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**Research Article**

# Analysis of Changes in the Phytochemical Content of Tartary Buckwheat Flowers and Seeds during the Post-flowering Growth

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## ABSTRACT

Buckwheat (*Fagopyrum esculentum*), which is a traditional Korean crop, has been known as a health food due to its rich nutrition. This study was conducted to evaluate the change in flavonoid content of flowers and seeds during post-flowering growth of Korean tartary buckwheat variety 'Hwanggeummiso', with the aim of providing basic data for the development of functional food and feed additive. Tartary buckwheat took 69 and 99 days from the sowing date to reach the flowering and maturity stages, respectively. As a result of examining the flavonoid components of each part of tartary buckwheat, chlorogenic acid, rutin, and isoquercitrin of flowers increased from the flowering period on 22 May (0 days after flowering) to 42 days after flowering, while quercetin increased until 21 days after flowering and then decreased thereafter. In seeds, chlorogenic acid, rutin, and isoquercitrin were most abundant at the time of seed-bearing on 14 days after flowering, and showed a decreasing tendency thereafter. On the other hand, quercetin showed a tendency to increase until 21 days after flowering and then decrease. Overall, the flavonoid content was higher in flowers than in seeds, with rutin being particularly prominent. Based on this, the possibility as food materials and feed additives was confirmed using buckwheat produced in Korea.

(**Key words**: Buckwheat, *Fagopyrum esculentum, Fagopyrum tataricum,* Flavonoid, Rutin)

## Ⅰ. INTRODUCTION

Buckwheat, a member of the *Fagopyrum* genus in the Polygonaceae family, comprises 21 known species, serving various purposes such as fodder, medicinal use, and green manure, and has recently gained prominence as a versatile food crop (Huda et al., 2021). Buckwheat boasts remarkable ecological adaptability, thriving even in harsh climates, and ensuring stable production. Two primary cultivated species, *Fagopyrum esculentum* Moench (common buckwheat) and *Fagopyrum tataricum* Gaertn (Tartary buckwheat), are widely grown worldwide, with common buckwheat primarily cultivated in regions like Europe, the United States, Canada, Brazil, South Africa, Australia, Japan, China (North and Central), and South Korea. In contrast, tartary buckwheat finds its niche in the mountainous areas of southwestern China, northern India, Bhutan, Nepal, among others (Huda et al., 2021). Although Tartary buckwheat has been traditionally used for centuries in traditional medicine and health products in China and India, it has been less popular than common buckwheat due to its higher husk content, shattering, and bitter taste. However, recent research has shed light on the superior flavonoid content and physiological activity of tartary buckwheat compared to common buckwheat, validating its potential as a source for pharmaceutical

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and nutraceutical materials (Kumar and Pandey, 2013). As of 2020, global buckwheat production reached approximately 4 million tons, with increasing attention and demand driven by the revelation of various bioactive compounds, including flavonoids, present abundantly in buckwheat (Zhu, 2021).

The feeding value of buckwheat can be influenced by various factors, including intake, chemical composition, and nutrient availability. Notably, the presence of elevated phytochemical content in buckwheat may introduce variability when incorporating it into ruminant diets, potentially resulting in diminished intake or compromised performance (Salem et al., 2014). Nevertheless, the judicious utilization of feed sources abundant in total phenolics can exert a beneficial impact on the quality of ruminant products by augmenting the phenolic content in both meat and milk (Kotsampasi et al., 2014; Kuhnen et al., 2014). This facet carries particular significance as phytochemical originating from botanical sources manifest robust antioxidant properties, effectively fortifying cellular defenses against oxidative damage and thereby mitigating the risk of various health ailments, notably those associated with cancer. Holasova et al. (2002) conducted research that revealed a significant correlation ($R^2$ = 0.99) between the total phenolic content in buckwheat and its antioxidant activity. Consequently, the incorporation of forage buckwheat into ruminant diets possesses the potential to yield heightened product quality, rendering it a promising dietary selection.

