Characterization and quantification of flavonoid glycosides in the *Prunus* genus by UPLC-DAD-QTOF/MS

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**KEYWORDS**

*Prunus*; Flavonol; Catechin; UPLC; QTOF-MS; Apricot

**Abstract**  Widely distributed in plants, flavonoids reduce the incidence of cancer and cardiovascular disease. In this study, flavonoid content and composition in members of the *Prunus* genus were evaluated using liquid chromatography with diode array and electrospray ionization mass spectrometric detection (UPLC-DAD-ESI/QTOF-MS). Flavonoids in plants of the *Prunus* genus include the basic structures of kaempferol, quercetin, and catechin, and exist as mono-, di-, or tri-glycoside compounds mono-acylated with acetic acid. A total of 23 individual flavonoids were isolated and confirmed, three of which appear to be newly identified compounds: quercetin 3-O-(2"-O-acetyl) neohesperidoside, quercetin 3-O-(4"-O-acetyl)rutinoside, and kaempferol 3-O-(4"-O-acetyl) rutinoside. Japanese apricot and Chinese plum contained the highest amounts of flavonoids in the *Prunus* genus. During the ripening stage of Japanese apricot, the total flavonol content was reduced, while the catechin content was increased.

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1. Introduction

Flavonoids are widely distributed in plants and are an important part of the diet due to their health-promoting benefits, including reduced risk of cancer and cardiovascular disease (Price and Rhodes, 1997; Zhishen et al., 1999; Lin and Harnly, 2008). Flavonoids are a large group of phytochemicals that are derived from multiple branches of the shikimic acid pathways, one of the most-characterized secondary metabolic routes in plant systems (Khanam et al., 2012; Wang et al., 2012). All food plants contain significant levels of these com-
pounds, which systematically identify glycosylated flavonoids (Price and Rhodes, 1997; Lin and Harrly, 2008).

The *Prunus* genus belongs to the Rosaceae family and consists of approximately 175 species distributed worldwide (Rashid et al., 2007), such as *P. armeniaca*, *P. mume*, *P. persica*, *P. salicina*, *P. domestica*, *P. spinosa*, *P. tomentosa*, *P. cerasus*, etc. Recent reports confirm that these plants contain high levels of flavonoids. *P. armeniaca* contains quercetin 3-O-rutinoside (rutin), quercetin 3-O-glucoside (isoquercitrin), and kaempferol 3-O-rutinoside (nicotiflorin), with rutin present at the highest levels (Schmitzer et al., 2011; Sanz et al., 2010; Rashid et al., 2007). Isoquercetin and quercetin derivatives were detected in the flowers of *P. mume*, and analysis of flavonoids in the fruits of *P. mume* using LC–MS identified glucoside, galactoside, and neohesperidoside (Nakamura et al., 2013; Yoshikawa et al., 2002; Yan, 2015). Flavonoids in *P. persica* were studied in a variety of plant parts including the leaves, stem bark, and peels (Buckhe et al., 2003; Tomas-Barberan et al., 2001). In the peels of *P. salicina* and *P. domestica*, the main flavonoids reported were quercetin 3-O-glucoside(isoquercitrin), quercetin 3-O-xylolide (reynoutrin), quercetin 3-O-rhamnoside (quercitrin), quercetin 3-O-galactoside (hyperoside), quercetin 3-O-rutinoside (rutin), quercetin 3-O-arabinobioside (gvaajverin), and isorhamnetin 3-O-glucoside (Tomas-Barberan et al., 2001; Treutter et al., 2012). Further, kaempferol 3-O-arabinofuranoside (juglanin) and quercetin 3-O-arabinofuranoside (avicularin) were isolated from extracts of *P. spinosa* flowers (Olszewska and Wolbis, 2001). Analysis of flavonoids from *P. cerasus* identified kaempferol, quercetin, quercetin 3-O-glucoside, and isorhamnetin 3-O-rutinoside (Piccolella et al., 2008), and catechin-type flavonoids were found to be distributed in the peels of *P. domestica*, peels and pulps of *P. persica*, and fruits of *P. mume* and *P. cerasus* (Tomas-Barberan et al., 2001; Piccolella et al., 2008; Treutter et al., 2012). Anthocyanins were reported mainly in the peels of fruits, and cyanidin 3-O-glucoside (chrysanthemin) and cyanidin 3-O-rutinoside (keracyanin) are the predominant anthocyanins present in *P. armeniaca*, *P. domestica*, *P. salicina*, and *P. persica* (Tomas-Barberan et al., 2001; Bureau et al., 2009; Treutter et al., 2012). Comparative evaluation is important for evaluating flavonoid characteristics in the various plants of the *Prunus* genus.

In this paper, flavonoid glycosides were characterized and quantified in plants of the *Prunus* genus, including *P. armeniaca* (apricot), *P. mume* (Japanese apricot), *P. persica* (peach), *P. salicina* (Chinese plum), *P. tomentosa*, and *P. cerasus* (cherry), using ultra performance liquid chromatography with diode array and quadrupole time-of-flight mass (UPLC-DAD/TOF-MS).

