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Flavonols and Carotenoids in Yellow Petals of Rose Cultivar (*Rosa* 'Sun City'): A Possible Rich Source of Bioactive Compounds

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ABSTRACT: Rose flowers have received increasing interest as rich sources of bioactive compounds. The composition of flavonols and carotenoids in yellow petals of *Rosa* 'Sun City' was determined by high-performance liquid chromatography coupled with photodiode array and mass spectrometric detectors (HPLC-PDA-MS). In total, 19 flavonols and 16 carotenoids were identified, some of which were first discovered in rose petals. Significant changes were observed in their profiles during seven blooming stages. Total flavonol contents showed the highest levels at stage 2 (S2; 1152.29 μg/g, FW). Kaempferol 7-*O*-glucoside and kaempferol 3-*O*-rhamnoside were the predominant individual flavonols. Total carotenoid concentration was highest at S4 (142.71 μg/g, FW). Violaxanthins with different geometrical configurations appeared as the major carotenoids across all blooming stages. These results indicated that 'Sun City' petals are rich sources of flavonols and carotenoids. Moreover, it is important to choose the appropriate harvest time on the basis of the targeted compounds.

KEYWORDS: *Rosa, petals, flavonols, carotenoids, HPLC, kaempferol, violaxanthin*

INTRODUCTION

Rosa spp. (family Rosaceae) is one of the most economically significant ornamental crops worldwide. The genus *Rosa* consists of more than 100 wild species as well as more than 30,000 modern rose cultivars.[1] They are highly popular as cut flowers, potted plants and garden ornamental plants. Most of them grow vigorously and can flower continuously under favorable environment. Moreover, rose flowers are extensively used in food service, cosmetics, and pharmaceutics.[2] The physiological functions of rose petals are mainly attributed to their abundance of natural antioxidants, such as flavonols, anthocyanins, and carotenoids.[3] Several reports have showed their potential as a source of food, pharmaceutical, or cosmetic applications.[2,4]

Rose flowers are rich in a great variety of flavonols, possessing several biochemical activities, such as antioxidant,[5] anticancer,[6] and antiinflammatory[7] properties. Therefore, flavonols have been extensively studied in rose species (containing many medicinal roses), such as *R. chinensis*, *R. damascena*, *R. bourboniana*, *R. rugosa*, and *R. gallica*.[8−10] There are two main flavonol aglycones detected in rose petals, namely, kaempferol and quercetin, which are glycosylated at the 3-, 7-, and 4′- positions generally by glucose, rhamnose, arabinose, or glucuronic acid and so on.[8,11] However, meticulous profound research on qualitation and quantitation of flavonols in petals of modern rose cultivars are limited.

Besides flavonols, yellow and orange rose petals also contain large amounts of carotenoids. Carotenoids play essential roles in human/animal nutrition. Animals including human beings cannot synthesize carotenoids de novo, so they have to absorb them from dietary food or supplements. Researches have indicated that carotenoids have the ability to decrease risk of vitamin A deficiency,[12] cancer, and cardiovascular diseases.[12,13] Accordingly, the worldwide demand for carotenoids has been markedly growing, and most people prefer to consume carotenoids extracted from natural sources. So far, there is only one published report of carotenoids in rose petals,[3] consisting mainly of qualitative analysis of carotenoid composition in petals of 40 old and modern rose cultivars. Thus, studies on identification and quantitation of carotenoids in rose petals during the blooming process have been scarce.

Therefore, the objective of this research was to obtain genuine information on the composition of flavonols and carotenoids in yellow petals of *Rosa* 'Sun City' during seven blooming stages by HPLC-PDA-MS analysis. Results of this study would provide new insights into the application potential of yellow rose petals in food service, cosmetics, and pharmaceutics.

MATERIALS AND METHODS

Reagents and Chemicals. Chromatographic grade methyl *tert*-butyl ether (MTBE), methanol, formic acid, ether, and hexane were supplied by Fisher Scientific (Fair Lawn, NJ). Chromatographic grade butylhydroxytoluene (BHT) and trifluoroacetic acid (TFA) were

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Figure 1. Flower phenotypes of modern rose cultivar *Rosa* 'Sun City' at seven blooming stages (S1, unopened bud stage; S2, semiopened stage; S3, fully opened stage; S4, initial bloom stage; S5, full bloom stage; S6, bloomed stage; and S7, senescent stage).

obtained from Sigma-Aldrich (St. Louis, MO). Analytical grade sodium chloride (NaCl) and potassium hydroxide (KOH) were acquired from Beijing Chemical Reagent Co. (Beijing, China). Flavonol standards and (all-*E*)-*β*-carotene were provided by Sigma-Aldrich (St. Louis, MO). Carotenoid standards such as (all-*E*)-zeaxanthin, (all-*E*)-antheraxanthin, and (all-*E*)-violaxanthin were supplied by CaroteNature (Ostermundigen, Switzerland).

Plant Materials and Sample Collection. *Rosa* 'Sun City' plants were grown in experimental greenhouses at Xiaotangshan, which is affiliated to Beijing Forestry University, Beijing, China. Rose flowers from seven development stages were harvested in June 2015. Rose flower development stages were classified by Wang et al.[14] with slight modifications. The characteristics of the seven blooming stages of *Rosa* 'Sun City' are shown in Figure 1. Fresh petals were placed in liquid nitrogen immediately. Then, all samples were preserved at −80 °C until the extraction of flavonols and carotenoids.

Extraction and HPLC Analysis of Flavonols. Samples of 0.2 g (fresh weight, FW) were fully ground in liquid nitrogen and then extracted in 1.8 mL solvents (methanol/water/formic acid/TFA, 70:27:2:1, v/v/v/v) in an ultrasonicator bath for 30 min.[15] The extracts were centrifuged (13 000 rpm, 10 min), and the supernatant was filtered (0.22 μm) into vials. The HPLC analyses were carried out on a Waters 2695 HPLC system connected with a 996 photodiode array detector (Waters, Milford, MA), which was set in the range of 200−600 nm. Data collection and processing was accomplished using Waters Empower software (version 3.0). The column used was a 150 mm × 4.6 mm i.d., 2.5 μm, XBridge BEH C18 (Waters, Milford, MA). As described by Liu et al.,[15] the mobile phases comprised 0.5% aqueous formic acid (A) and acetonitrile (B). The gradients were programmed as follows: 0 min, 5% B; 5 min, 10% B; 30 min, 19% B; 50 min, 40% B; and 50.01−60 min, 5% B. The column temperature, injection volume, and flow rate were set at 25 °C, 10 μL, and 0.5 mL/min, respectively. The chromatograms were extracted at 350 nm for flavonols. All samples were extracted in triplicate.

Extraction and HPLC Analysis of Carotenoids. Samples of 0.2−0.3 g (FW) were ground in liquid nitrogen and then extracted with 4 mL of methanol by shaking for 20−30 min (150 rpm, 23 °C, darkness) in a constant temperature incubator shaker. Then 4 mL hexane was added, and the mixture was shaken for 20−30 min under the same conditions. Next, 2 mL aqueous solution of NaCl (10%, w/v) was added. The mixture was briefly vortexed and left undisturbed for 5−10 min. Then the upper organic layer was collected and the aqueous phase was extracted again with 2 mL of hexane/ether (3:1, v/v) until it was colorless. The upper layer from the second extraction was collected and pooled with the organic solutions collected from the first extraction. The combined organic solutions were dried under nitrogen stream at room temperature by a sample concentrator.

