and cardiovascular events [Del Bo *et al*., 2019]. EF have a low-fat content and are rich in water similarly to leafy vegetables [González-Barrio *et al*., 2018; Rop *et al*., 2012]. It has also been demonstrated that many EF contain high amounts of phenolics, exceeding those found in fresh fruits and vegetables. For instance, *Rosa pendulina* petals have a total phenolic content of ~1,700 mg/100 g, more than double than blackcurrant (~800 mg/100 g) or blackberry (~600 mg/100 g) fruits [Demasi *et al*., 2021a; Pérez-Jiménez *et al*., 2010b]. Looking at single classes of phenolics, petals of *Dianthus pavonius* contain more than 2,000 mg/100 g of flavonols compared to ~100 mg/100 g in spinach, or *Paeonia officinalis* contains ~800 mg/100 g of benzoic acid compared to ~120 mg/100 g in raspberry, or finally *Taraxacum officinale* have ~800 mg/100 g of cinnamic

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acids compared to the 200 mg/100 g of globe artichoke [Demasi *et al*., 2021a; Pérez-Jiménez *et al*., 2010b]. It is therefore evident that it is important to deepen the knowledge on flowers composition in order to understand the role of their phenolics in human metabolism.

The quantity and quality of secondary metabolites and bioactive compounds in petals, similarly to other anatomical parts of plants, may be influenced by several factors. A wide variability in the amount and composition of metabolites in plants have been recorded depending on the genotype [Fiehn, 2002], the stage of development [Piccolella *et al*., 2018], the environmental conditions [Demasi *et al*., 2018], the cultivation practices [Caser *et al*., 2019a,b; Najar *et al*., 2019], the harvesting time [Pal & Singh, 2013], and storage [Demasi *et al*., 2021b].

To date, a few EF species have been investigated and this number is expected to increase [Fernandes *et al*., 2020; Pires *et al*., 2019]. Rose (*Rosa* spp.) is one of the most beloved and known ornamental plants, with a complex genus classification [Martínez *et al*., 2020; Smulders *et al*., 2019]. It is among the most frequently consumed EF worldwide [Fernandes *et al*., 2020], showing a high phenolic content and antioxidant activity according to the genotype [Demasi *et al*., 2021a; Fernandes *et al*., 2020; Guimarães *et al*., 2010; Li *et al*., 2014; Lu *et al*., 2016; Zheng *et al*., 2018]. At present, the contribution of EF to human metabolism *in vivo* is almost unexplored and data relative to phenolic urinary excretion after EF consumption in humans are lacking. We carried out an exploratory pilot study in a small group of healthy volunteers who were given a week away both a meal and the same meal with the addition of rose petals with different phenolic contents. Then we explored whether the fresh rose characteristics (total phenolic content, total anthocyanin content, and antioxidant activity) were associated with the excretion of phenolics in human urine, after standardization of the meals.

MATERIALS AND METHODS

Chemicals and apparatus

Sodium carbonate, sodium acetate, potassium chloride, potassium persulfate, hydrochloric acid, acetic acid, iron(III) chloride hexahydrate, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH radical), Folin–Ciocalteu phenol reagent, and gallic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). Oaxis MAX Cartridges were purchased from Waters (Milford, CT, USA). A Cary 60 UV-Vis spectrophotometer (Agilent, Santa Clara, CA, USA) was used to perform spectrophotometric readings.

Plant material

Fresh open flowers of Gourmet Roses™ were provided by the organic nursery RaveraBio® (Rzero Group di Orsini L. & C., Albenga, SV, Italy) once a week from 1st to 21st June 2019. From each of the three supplies, part of the fresh petals was used for the human experiment, and part was grinded in a mortar using liquid nitrogen, then prepared for the spectrophotometric analysis of total phenolic and total anthocyanin content, and antioxidant activity. One gram of flower powder was extracted with 50 mL of a water-methanol solution (1:1, *v/v*) at room temperature with ultrasound-assisted extraction (Sarl Reus, Drap, France) at 23 kHz for 30 min. Three different extractions were performed as replicates for each supply. The solution was filtered with one-layer of filter paper (Whatman No. 1, Maidstone, UK) and preserved at -20°C until the spectrophotometric analyses.