### Table 1 List of isolated flavonoids compounds and their mass spectrometric data in *Prunus* genus.

| Aglycones | Glycosides | Acylation | Individual flavonoids | MW | Fragment ions (m/z) | UV spectrum pattern (λ<sub>max</sub> → MeOH) |
|-----------|------------|-----------|-----------------------|----|--------------------|------------------------------------------|
| Kaempferol | Non Mono   | Mono      | Kaempferol            | 286| 287                | 265,294sh,324                           |
|           |            |           | Kaempferol 3-O-xylolide| 418| 441,287            |                                          |
|           |            | Ac        | Kaempferol 3-O-rhamnoside (aezelin)| 432| 455, 433, 287  | 265,294sh,324                           |
|           |            |           | Kaempferol 3-O-galactoside (trifolin)| 448| 471, 449, 287 | 266,301sh,346                           |
|           |            |           | Kaempferol 3-O-glucoside (astragalalin)| 448| 471, 287 | 266,298sh,346                           |
|           |            | Di        | Kaempferol 3-O-rutinoside (nicotiflorin)| 594| 617, 595, 449, 287 | 266,298sh,346                           |
| Quercetin  | Non Mono   | Mono      | Quercetin             | 302| 303                | 256,302sh,371                           |
|           |            |           | Quercetin 3-O-xylolide (reynoutrin) | 434| 457, 435, 303 | 257,266sh,296sh,356                      |
|           |            | Ac        | Quercetin 3-O-arabinobioside (gvaajverin)| 434| 457, 435, 303 | 257,265sh,300sh,354                      |
|           |            |           | Quercetin 3-O-rhamnoside (quercitrin) | 448| 471, 449, 303 | 256,307sh,351                           |
|           |            |           | Quercetin 3-O-galactoside (hyperoside) | 464| 487, 465, 303 | 257,265sh,298sh,355                      |
|           |            | Ac        | Quercetin 3-O-glucoside (isoquercitrin) | 464| 487, 465, 303 | 256,266sh,297sh,355                      |
|           |            | Di        | Quercetin 3-O-glucoside (astragalalin)| 506| 529, 507, 303 | 257,301sh,354                           |
|           |            | Ac        | Quercetin 3-O-neohesperidoside (cerakorin)| 610| 633, 611, 465, 449, 303 | 256,266sh,356                           |
|           |            | Tri       | Quercetin 3-O-rutinoside (rutin) | 610| 633, 611, 465, 449, 303 | 257,266sh,354                           |
|           |            | Ac        | Quercetin 3-O-(2"-O-acetyl)neohesperidoside (mumikotin B) | 652| 675, 653, 303 | 257,266sh,295sh,352                      |
|           |            | Ac        | Quercetin 3-O-(2"-O-acetyl)rutinoside (mumikotin A) | 652| 675, 653, 303 | 257,266sh,293sh,354                      |
|           |            | Ac        | Quercetin 3-O-(4"-O-acetyl)rutinoside (cerakorin)| 652| 675, 653, 303 | 257,301sh,354                           |
|           |            | Tri       | Quercetin 3-O-(2"-O-acetyl)rutinoside (cerakorin)| 756| 779, 757, 611, 465, 303 | 256,300sh,356                           |
|           |            | Tri       | Quercetin 3-O-(2"-O-acetyl)rutinoside (cerakorin)| 756| 779, 757, 611, 465, 303 | 256,299sh,355                           |
| Catechin  | Non        |           | (--)Epicatechin       | 290| 291                | 234,280                                  |