The dry residues were saponified with 1 mL of methanol containing 6% KOH (w/v) for about 12 h (150 rpm, 23 °C, darkness) in an incubator shaker. Then, the glass tubes were rapidly transferred to an ice bath, and 1 mL of NaCl solution (10%, w/v) followed by 1 mL of hexane/ether (3:1, v/v) was added to each tube. After extraction for 1 h under the same conditions, the upper organic solutions were gathered and the aqueous phase was re-extracted with hexane/ether (3:1, v/v). The mixed organic solutions were finally evaporated to dryness through a vacuum concentrator and stored at −80 °C. The dried carotenoids were dissolved in 1 mL of MTBE/methanol (1:1, v/v) immediately prior to HPLC analysis.

The HPLC method for the analysis of carotenoids was as described by Wibowo et al.[16] with minor modification. The column used was a 250 mm × 4.6 mm i.d., 5 μm, C30 (YMC, Tokyo, Japan), which was set at 25 °C. The mobile phases comprised methanol (A), MTBE (B) and ultrapure water (C) with the following gradient: 0−2 min, 95% A + 5% C; 10 min, 95% A + 3% B + 2% C; 21 min, 95% A + 5% B; 27 min, 90% A + 10% B; 37 min, 70% A + 30% B; 40 min, 50% A + 50% B; 40.01−50 min, 95% A + 5% C. The injection volume and flow rate were set at 20 μL and 1 mL/min, respectively. The chromatograms were extracted at 450 nm for carotenoids. To avoid oxidation and isomerization of carotenoids, all operations were made by organic solutions containing 0.1% BHT (w/v) under temperature not higher than 23 °C and dim light. All samples were extracted in triplicate.

LC−MS Analysis of Flavonols and Carotenoids. LC−MS analysis was performed on the same HPLC as above, interfaced with a micrOTOF Q quadrupole time-of-flight mass spectrometer (Bruker Daltonics, Bremen Germany) connected to either an electrospray ionization (ESI) or an atmospheric pressure chemical ionization source (APCI). The HPLC analysis conditions were the same as those described above. The mass signal range was *m*/*z* 50−1100. The ionization of flavonols was achieved with an ESI source in both positive and negative mode, and the parameters were set as followed: capillary voltage, 3500 V; end plate offset, 500 V; drying gas (nitrogen) flow, 8.0 L/min; drying gas temperature, 180 °C; collision rf, 200 Vpp; nebulizer pressure, 0.8 bar; prepulse storage, 8.0 μs; transfer time, 80.0 μs; and collision energy, 10.0 eV. For the carotenoid analysis, the ionization was performed by an APCI source in the positive mode. The parameters were set as followed: capillary voltage, 3000 V; end plate offset, 500 V; drying gas (nitrogen) flow, 8.0 L/min; drying gas temperature, 350 °C; collision rf, 300 Vpp; nebulizer pressure, 4.0 bar; prepulse storage, 8.0 μs; transfer time, 80.0 μs; and collision energy, 8.0 eV. The micrOTOF-Q control program (version 2.3) and Bruker Data Analysis package (version 4) was used to operate the equipment and analyzing the data, respectively.

Quantitation of Flavonols and Carotenoids. The quantitation analysis was conducted by external calibration of the corresponding standards from the areas of the chromatographic peaks detected by PDA at 350 nm for flavonols and 450 nm for carotenoids. All flavonol and carotenoid standards were dissolved in methanol and MTBE/methanol (1:1, v/v, containing 0.1% BHT), respectively, finally generating working standard solutions in the range of 0.05−500 and 0.05−100 μg/mL, respectively. Each standard solution was analyzed in triplicate. The following equations were obtained: quercetin 3-*O*-glucoside ($y = 67576x - 8477.3$, $R^2 = 0.9999$); kaempferol 3-*O*-rutinoside ($y = 37032x + 8438.8$, $R^2 = 0.9999$); kaempferol 3-*O*-glucoside ($y = 58747x - 8293$, $R^2 = 0.9999$); kaempferol ($y = 123185x - 19563$, $R^2 = 0.9999$); (all-*E*)-violaxanthin ($y = 77379x + 5724.3$, $R^2 = 0.9999$), and (all-*E*)-antheraxanthin ($y = 119525x + 11938$, $R^2 = 0.9998$). The quantitation of the cis isomers of carotenoids was conducted using the standard curve of the all-E counterparts. The content of compounds, which were unidentified or did not have corresponding standards, were calculated from the most suitable standard calibration curve.

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R_1 = rutinoside, R_2 = H: kaempferol 3-*O*-rutinoside, **5**
R_1 = glucoside, R_2 = H: kaempferol 3-*O*-glucoside, **6**
R_1 = glucuronide, R_2 = H: kaempferol 3-*O*-glucuronide, **7**
R_1 = xyloside, R_2 = H: kaempferol 3-*O*-xyloside, **10**
R_1 = H, R_2= glucoside: kaempferol 7-*O*-glucoside, **11**
R_1 = arabinoside, R_2 = H: kaempferol 3-*O*-arabinoside, **12**
R_1 = rhamnoside, R_2 = H: kaempferol 3-*O*-rhamnoside, **14**
R_1 = H, R_2= H: kaempferol, **20**

Figure 2. Representative structures of flavonols extracted from yellow petals of *Rosa* 'Sun City'.

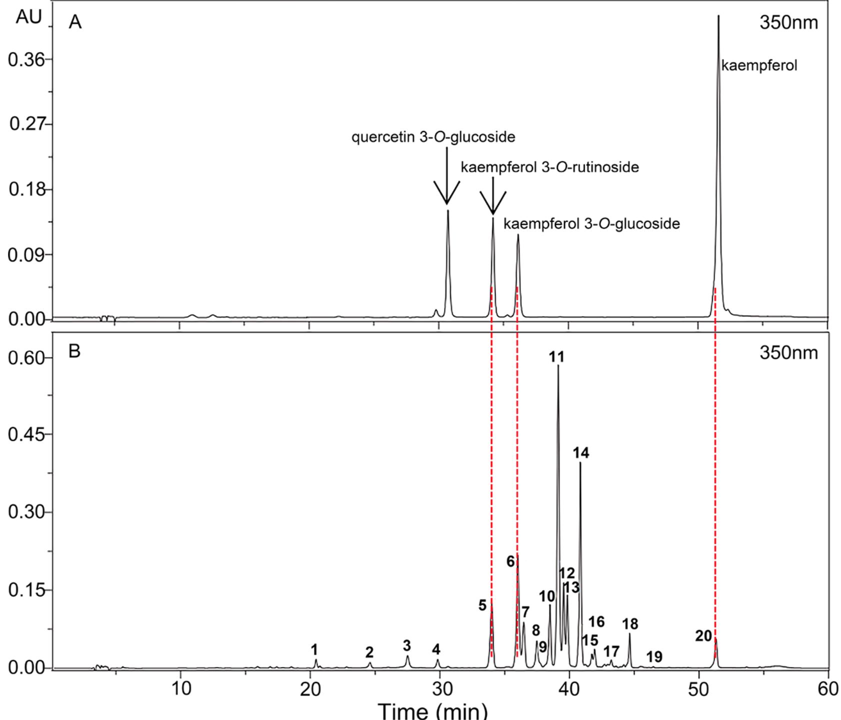

Figure 3. HPLC chromatogram of (A) a mix of standard flavonols and (B) flavonoids extracted from yellow petals of *Rosa* 'Sun City' at S3 (detection at 350 nm). Peak numbers were as shown in Table 1.