Total phenolic content of roses

The total phenolic content was analyzed using the Folin-Ciocalteu reagent [Demasi *et al*., 2021b], by mixing 750 μL of the reagent (diluted 1:10) with 150 μL of the rose extract and 600 μL of Na_2CO_3 (7.5%). The solution was left in a dark room at room temperature for 30 min. Then, its absorbance was read at 765 nm, and results were expressed as g of gallic acid equivalents (GAE) per kg of fresh flower (g GAE/kg).

Total anthocyanin content of roses

The pH-differential method was used for anthocyanin measurement [Demasi *et al*., 2020]. The rose extract (1 mL) was mixed with 9 mL of an aqueous buffer solution at pH 1 (4.026 g KCl + 12.45 mL HCl 37% in a 1 L water volume) in one flask. In another flask, 1 mL of the same rose extract was mixed with an aqueous buffer solution at pH 4.5 (32.82 g $C_2H_3NaO_2$ + 18 mL $C_2H_4O_2$ in a 1 L water volume). The solutions were kept in the dark for 20 min at room temperature, and their absorbance was read at 515 nm and 700 nm. Results were expressed as g of cyanidin 3-*O*-glucoside (C3G) per kg of fresh flower (g C3G/kg).

Antioxidant activity of roses

The antioxidant activity of roses was analyzed using different assays: the ferric reducing antioxidant power (FRAP), DPPH, and ABTS [Demasi *et al*., 2021b]. The FRAP assay was performed by mixing 30 μL of the rose extract with 90 μL of deionized water and 900 μL of the FRAP reagent. The solution was kept for 30 min at 37°C, and then its absorbance was measured at 595 nm, and results were expressed as millimoles of ferrous iron (Fe^{2+}) equivalents per kg (mmol Fe^{2+}/kg). The DPPH assay was performed with the following procedure: a $DPPH^{\bullet}$ solution was obtained by the reaction of 2 mg of $DPPH^{\bullet}$ with 50 mL of MeOH, up to the absorbance of 1.000 at 515 nm. Then, 3 mL of the $DPPH^{\bullet}$ solution was mixed with 40 μL of the rose extract. The mixture was left in the dark at room temperature for 30 min, and then its absorbance was measured at 515 nm. The ABTS assay was performed with the following procedure: the ABTS radical cation solution was obtained by the reaction of 7.0 mM ABTS with 2.45 mM $K_2S_2O_8$, incubated for 12–16 h in the dark at room temperature and diluted with distilled water until the absorbance of 0.70 had been achieved at 734 nm. Then, 2 mL of the diluted $ABTS^{\bullet+}$ solution was mixed with 30 μL of the rose extract. The mixture was left in the dark at room temperature for 10 min, and then its absorbance was measured at 734 nm. In both DPPH and ABTS methods the results were expressed as mmol of Trolox equivalents (TE) per kilogram (mmol TE/kg).

The water-methanol (1:1, *v/v*) extraction solution was used as control in each analysis instead of the rose extract.

The human experiment

Twenty healthy volunteers were enrolled for the experiment. Inclusion criteria were age 20–70 years, and a body mass index (BMI) 20–29 kg/m^2. Exclusion criteria were: treatment with any drugs and/or supplements, subjects in any dietary regimen, pregnant and/or lactating women, the presence of any known disease, active smoking, inability to express informed consent to the study, and known flower allergy. The study was conducted following a randomized cross-over design [Kuntz *et al*., 2015] and all participants received the same meal without (M; meal without EF) or with (EFM; meal + EF) the addition of 17 g of rose petals after 1 week of wash out. Participants were randomized to receive as a first meal either the meal without EF (M) or the same meal with the addition of EF (EFM). Meals were prepared by the same researcher in the same place; each meal consisted of 2 courses, and their composition is reported in Table 1.

