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Phenolics pattern of cut H3O rose flowers during floral development

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Abstract

An experiment was conducted to evaluate the phenolic acids and flavonoids contents from flower bud to flower senescence stages (7 stages) in cut H3O roses every other day. Some phenolic acids (Gallic, Caffeic, Caftaric, Chlorogenic, Chiconic, Coumaric, Sinapic, and Ferulic) and flavonoids (Catechin, Pelargonidin chloride, Cyanidin, Kaempferol, and Quercetin) were detected in the methanolic extract of rose petals by HPLC. Data analysis revealed that Gallic acid content did not significantly ($P \leq 0.05$) affects from the flower bud to the senescence stages. However, it's found that Caftaric acid, Chlorogenic acid, Coumaric acid, Ferulic acid, Chiconic acid, Quercetin, and Kaempferol (at $P \leq 0.05$), Catechin, Caffeic acid, Sinapic acid, Cyanidin, and Pelargonidin chloride contents (at $P \leq 0.01$) significantly affected during flower opening stages. The highest contents of phenolics during development of cut H3O rose flowers were as follow: day 1 [Coumaric acid], day 3 [Caftaric acid, Ferulic acid, and Kaempferol], day 5 [no compound was found], day 7 [Sinapic acid and Cyanidin], day 9 [Chlorogenic acid], day 11 [Chiconic acid and Quercetin], and day 13 [Caffeic acid, Catechin, and Pelargonidin chloride]. The results showed that flower development stages can be suggested to consider the phenolic compounds required to use in the therapeutic process.

Keywords: Chiconic acid, Cyanidin, HPLC, Pelargonidin, Secondary metabolites

Introduction

Reactive oxygen species (ROSs) such as singlet oxygen, peroxide radical, peroxyl, nitric oxide, and peroxynitrite are important factors affecting normal cellular functioning and thereby, induce cellular damage and cause human diseases (e.g. Cancer, inflammatory disorders, cardiovascular diseases, etc.) (Abdel-Hameed et al., 2012; Oyenihi and Smith, 2018). Some polyphenols, such as flavonoids and phenolic acids, are a group of phytochemicals with potent antioxidant properties virtues, may play a key role in reducing the risk of diseases via preventing oxidative damages (Kaisoon et al., 2011).

Rose (*Rosa hybrida* L.) From Rosaceae family, known as 'the queen of flowers' (Mohy, 2011; Chamani and Wagstaff, 2018) is the cornerstone of commercial floriculture due to its high demand all over the globe. In particular, some researchers reported that rose petals are a source of

polyphenols such as flavonoids, phenolic acids, and tannin with anti-proliferative activity (Daglia, 2012; Rusanov et al., 2014), and have the potential to add to the human diet (dos Santos et al., 2018).

In previous studies, HPLC/MS analysis detected 6 anthocyanins, 4 flavanols, 4 phenolic acids, and 31 flavonols in rose petals (Schmitzer et al., 2019). Elmastaş et al. (2017) reported that the content of phenolic compounds and flavonoids in some rose flowers changed during different times of harvesting. Flower opening happens variously in different plants and is generally controlled via a range of Physio-morphological and biochemical reactions such as cell expansion, hormonal regulation, solute accumulation, and apoplast sugar uptake (van Doorn and van Meeteren, 2003; van Doorn et al., 2013; van Doorn and Kamdee, 2014). These modifications might change the production of primary and secondary metabolites within tissues. Metabolic profiles in tea flowers and leaves were modified during flower development (Jia et al., 2016). In *Rosa damascena*, phenolic contents changed during flower development (Rasouli et al., 2018). Polyphenols contents changed in *Rosa damascena* 'Himroz' and *Rosa bourboniana* (Sood and Nagar, 2003), and *Helleborus niger* (Schmitzer et al., 2013). Oleuropein content, as the most important and abundant phenolic compound in olive trees, changed during flower development (Malik and Bradford, 2006). Further, the content and composition of phenolics were changed during flower development in *Origanum majorana* (Sellami et al., 2009). Pelargonidin, cyanidin, quercetin, catechin, gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, and *p*-coumaric acid was detected in flower extract of rose 'KORcrisett' (Schmitzer et al., 2009). Schmitzer et al. (2010) reported that quercetin and anthocyanin contents increased as flower open developed up to fully open flower in groundcover rose and then reduced in the senescent stage. Modifications in phenolics content and composition during flower development stages, could be due to gene expression changes (Wang et al., 2018),carbohydrates level (Yamada et al., 2007), change in cell membrane stability (Faragher et al., 1987), food remobilization, and total protein (Kumar et al., 2008). Efforts have been made to increase polyphenols quantity in order to use in the human diet (Mrad et al., 2012; Chamorro et al., 2012; Vallverdú-Queralt et al., 2013; Do et al., 2014), therefore selecting the best stage of flowering for polyphenol extraction is very important as a strategy for obtaining the highest polyphenols content. Although the phenolic content was studied in rose 'KORcrisett' (Schmitzer et al., 2009) and groundcover rose (Schmitzer et al., 2010), however, only four developmental stages, including flower bud, partially open flower, full open flower, and flower