RESULTS AND DISCUSSION

Identification of Flavonols. The identification of flavonols was based on the retention times, UV−vis absorption spectra (λ_{max}), MS data, and information reported in previous studies. Twenty flavonoids were thus successfully identified, and the representative structures are presented in Figure 2. The majority of these compounds were kaempferol and quercetin glycosides, which have been widely described in the genus *Rosa*.[8−11] The HPLC (350 nm) profiles of the identified flavonols are presented in Figure 3, and the chromatographic, spectroscopic and mass spectrometric features of the identified flavonols are shown in Table 1.

Among the 20 flavonoids, compounds **5**, **6**, and **20** were characterized as kaempferol 3-*O*-rutinoside, kaempferol 3-*O*-glucoside, and kaempferol, respectively, by the coelution with their corresponding standards, and their MS data further confirmed these results (Table 1). These compounds have been widely reported in flowers of rose species and cultivars.[8−11,17] Except for these three compounds, 13 kaempferol glycosides (peaks **1**, **7**, **8**, and **10**−**19**) were identified by the fragments at *m*/*z* 285 ($[Y0]^-$) in negative mode and *m*/*z* 287 ($[Y0]^+$) in positive mode. Similarly, three quercetin glycosides (peaks **2**, **3**, and **9**) were identified according to ions of aglycone (*m*/*z* 301/303). The remaining flavonoid (peak **4**), whose mass spectra was not detected, was tentatively assigned to a flavan-3-ol on the basis of its λ_{max} at 266.6 nm.[18]

Previous studies have found that the UV−vis spectra of kaempferol in methanol exhibited λ_{max} at approximately 266 (band II) and 367 (band I) nm, and the. λ_{max} values of quercetin were approximately 255 and 370 nm.[19] Furthermore, Singh et al.[20] demonstrated that the glycosylation of the 3-hydroxyls in both kaempferol and quercetin could result in band I λ_{max} hypsochromic shifts of about 12−17 nm, whereas glycosylation of the 7-hydroxyls would not change λ_{max} either in band I or II.

Based on these principles, the compounds (**1**, **7**, **8**, **10**, **12**−**16**, and **18**) were characterized as kaempferol 3-*O*-glycosides due to their λ_{max} values at 263−267 nm (band II) and 341−348 nm (band I). On the other hand, peaks **11** and **17** were characterized as kaempferol 7-*O*-glycosides because of their λ_{max} values at approximately 266 and 361 nm. Similarly, peaks **3** and **9** were assigned to quercetin 7-*O*-glycosides according to their UV−vis absorption spectra.

During mass analysis in the positive mode, Shahat et al.[21] found that the flavonol 3,7-di-*O*-glycosides were more readily fragmented at position 3. In the present study, peak **1** generated fragments at *m*/*z* 595 ($[M + H]^+$), *m*/*z* 449 (loss of rhamnosyl at 3-position, 146 Da), and *m*/*z* 287 (successive loss of glucosyl at the 7-position). Therefore, peak **1** was tentatively identified

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Table 1. Chromatographic, Spectroscopic, and Mass Spectrometric Features of Flavonoids Detected in Yellow Petals of *Rosa* 'Sun City'

				negative ion mode		positive mode		
peak	identification	Rt (min)	λ_{max} (nm)[a]	$[M-H]^-$	MS/MS (m/z)	$[M+H]^+$	MS/MS (m/z)	refs
1	kaempferol 3-*O*-rhamnoside-7-*O*-glucoside	20.45	263.0, 341.5	593.1424	478.9144	595.1369	449.1019, 287.0479	9
2	quercetin 3-*O*-glycoside	24.61	252.4, 359.9	nd	nd	nd	nd	19,20
3	quercetin 7-*O*-glucoside	27.51	252.4, 365.1	463.0237	301.0243	465.0798	303.0169	11
4	flavan-3-ol derivative	29.84	266.6	nd	nd	nd	nd	18
5	kaempferol 3-*O*-rutinoside	34.01	264.2, 347.5	593.1426	447.0618, 285.0714	595.1736	449.1134, 287.0553	standard
6	kaempferol 3-*O*-glucoside	36.02	264.2, 346.3	447.0851	285.0328	449.1206	287.0553	standard
7	kaempferol 3-*O*-glucuronide	36.48	264.2, 346.3	461.0627	285.0324	463.0936,	287.0561	22
8	kaempferol 3-*O*-(galloyl)-glucoside	37.49	266.6, 352.3	599.0923	447.0728, 285.0325	601.0135	449.1017, 287.0571	8
9	quercetin 7-*O*-rhamnoside	37.89	252.4, 362.9	447.0846	301.0247	449.1119	303.0165	20
10	kaempferol 3-*O*-xyloside	38.50	265.4, 347.5	417.0743	285.0147	419.0567	287.0571	23
11	kaempferol 7-*O*-glucoside	39.14	265.4, 318.8, 360.9	447.0845	285.0374	449.1114	287.0548	20
12	kaempferol 3-*O*-arabinoside	39.56	263.0, 342.7	417.0734	285.0547	419.1072	287.0547	23
13	kaempferol 3-*O*-hexoside	39.85	264.2, 346.3	447.0847	285.0376	449.1116	287.0549	9
14	kaempferol 3-*O*-rhamnoside	40.84	263.0, 342.7	431.0894	285.0284	433.1109	287.0546	8,10
15	kaempferol 3-*O*-glycoside 1	41.73	265.4, 347.5	nd	nd	nd	nd	19,20
16	kaempferol 3-*O*-glycoside 2	41.95	264.2, 347.5	nd	nd	nd	nd	19,20
17	kaempferol 7-*O*-(galloyl)-glucoside	43.23	266.6, 362.9	599.0925	447.0728, 285.0325	nd	287.0587	8
18	kaempferol 3-*O*-glycoside 3	44.65	263.0, 342.7	nd	nd	nd	nd	20
19	kaempferol 3-(p-coumaroyl)-glucoside	46.48	266.6, 315.2	593.1059	447.0352, 285.0678	595.0969	449.1015, 287.0382	18
20	kaempferol	51.31	265.4, 363.9	285.0324	nd	287.0562	nd	standard

[a] λ_{max} (nm) in the mobile phase (acidified water−acetonitrile), detected by photodiode array detector.

to be kaempferol 3-*O*-rhamnoside-7-*O*-glucoside, which has been described in flowers of *R. damascena*, *R. bourboniana*, and *R. brunonii*.[9]