Polyphenolic compounds, flavonoids, and anthraquinones are regarded as the most important phytochemicals found in buckwheat seeds (Zhu, 2016). Prominent flavonoids like rutin, quercetin, and quercitrin are known to be present in buckwheat seeds, with recognized physiological effects such as antioxidant, anticancer, antihypertensive, antidiabetic, antimicrobial, anti-inflammatory, and neuroprotective properties. Rutin, the major flavonoid component constituting 70-85% of the total flavonoids in buckwheat, exhibits particularly high concentrations in tartary buckwheat compared to common buckwheat. Similarly, quercetin content is also higher in Tartary buckwheat than in common buckwheat (Borovaya and Klykov, 2020; Yao et al., 2020). Various flavonoids, approximately 20 different types, have been isolated and confirmed in different parts of Tartary buckwheat, including seeds, leaves, sprouts, and flowers, along with the detection of a limited number of anthraquinones in the seeds (Kim et al., 2008; Peng et al., 2013; Ren et al., 2013; Wu et al., 2015). Beyond flavonoids, buckwheat cultivars contain a spectrum of other bioactive compounds such as phenolic acids, tannins, alkaloids, triterpenoids, organic acids, steroids, and stilbenes, contributing to its status as an excellent health food.

As of July 2023, the Korea Seed & Varieties Service (KSVS) has only registered three common buckwheat varieties (Dayon, Sunbaek, and Takane Ruby 2011) and one tartary buckwheat variety (Hwanggeummiso) under plant variety protection in South Korea. Thus, through this research, we aim to evaluate the changes in the content of valuable components in Tartary buckwheat, specifically variety Hwanggeummiso, according to its growth stage and plant part. Therefore, in this study, our objective is to evaluate the phytochemicals present in Korean tartary buckwheat. This evaluation provides a basis

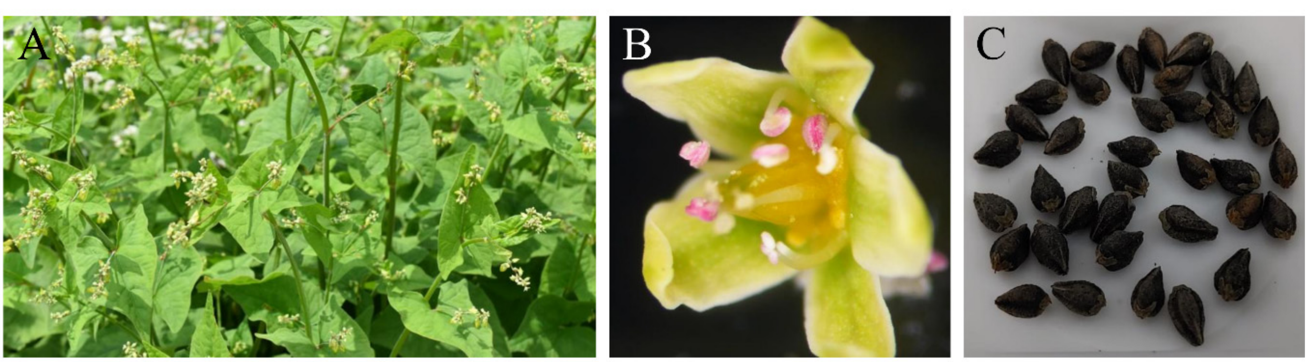


Fig. 1. Morphology of tartary buckwheat (*Fagopyrum tataricum*) cultivar 'Hwanggeummiso'. Plant, flower, and seed of tartary buckwheat. Plant (A) depicts the entire plant during the flowering season, flower (B) illustrates the morphology of the flower at 0 DAF (days after flowering), when the initial sampling occurred, and seed (C) depicts the morphology of the seed at 42 DAF, representing full maturity.

for exploring the potential of tartary buckwheat as a functional material and feed additive.

## Ⅱ. MATERIALS AND METHODS

### 1. Cultivation and characterization of Tartary Buckwheat

This study was conducted using the tartary buckwheat variety ‘Hwanggeummiso’ developed by the National Institute of Crop Science (NICS), Rural Development Administration (RDA) (Fig. 1). In 2020, tartary buckwheat seeds harvested in 2019 from the NICS Central Crop Research Farm located in Seodun-dong, Suwon, Gyeonggi-do, South Korea (37.26234 N, 126.99206 E) were sown on March 14, 2020, using a seed drill (40 cm row spacing, 0.4 kg/ha). From May 22 (0 days after flowering, DAF), samples were collected at 7-day intervals until June 29 (42 DAF), resulting in a total of 7 sampling occasions, during which flowers and seeds were separated. The separated samples were freeze-dried at -70°C for 96 hours and then ground using a grinder (BL142AKR, Tefal, France). To remove large particles and debris and achieve a powder with a uniform particle size, the ground sample was sieved through a 20-mesh sieve and then used as the analytical sample. Agricultural characteristics evaluation of tartary buckwheat resources included measurements of plant height, stem diameter, main stem nodes, branching ratio, seed count, fresh weight, dry weight, and 100-grain weight, following the guidelines of the RDA (2012).

