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Research Article

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# Effect of the Nutritive Components of Mulberry Fruits From Two Cultivars Based on Irrigation Scheduling

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## Abstract

**BACKGROUND:** The mulberry cultivars 'Daeshim' and 'Shimgang' were developed in RDA in 2014 and 2017, respectively. 'Daeshim' yields a fruit size bigger than that of other varieties and has a productivity of over 70%, whereas 'Shimgang' has a high yield and a special characterization against the mulberry popcorn disease. In our study, a compositional comparison of these popular cultivars in Korea was undertaken to explore the nutrient profiles of mulberry fruit and promote the development of the rich minerals and flavonoids in mulberry fruit as performing each other irrigation time.

**METHODS AND RESULTS:** These two cultivars were collected from the Sericulture and Apiculture Division, RDA, in Korea to investigate their amounts, weights, minerals, and flavonoid content using each other instrument. After 6 h of irrigation treatment, the amount of fruit (kg/tree) from Daeshim and Shimgang increased by 17.5 and 15.2 kg/tree, respectively. The total flavonoid content from Daeshim and Shimgang was determined to be 132.9 mg and 36.3 mg, respectively, after the 6 h irrigation treatment.

**CONCLUSION:** Appropriate irrigation treatment methods such as water scheduling and volume will help increase fruit quantities and farmer incomes. It would be interesting to conduct further in-depth research on these fruits so that consumers can benefit from them as a food additive.

**Key words:** Flavonoids, Irrigation, Minerals, Mulberry

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## Introduction

Mulberry (Morus spp.) trees belong to the order Rosales, family Moraceae [1]. The genus Morus is distributed worldwide, including Asia, Europe, North and South America, and Africa [2]. It has historically been used for leaf yield in sericulture, and its fruit, leaves, branches, and roots, which contain good sources of bioactive compounds, have been used in traditional medicine to treat diabetes, hypotension, anemia, and arthritis [3]. Mulberries are also known as a food source, and mulberry leaves have been used as feed for silkworms. Nowadays, mulberry fruit is known to be a by-product that is produced from mulberry trees after harvesting the tree leaves for silkworm rearing; as the yield and consumption of mulberry fruit is increasing, it has become a new income crop.

Recently, numerous studies have revealed that edible plants are good sources of phytochemicals and play a prominent part in the maintenance of human health [4]. It is known that protein, minerals, carbohydrates, vitamins, microelements, and dietary fiber are the main factors that influence the nutritional quality of edible

plants. Both the nutrients and bioactive compounds of these plants could have synergistically beneficial effects on human health. In particular, mulberry fruits have a high content of polyphenols, including flavonoids (anthocyanin, rutin, quercetin, and isoquercetin), steroids, and amino acids, which are known to have multi-bioactive functions with neuroprotective effects [5,6]. In a previous study, the profile of flavonoids was analyzed using HPLC in M. nigra and M. alba fruits, identifying the compounds using NMR and ESI-MS [7]; this study also found that the red pigment of M. nigra fruits contained four anthocyanins identified as cyanidin 3-O-glucoside, cyanidin 3-O-rutinoside, pelargonidin 3-O-glucoside, and pelargonidin 3-O-rutinoside using HPLC/PDA/ESI-MS analyses.

Mulberries have been recognized as a source owing to their biologically active compounds, minerals, and flavonols [8]. The nutritional and functional components of mulberry fruits may be significantly influenced by the physiological and environmental factors of the area, such as the soil chemistry, cultivar, cultivation technique, harvesting time, and degree of fruit maturity [9-11]. For example, in the case of mulberry leaves, juvenile leaves contain more active substances than mature leaves [12]. In addition, the ascorbic acid, carotenoid, total soluble protein, total soluble sugar, fructose, and sucrose content in the tender shoots of eight mulberry cultivars were investigated and it was found that there were obvious differences in some nutrients and flavor components among the different mulberry cultivars [11]. The phenolic content of mulberry leaves also varied with cultivars and harvesting time. Mulberry leaves collected in May are considered to be good sources of phenolic compounds [10,11].