In a random order, 7, 8, and 5 participants received the EFM supplied respectively in the first, second, and third week. Randomization sequence was computer-generated by a statistician. Participants were to consume either the M or the EFM in 60-min under researchers' supervision at 1:00 pm at the kitchen of the School of Dietetics (University of Turin). Volunteers had to eat both the two courses each of the two days of the experiment. During each meal, only water was allowed. The same dietary recommendations for the 24-h before each test and the 24-h after each test were given to all participants. The energy content was calculated according to the participant's energy need (range 1500–1900 kcal); the dietary composition was 20 g/100 g proteins, 30 g/100 g lipids, 50 g/100 g carbohydrates. Fiber intake was restricted to 17 g/day, by reducing phenolic-rich foods (no more than 250 g fruit/day -only peeled apple and banana allowed-, no more than 200 g vegetables/day -only lettuce and zucchini allowed-, no wine, no tea, no coffee, no cocoa) in order to avoid interference from dietary polyphenols. The dietary phenolic intake was calculated according to the published database [Neveu *et al*., 2010; Rothwell *et al*., 2013]. Each participant completed both a 1-day food record to collect data relative to food consumption 24-h before each experiment and a validated food-frequency questionnaire to obtain data relative to usual dietary habits. Diet adherence was verified both by the 1-day food recall and by telephone interview with each participant the day before the experiment. Urine collection began from 4:00 pm of the day of the test until 4:00 pm of the day after the test. Each volunteer was asked to urinate before the meal and then to wait until 4:00 pm before the next voiding. All procedures were in agreement with the principles of the Helsinki Declaration; the study protocol, the questionnaires used, the informed consent, the information for the participant and the *curriculum vitae* of the researchers were submitted to the attention of the Local Bioethics Committee of the University of Turin on 25 March 2019. The study protocol was approved by the Local Bioethics Committee of the University of Turin (No.176859, Turin, Italy) on 2 May 2019. Informed consent was obtained from all individual participants included in the study.

Total phenolic content of urine

Urine samples were collected into sterilized 1.5 L bottles, acidified with HCl to preserve the phenolic compounds in line with literature [Roura *et al*., 2006], and processed the following morning to avoid formation of artefacts and loss of phenolic content. The total phenolic content excreted in urine after consumption of the test meals was determined with Folin-Ciocalteu assay after purifying the samples by solid-phase extraction according to literature [Medina-Remón *et al*., 2009], with the difference that the solid-phase extraction was carried out through Oaxis MAX Cartridge Waters containing the same stationary phase as micro titer 96-well plate cartridges. Briefly, 1 mL of acidified urine was applied to an activated Waters Oasis MAX cartridge. The cartridge was rinsed with 4 mL of sodium acetate 50 mM pH 7/5% methanol. The phenolics were eluted with 1.8 mL of 2% (*v/v*) formic acid in methanol. Then, 30 μL of the eluted fractions were mixed with 340 μL of deionized water adding 25 μL

TABLE 1. Composition of the meal (M) received by the participants. The meal with edible flowers (EFM) was the same with the addition of 17 g of rose petals.

Meal	Ingredient	Quantity (g)	Proteins (g)	Lipids (g)	Carbohydrates (g)	Fiber (g)	Total kcal
Vegetable noodles	Noodles	80	7.3	1.7	39.5	1.81	
	Courgettes	60	0.81	0	0.87	0.87	
	Carrots	50	0.55	0	3.8	1.55	
	Ricotta	40	3.5	4.37	1.4	0	
	Olive oil	10	0	10	0	0	
Baked fish fillet with yogurt sauce	Cod	100	17	0.3	0	0	
	Natural yogurt	50	1.9	1.95	2.15	0	
	Cucumbers	15	0.1	0	0.3	0.11	
	Olive oil	5	0	5	0	0	
Intake (*per capita*)			30	23	48	4.34	520

of the Folin-Ciocalteu reagent and 60 μL of sodium carbonate (200 g/L). The mixture was incubated for 1 h at room temperature in the dark. Later, 145 μL of deionized water was added. Absorbance was measured at 765 nm. Results were expressed as mg gallic acid equivalent per liter (mg/L) and per day (mg/day).