* Ac, Acetic acid.
| No. | Compound names                          | MW | UV spectrum pattern | States Used | Used parts | Plant resources | References                                                                 |
|-----|----------------------------------------|----|--------------------|-------------|------------|----------------|---------------------------------------------------------------------------|
| 1   | Kaempferol                             | 286|                    | Flower e   | spinosa e  | Prunus spinosa, Prunus cerasus | Olszewska and Wolbs (2001) and Piccolella et al. (2008)                  |
| 2   | Quercetin                              | 302|                    | Flower e   | spinosa e  | Prunus spinosa, Prunus cerasus | Olszewska and Wolbs (2001) and Piccolella et al. (2008)                  |
| 3   | Kaempferol 3-O-xyloside                | 418| (4)265,296sh,348  | Flower c   |             | Prunus spinosa, Prunus cerasus f | Olszewska and Wolbs (2001)                                               |
| 4   | Kaempferol 3-O-arabinofuranoside       | 418| (4)266,300sh,348  | Flower e   | spinosa e  | Prunus spinosa, Prunus cerasus | Olszewska and Wolbs (2001)                                               |
| 5   | Kaempferol 3-O-rhamnoside (aezulin)    | 432| (4)260,295sh,346  | Flower e   | spinosa e  | Prunus spinosa, Prunus cerasus | Olszewska and Wolbs (2001)                                               |
| 6   | Kaempferol 7-O-rhamnoside quercetin 3-O-xyloside | 434| (11)254,355       | Peels d    | salicina d | Prunus spinosa, Prunus cerasus | Tomas-Barberan et al. (2001)                                             |
| 7   | quercetin 3-O-xyloside (reynoutrin)    | 434|                    | Peels b    | domestica b| Prunus spinosa, Prunus cerasus | Treutter et al. (2012)                                                   |
| 8   | quercetin 3-O-arabinoside (gvajaverin) | 434|                    | Peels e    |             | Prunus spinosa, Prunus cerasus | Olszewska and Wolbs (2001)                                               |
| 9   | quercetin 3-O-arabinofuranoside (avicularin) | 434| (4)256,269sh,300sh,358 | Flower c         |              | Prunus spinosa, Prunus cerasus | Olszewska and Wolbs (2001)                                               |
| 10  | quercetin 3-O-rhamnoside (quercitrin)  | 448| (11)254,355       | Peels d    | salicina d | Prunus spinosa, Prunus cerasus | Tomas-Barberan et al. (2001)                                             |
| 11  | Kaempferol 3-O-glucoside (astragalin)  | 448| (6)265,300sh,351  | Leaves e   | persica e  | Prunus spinosa, Prunus cerasus | Backheet et al. (2003)                                                   |
| 12  | Kaempferol 3-O-galactoside (trifolin)  | 448| (6)265,289sh,351  | Leaves e   | persica e  | Prunus spinosa, Prunus cerasus | Backheet et al. (2003)                                                   |
| 13  | Isorhamnetin 3-O-rhamnoside            | 462|                    | Flowers b   | mume b     | Prunus spinosa, Prunus cerasus | Yoshikawa et al. (2002)                                                  |
| 14  | Quercetin 3-O-glucoside (isoquercitrin)| 464| (5)258,354        | Peels abde, | armeniaca a, | Prunus spinosa, Prunus cerasus | Backheet et al. (2003), Piccolella et al. (2008), Schmitzer et al. (2011), Tomas-Barberan et al. (2001), Treutter et al. (2012) and Yan (2015) |
| 15  | Quercetin 3-O-galactoside (hyperoside) | 464| (11)254,355       | Peels b    | domestica b| Prunus spinosa, Prunus cerasus | Tomas-Barberan et al. (2001) and Treutter et al. (2012)                 |
| 16  | Isorhamnetin 3-O-glucoside             | 478|                    | Peels b    |              | Prunus spinosa, Prunus cerasus | Nakamura et al. (2013)                                                   |
| 17  | Isorhamnetin 3-O-galactoside           | 478|                    | Flowers b   |              | Prunus spinosa, Prunus cerasus | Nakamura et al. (2013)                                                   |
| 18  | Quercetin 3-O-(2''-O-acetyl)glucoside  | 506|                    | Flowers b   | mume b     | Prunus spinosa, Prunus cerasus | Nakamura et al. (2013)                                                   |
| 19  | Quercetin 3-O-(6''-O-acetyl)glucoside  | 506|                    | Flowers b   | mume b     | Prunus spinosa, Prunus cerasus | Nakamura et al. (2013)                                                   |
| 20  | Isorhamnetin 3-O-(3''-O-acetyl)glucoside (mumellavonoside A) | 520| (Presumed)         | Flowers b   | mume b     | Prunus spinosa, Prunus cerasus | Nakamura et al. (2013), Sanz et al. (2010) and Schmitzer et al. (2011) Nakamura et al. (2013) |
| 21  | Kaempferol 3-O-(2''-O-p-coumaroyl)      | 564| (4)268,300sh,316,360 | Flower e   | spinosa e  | Prunus spinosa, Prunus cerasus | Olszewska and Wolbs (2007)                                               |
| 22  | Quercetin 3-O-(6''-O-benzoyl)glucoside | 568|                    | Flowers b   | mume b     | Prunus spinosa, Prunus cerasus | Nakamura et al. (2013)                                                   |
| 23  | Kaempferol 3-O-rutinoside (nicotifolin) | 594|                    | Peels e    | armeniaca a| Prunus spinosa, Prunus cerasus | Sanz et al. (2010)                                                       |
| 24  | Kaempferol 3-O-glucosyl(1 → 4)galactoside | 610| (6)267,289sh,350  | Leaves e   | persica e  | Prunus spinosa, Prunus cerasus | Backheet et al. (2003)                                                   |
| 25  | Quercetin 3-O-rutinoside (rutin)       | 610| (5)258,355        | Peels abde, | armeniaca a, | Prunus spinosa, Prunus cerasus | Sanz et al. (2010), Schmitzer et al. (2011), Slimestad et al. (2009), Tomas-Barberan et al. (2001), Treutter et al. (2012), Yan (2015) and Yoshikawa et al. (2002) |
| No. | Compound names                                                                 | MW  | UV spectrum pattern $i_{\text{max}}$ | States | Used parts | Plant resources | References                  |
|-----|--------------------------------------------------------------------------------|-----|-------------------------------------|--------|------------|----------------|---------------------------|
| 26  | Quercetin 3-O-neohesperidoside                                                  | 610 | NMR, MS                            | Flowers $^\text{g}$, Fruits $^\text{g}$     | mume $^\text{g}$        | Yan (2015) and Yoshikawa et al. (2002) |