Peak **7** was presumed to be kaempferol 3-*O*-glucuronide because it has fragments at m/z 461 ($[M-H]^-$) and 285 ($[M-H-176]^-$, loss of glucuronic acid at the 3-position). Kaempferol 3-*O*-glucuronide has been published in leaves of *Rosa* species.[22] Peaks **10** and **12** had the same fragments at m/z 417 ($[M-H]^-$) and 285 ($[M-H-132]^-$). Considering their elution order, peaks **10** and **12** were tentatively postulated to be kaempferol 3-*O*-xyloside and kaempferol 3-*O*-arabinoside, respectively, which have been published previously in rose petals.[23] Peak **13** shared the same deprotonated molecule with kaempferol 3-*O*-glucoside. This compound was suggested as kaempferol 3-*O*-hexoside, which has been reported in petals of *Rosa* species.[9] Peak **14** was tentatively postulated to be kaempferol 3-*O*-rhamnoside based on the MS data, UV−vis spectrum, and the literatures.[8,10] Owing to a lack of MS data, peaks **15**, **16**, and **18** were tentatively characterized as kaempferol 3-*O*-glycosides according to their λ_{max}.[19,20] Similarly, peak **2** was tentatively assigned to be quercetin 3-*O*-glycoside.[19,20]

Peak **11** shared the same MS fragments with kaempferol 3-*O*-glucoside. Considering its characteristic absorption peaks at 265.4 and 360.9 nm, peak **11** was tentatively postulated to be kaempferol 7-*O*-glucoside. In the same manner, peak **3** was attributed to be quercetin 7-*O*-glucoside. These two compounds have been detected in 94% and 90%, respectively, of the surveyed members of the subgenus *Rosa*.[11] Peak **9** generated deprotonated fragments at m/z 447 and 301, corresponding to the loss of rhamnosyl. It was tentatively postulated to be quercetin 7-*O*-rhamnoside, which was shown to have hepatopreventative effects.[24] Quercetin 7-*O*-rhamnoside has not been found in *Rosa* species and cultivars.

The flavonol glycosides acylated with p-coumaroyl can be easily recognized from the λ_{max} at 310−312 nm and an increase of 146 Da in molecular weight. As an example, peak **19** had mass fragments at m/z 593 ($[M-H]^-$), 447 (loss of p-coumaroyl, 146 Da), and 285 (sequential loss of glucosyl at 3-position, 162 Da). Furthermore, this compound exhibited λ_{max} at 266.6 and 315.2 nm, being in agreement with previous reports.[18] Therefore, peak **19** was tentatively suggested to be kaempferol 3-(p-coumaroyl)-glucoside, which was found for the first time in *Rosa* species and cultivars.

Peaks **8** and **17** displayed the same molecular masses at m/z 599 ($[M-H]^-$), 447 ($[M-H-152]^-$), and 285 ($[M-H-314]^-$), corresponding to the loss of gallic acid (152 Da) and glucosyl. They were easily distinguished by the UV−vis spectra. Peak **8** exhibited λ_{max} at 266.6 and 352.3 nm, indicating glycosylation of the 3-hydroxyls. Peak **8** was tentatively proposed to be kaempferol 3-*O*-(galloyl)-glucoside, which has been previously described in petals of *Rosa* species.[8] However, peak **17** had its λ_{max} at 266.6 and 362.9 nm, suggesting it was glycosylated at the 7-position. Therefore, peak **17** was proposed as kaempferol 7-*O*-(galloyl)-glucoside.

In summary, 19 flavonols were successfully identified, including 13 kaempferol glycosides, 3 quercetin glycosides, and 3 acylated kaempferol glycosides. Of these 19 compounds, quercetin 7-*O*-rhamnoside, kaempferol 7-*O*-(galloyl)-glucoside, and kaempferol 3-(p-coumaroyl)-glucoside were discovered for the first time in the genus *Rosa*.

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Table 2. Content of Flavonols in Yellow Petals of *Rosa* 'Sun City' at Seven Blooming Stages (S1−S7)

		content (μg/g FW)[a]						
peak	identification	S1	S2	S3	S4	S5	S6	S7
1	kaempferol 3-*O*-rhamnoside-7-*O*-glucoside	37.08 ± 0.72 a	35.51 ± 0.63 ab	19.43 ± 0.42 d	31.72 ± 2.72 b	24.98 ± 4.08 c	15.45 ± 2.26 e	19.68 ± 0.47 d
2	quercetin 3-*O*-glycoside	4.62 ± 0.9 abc	6.48 ± 2.54 a	2.92 ± 0.64 bc	1.58 ± 0.38 c	2.04 ± 0.16 c	3.33 ± 1.32 bc	5.34 ± 2.96 ab
3	quercetin 7-*O*-glucoside	6.57 ± 3.75 a	6.95 ± 2.54 a	1.24 ± 0.79 b	nd[b]	0.45 ± 0.06 b	1.06 ± 0.19 b	nd
5	kaempferol 3-*O*-rutinoside	67.5 ± 2.64 b	70.85 ± 2.25 b	95.73 ± 3.03 a	92.06 ± 7.94 a	37.88 ± 1.36 c	37.44 ± 1.45 c	38.46 ± 1.06 c
6	kaempferol 3-*O*-glucoside	82.82 ± 2.53 b	80.76 ± 2.27 b	95.05 ± 2.4 a	48.43 ± 4.16 c	31.88 ± 1.45 e	39.62 ± 1.59 d	23.03 ± 2.29 f
7	kaempferol 3-*O*-glucuronide	33.72 ± 1.06 c	57.88 ± 1.93 b	57.72 ± 2.62 b	87.86 ± 7.64 a	8.57 ± 0.29 d	28.66 ± 0.37 c	11 ± 1.07 d
8	kaempferol 3-*O*-(galloyl)-glucoside	32.08 ± 1.1 c	42.74 ± 1.25 a	33.07 ± 0.93 bc	35.34 ± 3.04 b	22.58 ± 0.79 d	21.24 ± 1.14 d	16.38 ± 0.56 e
9	quercetin 7-*O*-rhamnoside	4.24 ± 0.13 c	2.86 ± 0.24 d	5.01 ± 0.22 c	6.54 ± 0.67 b	2.49 ± 0.18 d	3.07 ± 0.15 d	11.63 ± 0.82 a
10	kaempferol 3-*O*-xyloside	37.99 ± 0.24 e	67.35 ± 1.04 a	58.28 ± 0.62 bc	58.89 ± 5.06 bc	59.31 ± 1.11 b	55.15 ± 1.82 c	43.27 ± 0.23 d
11	kaempferol 7-*O*-glucoside	224.03 ± 4.82 d	348.93 ± 2.78 b	397.75 ± 8.75 a	252.88 ± 22.34 c	226.04 ± 4.26 d	213.94 ± 3.44 d	262.37 ± 7.57 c
12	kaempferol 3-*O*-arabinoside	nd	nd	18.51 ± 1.42 d	39.41 ± 3.47 b	69.04 ± 1.22 a	31.85 ± 1.47 c	19.25 ± 0.32 d
13	kaempferol 3-*O*-hexoside	33.39 ± 0.75 c	79.75 ± 1.52 a	76.18 ± 1.43 a	76.07 ± 6.77 a	69.29 ± 1.8 b	64.27 ± 2.33 b	31.26 ± 2.47 c
14	kaempferol 3-*O*-rhamnoside	135.67 ± 1.35 e	320.68 ± 1.93 a	176.18 ± 3.47 d	196.35 ± 17.35 c	276.81 ± 6.08 b	271.77 ± 6.09 b	193.67 ± 3.27 c
15	kaempferol 3-*O*-glycoside 1	4.56 ± 0.23 e	14.25 ± 0.87 c	8.31 ± 0.06 d	14.9 ± 1.49 c	23.96 ± 1.35 a	20.1 ± 1.65 b	14.56 ± 0.83 c
16	kaempferol 3-*O*-glycoside 2	nd	4.69 ± 2.04 b	0.66 ± 0.05 c	7.13 ± 0.63 a	nd	nd	nd
17	kaempferol 7-*O*-(galloyl)-glucoside	2.54 ± 0.68 de	nd	6.87 ± 0.81 c	2.74 ± 0.24 de	28.89 ± 3.21 a	14.88 ± 2.63 b	3.61 ± 0.37 d
18	kaempferol 3-*O*-glycoside 3	2.75 ± 0.05 e	11.16 ± 2.78 d	19.68 ± 1.12 b	24.74 ± 2.18 a	16.61 ± 0.11 c	9.53 ± 0.07 d	8.61 ± 0.33 d
19	kaempferol 3-(p-coumaroyl)-glucoside	nd	nd	1.12 ± 0.08 a	nd	0.92 ± 0.11 b	1.21 ± 0.11 a	nd
20	kaempferol	0.58 ± 0.11 b	1.43 ± 0.31 a	1.14 ± 0.29 a	1.37 ± 0.13 a	1.32 ± 0.44 a	1.16 ± 0.11 a	1.3 ± 0.02 a
total quercetins		15.43 ± 4.59 a	16.3 ± 4.01 a	9.17 ± 1.05 b	8.12 ± 1 b	4.98 ± 0.4 b	7.46 ± 1.65 b	16.97 ± 3.55 a
total kaempferols		694.7 ± 14.13 f	1135.99 ± 17.58 a	1065.68 ± 25.21 b	969.9 ± 84.79 c	898.07 ± 17.18 d	826.27 ± 20.66 e	686.44 ± 7.4 f
total flavonols		710.13 ± 17.69 f	1152.29 ± 16.18 a	1074.85 ± 25.98 b	978.02 ± 85.34 c	903.05 ± 16.99 d	833.74 ± 19.04 e	703.41 ± 7.84 f