Statistical analyses

Flowers' variables and human data were reported as means ± standard deviations. For each rose's phytochemical parameter, differences between the three samples were analyzed by non-parametric analysis of variance using Kruskal-Wallis test, with stepwise comparison, and by Spearman's correlation analyses. Between-group differences in urinary phenolic excretion were analyzed by Kruskal-Wallis test. Analyses were performed using SPSS 24.0 Inc. software (SPSS Inc., Chicago, IL, USA). Crude and adjusted linear regression models were used to examine the urinary phenolic excretion (dependent variable) in relation to each compositional characteristics and antioxidant activity of edible rose flowers: (a) the flower total phenolic, (b) the total anthocyanin contents, and (c) the antioxidant activity. A multiple regression model adjusted for age, sex, BMI, and dietary phenolic intake was estimated for each phytochemical characteristic (a, b, c). These analyses were done with Statistica software (ver. 7.0; StatSoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Bioactive compounds in rose petals

The content of phenolics and anthocyanins in rose petals and their antioxidant activity are reported in Table 2. The total phenolic content (6.53, 8.01, and 11.71 g GAE/kg) and the FRAP (298.23, 407.41, 564.77 mmol Fe^{2+}/kg) significantly differed in each sample, increasing from the first to the third week of EF supply. Similarly, anthocyanin content (0.86–1.36 g C3G/kg), DPPH$^{\bullet}$ (26.67–45.58 mmol TE/kg), and $ABTS^{\bullet+}$ (9.49–14.12 mmol TE/kg) scavenging activities were higher in the third sample. All the evaluated parameters of EF were positively highly correlated with each other (p values were always lower than 0.01), as reported in Table 3.

Rosa is an extremely wide and complex genus of plant and comprises more than 150 species and 30,000 cultivars [Smulders *et al*., 2019]. Some of them have already been studied as edible flowers and source of bioactive compounds (*e.g. Rosa* × *hybrida*, *Rosa* × *odorata*, *Rosa centifolia*, *Rosa chinensis*, *Rosa gallica*, *Rosa micrantha*, *Rosa damascena*, *Rosa bourboniana*, *Rosa brunonii,* and *Rosa rugosa*), and results showed a wide variability according to the species [Chen *et al*., 2018; Guimarães *et al*., 2010; Kumar *et al*., 2009; Li *et al*., 2014; Lu *et al*., 2016; Mohsen *et al*., 2020; Rop *et al*., 2012; Zhang *et al*., 2014; Zheng *et al*., 2018]. Different analytical assays

TABLE 2. Total phenolic and total anthocyanin contents, and antioxidant activity measured as ferric reducing antioxidant power (FRAP) and as DPPH$^{\bullet}$ and $ABTS^{\bullet+}$ scavenging activities of edible roses (Gourmet Roses™) supplied in the first, second, and third week of June 2019.

Rose supply	Total polyphenol content (g GAE/kg)	Total anthocyanin content (g C3G/kg)	FRAP (mmol Fe^{2+}/kg)	DPPH$^{\bullet}$ scavenging activity (mmol TE/kg)	$ABTS^{\bullet+}$ scavenging activity (mmol TE/kg)
n.1	6.53 ± 0.70^{c}	0.86 ± 0.09^{b}	298.23 ± 35.75^{c}	26.67 ± 1.47^{b}	9.49 ± 1.92^{b}
n.2	8.01 ± 0.19^{b}	0.97 ± 0.02^{b}	407.41 ± 16.53^{b}	28.36 ± 2.62^{b}	9.96 ± 0.49^{b}
n.3	11.71 ± 0.40^{a}	1.36 ± 0.08^{a}	564.77 ± 6.45^{a}	45.58 ± 0.82^{a}	14.12 ± 0.17^{a}
p	0.00003	0.0004	0.00002	0.00002	0.005

Results are expressed as mean of three replicates ± standard deviation; different letters indicate significant differences in a column according to Kruskal-Wallis stepwise comparisons ($p \leq 0.05$). GAE – gallic acid equivalent; C3G – cyanidin 3-*O*-glucoside; TE – Trolox equivalent.