senescent were studied and changes in metabolic pattern over a time lapse from bud stage to senescent did not study. Here we have attempted to study the metabolic changes in cut H3O rose flowers with a two-day interval to profile the phenolic content in order to obtain the highest content of pre-specified compound at the correct time, in order to use in medicine, cosmetics, and/or human diet.

Material and methods

Cut H3O rose flowers were obtained from MM Flower Factory in Cambridge, UK (at the stage of petal starting to reflex), transferred to the laboratory (Reading University, UK), and held at 4°C. Afterward, flowers placed in the bucket containing 10 ppm chlorine and transferred to the Phytotrons (The phytotrons condition was 22±2°C, 60% RH, 10 μmol. M^{-2}. S^{-1} irradiance with a 12 h photoperiod) and phenolics pattern was studied during flower open stages. Methanolic extract of cut H3O rose petals were prepared from flower bud to flower senescence stages (7 stages as shown in Figure 1) every other day (day 1:D1, day2: D2,….and day 13:D13) with four replications, including four flowers at each repetition. The prepared extracts were injected into the HPLC and the phenolics pattern of cut H3O rose flowers were determined.

Fig. 1. Cut H3O rose flower development stages from flower bud to senescence with two-day intervals from day 1 to day 13

The standard solution of polyphenols including Gallic acid, Catechin, Kaempferol, Quercetin, Pelargonidin chloride, Cyanidin, Chlorogenic acid, Caffeic acid, Caftaric acid, Chiconic acid,

Coumaric acid, Sinapic acid, and Ferulic acid was prepared (0.25 mg. L^{-1}). Freeze-dried flowers were ground by using fine powder and liquid nitrogen. A sample of 500 mg was extracted with 2 ml methanol containing 3% (v/v) formic acid (HCOOH) and 1% (w/v) 2, 6-di-*tert*-butyl-4-ethylphenol (BHT) in an ultrasonic bath for 1 h. The treated samples were centrifuged for 7 min at 12000 rpm. The chromafil AO-20/25 filter (Macherey-Nagel Düren, Germany) was used to filter the supernatant and transferred to a vial prior to injection into the HPLC. The samples were analyzed using an HPLC system (Agilent 1100, USA) with a diode array detector at 254, 280, and 520 nm according to absorption maxima of analyzing compounds. A Nova pack C18 (250*4.6; 4mm) HPLC column at 25°C was used. The injection volume was regulated as 20 µL and the flow rate was 1 ml. min^{-1}. The elution solvents were aqueous 1% formic acid (A) and acetonitrile (B). The samples were eluted according to the linear gradient described by Marks et al. (2007).

The mobile phase involved 1% aq. Solvent A (Acetic acid) solution and Solvent B (acetonitrile). The gradient elution was modified from 10 % to 40% B for the duration of 28 min, from 40 to 60 % B in 39 min, from 60 to 90 % B in 50 min. The mobile phase composition back to the initial condition (solvent B: solvent A: 10: 90) in 55 min and allowed to run for another 10 min, before the injection of another sample.

Data analysis

Data analyzed by SAS V9.2 software. Mean comparisons done using Duncan's multiple test range at 1 and 5% levels.

Results and discussion

Phenolic acids

Flower development in cut H3O roses was studied during a period of time from flower bud to flower senescence at 7 stages (every other day) and chromatograms were obtained using HPLC (Figure 2). The result showed that Gallic acid content did not significantly ($P \leq 0.05$) affect from the flower bud to the senescence stages. However, the highest content of Gallic acid was produced in D7 (6.85±0.45 a) followed by D9 (5.99±0.3 a: Figure 3A). Caftaric acid content was increased from D1 (15.19±0.54 e) to D7, where the highest content of Caftaric acid was produced (43.88±1.2 a) (Figure 3B).