| 27  | Isorhamnetin 3-O-rutinoside (narcissin)                                        | 624 | NMR, MS                            | Fruits $^\text{f}$                           | cerasus $^\text{f}$     | Piccolella et al. (2008)   |
| 28  | Kaempferol 3-O-(4″-O-acetyl) rutinoside (cerakorin)                            | 636 | MS (Presumed)                      | Fruits $^\text{f}$                           | cerasus $^\text{f}$     |
| 29  | Quercetin 3-O-(2″-O-acetyl) rutinoside (2″-O-acetylrutin) (mumikotin A)         | 652 | $^{(10)}258,270$sh,354             | NMR, MS                             | mume $^\text{g}$        | Yoshikawa et al. (2002)   |
| 30  | Quercetin 3-O-(2″-O-acetyl) neohesperidoside (mumikotin B)                     | 652 | MS (Presumed)                      | Fruits $^\text{f}$                           | mume $^\text{g}$        |
| 31  | Isorhamnetin 3-O-(2″-O-acetyl) rutinoside (2″-O-acetylnarcissin)               | 666 | $^{(10)}254,269$sh,354             | NMR, MS                             | mume $^\text{g}$        | Yoshikawa et al., 2002    |
| 32  | 3,5,7,4′-tetrahydroxy-3′,5′-dimethoxy flavone 3-O-robinobioside                | 654 | $^{(1)}252,357$                    | NMR, MS                             | mume $^\text{g}$        | Rashid et al., 2007      |
| 33  | Isorhamnetin 3-O-(2″-O-acetyl) rutinoside (2″-O-acetylparaxanthin)              | 666 | $^{(10)}254,269$sh,354             | NMR, MS                             | mume $^\text{g}$        | Yoshikawa et al., 2002    |
| 34  | quercetin 3-O-(2″,6″-di-O-Rhamnosyl) glucoside                                 | 756 | MS (Presumed)                      | Fruits $^\text{f}$                           | mume $^\text{g}$        | Yoshikawa et al. (2002)   |
| 35  | Quercetin 3-O-(2″,6″-di-O-Rhamnosyl) galactoside                                | 756 | MS (Presumed)                      | Fruits $^\text{f}$                           | mume $^\text{g}$        | Yoshikawa et al. (2002)   |
| 36  | Apigenin 5-O-glucoside                                                         | 432 | $^{(8)}258,329$                    | MS                                | bark $^\text{f}$                    | cerasus $^\text{f}$     | Geibel et al. (1991)      |
| 37  | Luteolin 5-O-glucoside                                                         | 448 | $^{(8)}289,324$                    | MS                                | bark $^\text{f}$                    | cerasus $^\text{f}$     | Geibel et al. (1991)      |
| 38  | Apigenin 7-O-mannosyl (1 → 2)galactoside                                       | 594 | $^{(1)}272,333$                   | NMR, MS                             | mume $^\text{g}$        | Rashid et al. (2007)      |
| 39  | Tectochrysin 5-O-glucoside                                                     | 430 | $^{(9)}243$sh,258,304              | NMR, MS                             | bark $^\text{f}$                    | cerasus $^\text{f}$     | Geibel et al. (1990, 1991) |
| 40  | Genkwanin 5-O-glucoside                                                        | 446 | $^{(8)}257,326$                    | MS                                | bark $^\text{f}$                    | cerasus $^\text{f}$     | Geibel et al. (1991)      |
| 41  | Naringenin                                                                     | 272 | $^{(6)}291,328$sh                 | NMR, MS                             | bark $^\text{f}$                    | persica $^\text{f}$     | Backheet et al. (2003)    |
| 42  | Eriodictyol                                                                    | 288 | $^{(6)}289,324$sh                 | NMR, MS                             | bark $^\text{f}$                    | persica $^\text{f}$     | Backheet et al. (2003)    |
| 43  | Dihydrokaempferol (aromadendrin)                                               | 288 | $^{(6)}290,327$sh                 | NMR, MS                             | bark $^\text{f}$                    | persica $^\text{f}$     | Backheet et al. (2003)    |
| 44  | Hesperitin 5-O-glucoside                                                       | 464 | $^{(6)}281,325$                   | NMR, MS                             | bark $^\text{f}$                    | persica $^\text{f}$     | Backheet et al. (2003)    |
| 45  | 5,3′-dihydroxy-7,4′-dimethoxy flavanone (persicogenin)                          | 316 | $^{(6)}285,332$                   | NMR, MS                             | bark $^\text{f}$                    | persica $^\text{f}$     | Backheet et al. (2003)    |
| 46  | Pinostrobin 5-O-glucoside                                                      | 432 | $^{(8)}279,305$sh                 | MS                                | bark $^\text{f}$                    | cerasus $^\text{f}$     | Geibel et al. (1991)      |
| 47  | Sakurarin                                                                       | 448 | $^{(8)}286,332$                   | MS                                | bark $^\text{f}$                    | persica $^\text{f}$     | Geibel et al. (1991)      |
| 48  | Persicogenin 3′-O-glucoside                                                     | 478 | $^{(8)}286,332$                   | MS                                | bark $^\text{f}$                    | persica $^\text{f}$     | Backheet et al. (2003)    |
| 49  | Neosakurarin (1′-O-methyl)                                                     | 448 | $^{(8)}254$sh,310sh,364           | MS                                | bark $^\text{f}$                    | persica $^\text{f}$     | Tomas-Barberan et al. (2001) and Treutter et al. (2012) |
| 50  | (-)-Catechin                                                                   | 290 | $^{(11)}280$                      | MS                                | bark $^\text{f}$                    | persica $^\text{f}$     | Tomas-Barberan et al. (2001), Treutter et al. (2012) and Yan (2015) |
| 51  | (-)-Epicatechin                                                                | 290 | $^{(11)}280$                      | NMR, MS                             | Fruits $^\text{f}$                   | cerasus $^\text{f}$     | Piccolella et al. (2008)   |
| 52  | (-)-Epicatechin 3-O-malate                                                     | 406 | $^{(7)}217$                       | NMR, MS                             | Fruits $^\text{f}$                   | cerasus $^\text{f}$     | Piccolella et al. (2008)   |
| 53  | (-)-Epicatechin 3-O-(1″-O-methyl)malate                                         | 420 | $^{(7)}216$                       | NMR, MS                             | Fruits $^\text{f}$                   | cerasus $^\text{f}$     | Piccolella et al. (2008)   |