TABLE 3. Spearman's correlation coefficients between total phenolic content, total anthocyanin content, and antioxidant activity measured as ferric reducing antioxidant power (FRAP) and as DPPH$^{\bullet}$ and $ABTS^{\bullet+}$ scavenging activities of edible roses (Gourmet Roses™).

		FRAP	DPPH$^{\bullet}$ scavenging activity	$ABTS^{\bullet+}$ scavenging activity	Total anthocyanin content
Total phenolic content	Correlation coefficient	0.983	0.850	0.803	0.833
	p	0.000	0.004	0.009	0.005
FRAP	Correlation coefficient		0.883	0.820	0.850
	p		0.002	0.007	0.004
DPPH$^{\bullet}$ scavenging activity	Correlation coefficient			0.887	0.800
	p			0.001	0.010
$ABTS^{\bullet+}$ scavenging activity	Correlation coefficient				0.937
	p				0.000

(*e.g.* FRAP, DPPH, and ABTS, or total phenolic content) are necessary to evaluate the antioxidant activity of matrices. However, different extraction and analytical methods, and sample preparation could make the comparison among different studies difficult [Santos-Buelga *et al*., 2012]. The range of total phenolic content has been reported to vary from 5.00 to 24.00 g GAE/kg in *R.* × *hybrida* and *R.* × *odorata* [Li *et al*., 2014; Rop *et al*., 2012], while other authors [Chen *et al*., 2018] found 74 g GAE/kg in pink *R. rugosa* (on dry weight). Twelve rose cultivars are reported to have a total anthocyanin content ranging from 0 to 2.50 g C3G/kg [Friedman *et al*., 2010]. In four rose species [Zheng *et al*., 2018], ranges of 360–3620 mmol Fe^{2+}/kg were recorded with FRAP, and 239–1037 mmol TE/kg with ABTS assay. $ABTS^{\bullet+}$ scavenging activities of 2–36 mmol TE/kg and 653 mmol TE/kg were reported in fresh flowers of 12 rose cultivars [Friedman *et al*., 2010] and in dry flowers of *R. rugosa* [Zhang *et al*., 2014], respectively. In turn, 101 mmol TE/kg (by ABTS assay) and 451 mmol Fe^{2+}/kg (by FRAP) were recorded in *R.* × *hybrida* petals [Li *et al*., 2014]. Our results on Gourmet Roses™ petals are consistent with the above-mentioned ranges, except for the DPPH assay, which detected lower values than those reported in literature (243–520 mmol TE/kg) [Chen *et al*., 2018; Zheng *et al*., 2018].

The petals of roses harvested at one-week distance during the month of July showed an increasing content over time in bioactive compounds, namely total phenolics, and anthocyanins. Consequently, an increased antioxidant activity was found. These parameters were positively correlated, confirming previous results on edible plants and wildflowers [Demasi *et al*., 2021a; Li *et al*., 2014]. The secondary metabolite content in roses varies among species and may be triggered by various stimuli, which commonly occur because of seasonal variations, or biotic and abiotic stresses. Similarly, also the phenological stage and senescence of the plant could have determined an increased production of antioxidants, such as phenolic compounds, as a defense system that can lead to increments both in the phenolic content and the biological activity over time [Piccolella *et al*., 2018].

Urinary phenolic content

Twenty volunteers participated in the study (12 males, 8 females); their mean age and BMI were 41.2±10.8 years and 22.6±3.2 kg/m^2, respectively. Their usual mean phenolic dietary intake was 931.3±219.1 mg/day. The 24-h before each experiment, the mean phenolic dietary intake was lower (784.5±230.4 mg/day) in line with the given dietary recommendations for those days. Diet adherence was evaluated by 1-day food record and telephone interview the day before each experiment and resulted to be adequate. Out of them, 2 volunteers (1 male and 1 female, both receiving the third EF sample) did not perform a correct 24-h urine collection. Therefore, the urine samples of 18 subjects were analyzed. No adverse effects related to the EF assumption were reported by participants.