However, no conclusive and comprehensive investigation has been conducted on the nutritional composition of mulberry cultivars from Korea in previous studies. Furthermore, the correlations between antioxidant activities and major active components in mulberry fruits remain unknown. In our study, a compositional comparison of typical mulberry cultivars 'Daeshim' and 'Shimgang' from Korea was undertaken to explore the nutrient profiles of mulberry fruit and promote the development of the rich minerals in mulberry fruit as performing each other irrigation time. In addition, this study aimed to identify the flavonols and evaluate their content in these cultivars.

## Materials and Methods

### Preparation of samples, mineral assessment and proximate analysis methods

Daeshim and Shimgang fruits were collected from the Sericulture and Apiculture Division for the Department of Agricultural Biology, Rural Development Administration (RDA), Wan-Ju, Republic of Korea. To promote the development of the rich minerals and flavonoid content in mulberry fruit, each mulberry cultivar was cultivated in a rain-sheltered house with drip-watering facilities (Fig. 1). They were irrigated once weekly with 2 h, 4 h, and 6 h treatments for two months (From April to May), and the volume irrigated for 2 h was 21 L. The physicochemical properties of the soil were investigated before conducting this experiment (Table 1). The fruit samples were cleaned and dried in a lyophilizer. All the dried samples were pulverized and stored below −18 ℃ prior to the proximate analysis of minerals.

The minerals (nine types of inorganic elements) were determined using the methods described by the Association of Official Analytical Chemists (AOAC, 1990), and were analyzed using an inductively coupled plasma optical emission spectrometer. All the proximate analyses were reported per 100 g of fruits. Additionally, the fruit amounts and fruit weight were checked as

Fig. 1. Rain-sheltered house with irrigation (drip-watering facilities).

Table 1. Physicochemical properties of the soil used for the experiment

| A type of soil | pH (1:5) | EC (dS/m) | Organic Matter(%) | Av.$P_2O_5$ (mg/kg) | Ex. Cation (cmol+/kg) | | |
|---|---|---|---|---|---|---|---|
| | | | | | K | Ca | Mg |
| Rain-sheltered house Soil | 6.54 | 0.53 | 2.64 | 659.52 | 0.6 | 6.24 | 1.68 |
| Optimum Ranges | 6.0-6.5 | - | 2.0-3.0 | 300-500 | 0.5-0.6 | 5.0-6.0 | 1.5-2.0 |

amounts (kg/fresh mulberry fruit) per tree and weight per mulberry fruit, respectively. The sugar content was determined using a saccharometer. All data are expressed as mean ± standard deviation from three independent experiments.

### Preparation and determination of samples for flavonoids contents

A sample extraction was conducted according to the sample extraction method with minor modifications [13]. The powdered fruits (1 g) were mixed with 10 mL of an acidified hydro-alcoholic solvent (methanol: water:formic acid (50:45:5, v/v/v) containing 100 ppm of galangin as an internal standard). The mixture was first stirred using a shaker for 5 min at 200 rpm and then centrifuged for 15 min at 3,000 rpm and 10℃. The supernatant was filtered using a syringe filter (0.45 μm, PTFE, Whatman, Kent, England) and 0.5 mL of the filtrate was diluted with water to reach a final volume of 5 mL. The flavonoid extract was purified and isolated with the solid phase extraction method using Sep-Pak C-18 (Waters Co., Milford, MA, USA). Sep-Pak activation was performed by washing the cartridge with 2 mL of methanol, followed by 2 mL of water for conditioning. Then, the diluted extract was loaded on the Sep-Pak and impurities were removed by washing the extract with 2 mL of water. Finally, the total flavonoids mixture was eluted from Sep-Pak by using 3 mL of methanol. The purified extract was concentrated using $N_2$ gas, and then re-dissolved with 0.5 mL of the extract solvent without an internal standard prior to the instrument analysis.