[a]Data are expressed as mean ± SD (n = 3). Different letters represent significant ($P < 0.05$) differences. [b]nd is not detected.

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Quantitation of Flavonols. Quantitation of the flavonols in yellow petals of *Rosa* 'Sun City' at seven blooming stages is shown in Table 2. There are significant differences ($P < 0.05$) in the content of flavonols in rose petals at different development stages. The total flavonols content was obtained through the sum of the concentrations of the 19 identified flavonols. During the flowering periods from S1 to S7, the total flavonols content increased first and then decreased. The content reached the highest level at S2 (1152.29 μg/g, FW), followed by S3 (1074.85 μg/g, FW) and S4 (978.02 μg/g, FW), and showed the lowest level at S7 (703.41 μg/g, FW). From S2 to S7, the content of total flavonols exhibited a continuous decreasing trend, showing a 2-fold decrease. A hypothesis explaining this fact is the rapid expansion of petals, the slowing down of flavonol synthesis, and the acceleration of flavonol degradation.

Many preclinical researches have indicated that kaempferol glycosides have a wealth of pharmacological activities, such as antioxidant, cardioprotective, anticancer, etc.[25−27] At all blooming stages, the main flavonols were kaempferol glycosides in rose petals accounting for 97−99% of the total flavonols content. This behavior is in accord with the discovering of Biolley et al.,[28] who found that 50 cultivars of *R.* × *hybrida* almost exclusively accumulated kaempferol glycosides. The change in the total kaempferol content is similar to that of total flavonol content, with the highest level at S2 (1135.99 μg/g, FW) and the lowest level at S7 (686.44 μg/g, FW).

Among the kaempferol glycosides, kaempferol 7-*O*-glucoside and kaempferol 3-*O*-rhamnoside were the predominant components in petals at all surveyed stages, and their highest levels were found in petals at S3 and S2, with 397.75 and 320.68 μg/g (FW), respectively. A remarkable feature of these samples analyzed here is their very high content of kaempferol 7-*O*-glucoside and kaempferol 3-*O*-rhamnoside, as compared to previous studies.[9,29] Schieber et al.[29] found that kaempferol 3-*O*-glucoside was by far the predominant flavonol in petals of *R. damascena* Mill, and Kumar et al.[9] subsequently found that the most abundant flavonols in petals of *R. boubouniana* and *R. brunonii* were quercetin 3-*O*-rhamnoside and quercetin 3-*O*-hexoside, respectively. Kaempferol 7-*O*-glucoside was demonstrated to have antiviral activity, including anti-HSV and anti-HIV-1.[26,27] These results demonstrate that yellow petals of 'Sun City' could be considered as potential candidates for developing anti-AIDS drugs.

Except for kaempferol 7-*O*-glucoside and kaempferol 3-*O*-rhamnoside, the concentration of kaempferol 3-*O*-rutinoside, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-glucuronide, kaempferol 3-*O*-xyloside, kaempferol 3-*O*-arabinoside, and kaempferol 3-*O*-hexoside was considerable, showing the highest levels at S3 (95.73 μg/g, FW), S3 (95.05 μg/g, FW), S4 (87.86 μg/g, FW), S5 (59.31 μg/g, FW), S5 (69.54 μg/g, FW), and S2 (79.75 μg/g, FW), respectively. The pattern of variation in content of individual flavonol during the blooming process were not the same. Therefore, it is important to choose appropriate harvest time on the basis of the targeted bioactive compounds.

Identification of Carotenoids. Until now, only one study has analyzed the carotenoids from petals of the yellow rose species and cultivars.[3] In that study, the crude extracts of carotenoids required to be purified by classical adsorption chromatography before HPLC and/or high-field NMR analysis. However, in this experiment, the crude extracts of carotenoids from yellow rose petals were directly separated and detected by HPLC-PDA-MS.

Carotenoids were identified by comparing elution orders, UV−vis spectrum and MS data with commercial standards and previously reported data. Besides λ_{max}, the ratio of peak heights (% III/II) was calculated.[30] The geometrical isomers were identified mainly by their spectroscopic features, such as the presence of a cis peak, the reduction in absorbance intensity, a minor hypsochromic shift in λ_{max}, and the % AB/AII value.[30−33] Furthermore, Melendez-Martinez et al.[34] found that the % AB/AII value increased with the cis-double bond closer to the center of the carotenoid. In this study, there were altogether 17 carotenoids detected in yellow petals of *Rosa* 'Sun City' by HPLC-PDA-MS analysis (Figures 4 and 5). The corresponding chemical information is listed in Table 3.