### 2. Extraction methods

For the extraction of bioactive compounds from tartary buckwheat flowers and seeds, 0.1 g of ground sample was mixed with 10 mL of 70% methanol and subjected to ultrasonication extraction (UE), shaking extraction (SE), and combined extraction (ultrasonication followed by shaking extraction, UESE) (Table 1). Ultrasonication extraction was performed by placing the sample and solvent in a 5 mL Eppendorf tube and extracting for 5 min using an ultrasonic bath (WUC-D10H, Daihan scientific Co., Gangwon, Korea) filled with water at 50 Hz. Shaking extraction was carried out at room temperature using a vortex mixer (VIBA X. 30V, Collomix, Gaimersheim, Germany) for 5 min. Combined extraction involved performing ultrasonication extraction followed by shaking extraction.

**Table 1. The conditions for flavonoid extraction from tartary buckwheat as a pre-treatment step for UPLC analysis**

| Extraction method | Abbreviation | Number of extraction step | Extraction time (min) |
|---|---|---|---|
| Ultrasonification extraction | UE | 1 | 5 |
| Shaking extraction | SE | 1 | 5 |
| Ultrasonification after shaking extraction | UESE | 2 | 10 |

**Table 2. UPLC analysis conditions for flavonoids in tartary buckwheat**

| Model | Acquity UPLC I-Class, Waters Co., USA |
|---|---|
| Wavelength | 254 nm |
| Column | Acquity UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm) |
| Injection volume | 2 μL |
| Column Temp. | 35℃ |
| Mobile phase | Solvent A: 0.1% formic acid in water Solvent B: 0.1% formic acid in acetonitrile |
| Flow rate | 0.3 mL/min |
| Gradient condition | 0.0-2.0 min, solvent B 10%→10%<br>2.0-3.0 min, solvent B 10%→20%<br>3.0-5.0 min, solvent B 20%→20%<br>5.0-6.0 min, solvent B 20%→30%<br>6.0-8.0 min, solvent B 30%→30%<br>8.0-9.0 min, solvent B 30%→40%<br>9.0-10.0 min, solvent B 40%→40%<br>10.0-10.1 min, solvent B 40%→90%<br>10.1-11.0 min, solvent B 90%→90%<br>11.0-11.1 min, solvent B 90%→10%<br>11.1-13.0 min, solvent B 10%→10% |

### 3. Sample preparation and analytical conditions for flavonoid content analysis

To quantify flavonoid content in ground tartary buckwheat seeds and flowers, 0.1 g of sample was weighed and placed in a 50 mL conical tube. Then, 10 mL of 70% methanol was added, and the mixture was extracted for 1 hour. After extraction, the samples were filtered through a syringe filter (0.45 ㎛, advantec, Japan) and used as analytical samples. Flavonoid quantification was carried out using a Waters Acquity UPLC system (Acquity UPLC I-Class, Waters Co., USA) equipped with a UV detector, and the instrument parameters are presented in Table 2. Standard substances, including chlorogenic acid, rutin, isoquercitrin, and quercetin, were purchased from ChemFaces (Wuhan, Hubei, China). Standard solutions for each compound were prepared at concentrations of 1, 5, 10, 25, 50, and 100 ㎍/mL. Quantification curves were generated by analyzing the standard solutions using UPLC to calculate regression equations and coefficients of determination (r). In the regression equation (y=ax+b), y represents the peak area, x is the concentration of the standard solution, a is the slope, and b is the intercept. The flavonoid content in tartary buckwheat was quantified and expressed as mg/kg of dried weight (DW).

### 4. Statistical analysis

All analyses were performed in triplicate, and results are presented as mean ± standard deviation. Basic statistics and one-way analysis of variance (One-way ANOVA) were conducted using JAMOVI v1.2.27 (jamovi.org).