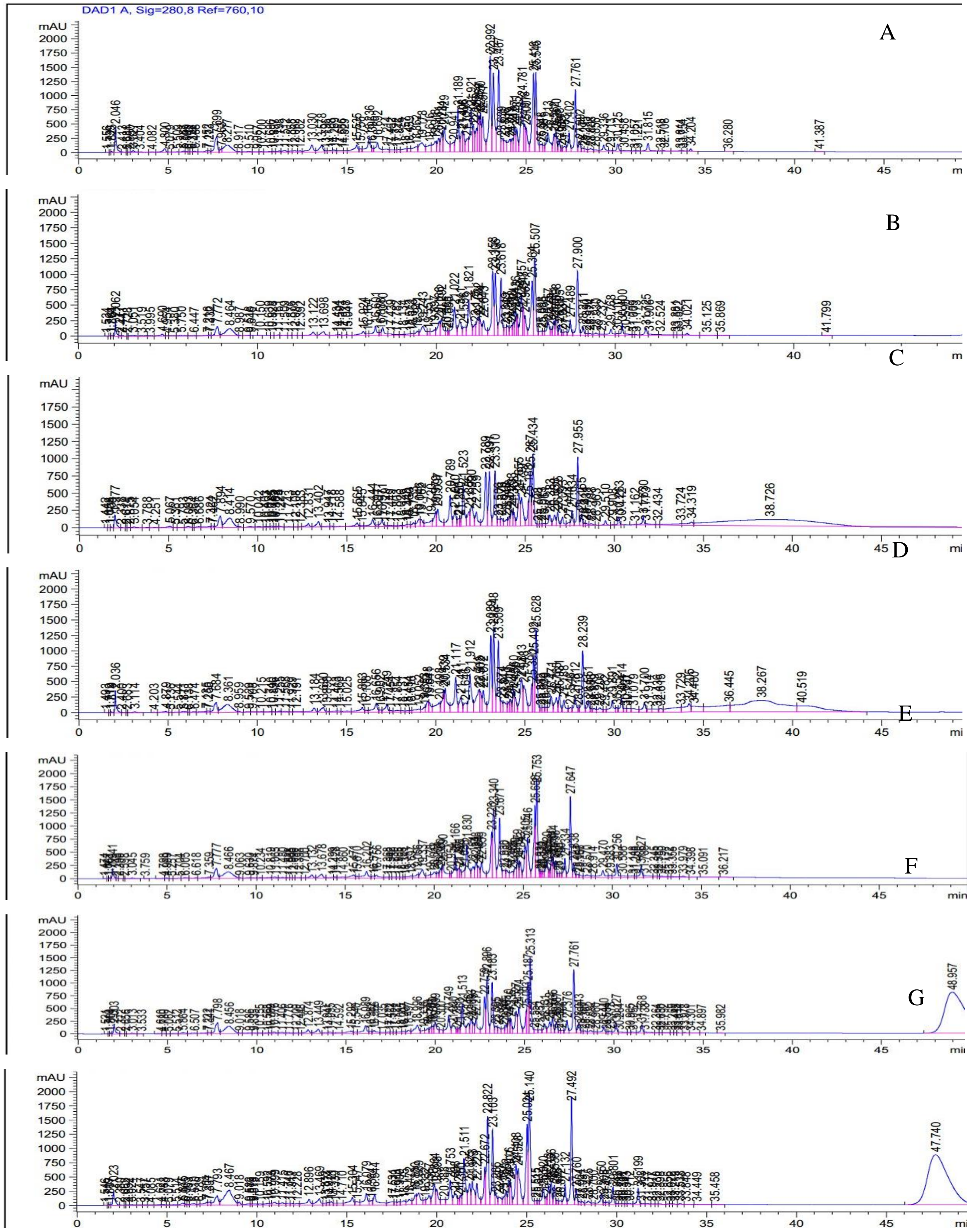

Fig. 2. Chromatography of phenolics in cut H3O rose flowers during flower open development. (A: day 1, B: day 3, C: day 5, D: day 7, E: day 9, F: day 11, and G: day 13)

Caffeic acid content was reduced as flower open developed and reached the lowest content at full open flower stage, then start to increase. However, the highest level of Caffeic acid produced in senescent flowers (Figure 3B). Chlorogenic acid content was increased from D1 to-day D9 and then start to decrease (Figure 3C). The Chiconic acid content was start to reduce as flower open developed and the lowest content produced at D11, then slightly increased during senescence (Figure 3C).