Figure 4. Representative structures of carotenoids extracted from yellow petals of *Rosa* 'Sun City'.

Among the 17 carotenoids, peaks **3**, **8**, **10**, **11**, and **16** were identified as (all-*E*)-violaxanthin, (all-*E*)-antheraxanthin, (all-*E*)-lutein, (all-*E*)-zeaxanthin, and (all-*E*)-β-carotene, respectively, by the coelution with their corresponding standards, and their λ_{max} and MS data further confirmed these results.

Peaks **1**, **2**, and **7** yielded the same mass fragments to that of peak **3**, but their UV−vis spectrum showed a slight hypsochromic shift and displayed a new band at about 327 nm. Among them, peaks **1** and **7** were identified as (13*Z*)-violaxanthin and (9*Z*)-violaxanthin, respectively, according to % AB/AII values (45.80 and 12.50, respectively) and the hypsochromic shift in λ_{max} (8 and 5 nm, respectively), which was in agreement with previous investigations.[31,35] Unlike peaks **1** and **7**, it is even harder to identify peak **2**, because its λ_{max} and % AB/AII value indicated that there were seemingly

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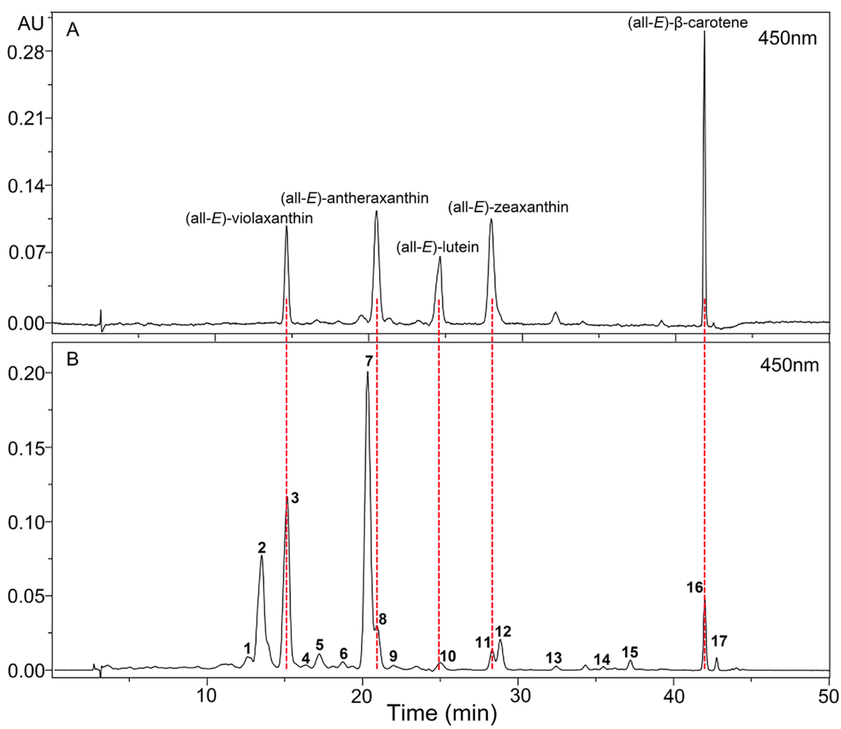

Figure 5. HPLC chromatogram of (A) a mix of standard carotenoids and (B) carotenoids extracted from yellow petals of *Rosa* 'Sun City' at S4 (detection at 450 nm). Peak numbers were as shown in Table 3.

Table 3. Chromatographic, Spectroscopic, and Mass Spectrometric Features of Carotenoids Detected in Yellow Petals of *Rosa* 'Sun City'

peak	identification	Rt (min)	λ_{max} (nm)[a]	% III/II[b]	% AB/AII[c]	$[M + H]^+$ (m/z)	MS/MS(m/z)	refs
1	(13Z)-violaxanthin	12.61	327.2, 408.4, 430.2, 458.0	63.38	48.82	601.4250	583.4157, 565.4016, 491.3513	31,35
2	(13Z) + (di-Z)-violaxanthin	13.52	327.2, 410.2, 430.2, 456.8	45.35	27.48	601.4253	583.4143, 565.4053, 491.3518	31,35
3	(all-E)-violaxanthin	15.16	416.1, 438.6, 467.7	96.90		601.4257	583.4179, 565.4042, 491.3502	standard
4	(13/13′Z)-antheraxanthin	16.33	329.6, 415.8, 438.7, 465.3	45.45	22.73	585.4381	567.4331, 549.4172	37
5	(all-E)-luteoxanthin	17.22	309.3, 399.1, 420.5, 447.1	99.70		601.4298	583.4162, 565.4045	38,39
6	(13/13′Z)-neoxanthin	19.34	329.6, 415.2, 438.6, 466.5	66.67	37.19	601.4233	583.4159, 565.4051	40
7	(9Z)-violaxanthin	20.33	327.2, 410.9, 433.5, 464.1	90.14	12.50	601.4257	583.4151, 565.4052, 509.5106, 491.3509	31,35
8	(all-E)-antheraxanthin	20.92	420.8, 443.5, 470.1	56.76		585.4339	567.4326, 549.4165	standard
9	xanthophyll 1	21.96	394.4, 416.9, 443.5	94.74		601.4320	583.4163	
10	(all-E)-lutein	25.02	444.7, 472.6	67.56		569.4314	551.4226, 533.5631	standard
11	(all-E)-zeaxanthin	28.34	423, 449.5, 476.2	37.29		569.4314	551.4237, 533.5624	standard
12	(9/9′Z)-lutein epoxide	28.86	329.6, 416.2, 441.1, 467.7	65.48	10.19	585.4330	567.4356, 549.4173	42,43
13	(all-E)-cryptoxanthin 5,6-epoxide	32.45	417.1, 443.5, 472.6	59.70		569.4211	551.4232	45
14	(all-E)-alloxanthin	35.49	424.4, 449.5, 477.4	45.45		565.5669	547.4239	33
15	(13Z)-β-carotene	37.22	339.1, 419.3, 444.7, 470.1	22.22	43.11	537.4499	445.4425	34
16	(all-E)-β-carotene	42.01	425.1, 452.1, 478.6	30.95		537.4487	445.4431	standard
17	(9Z)-β-carotene	42.77	341.5, 420.2, 447.1, 472.6	31.91	9.76	537.4605	445.4535	34

[a] λ_{max} (nm) in the mobile phase (MTBE-methanol−water), detected by photodiode array detector. [b] % III/II is the ratio of the peak height of band III to that of band II. [c] % AB/AII is the ratio of the height of the cis-peak band to that of band II.

different geometrical isomers coeluted. The λ_{max} of peak **2** was very similar to that of (13*Z*)-violaxanthin, but their corresponding %AB/AII values (27.48 and 45.80 for peak 2 and (13*Z*)-violaxanthin, respectively) differed clearly. Thus, peak **2** was tentatively proposed to be a mixture of (13*Z*)-violaxanthin and a possible (di-*Z*)- violaxanthin.