(continued on next page)
Table 2 (continued)

| No. | Compound names | MW * | UV spectrum pattern | States | Used parts | Plant resources | References |
|-----|----------------|------|---------------------|--------|------------|----------------|------------|
| 54  | Genistein 5-O-glucoside | 432  | *(9)252 *(9)253     | NMR,MS | Bark f     | cerasus f       | Bureau et al. (2009), Sanz et al. (2010), Slimestad et al. (2009), Tomas-Barberan et al. (2001) and Treutter et al. (2012) |
| 55  | Prunetin 5-O-glucoside (prunetinoside) | 446  | *(9)252 *(9)253     | NMR,MS | Bark f     | cerasus f       | Bureau et al. (2009), Sanz et al. (2010), Slimestad et al. (2009), Tomas-Barberan et al. (2001) and Treutter et al. (2012) |
| 56  | Cyanidin 3-O-glucoside (chrysanthemin) | 449  | *(2)280,517 *(5)280,517 *(3)280,520 *(11)280,520 | MS     | Peels^d, Pulps^d e, Fruits^d | armeniaca^a, domestica^b, salicina^d, persica^d | Tomas-Barberan et al. (2001) and Treutter et al. (2012) |
| 57  | Cyanidin 3-O-galactoside (idaein) | 449  | *(11)280,520       | MS     | Peels^d | salicina^d | Tomas-Barberan et al. (2001) |
| 58  | Peonidin 3-O-glucoside | 463  | *(5)519            | MS     | Peels^b, Fruits^b | domestica^b | Slimestad et al. (2009) |
| 59  | Cyanidin 3-O-(6"-O-acetyl)glucoside | 491  | *(11)280,520       | MS     | Peels^d | salicina^d | Tomas-Barberan et al. (2001) |
| 60  | Cyanidin 3-O-rutinoside (keracayanin) | 595  | *(2)280,519 *(5)281,518 *(3)280,520 *(11)280,520 | MS     | Peels^a, Pulps^a e, Fruits^a f | armeniaca^a, domestica^b, salicina^d, persica^d, cerasus^f | Bureau et al. (2009), Sanz et al. (2010), Simunic et al. (2005), Slimestad et al. (2009), Tomas-Barberan et al. (2001) and Treutter et al. (2012) |
| 61  | Peonidin 3-O-rutinoside | 609  | *(2)280,519 *(3)274,520 | MS     | Peels^a, Fruits^a | cerasus^f | Bureau et al. (2009), Slimestad et al. (2009) and Treutter et al. (2012) |
| 62  | Cyanidin 3-O-(2"-O-glucosyl)rutinoside | 757  |                   | MS     | Fruits^f | cerasus^f | Simunic et al. (2005) |

*MW (mass/charge); UV: UV spectrum pattern, (1)(2)(4)(5)(6)(7)(8)(9)(10)MeOH, (3)(11)80%MeOH.

2. Materials and methods

2.1. Materials

For this study, P. armeniaca (apricot), P. persica (peach) (white, heavenly, and yellow), P. salicina (Chinese plum), and P. tomentosa (Korean cherry, sweet cherry, and cherry) were purchased in 2015 from Research Center, was in accordance with three different harvest times. These samples were freeze dried and finely ground with a sample mill for use as analytical samples.

2.2. Instrumentation and reagents

The instruments used during the pretreatment process included a refrigerated multi-purpose centrifuge (Hanil Science Industrial Co. Ltd., Korea) and a digital precise shaking bath (Dainhan Scientific Co. Ltd., Korea). Acetonitrile, methanol, and water were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid was provided by Junsei Chemical Co., Ltd., Japan. Galangin (Sigma, St. Louis, MO, USA) was used as the internal standard solution.

2.3. Extraction

Ground samples (1 g) in conical tubes (50 mL) were centrifuged (3000 rpm, 10 min, 4 °C) following extraction with 10 mL of methanol:water:formic acid (50:45:5, v/v/v) containing internal standard (galangin) in a shaking bath at room temperature for 5 min. The supernatant was immediately filtered with a syringe filter (PVDF, 0.2 μm, 25 mm; Whatman), and 1 mL of supernatant was concentrated with N2 gas. The extract was dissolved with 0.5 mL of methanol:water:formic acid (50:45:5, v/v/v) and diluted with 4.5 mL of water. A Sep-Pak C18 cartridge (Waters Co., Milford, MA, USA) was flushed with methanol and water for activation, and 1 mL of the diluted supernatant was loaded onto the cartridge. The cartridge was then washed with water and eluted with 1 mL of methanol. The extract was concentrated using N2 gas, and then re-dissolved in 0.5 mL of methanol:water:formic acid (50:45:5, v/v/v) prior to analysis by UPLC-DAD-ESI/QTOF-MS.

2.4. Quantitative and qualitative analysis of flavonoids by UPLC-DAD-ESI/QTOF-MS

Flavonoids in Prunus genus samples were identified and quantified using an UPLC-DAD-ESI/QTOF-MS system (Waters...
Table 3  Comparison of flavonoids composition and contents from extracts of the fruits in *Prunus* genus.\(^a\)