Ultra performance liquid chromatography (UPLC) was performed with a photo diode array detector set at 280 and 320 nm coupled with a quadrupole time-of-flight mass spectrometer (Waters Co., Milford, MA, USA). UV spectra were obtained in the region of 210-600 nm. The following chromatographic conditions were used: column, Luna Omega 1.6 μm C18, 150 × 2.1 mm, Phenomenex; Pre-column: SecurityGuard ULTRA Cartridges, UHPLC C18 for 2.1 ID column, Phenomenex, column temperature 30℃; mobile phase used was 0.5% formic acid in water (A) and 0.5% formic acid in acetonitrile (B); flow rate 0.3 ml/min; injection volume 5 μL; total running time 60 min. The gradient elution had the following profile: 0-2 min 7% B, 24 min 15% B, 40 min 30% B, 48-50 min 60% B, 53-54 min 90% B, and 55-60 min 7% B. All data are expressed as mean ± standard deviation from three independent experiments.

## Results and Discussion

### Changes of properties and mineral composition about mulberry fruits based on irrigating treatments

During the irrigation treatments, there was a considerable increase in the amount and weight of fruits and their sugar content. Generally, the weight of fruit (g/fresh weight) from Daeshim and Shimgang was 4.2 g and 2.1 g, respectively. In this study, the highest increase in Daeshim fruit amounts (17.5 kg/tree) and fruit weights (5.2 g/fresh fruit) occurred after the 6 h irrigation treatment (Table 2). In contrast, the sugar content was not affected by the irrigation treatment. Additionally, although the data is not shown, the amount, weight, and mineral composition of the fruits were similar between irrigation periods of 6 h and over

Table 2. The mulberry fruits amounts, weights, sugar contents based on irrigation

| Treatment | Daeshim | | | Shimgang | | |
|---|---|---|---|---|---|---|
| | Amounts (kg/onetree) | Weights (g/onefreshfruit) | Sugar contents (Brix) | Amounts (kg/onetree) | Weights (g/onefreshfruit) | Sugar contents (Brix) |
| 2h | 15.7±1.21 | 3.9±0.14 | 12.7±0.13 | 13.5±1.44 | 2.1±0.07 | 14.2±0.15 |
| 4h | 16.8±0.54 | 5.1±0.01 | 12.9±0.22 | 14.7±1.31 | 2.9±0.04 | 14.1±0.13 |
| 6h | 17.5±0.42 | 5.2±0.02 | 12.8±0.12 | 15.2± 1.83 | 3.1±0.06 | 14.3±0.16 |

*Each value calculated as means ± SD of three replicates.

Table 3. Mineral content of Daeshim and Shimgang mulberry fruits

(mg/100g)

| Cultivar | Treatment | K | P | Ca | Mg | Na | Cu | Fe | Mn | Zn | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Daeshim | 2h | 1488.4±78.4 | 300.67±21.5 | 284.58±9.5 | 94.94±5.4 | 14.62±1.5 | 0.16±0.0 | 3.39±0.5 | 0.63±0.1 | 0.72±0.1 | 2188.11±117 |
| | 4h | 1531.2±82.5 | 327.19±22.5 | 306.88±15.5 | 105.12±6.7 | 16.7±1.7 | 0.2±0.0 | 3.65±0.4 | 0.7±0.1 | 0.93±0.1 | 2292.57±129.5 |
| | 6h | 1878.9±102.8 | 370.36±24.1 | 304.65±12.1 | 119.88±6.9 | 17.55±1.6 | 0.18±0.0 | 3.7±0.4 | 0.92±0.1 | 1.22±0.1 | 2697.36±148.1 |
| Shimgang | 2h | 1198.2±52.1 | 242.1±15.8 | 272.5±12.5 | 75.3±4.1 | 13.8±0.9 | 0.1±0.0 | 2.61±0.3 | 0.53±0.1 | 0.65±0.1 | 1805.79±85.9 |
| | 4h | 1252.1±53.4 | 251.3±16.4 | 294.1±15.4 | 85.7±5.8 | 15.1±1.5 | 0.16±0.0 | 2.98±0.4 | 0.68±0.1 | 0.98±0.1 | 1903.1±93.1 |
| | 6h | 1321.3±62.9 | 275.2±18.5 | 302.1±13.2 | 95.2±5.3 | 15.3±1.8 | 0.17±0.0 | 3.24±0.4 | 0.75±0.1 | 1.13±0.1 | 2014.39±102.3 |