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Table 4. Content of Carotenoids in Yellow Petals of *Rosa* 'Sun City' at Seven Blooming Stages (S1−S7)

peak	identification	content (μg/g FW)[a]						
		S1	S2	S3	S4	S5	S6	S7
1	(13*Z*)-violaxanthin	0.33 ± 0.02 c	0.55 ± 0.13 bc	0.36 ± 0.18 c	0.78 ± 0.21 ab	0.83 ± 0.21 a	0.84 ± 0.05 a	nd[b]
2	(13*Z*) + (di-*Z*)-violaxanthin	1.52 ± 0.23 c	9.13 ± 1.88 b	13.52 ± 1.46 a	15.28 ± 1.14 a	9.88 ± 0.19 b	7.83 ± 1.52 b	1.33 ± 0.17 c
3	(all-*E*)-violaxanthin	2.48 ± 0.22 e	12.14 ± 1.99 d	21.64 ± 1.74 b	32.09 ± 1.92 a	16.82 ± 0.85 c	11.35 ± 0.72 d	3.43 ± 0.16 e
4	(13/13′*Z*)-antheraxanthin	nd	2.99 ± 0.32 bc	7.13 ± 1.83 a	2.36 ± 0.59 c	4.04 ± 0.5 b	nd	nd
5	(all-*E*)-luteoxanthin	0.25 ± 0.04 b	2.27 ± 0.77 a	2.05 ± 0.27 a	2.1 ± 0.55 a	1.58 ± 0.81 a	1.42 ± 0.65 a	0.33 ± 0.09 b
6	(13/13′*Z*)-neoxanthin	nd	0.2 ± 0.05 bc	0.7 ± 0.08 a	0.31 ± 0.04 bc	0.34 ± 0.23 b	0.93 ± 0.37 a	nd
7	(9*Z*)-violaxanthin	5.74 ± 0.46 d	40.61 ± 4.94 b	68.57 ± 8.02 a	65.89 ± 5.51 a	35.72 ± 1.57 b	18.92 ± 1.2 c	1.51 ± 0.55 d
8	(all-*E*)-antheraxanthin	1.04 ± 0.15 d	1.13 ± 0.39 bcd	1.48 ± 0.22 b	1.88 ± 0.1 a	1.45 ± 0.08 bc	1.1 ± 0.15 cd	0.95 ± 0.17 d
9	xanthophyll 1	nd	0.52 ± 0.1 b	0.94 ± 0.25 a	0.34 ± 0.14 b	0.32 ± 0.17 b	nd	nd
10	(all-*E*)-lutein	0.42 ± 0.09 b	0.89 ± 0.17 a	0.85 ± 0.07 a	0.24 ± 0.06 c	0.48 ± 0.23 b	nd	nd
11	(all-*E*)-zeaxanthin	0.98 ± 0.02 d	2.2 ± 0.24 c	2.65 ± 0.06 bc	6.29 ± 1.26 a	3.24 ± 0.29 b	0.8 ± 0.24 de	nd
12	(9/9′*Z*)-lutein epoxide	0.92 ± 0.06 d	4.42 ± 0.79 b	5.79 ± 0.57 a	5.35 ± 0.83 a	3.33 ± 0.2 c	0.93 ± 0.32 d	nd
13	(all-*E*)-cryptoxanthin 5,6-epoxide	nd	nd	nd	0.25 ± 0.07 a	0.21 ± 0.12 a	nd	nd
14	(all-*E*)-alloxanthin	nd	nd	nd	0.58 ± 0.08 b	0.68 ± 0.02 a	nd	nd
15	(13*Z*)-β-carotene	0.16 ± 0.01 bc	0.22 ± 0.05 bc	0.42 ± 0.26 b	1.55 ± 0.32 a	0.4 ± 0.19 b	nd	nd
16	(all-*E*)-β-carotene	0.19 ± 0 d	3.17 ± 0.36 c	6.56 ± 0.39 b	11.96 ± 2.6 a	3.94 ± 0.11 c	0.37 ± 0.07 d	0.13 ± 0 d
17	(9*Z*)-β-carotene	nd	nd	0.12 ± 0.02 b	0.82 ± 0.23 a	0.71 ± 0.17 a	nd	nd
total epoxycarotenoids		11.37 ± 0.81 d	69.03 ± 8.35 b	115.44 ± 11.43 a	120.93 ± 9.06 a	70.87 ± 3.69 b	42.38 ± 3.61 c	7.55 ± 0.65 d
total hydroxycarotenoids		1.39 ± 0.11 d	3.09 ± 0.13 c	3.5 ± 0.05 c	7.11 ± 1.27 a	4.4 ± 0.18 b	0.8 ± 0.24 de	nd
total xanthophylls		12.76 ± 0.93 d	72.64 ± 8.4 b	119.88 ± 11.24 a	128.37 ± 10.43 a	75.6 ± 3.65 b	43.17 ± 3.38 c	7.55 ± 0.65 d
total hydrocarbons		0.35 ± 0.01 d	3.38 ± 0.35 c	7.1 ± 0.66 b	14.33 ± 3.13 a	5.05 ± 0.45 bc	0.37 ± 0.07 d	0.13 ± 0 d
total carotenoids		13.11 ± 0.93 e	76.02 ± 8.74 c	126.98 ± 11.62 b	142.71 ± 13.56 a	80.65 ± 3.96 c	43.54 ± 3.4 d	7.68 ± 0.65 e
ratio of E/Z		1:1.62	1:2.6	1:2.67	1:1.66	1:1.92	1:1.96	1:0.59

[a]Data are expressed as mean ± SD (n = 3). Different letters represent significant (P < 0.05) differences. [b]nd is not detected.

In the same manner, peaks **4**, **15**, and **17** were attributed to *Z*-isomers of the (all-*E*)-antheraxanthin and (all-*E*)-β-carotene, respectively, on the basis of the slight hypsochromic shift, the height of the cis peak and the identical fragments with peak **8** and **16**, respectively. Turcsi et al.[36] found that the elution order of geometrical isomers was 15/15′Z < 13/13′Z < all-E < 9/9′Z on C30 stationary phase. Furthermore, comparing their % AB/AII values (i.e., 22.73, 43.11, and 9.76 for peaks **4**, **15**, and **17**, respectively) with the literature,[34,37] peaks **4**, **15**, and **17** were identified more precisely as (13/13′*Z*)-antheraxanthin, (13*Z*)-β-carotene, and (9*Z*)-β-carotene, respectively.