## Ⅲ. RESULTS AND DISCUSSION

### 1. Growth characteristics of tartary buckwheat variety ‘Hwanggeummiso’

Hwanggeummiso, a Korean tartary buckwheat variety, is known for its erect, medium-late type with long stems, and it has more main stem nodes and branching ratios than common buckwheat. Generally, buckwheat is cultivated in both spring and autumn, with the flowering period of tartary buckwheat reported to be from early to mid-June during spring cultivation, which is about 10 days later than common buckwheat (Yao et al., 2006). The agronomic characteristics of buckwheat were investigated in accordance with RDA (2012) and based on the timing of the investigation. In this study, it took 69 days from sowing on March 14, 2020, to the flowering stage. Growth characteristics related to yield included plant height (135 ± 2.0 cm), stem diameter (6.7 ± 0.1 mm), main stem nodes (14.7 ± 0.9), branching ratio (4.1 ± 0.3), seed count (122.5 ± 9.9), fresh weight (28.9 ± 0.9 g), dry weight (5.9 ± 0.5 g), and 100-grain weight (20.9 ± 2.5 g), as shown in Table 3. Buckwheat is known to exhibit differences in yield-related growth characteristics between spring and autumn cultivation, with higher growth characteristics associated with better yield in spring cultivation due to active vegetative growth (Yao et al., 2006). Hwanggeummiso is a crop that can be cultivated twice a year, in both spring and autumn, which is advantageous for integrating it into cropping systems with other crops. Its yield is approximately 21% higher than that of the existing buckwheat variety Yangjul, thereby contributing to increased farmers' productivity. Additionally, it can be mechanically harvested due to its resistance to falling in the rain and wind, and it can be cultivated in an eco-friendly manner because of its strong powdery mildew resistance. Livestock farmers can set up cropping systems that turn corn and buckwheat into silage, and mechanisation can increase production efficiency. Moreover, its robust disease resistance allows for reduced pesticide use, making a significant contribution to preserving the

**Table 3. Growth characteristics of tartary buckwheat**

| Characteristics | Values |
|---|---|
| Plant height (cm/plant) | 135±2.02 |
| Stem diameter (mm/plant) | 6.7±0.1 |
| No. of node (no./plant) | 14.7±0.9 |
| No. of branch (no./plant) | 4.1±0.3 |
| No. of seed (no./plant) | 122.5±9.9 |
| Fresh weight (g/plant) | 28.9±0.9 |
| Dry weight (g/plant) | 5.9±0.5 |
| 1000-seed weight (g) | 20.9±2.5 |

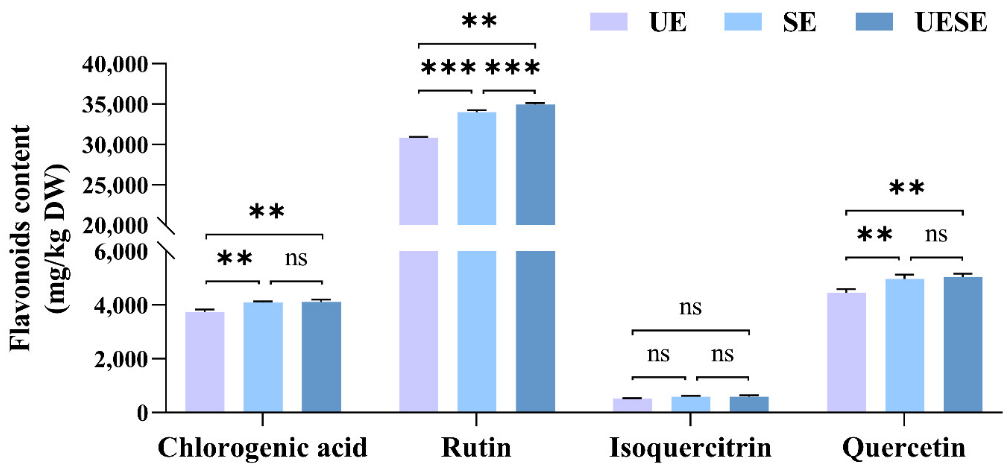


Fig. 2. Comparison of three extraction methods for determining flavonoid contents in tartary buckwheat. All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation. Differences in flavonoid content were analyzed by one-way ANOVA at $p < 0.05$. Differences in flavonoid content between extraction methods were determined using Tukey's honestly significant difference post hoc test. The abbreviations UE, SE, and UESE stand for ultrasonification extraction, shaking extraction, and ultrasonification after shaking extraction, respectively.