The differences in urinary phenolic excretion (expressed as mg/L or mg/day) between the participant consuming EFM and M were -1.8±11.4, 7.0±14.6, 59.1±94.6 mg/L (p=0.15 according to Kruskal-Wallis) and -4.5±20.2, 6.8±10.9, 72.7±124.1 mg/day (p=0.15), respectively from the first, second, and third supply of roses. The associations between the urinary human phenolic excretion (dependent variable) and the EF characteristics, namely total phenolic and total anthocyanin content, and antioxidant activity are shown in Table 4. Direct significant associations were found with all the EF characteristics in both crude and adjusted models, in which four variables potentially impacting the intake or excretion of phenolics (age, gender, body mass index, and dietary phenolic intake) were considered. It is reassuring that the associations remain statistically significant in the adjusted model, despite the small size of the sample (20 participants). This might suggest a potential influence of the rose characteristics on the human absorption of the rose phenolic content.

TABLE 4. Parameters of a regression model for relationships between differences in 24-h urinary phenolic excretion of participants consuming meal with and without edible flower, and the roses (Gourmet Roses™) characteristics.

Characteristics	Model	β_1	SE_1	p_1	β_2	SE_2	p_2
Total phenolic content (g GAE/kg)	Crude	0.11	0.048	0.031	0.15	0.063	0.032
	Adjusted[a]	0.16	0.063	0.030	0.19	0.084	0.044
Total anthocyanin content (g C3G/kg)	Crude	1.21	0.50	0.027	1.58	0.65	0.027
	Adjusted	1.65	0.65	0.026	2.03	0.86	0.035
Ferric reducing antioxidant power (mmol Fe^{2+}/kg)	Crude	0.20	0.09	0.046	0.26	0.12	0.049
	Adjusted	0.28	0.12	0.047	0.32	0.17	0.07
$DPPH^{\bullet}$ scavenging activity (mmol TE/kg)	Crude	3.10	1.24	0.024	4.09	1.62	0.022
	Adjusted	4.27	1.64	0.023	5.40	2.12	0.026
$ABTS^{\bullet+}$ scavenging activity (mmol TE/kg)	Crude	12.85	5.15	0.024	16.90	6.70	0.023
	Adjusted	17.67	6.78	0.023	22.24	8.79	0.026

[a] adjusted – multiple regression model adjusted for age, gender, body mass index, and dietary polyphenol intake. β_1, ES_1, p_1 – parameters for urinary phenolic excretion expressed in mg/L; β_2, ES_2, p_2 – parameters for urinary phenolic excretion expressed in mg/day. β – regression coefficient; SE – standard error; GAE – gallic acid equivalent; C3G – cyanidin 3-*O*-glucoside; TE – Trolox equivalent.

Polyphenols bioavailability varies widely among different classes of phenolics and they can be absorbed and metabolized differently according to their chemical structure [Teng & Chen, 2019]. Briefly, part of them are absorbed by the small intestine, while part are metabolized by microbiota. The metabolites reach the liver to be absorbed by tissues and cells or the kidneys, to be ultimately excreted through urine. Phenolic intake from food has been therefore associated with human total urinary phenolic excretion [Nielsen *et al*., 2002; Mennen *et al*., 2006; Pérez-Jiménez *et al*., 2010a; Roura *et al*., 2006; Spencer *et al*., 2008; Zamora-Ros *et al*., 2011]. A systematic review has suggested that urinary phenolics might be considered as an indicator of phenolic intake [Pérez-Jiménez *et al*., 2010a]. An increased consumption of phenolic compounds with diet has been reported to reduce cardiovascular risk factors [Guo *et al*., 2016; Medina-Remón *et al*., 2017], the incidence of type 2 diabetes mellitus [Wedick *et al*., 2012], cardiovascular events, and all-cause mortality [Agudo *et al*., 2007; Alonso *et al*., 2004; Covas *et al*., 2001; Grassi *et al*., 2005; Manach *et al*., 2005; Tresserra-Rimbau *et al*., 2014a,b], and to decrease blood concentrations of inflammatory biomarkers [Medina-Remón *et al*., 2017]. The beneficial effects of dietary polyphenols may also be due to a bi-directional relationship with the gut microbiota: polyphenols can favorably affect the gut microbiota composition, and the gut microflora could metabolize polyphenols into beneficial bioactive compounds, such as chlorogenic acid and the derived compounds [Fraga *et al*., 2019; Liu *et al*., 2020a; Ozdal *et al*., 2016; Tomas-Barberan *et al*., 2014].