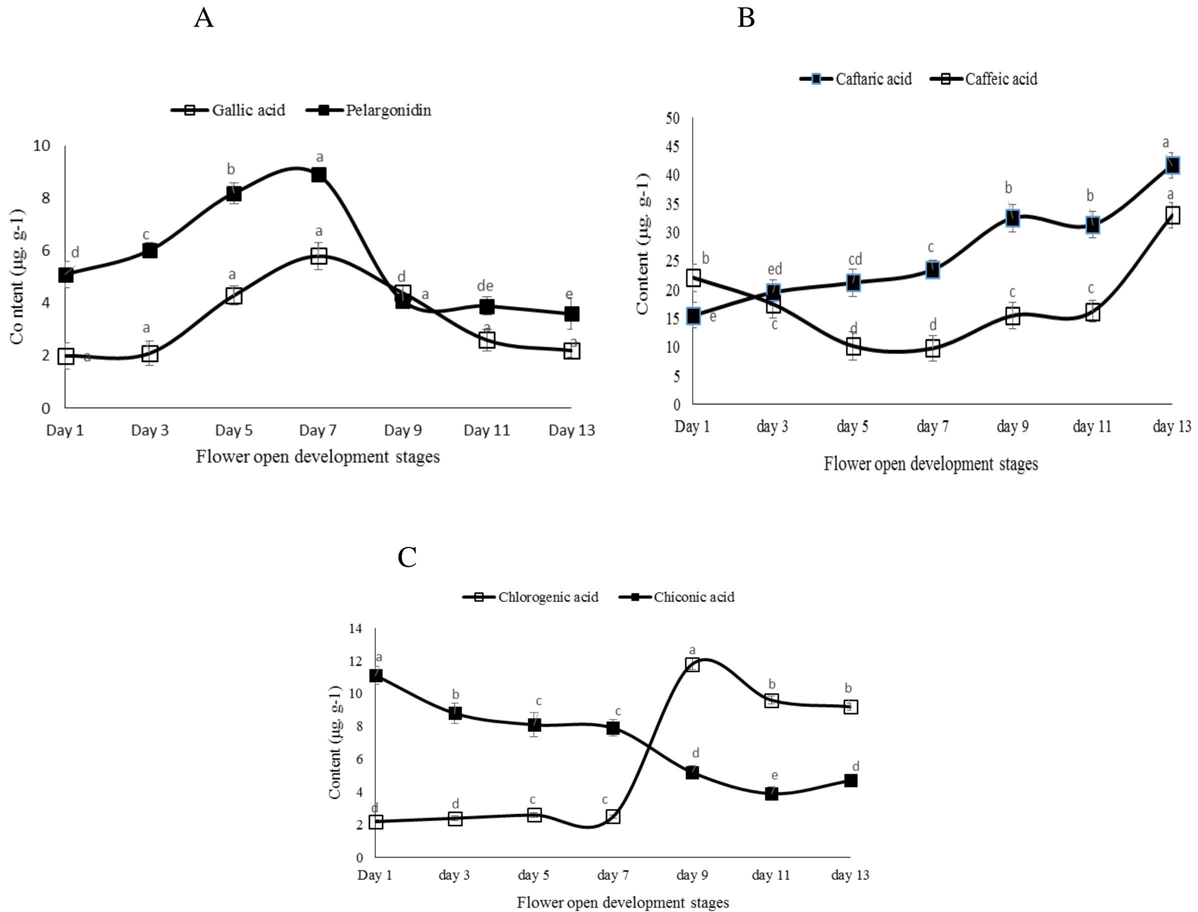

Fig. 3. Some phenolic acids and flavonoids content (A, B, and C) in cut H3O roses during flower open development

Different letters in each row indicate significant differences determined using a Duncan's multiple range test (*$P < 0.01$*). Error bars = SE (n = 4).