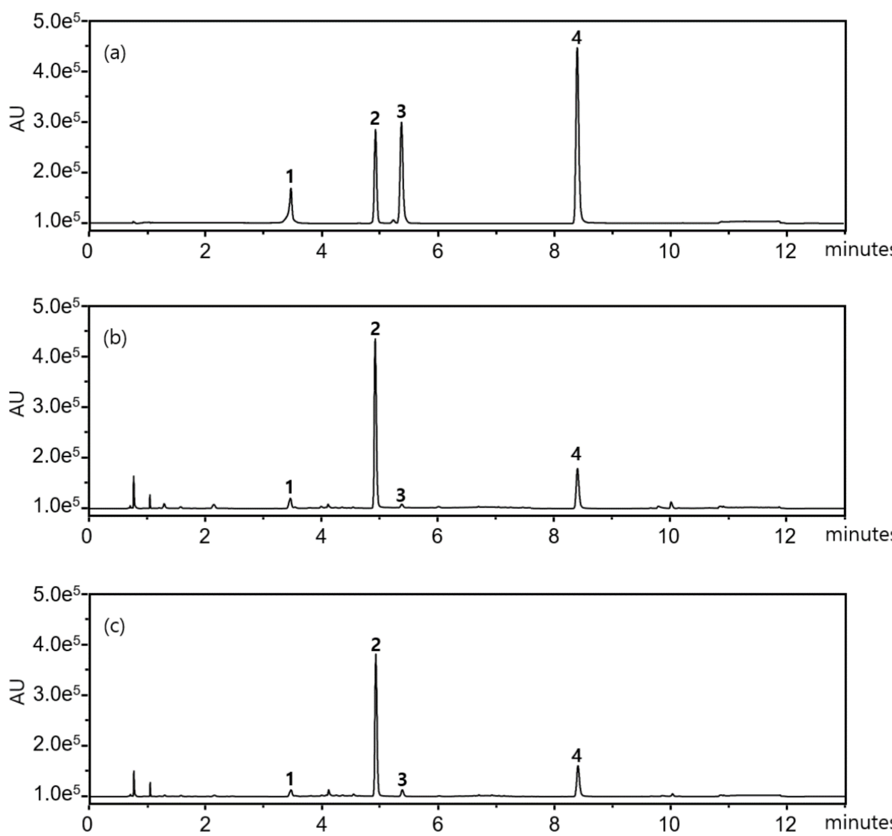


Fig. 3. UPLC Chromatogram of chlorogenic acid, rutin, isoquercitrin and quercetin in flower and seed extracts. (a) Standard (chlorogenic acid, rutin, isoquercitrin, quercetin), (b) Tartary buckwheat flower, and (c) Tartary buckwheat seed. In the chromatogram, 1, 2, 3, and 4 represent chlorogenic acid, rutin, isoquercitrin, and quercetin, respectively.

agricultural environment and promoting sustainable agriculture.

## 2. Comparison of Flavonoid Content in Tartary Buckwheat Seeds According to Extraction Methods

The major flavonoids in tartary buckwheat seeds include rutin, orientin, isoorientin, vitexin, isovitexin, and quercetrin. Rutin is particularly abundant in tartary buckwheat seeds (Almuhayawi et al., 2021). While ambient temperature stirring extraction is commonly used as an extraction method for bioactive compounds, it has the drawback of varying extraction efficiency depending on the extraction time and indoor conditions (Park et al., 2005a; Kim et al., 2010; Kim et al., 2013). Therefore, this study compared and analyzed the contents of chlorogenic acid, rutin, isoquercitrin, and quercetin in tartary buckwheat seeds using three different extraction methods: ambient temperature stirring extraction, ultrasonication extraction, and combined extraction (ultrasonication followed by stirring extraction) (Fig. 2). Among the four flavonoids in tartary buckwheat seeds, rutin content was the highest. The UESE yielded the highest rutin content at 34,930.0 ± 172.0 mg/kg DW, while isoquercitrin showed no statistically significant differences between the three extraction methods at $p < 0.05$. UE yielded the lowest extraction efficiency among the three methods, with chlorogenic acid, rutin, isoquercitrin, and quercetin content being approximately 9.2%, 11.8%, 12.5%, and 11.5% lower, respectively, compared to the UESE. SE resulted in chlorogenic acid, rutin, isoquercitrin, and quercetin content approximately 0.4%, 2.7%, 0.5%, and 1.4% lower, respectively, than the UESE. In conclusion, the UESE following ultrasonication was confirmed as an effective extraction method for analyzing chlorogenic acid, rutin, isoquercitrin, and quercetin in tartary buckwheat seeds.