Peak **5**, which eluted at 17.22 min, was tentatively proposed as (all-*E*)-luteoxanthin owing to its λ_{max} (399.1, 420.5, and 447.1 nm), its protonated molecule $[M + H]^+$ at m/z 601, and characteristic fragments $[M + H - 18]^+$ at m/z 583 and $[M + H - 18 - 18]^+$ at m/z 565, consistent with previous reports.[38,39]

Peak **6** was tentatively assigned to (13/13′*Z*)-neoxanthin on the basis of its λ_{max} at 329.6, 415.2, 438.6, and 466.5 nm, its % III/II value at 66.67, its % AB/AII value at 37.19, its MS data at m/z 601, 583, and 565, identical with previous reports.[40] The (all-*E*)-isomer of peak **6** has been reported previously in rose hips and petals.[3,41]

Peak **12**, which yielded protonated molecule $[M + H]^+$ at m/z 585, showed a hypsochromic shift with a decrease of the fine structure compared to (all-*E*)-lutein epoxide reported by Buchecker et al.[42] and by Meléndez-Martínez et al.[43] Given that (all-*E*)-isomer of this compound has been found in rose hip fruits of different species,[44] peak **12** was tentatively postulated to be (9/9′*Z*)-lutein epoxide.

Peak **13** was tentatively proposed to be (all-*E*)-cryptoxanthin 5,6-epoxide according to its λ_{max} at 417.1, 443.5, and 472.6 nm, its fine structure and its protonated molecule $[M + H]^+$ at m/z 569, as well established by previous research.[45] This compound was found for the first time in *Rosa* species and cultivars.

Peak **14** was tentatively characterized as (all-*E*)-alloxanthin according to its protonated molecule $[M + H]^+$ at m/z 565. This compound showed UV−vis absorption maxima at 424.4, 449.5, and 477.4 nm with % III/II value of 45.45, in line with reports by Britton,[33] but this compound has not been detected in the genus *Rosa*.

In this study, 16 carotenoids were identified, including three hydrocarbons, three hydroxycarotenoids, and 10 epoxycarotenoids (Table 3). These carotenoids were also verified in terms of geometrical configuration. Eight naturally occurring (mono-*Z*) and (di-*Z*)-carotenoids were determined in the present study. Among the carotenoids identified, 10 carotenoids were found for the first time in petals of *Rosa*, and 5 carotenoids were discovered for the first time in both petals and fruits of *Rosa*.

Quantitation of Carotenoids. Quantitation of the carotenoids identified is reported in Table 4. The content of carotenoids showed significant differences (P < 0.05) at different blooming stages. From S1 to S7, the content of many carotenoid compounds, e.g., total carotenoids, xanthophylls, hydrocarbons, epoxycarotenoids, hydroxycarotenoids, (13*Z*) + (di-*Z*)-violaxanthin, (9*Z*)-violaxanthin, (all-*E*)-violaxanthin, (all-*E*)-antheraxanthin, xanthophyll 1, (all-*E*)-zeaxanthin, (9/9′*Z*)-lutein epoxide, (all-*E*)-cryptoxanthin 5,6-epoxide, (all-*E*)-alloxanthin, (13*Z*)-β-carotene, (all-*E*)-β-carotene, and (9*Z*)-β-carotene, increased initially and then decreased. Except for (9*Z*)-violaxanthin, xanthophyll 1 and (9/9′*Z*)-lutein epoxide reached the highest levels at S3, (all-*E*)-alloxanthin being highest at S5, all of the other aforementioned carotenoids

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peaked at S4. The remainder of the carotenoids had an alternating trend of increase-decrease-increase-decrease.

The concentration of total carotenoid was highest at S4 (142.71 μg/g, FW), which was 18 times higher than that at S7 (7.68 μg/g, FW). At all blooming stages, xanthophylls accounted for 90−99% of total carotenoids, while hydrocarbons only accounted for 1−10%. Xanthophylls were found to comprise 84−98% of epoxycarotenoids and 0−11% of hydroxycarotenoids. These results support the view of Eugster and Märki Fischer,[3] speculating that the modern yellow roses mainly contained oxycarotenoid, such as epoxycarotenoids and hydroxycarotenoids.

At S4, (9*Z*)-violaxanthin (comprising 46% of total carotenoids) was the most abundant individual carotenoid in yellow rose petals, followed by (all-*E*)-violaxanthin (22%), (13*Z*) + (di-*Z*)-violaxanthin (11%), (all-*E*)-*β*-carotene (8%), (all-*E*)-zeaxanthin (4%), and small proportions of other identified carotenoids. Results showed that the concentration of violaxanthins including different geometrical isomers was at high levels with 114.04 μg/g (FW), accounting for 80% of the total carotenoids. Except for S4, petals at other stages contained 6.27−104.09 μg/g (FW) violaxanthins with different geometrical configurations, accounting for 77−89% of total carotenoids. Similar results were reported for carotenoid compositions in flowers of *R. fetida*, yellow modern rose cultivar 'Soleil d'Or' and 'Star of Persia', where violaxanthin was the most abundant carotenoid, accounting for 58.3, 23.7, and 40% of the total carotenoids, respectively.[3] These results suggested a higher rate of epoxidation of carotenoid in yellow rose petals. In comparison to other violaxanthin-rich flowers, vegetables, and fruits, the total violaxanthin content (114.04 μg/g, FW) in petals of *Rosa* 'Sun City' are much higher than those in petals of *Lotus japonicus* (70 μg/g, FW),[46] leaves of water convolvulus *Ipomoea acquatica*, and pulps of mango *Mangifera indica* (37 μg/g, FW).[47] Therefore, based on the criterion of good sources of carotenoids introduced by Britton et al.,[48] the yellow petals of *Rosa* 'Sun City' can be classified as a very high (>20 μg/g) carotenoid-containing natural source. Furthermore, Araki et al.[49] found that the lipid peroxidation inhibitory activities of (all-*E*)-violaxanthin and (9*Z*)-violaxanthin were better than those of *β*-carotene and astaxanthin. These findings indicated that the yellow petals of *Rosa* "Sun City' may be good candidates as antioxidants for protecting lipids.

At all blooming stages, carotenoids with *Z*-configuration accounted for 37−76% of the total carotenoid content in petals of *Rosa* 'Sun City'. As a major carotenoid with *Z*-configuration, (9*Z*)-violaxanthin made up 20−54% of the total carotenoid content. Considering there was no heat treatment or light exposure, these results suggested that these cis-isomers were very likely derived from biosynthesis.

In conclusion, this is the first report on systematic identification and quantification of flavonols and carotenoids in petals of *Rosa* 'Sun City' during seven blooming stages by HPLC-PDA-MS. A total of 19 flavonols and 16 carotenoids were identified, among which three flavonols and 10 carotenoids were found for the first time in rose petals. The petal of *Rosa* 'Sun City' was shown to be a good source of flavonols and carotenoids. Because these compounds have numerous biological activities, the yellow rose petals display great potential as sources of bioactive compounds, mainly kaempferol 7-*O*-glucoside, kaempferol 3-*O*-rhamnoside, and violaxanthin. Further studies regarding the isolation and application of these compounds are also necessary.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b01509.

Figure S1, UV−vis absorption spectra (λ_{max}) of flavonols extracted from yellow petals of *Rosa* 'Sun City'; Figure S2, UV−vis absorption spectra (λ_{max}) of carotenoids extracted from yellow petals of *Rosa*'Sun City'. (PDF)

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Notes

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■ ABBREVIATIONS USED

HPLC-PDA-MS, high-performance liquid chromatography coupled to photodiode array and mass spectrometry detectors; R^2, square correlation coefficient; HIV-1, human immunodeficiency virus I; HSV1, herpes simplex virus 1

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