At present, only *in vitro* and animal studies evaluated the effects of EF phenolics. The anti-inflammatory property of extract of *R. canina,* tested on the carrageenin-induced rat paw edema assay, was demonstrated by the inhibition of carrageenin-induced edema, similarly to the effect of indomethacin [Lattanzio *et al*., 2011]. Flower compounds have been reported both to induce cell apoptosis *via* the p53 signalling and p38 MAPK/FasL (mitogen-activated protein kinase--FAS ligand) cascade pathways [Lin *et al*., 2005; Lo *et al*., 2007] and to ameliorate the ROS-mediated mitochondrial dysfunction pathway [Hou *et al*., 2005; Lin *et al*., 2005; Lo *et al*., 2007]. Hibiscus acid from *Hibiscus sabdariffa* (roselle) and (+)-epimagnolin A and (+)-magnolin from *Magnolia denudata* induced weight loss in animals and in *in vitro* experiments, by acting on fat metabolism-related enzymes, down-regulating adipocyte differentiation *via* the modulation of the PI3K (phosphoinositide 3-kinase) and MAP-kinase pathways and inhibiting α-amylase activity and sugar/starch absorption [Hansawasdi *et al*., 2001; Kim *et al*., 2007; Kong *et al*., 2011; Preuss *et al*., 2007]. Similarly, the methanol extract of *Nymphaeaceae* inhibited the lipid storage in adipocytes by promoting lipolysis [Hansawasdi *et al*., 2001; Lee *et al*., 2010; Kong *et al*., 2011; Velusami *et al*., 2013]. *Rosa* spp. are very rich in quercetin, which has been shown to inhibit both α-glucosidase and α-amylase, thus reducing the intestinal absorption of glucose [Lu *et al*., 2016; Oboh *et al*., 2015]. However, different EF showed great variability in their phenolic composition and bio-accessibility through an *in vitro* digestion model coupled to a simulated intestinal barrier [de Morais *et al*., 2020]. Thus, *in vivo* and human studies are needed to define the potential role of EF on human health.

This is the first human study analyzing the relationship between the dietary content of phenolics from EF and the urinary phenolic excretion in healthy volunteers. According to the multiple regression analysis, we found a direct relationship between the increasing rose phenolic content and the phenolic excretion, meaning that phenolics have been absorbed and metabolized by the body [Pérez-Jiménez *et al*., 2010a].

CONCLUSIONS

The preliminary data of this pilot explorative study suggest the importance of carrying out further human trials to characterize the absorption of the phenolics contained in the EF and their impact on the human oxidative status. Indeed, edible roses were confirmed as a rich source of bioactive compounds (total phenolics and total anthocyanins) with high antioxidant activity, and the increasing values of these parameters in flowers corresponded to an increase of the urinary phenolic excretion in healthy volunteers. Interestingly, the amount of EF used in the recipes (17 g) provided an amount of phenolics (111–199 mg GAE) proper to fill the gap between the dietary recommendations during the trial (784 mg/day) and the usual mean dietary intake of the participants (931 mg/day), suggesting that no other supplemented phenolics are required to meet their needs. Though, we cannot exclude the possibility that an absorption plateau could be reached at a specific threshold of phenolic intake by EF supplementation. In order to reduce the risk of bias, the conditions were standardized as much as possible before and during the human experiment and the participants reported an adequate compliance to the given dietary recommendations. Even though for the purposes of a pilot study any sample size from 12 upwards has been considered adequate [Julious, 2005], a larger sample of participants will be needed for future studies to specifically test a dose-response relationship [Shader, 2015]. Moreover, the evaluation, besides urine, also of blood markers of oxidative stress, inflammation, kidney and liver function would be of interest to define the efficiency of phenolics absorption and the potential benefits and safety of adding specific EF in daily diet.

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CONFLICT OF INTEREST

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