*Each value calculated as means ± SD of three replicates

6 h (6 h-10 h). The relationships between irrigation and crop rotation treatments affect the water use efficiency of crops, influencing agronomic quality, and frequent irrigations are beneficial and often increase growth and yield in many horticultural crops [14]. A previous study reported that frequent drip irrigation in peaches increased fruit size and yield compared to that using other irrigation methods by maintaining a higher tree water status between irrigations [15]. In addition, irrigation is a determinant factor in the growth and production of blueberries because their root system is superficial and confined, with a small amount of root hairs, which restricts their water uptake capacity. An increased fruit size induced by fruit thinning under various irrigation regimes can probably be attributed to the enhanced availability of assimilates to individual fruits and the maintenance of turgor for expansion [16].

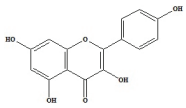
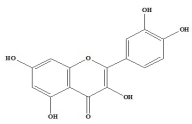
The results regarding the changes in the mineral composition levels of mulberry fruits based on the irrigation treatments showed that the highest concentrations of potassium were found when the cultivars were irrigated for 6 h, with 1878.9 mg/100 g and 1321.3 mg/100 g of dry weight in each cultivar, respectively (Table 3). Generally, the potassium levels of the mulberry cultivars M. alba, M. nigra, and M. rubra ranged from 1272.3-1673.7 mg/100 g without irrigation treatment [17]. Potassium is one of the most important nutrients for controlling human blood pressure; therefore, an abundance of potassium may be useful for those suffering from hypertension [4]. In addition, the quantity of phosphorus, calcium, and magnesium levels increased slightly after the 6 h treatment as well. The Ca levels of the Daeshim and Shimgang cultivars after the 6 h treatment increased to 304.65 mg/100 g and 302.1 mg/100 g, respectively. The recommended daily calcium intake for adults ranges from 1000 mg to 1500 mg. It is also recommended to take supplements with food to aid absorption. Compared with other metals, the calcium ion and most of its compounds have low toxicity [18].

However, irrigators, particularly those using drip-watering facilities, should be carefully supervised to avoid the temptation to over-irrigate. Over-irrigation depletes the root zone of much-needed oxygen, thus reducing both root growth and nutrient uptake and leading to a host of potential root disease problems. Therefore, the variations might be due to growth conditions and geographical variations; however, appropriate irrigation treatment methods such as water scheduling and volume will help increase fruit quantities and production for farmers' incomes. In addition, this study show that mulberry fruits are promising sources with high energy value and essential micronutrients such as K, Mg, Ca, and P. This will ensure dietary diversity and food security in different parts of the world.

### Identification of flavonoids and their content in fruits of the two cultivars

Mulberries contain various bioactive components, such as alkaloids and flavonoids [12]. Nine flavonoids from the mulberry fruit of the Daeshim and Shimgang cultivars were isolated and analyzed (Table 4). We identified nine flavonoids from mulberry fruit: Peak 1, quercetin 3-O-rutinoside-7-O-glucoside (morkotin A); Peak 2, quercetin 3,7-di-O-glucoside; Peak 3, quercetin 3-O-rutinoside (rutin); Peak 4, quercetin 3-O-glucoside (isoquercitrin); Peak 5, quercetin 3-O-(6″-O-malonyl) glucoside; Peak 6, kaempferol 3-O-rutinoside; Peak 7, kaempferol 3-O-glucoside; Peak 8, Kaempferol 3-O-(6″-O-malonyl) glucoside; and Peak 9, quercetin 3-O-(2″-O-malonyl) glucoside (morkotin C). In particular, quercetin 3-O-rutinoside-7-O-glucoside (morkotin A) and quercetin 3-O-(2″-O-malonyl) glucoside (morkotin C) were identified as new compounds and further research will be needed to purify these compounds and