| Peak No. | Compound                                      | Apricot   | Japanese apricot (suyangmae) | White peach | Heavenly peach | Yellow peach | Chinese plum | Korean cherry | Sweet cherry | Cherry |
|---------|-----------------------------------------------|-----------|-------------------------------|-------------|----------------|-------------|--------------|---------------|--------------|--------|
| 1       | (−)-Epicatechin                               | 32.5 ± 0.4\(^d\) | 50.9 ± 1.0\(^b\) | ND          | ND             | ND          | 77.3 ± 13.0\(^d\) | ND           | ND           | ND      |
| 2       | Quercetin 3-O-(2\(^\prime\),6\(^\prime\)-di-O-rhamnosyl)galactoside | ND        | 18.2 ± 0.1\(^b\)         | ND          | ND             | ND          | ND           | ND            | ND           | ND      |
| 3       | Quercetin 3-O-(2\(^\prime\),6\(^\prime\)-di-O-rhamnosyl)glucoside | ND        | 18.8 ± 0.1\(^b\)         | ND          | ND             | ND          | ND           | ND            | ND           | ND      |
| 4       | Quercetin 3-O-neohesperidoside                | ND        | 6.9 ± 0.0\(^c\)          | ND          | ND             | ND          | ND           | ND            | ND           | ND      |
| 5       | Quercetin 3-O-rutinoside (rutin)             | 31.0 ± 0.6\(^c\) | 22.4 ± 0.0\(^b\) | 0.3 ± 0.0\(^d\) | 1.1 ± 0.1\(^c\) | 0.8 ± 0.0\(^e\) | 17.1 ± 0.3\(^b\) | 1.7 ± 0.1\(^d\) | 6.3 ± 0.1\(^c\) | 8.2 ± 1.4\(^c\) |
| 6       | Quercetin 3-O-galactoside (hyperoside)       | ND        | 3.5 ± 0.3\(^b\)          | 1.4 ± 1.7\(^b\) | 4.0 ± 0.1\(^e\) | 0.5 ± 0.0\(^d\) | 0.5 ± 0.0\(^b\) | 32.7 ± 0.5\(^e\) | 0.4 ± 0.0\(^b\) | 0.3 ± 0.0\(^d\) | 0.3 ± 0.0\(^a\) |
| 7       | Quercetin 3-O-glucoside (isoquercitrin)      | 1.7 ± 0.0\(^b\) | 4.9 ± 0.0\(^e\)          | 0.5 ± 0.0\(^c\) | 4.6 ± 0.2\(^f\) | 0.5 ± 0.0\(^b\) | 32.7 ± 0.5\(^e\) | 0.4 ± 0.0\(^b\) | 0.3 ± 0.0\(^c\) | 0.3 ± 0.0\(^a\) |
| 8       | Quercetin 3-O-xylloside (reynoutrin)         | ND        | ND                           | ND          | 2.4 ± 0.1\(^h\) | ND           | ND           | ND            | ND           | ND      |
| 9       | Kaempferol 3-O-galactoside (trifolin)        | ND        | ND                           | 0.1 ± 0.0\(^a\) | 0.9 ± 0.1\(^b\) | 0.4 ± 0.0\(^e\) | ND           | ND            | ND           | ND      |
| 10      | Kaempferol 3-O-rutinoside (nicotiflorin)     | 0.6 ± 0.0\(^a\) | ND                          | 1.2 ± 0.1\(^d\) | 0.4 ± 0.1\(^a\) | 0.8 ± 0.2\(^e\) | ND           | 2.6 ± 0.1\(^f\) | 3.0 ± 0.1\(^b\) | 2.1 ± 0.4\(^b\) |
| 11      | Quercetin 3-O-arabinoside (gvaajarverin)     | ND        | ND                           | ND          | ND             | 13.0 ± 0.3\(^k\) | ND           | ND            | ND           | ND      |
| 12      | Kaempferol 3-O-glucoside (astragalin)        | 0.4 ± 0.0\(^a\) | ND                          | 2.1 ± 0.2\(^e\) | 2.5 ± 0.4\(^d\) | 2.5 ± 0.1\(^e\) | ND           | 1.1 ± 0.0\(^c\) | ND           | 0.2 ± 0.1\(^a\) |
| 13      | Quercetin 3-O-rhamnoside (quercitrin)        | ND        | ND                           | ND          | ND             | 4.5 ± 0.1\(^i\) | ND           | 31.2 ± 0.8\(^b\) | ND           | ND      |
| 14      | Quercetin 3-O(6\(^\prime\)-O-acetyl)glucoside | 1.8 ± 0.1\(^b\) | 4.4 ± 0.1\(^c\)         | ND          | ND             | 1.4 ± 0.3\(^b\) | ND           | ND            | ND           | ND      |
| 15      | Kaempferol 3-O-xylloside                     | ND        | ND                           | ND          | ND             | ND           | 0.4 ± 0.0\(^b\) | ND            | ND           | ND      |
| 16      | Quercetin 3-(O-2\(^\prime\)-O-acetyl)neohesperidoside (mumikotin B) | ND | 5.7 ± 0.1\(^c\) | ND          | ND             | ND           | ND           | ND            | ND           | ND      |
| 17      | Quercetin 3-(O-2\(^\prime\)-O-acetyl)rutinoside (mumikotin A) | ND | 22.8 ± 0.1\(^j\) | ND          | ND             | ND           | ND           | ND            | ND           | ND      |
| 18      | Kaempferol 3-O-rhamnoside (afzelin)          | ND        | ND                           | ND          | ND             | ND           | 23.6 ± 0.6\(^b\) | ND           | ND           | ND      |
| 19      | Quercetin 3-(O-2\(^\prime\)-O-acetyl)glucoside | ND        | 1.3 ± 0.0\(^c\)          | ND          | ND             | ND           | ND           | ND            | ND           | ND      |
| 20      | Quercetin 3-O-(4\(^\prime\)-O-acetyl)rutinoside (cerakocetin) | ND | ND                         | ND          | ND             | 6.0 ± 0.1\(^i\) | ND           | ND            | ND           | ND      |
| 21      | Kaempferol 3-O-(4\(^\prime\)-O-acetyl)rutinoside (cerakorin) | ND | ND                         | ND          | ND             | ND           | 42.0 ± 0.9\(^b\) | ND           | ND           | ND      |
| 22      | Quercetin                                   | ND        | ND                           | ND          | 0.2 ± 0.0\(^a\) | ND           | ND           | ND            | ND           | ND      |
| 23      | Kaempferol                                  | ND        | ND                           | ND          | 0.6 ± 0.1\(^b\) | ND           | ND           | ND            | ND           | ND      |

ND, not detected.