Changes in Coumaric acid content showed a sinus curve, where the highest content was produced in flower bud stage (Table 1; 39.9±0.4 a) and then reduced as flower open developed.

Ferulic acid content as a phenolic phytochemical found in plant cell wall was changed from flower bud stage to senescence in cut H3O rose flowers. The highest content of Ferulic acid was produced during D5 stage and in D13 the lowest content was produced (Table 1). Cut H3O rose flowers produced the highest content of Sinapic acid in D13 (148.3±5.4 a) and the lowest content in day 1 (38.0±3.6 e) (Table 1).

Table 1. Phenolics content in cut H3O roses during flower open development

	$\mu g. g^{-1}$				
Flower open stage	Catechin	Cyanidin	Coumaric acid	Ferulic acid	Sinapic acid
Day 1	139.5[††]±3.2 b[†]	45.9±2.1 c	25.3±0.4 a	55.4±4.1 ab	49.6±3.6 b
Day 3	85.8±2.4 b	50.6±3.6 c	7.6±0.1 b	79.6±4.6 a	133.2±4.5 a
Day 5	14.7±0.7 c	111.5±4.7 b	20.1±0.1 a	72.2±3.8 a	45.2±1.8 b
Day 7	134.2±3.1 b	172.1±5.1 a	20.9±0.4 a	27.4±2.1 b	164.9±2.0 a
Day 9	124.3±2.9 b	159.7±4.9 a	18.0±1.3 ab	57.3±1.5 ab	120.3±4.7 a
Day 11	121.6±2.5 b	46.6±2.8 c	16.4±0.8 ab	20.6±1.6 b	157.4±5.1 a
Day 13	250.9±4.1 a	128.1±4.1 b	7.6±0.8 b	59.4±1.8 ab	129.6±5.4 a

[†]Different letters in each row indicate significant differences determined using Duncan's multiple range test ($P < 0.01$)
[††] Mean ±SE, n=4

Flavonoids

Catechin content was start to increase from D1 to D7, where the highest content was produced (171.9±3.1 a), and then was reduced (Table 1). The highest content of Pelargonidin chloride was produced in day 7 (9.7±0.43 a) followed by day 5 (8.92±0.4 b) (Figure 3A).

The highest content of Cyanidin was produced in D11 (177.2±2.8 a), followed by D9 (172.9±4.9 a) and the lowest content was in day 1 (Table 1). The content of Quercetin and kaempferol were low in cut H3O reses during flower development. The highest content of Quercetin was produced in D11 after cutting (41.6±2.1 a; Figure 4). During D5, the highest content of Kaempferol was produced (7.8±1.1 a) and Kaempferol did not detect in cut H3O rose petals extract after day 5 and in flower bud (Figure 4).

Versatile health benefits of phenolic compounds have led the researchers to focus on the study of their products in different plants. Phenolic compounds importance encompasses our life from

health, beauty, and dietary supplements to cosmetic (Yáñez et al., 2013). Recognizing the best stage of plant growth and/or flowering in order to extract the highest quantity of phenolics in plants could be one of the most effective ways to obtain a higher volume of these compounds. In *Ziziphora clinopodioides*, total phenolic contents varied between 9.91-12.80 mg. g^{-1} in different developmental stages (Tian et al., 2011). Further, the UPLC–MS/MS analysis method revealed that the flavonoids content were varied in a different part of *Abelmoschus manihot* (Pan et al., 2017). Jia et al. (2016) reported that the content of flavonoids was changed during flower development in *Camellia sinensis*. The difference in the level of phenolics during flower open stages have been previously reported in olive cultivars (Malik and Bradford, 2006; Taamalli et al., 2013), rose 'KORcrisett' (Schmitzer et al., 2009), groundcover rose (Schmitzer et al., 2010), *Helleborus niger* (Christmas rose) (Schmitzer et al., 2013).