Table 4. Flavonoids isolated from mulberry fruits of two Korean mulberry cultivars and their mass spectrometric data

| Aglycones | Peak no. | Individual flavonols (Retention Time) | MW | Retention Time(min) | Fragment ions (*m/z*) |
|---|---|---|---|---|---|
| Kaempferol (*m/z* 287)  | 7 | Kaempferol 3-*O*-glucoside (astragalin) | 448 | 16.07 | 471[M+Na]$^+$,449[M+H]$^+$,287 |
| | 8 | Kaempferol 3-*O*-(6″-*O*-malonyl)glucoside | 534 | 16.21 | 557[M+Na]$^+$,535[M+H]$^+$,287 |
| | 6 | Kaempferol 3-*O*-rutinoside (nicotiflorin) | 594 | 15.50 | 617[M+Na]$^+$,595[M+H]$^+$,449,287 |
| Quercetin (*m/z* 303)  | 4 | Quercetin 3-*O*-glucoside (isoquercitrin) | 464 | 14.01 | 487[M+Na]$^+$,465[M+H]$^+$, 303[quercetin+H]$^+$ |
| | 5 | Quercetin 3-*O*-(6″-*O*-malonyl)glucoside | 464 | 15.42 | 465[M+H]$^+$,303[quercetin+H]$^+$ |
| | 9 | Quercetin 3-*O*-(2″-*O*-malonyl)glucoside (morkotin C)* | 550 | 17.88 | 573[M+Na]$^+$,551[M+H]$^+$, 303[quercetin+H]$^+$ |
| | 3 | Quercetin 3-*O*-rutinoside (rutin) | 610 | 13.50 | 633[M+Na]$^+$,611[M+H]$^+$, 465,449,303 |
| | 2 | Quercetin 3,7-di-*O*-glucoside | 626 | 10.99 | 649[M+Na]$^+$,627[M+H]$^+$,465,303 |
| | 1 | Quercetin 3-*O*-rutinoside-7-*O*-glucoside (morkotin A)* | 772 | 10.25 | 795[M+Na]$^+$,773[M+H]$^+$, 627, 611, 465, 303 |

All samples analyzed in positive ion mode ([M+H]$^+$) using UPLC-DAD-QTOF/MS.
*New flavonoid identified in mulberry fruit.

evaluate their biological activity. Although we did not research this in the current study, anthocyanins, which are a group of naturally occurring phenolic compounds that are responsible for color attributes and biological activities such as antioxidant and antimicrobial properties, are the most important constituents of mulberry fruit [19-21]. In contrast, blueberries are a very popular fruit in Korea and have been known to have a high content of antioxidants. In a previous study on blueberries, it was reported that the flavonoids of blueberry fruit analyzed using HPLC were myricetin 3-arabinoside, quercetin 3-galactoside, quercetin 3-glucoside, delphinidin 3-galactoside, delphinidin 3-glucoside, cyanidin 3-galactoside, delphinidin 3-arabinoside, petunidin-3-galactoside, petunidin 3-glucoside, petunidin 3-arabinoside, malvidin 3-galactoside, malvidin 3-galactoside, and malvidin 3-arabinoside. Blueberry fruit contained four major anthocyanins: delphinidin, cyanidin, petunidin, and malvidin [22]. The malvidin-based anthocyanin content in blueberries was much higher than that of petunidin-, delphinidin-, or cyanidin-based anthocyanins [23]. In conclusion, mulberry fruits had various other flavonoids besides anthocyanins compared with blueberries' functional materials.