\(^a\) mg per 100 g dry weight (DW); each value calculated as means ± SD of three replicates using internal standard (galangin).
kaempferol 3-
Although astragalin was detected previously in leaves of peach galin in apricot (0.4 mg/100 g DW). The compound fragment temperature of 500
ranoside, xyloside (terns, in which acylated phenolic acids such as acetic acid ual flavonoids were determined by analysis of fragment pat-
Prunus the these samples contained the highest amounts of flavonoids in
vonol glycosides as well as catechin-type flavonoids, and nese plum. Japanese apricot and Chinese plum contained fla-
epicatechin, only showed apricot, Japanese apricot, and Chi-
chemical structures of the individ-
A library containing 62 compounds identified in previous stud-
2.5. LC–MS library for qualitative analysis of flavonoids
Based on a variety of literature sources, a LC–MS library of 35
flavonoids, five flavones, eight flavanones, one chalcone, four
flavanols, two isoflavones, and seven anthocyanins was created and used for the identification of individual flavonoid components.
3. Results and discussion
A library containing 62 compounds identified in previous studies was used for identification of flavonoids (Table 1). A total of 23 different compounds, including four unknown compounds, were isolated and identified by UPLC-DAD-QTOF/ MS with reference to the LC–MS library of Prunus genus flavonoids (Table 2). These detected compounds included seven kaempferol derivatives, 15 quercetin derivatives, and (−)-epicatechin (Table 3). The chemical structures of the individual flavonoids were determined by analysis of fragment patterns, in which acylated phenolic acids such as acetic acid (m/z 42) were cut out from their structures with glucose, galactose (m/z 162), rhamnoside (m/z 146), arabinofuranose, arabinofuranose, xyloside (m/z 132), rutinoside, and neohesperidoside (m/z 308) found to appear independently were cut off from whole structure step by step (Backheet et al., 2003; Piccolella et al., 2008; Olszewska and Wolbis, 2001; Slimestad et al., 2009; Nakamura et al., 2013).
Previous studies reported the isolation of (−)-epicatechin from peach, plum, and Japanese apricot (Tomas-Barberan et al., 2001; Treutter et al., 2012; Yan, 2015). Tomas-Barberan et al., 2001, isolated (−)-epicatechin from the peels and pulps of peach, but this study was not isolated (−)-epicatechin, only showed apricot, Japanese apricot, and Chinese plum. Japanese apricot and Chinese plum contained flavonol glycosides as well as catechin-type flavonoids, and these samples contained the highest amounts of flavonoids in the Prunus genus (Table 4).
We did not detect any kaempferol-type flavonoids in Japanese apricot, but kaempferol 3-O-rutinoside (nicotiflorin) and kaempferol 3-O-glucoside (astragalin) were detected in apricot. Although astragalin was detected previously in leaves of peach (Backheet et al., 2003), this study was the first to detect astragalin in apricot (0.4 mg/100 g DW). The compound fragment

### Table 4. Comparison of flavonoids composition and contents in Japanese apricot (Prunus mume) by variety and during ripening.

| No. | Compound | Imju | Namgo | Suyangmae |
|-----|----------|------|-------|-----------|
| 1   | (−)-Epicatechin | 20.3 ± 1.8 | 30.1 ± 0.7 | 50.9 ± 1.0 |
| 2   | Quercetin 3-O-β-D-glucoside | 9.9 ± 0.2 | 5.8 ± 0.4 | 13.2 ± 1.0 |
| 3   | Quercetin 3-O-rutinoside | 11.8 ± 0.2 | 10.9 ± 0.2 | 11.3 ± 0.3 |
| 4   | Quercetin 3-O-neohesperidoside | 10.9 ± 0.4 | 5.0 ± 0.3 | 13.2 ± 0.3 |
| 5   | Quercetin 3-O-glucoside (hyperoside) | 3.7 ± 0.1 | 1.4 ± 0.1 | 1.8 ± 0.1 |
| 6   | Quercetin 3-O-neohesperidoside (mumikotin B) | 3.7 ± 0.1 | 3.3 ± 0.1 | 1.3 ± 0.1 |
| 7   | Quercetin 3-O-rutinoside (mumikotin A) | 6.9 ± 0.1 | 3.9 ± 0.1 | 1.8 ± 0.1 |
| 8   | Quercetin 3-O-glucoside | 0.7 ± 0.1 | 0.6 ± 0.1 | 1.3 ± 0.1 |
| 9   | Quercetin 3-O-rutinoside | 0.7 ± 0.1 | 0.6 ± 0.1 | 1.3 ± 0.1 |
| 10  | Quercetin 3-O-neohesperidoside | 0.7 ± 0.1 | 0.6 ± 0.1 | 1.3 ± 0.1 |

| Total flavonoids contents | 128.9 ± 6.6 | 129.4 ± 7.3 | 122.8 ± 2.7 |
| ND, not detected. | | | |
ion pattern was [M+Na]+ at m/z 471, [M+H]+ at m/z 449, and [M+H-Glu]+ at m/z 287 (Table 2). In addition, nicotiflorin was isolated from peels of apricot in a previous report (Sanz et al., 2010).