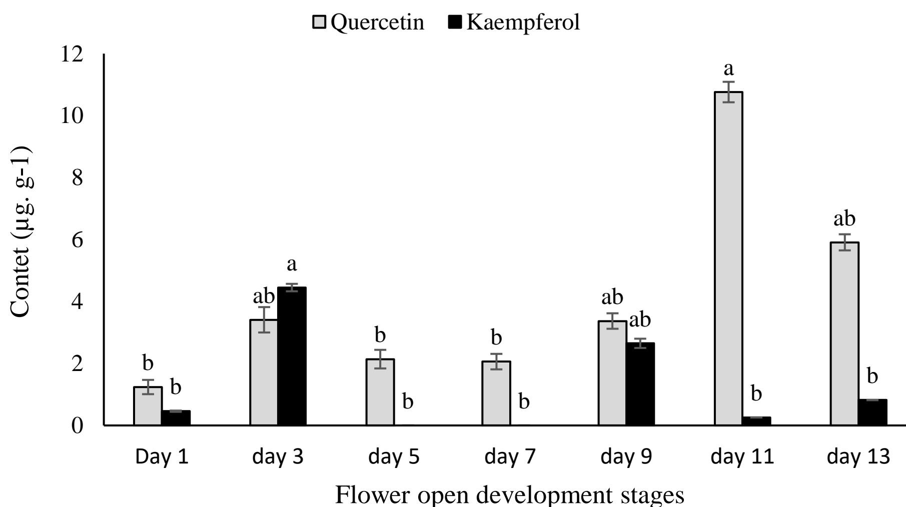

Fig. 4. Quercetin and Kaempferol content in cut H3O roses during flower open development

Different letters indicate significant differences determined using Duncan's multiple test range ($p < 0.05$).

Error bars = SE (n = 4).

Here, phenolic acids content (Gallic, Caffeic, Caftaric, Chlorogenic, Chiconic, Sinapic, Ferulic, and Coumaric) and flavonoids (Catechin, Cyanidin, Pelargonidin chloride, Quercetin, and Kaempferol) were significantly changed during flower open stages. The content of Cyanidin was the highest at full open flower stages (D7, D9, and D11), while the highest content of Pelargonidin

chloride was produced at D7 and then declined. Cyanidin and Pelargonidin were two anthocyanins made up in ‘Jaguar’ rose flowers (Dela et al., 2003) and hybrid tea rose petals (Barnes and Schug, 2011). The flavonoid 3′-hydroxylase enzyme (F3′H) determines the shift from Cyanidin to Pelargonidin synthase in anthocyanin pathway (Tanaka and Brugliera, 2013). Change in F3′H enzyme activity level, may be the reason for petal color change in ‘H3O’ rose flowers during flower open development. All detected phenolics showed the variable patterns during flower open. The content of Quercetin was increased and reached the highest level at D11, before senescing (Table 1) which is consistent with the report of Schmitzer et al. (2010) on groundcover rose.

The differences among phenolic contents in various plants and during different flower development stages could be due to crosslinking among polyphenols and carbohydrates, lipids, and proteins (Kennedy et al., 2000; Jakobek, 2015), changes in petals water content, different enzymes activity during flower open stages, especially phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), and flavonol synthase (FLS), petal sap cell pH, minerals profile, and different genes expression (Schmitzer et al., 2009; Burin et al., 2010; Schmitzer et al., 2013; Jia et al., 2016; Lucini et al., 2016; Kanani and Nazarideljou, 2017), and cell membrane stability (Faragher et al., 1987). Our results indicated that based on phenolic compound quantity during flower opening stages, and required to use in the therapeutic process, food additive and/or beauty and cosmetics, using of flowers at different opening stages can be an effective strategy.

Conclusion

Cut H3O roses produced different phenolic patterns during flower open after cutting with a two-day intervals study. Gallic acid, Caffeic acid, Caftaric acid, Chlorogenic acid, Chiconic acid, Sinapic acid, Ferulic acid, Coumaric acid, Catechin, Cyanidin, Pelargonidin chloride, Quercetin, and Kaempferol were the main phenolic compounds detected in methanolic extract of cut H3O roses. The content of phenolics significantly ($P \leq 0.05$) affected during flower open stages. The highest content of Chiconic acid (10.7±0.4 a) and Coumaric acid (39.9±0.4 a) were produced in bud stage, where Pelargonidin chloride (3.92±0.3 e), Coumaric acid (8.1±0.8 f), and Ferulic acid (16.6±1.8 e) were produced in senescent flowers. Range on physio-chemical parameters as well as genetic background can change during flower open. These changes have affected phenolics content

in cut H3O rose petals. Results indicated that, according to the needed phenolic compound, extraction at specified time of flower open development can be proposed.

Disclosure statement

No potential conflict of interest was reported by the authors.

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