To determine the content of these flavonols, the content of nine flavonoids from the two cultivars were measured. Undoubtedly, flavonol glycosides are the second major type of mulberry polyphenols, with the content fluctuating from 55.62 to 432.38 mg kg$^{-1}$ fw in different species [24]. As shown in Table 5, the total flavonoid content from Daeshim and Shimgang was determined to be 132.9 mg and 36.3 mg, respectively, in the 6 h irrigation treatment. This difference might be In particular, quercetin 3-O-rutinoside (Peak 3) and quercetin 3-O-(6″-O-malonyl) glucoside (Peak 5) were the most common flavonols. It was reported that the major flavonoid compounds in the eight cultivars of mulberry fruits were quercetin (5.36-58.42 mg/100 g DW) and rutin (18.73-26.90 mg/100 g DW) [25]. Our study confirmed that the major flavonoid in mulberry fruits was quercetin 3-O-rutinoside (rutin), and the content of this compound in fruit was high. Additionally, in a previous study, it was reported that the total flavonoid amount from the Korean mulberry cultivar 'Suhyang' was determined to be 79.6 mg [26]. Blueberries contain flavonols including kaempferol, quercetin, isorhamnetin, myricetin, and syringetin aglycones. Isorhamnetin 3-O-robinobioside, kaempferol 3-O-(6″-O-acetyl) glucoside, quercetin, quercetin 3-O-arabinofuranoside (avicularin), quercetin 3-O-(6″-O-malonyl) glucoside, and quercetin 3-O-robinobioside were detected for the first time in blueberries [27]. Generally, it is known that rutin and isoquercitrin comprise the main flavonoids in mulberry fruit. Isoquercitrin is a natural flavonoid glucoside that is distributed in medicinal and dietary plants, such as vegetables, herbs, and flowers, and together with rutin, is one of the major glycosidic forms of the natural flavonol quercetin [28]. In the case of these two cultivars, quercetin 3-O-(6″-O-malonyl) glucoside was more abundant than isoquercitrin. Further studies will be

Table 5. Contents (mg/100g DW) of isolated 9 flavonoids in the mulberry fruit

| Peak No. | Individual flavonols/Treatment | Daeshim (mg/100 g) | Shimgang (mg/100 g) |
|---|---|---|---|
| 1 | Quercetin 3-*O*-rutinoside-7-*O*-glucoside (morkotin A) | 1.6 ± 0.2 | 0.6 ± 0.1 |
| 2 | Quercetin 3,7-di-*O*-glucoside | 0.6 ± 0.1 | 0.1 ± 0.0 |
| 3 | Quercetin 3-*O*-rutinoside (rutin) | 76.1 ± 5.1 | 24.8 ± 4.2 |
| 4 | Quercetin 3-*O*-glucoside (isoquercitrin) | 12.0 ± 0.5 | 3.0 ± 0.4 |
| 5 | Quercetin 3-*O*-(6″-O-malonyl)glucoside | 28.7 ± 2.4 | 5.2 ± 0.9 |
| 6 | Kaempferol 3-*O*-rutinoside (nicotiflorin) | 5.3 ± 0.3 | 0.5 ± 0.1 |
| 7 | Kaempferol 3-*O*-glucoside (astragalin) | 3.1 ± 0.4 | 1.3 ± 0.2 |
| 8 | Kaempferol 3-*O*-(6″-*O*-malonyl)glucoside | 4.1 ± 0.5 | 0.3 ± 0.1 |
| 9 | Quercetin 3-*O*-(2″-*O*-malonyl)glucoside (morkotin C) | 1.4 ± 0.1 | 0.5 ± 0.1 |
| Total | | 132.9 ± 9.0 | 36.3 ± 6.1 |

*Each value calculated as means ± SD of three replicates using internal standard (galangin).

needed to evaluate the biological activity of quercetin 3-O-(6″-O-malonyl) glucoside.

In this study, we managed to change the properties of two cultivars based on irrigation treatments. These variations might be due to the growth conditions and geographical variations, but the amounts of mulberry fruit, minerals, and flavonoid content increased using appropriate irrigation treatments. Further research will be devoted to evaluate their biological activity. In conclusion, various methods of cultivation such as irrigation and fertilization will promote the development of rich minerals and functional materials. It will be interesting to conduct more in-depth research on the leaves so that consumers can benefit from them as a food additive or in nutraceutical and biopharmaceutical industries. In addition, this study provides a possible industrial use of mulberry fruit, and holds promise to enhance the overall profitability of sericulture.

## Note

The authors declare no conflict of interest.

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