The flavonol glycoside contents of the different peach varieties are shown in Table 3. The flavonol contents in the heavenly peach were generally three times higher than in white and yellow peaches, and the amounts of quercetin 3-O-galactoside (hyperoside) and quercetin 3-O-glucoside (isoquercitrin) were greater than those of other compounds in the heavenly peach. In previous studies, flavonols were found mainly in the peels of peaches (Tomas-Barberan et al., 2001) and the leaves and stem bark when analyzed by NMR (Backheet et al., 2003). Accordingly, the composition and content of flavonol glycosides will need studying depending on the cultivars and parts of the plant in the peach. Analysis of Chinese plum revealed ([M+H]+ at m/z 303) and kaempferol ([M+H]+ at m/z 287) were the 3,5,7,3',4',5'-pentahydroxyflavone and 3,5,7,4'-tetrahydroxyflavone, respectively (Olszewska and Wolbis, 2001; Piccolella et al., 2008). In Fig. 1, the UV data (λmax 257,266sh,293sh,354 nm) and MS data ([M+Na]+ at m/z 675, [M+H]+ at m/z 653, [M+H-Ac-rut]+ at m/z 303) from analysis of peak 17 suggested this was quercetin 3-O-(200-O-acetyl)rutinoside. Peak 16 (tR = 18.10 min, λmax 257,266sh,295sh,352 nm, [M+Na]+ at m/z 675, [M+H]+ at m/z 653, [M+H-Ac-Neo]+ at m/z 303) was identified as a quercetin 3-O-(200-O-acetyl)neohesperidoside. These compounds are novel flavonoids, identified for the first time in Japanese apricot. Quercetin 3-O-(200-O-acetyl) rutinoside and quercetin 3-O-(200-O-acetyl) neohesperidoside were named mumikotin A and B, respectively, by combining the scientific name of *Prunus mume*, ‘Korea’, and ‘rutinoside’. Peak 20 (tR = 19.42 min, λmax 257,301sh,354 nm, [M+Na]+ at m/z 675, [M+H]+ at m/z 653, [M+H-Ac-NeO]+ at m/z 303) was identified as a quercetin 3-O-(40-O-acetyl)rutinoside. Furthermore, UV data (λmax 265,294sh,320sh,343 nm) and MS data ([M+Na]+ at m/z...
659, \([M + H]^+\) at \(m/z\) 637, \([M + H-Na]^+\) at \(m/z\) 297) from analysis of peak 21 suggested this was a kaempferol 3-O-(4′-O-acyl)rutinoside (Fig. 1). These compounds are also novel compounds isolated for the first time, and the kaempferol 3-O-(4′-O-acyl)rutinoside was the major flavonol in Korean blackberry (42.0 mg/100 g DW). These compounds were named by combining ‘ceras’ from the scientific name Prunus cerasus, ‘ko’ of Korea, and ‘cetin’ of quercetin; hence, carakocetin (kaempferol 3-O-(4′-O-acyl)rutinoside) and cerakorin (kaempferol 3-O-(4′-O-acyl)rutinoside) (Geibel and Feucht, 1990, 1991; Babaei et al., 2008; Fischer et al., 2007; Jaisswal et al., 2013).

Among the Prunus genus, the Japanese apricot contained the greatest amount of flavonoids (Table 2). The flavonoids detected in Japanese apricot were (−)-epicatechin and quercetin derivatives, and the most predominant flavonoids were (−)-epicatechin, quercetin 3-O-(2′,6′-di-O-rhamnosyl)galactoside, quercetin 3-O-rutinoside (rutin), and quercetin 3-O-(2′-O-acyl)rutinoside (mumikotin A) (Table 4). Importantly, the composition and amounts of flavonols in Japanese apricot varied in accordance with the variety and stage of ripening (Table 4). When comparing varieties, the suyangmae variety showed the highest flavonol contents. When analyzed based on ripening stage, although overall flavonol contents decreased upon ripening, levels of (−)-epicatechin increased. Thus, it appears that catechins are synthesized from flavonols during maturation. In a previous study, quercetin 3-O-(2′,6′-di-O-rhamnosyl)galactoside was identified in flowers of the Japanese apricot by NMR (Yoshikawa et al., 2002). In the present study, peak 3 was confirmed to be the same compound based on MS fragment data (\(m/z\) 779, \([M + H]^+\) at \(m/z\) 757, \([M + H-H-Rham]^+\) at \(m/z\) 611, \([M + H-2Rham]^+\) at \(m/z\) 465, \([M + H-Gal-2Rham]^+\) at \(m/z\) 303). Furthermore, peak 2 produced the same MS fragment profile as peak 3, and this was estimated to galactose (\(m/z\) 162) instead of glucose (\(m/z\) 162). If so, this compound would be identified as quercetin 3-O-(2′,6′-di-O-rhamnosyl)glucoside and will have been first discovered in the fruit of the Japanese apricot.

4. Conclusions

A total of 23 different compounds were isolated from members of the Prunus genus and identified by UPLC-DAD-QTOF/MS. Galangin was used as an internal standard solution for flavonoid quantification. The Prunus genus flavonoids include the basic structures of kaempferol, quercetin, and catechin, and exist as mono-, di-, or tri-glycoside compounds monoacylated with acetic acid. In this study, four flavonoid species were detected for the first time in the Japanese apricot and Korean cherry. The Japanese apricot and Chinese plum contained flavonol glycosides as well as catechin-type flavonoids, and these two plants contained the highest amounts of flavonoids in the Prunus genus. During ripening of the Japanese apricot, although the overall flavonol contents decreased, the amount of catechin-type flavonoids increased. Thus, it appears that catechins are synthesized from flavonols during maturation. Future studies are needed to determine the bioactive properties of each flavonoid compound and promote the use of extracts derived from members of the Prunus genus.
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