## 3. Changes in flavonoid content in tartary buckwheat flowers and seeds after flowering

To compare the flavonoid content in different parts (flowers and seeds) of tartary buckwheat at different growth stages, four types of flavonoids (chlorogenic acid, rutin, isoquercitrin, and quercetin) were analyzed, and the chromatogram is shown in Fig. 3. The retention times for each component were in the order of chlorogenic acid, rutin, isoquercitrin, and quercetin. Rutin was found to be the most abundant flavonoid in both flowers and seeds.

Flowers were sampled at 7-day intervals from May 22 (0 days after flowering, DAF) to June 29 (42 DAF), and the flavonoid contents were examined (Fig. 4). The highest contents

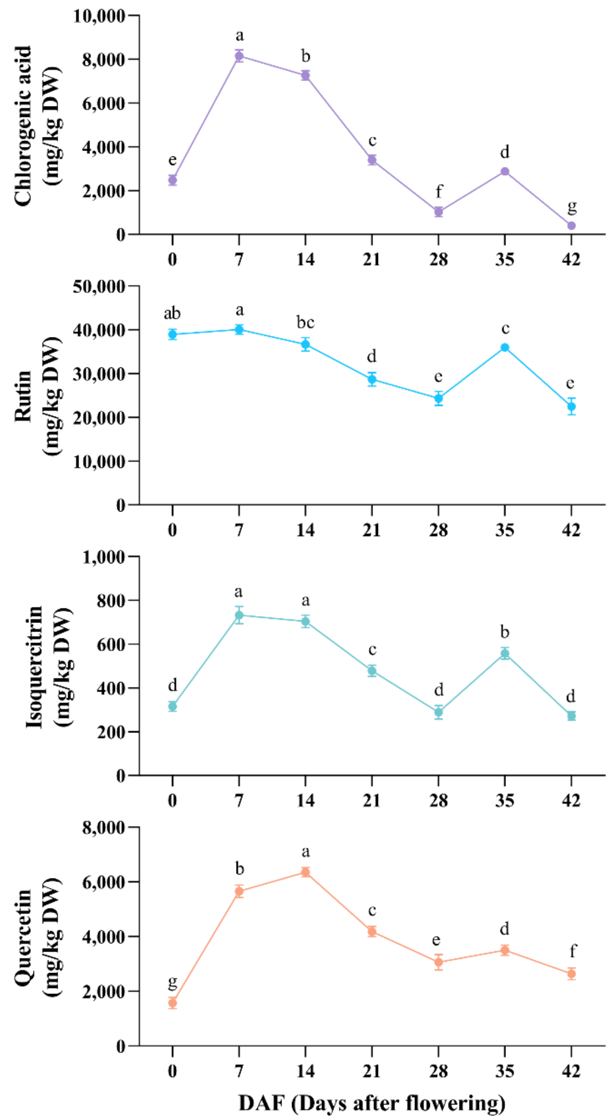


Fig. 4. Comparison of flavonoid content in tartary buckwheat flower during the post-flowering growth. The experiments were performed in triplicate and the values are presented as mean ± standard deviation. Flavonoid contents were assessed by one-way ANOVA at a significance level of $p < 0.05$. Additionally, differences in the flavonoid content of flowers, concerning post-flowering growth, were determined using Tukey's honestly significant difference post hoc test, and these differences are indicated by different letters.

of chlorogenic acid (8,151.8 ± 279.8 mg/kg DW), rutin (40,061.5 ± 1,084.2 mg/kg DW), and isoquercitrin (732.5 ± 39.0 mg/kg DW) were observed at 7 DAF, while quercetin showed the highest content at 14 DAF, with 6355.5±170.5 mg/kg DW. The decrease in flavonoid content after 7 days from flowering is attributed to the development of seeds, which is closely related to flavonoid content (Park et al., 2005b). Flavonoid content varied from 1.8-fold to 6.2-fold throughout the growth stages, with rutin showing the least variation at 1.8-fold and chlorogenic acid exhibiting the largest variation at 6.2-fold.

Seeds were sampled at 7-day intervals from June 1 (14 DAF), the period when seeds start forming, and the flavonoid content was analyzed (Fig. 5). Rutin content in tartary buckwheat seeds is generally known to be more than 100 times higher than that in common buckwheat, which contains only 130–150 mg/kg (Yoon et al., 2006; Kim et al., 2020). In this study, the highest content of chlorogenic acid, rutin, isoquercitrin, and quercetin in tartary buckwheat seeds occurred at 14 DAF, with values of 4941.4 ± 182.1, 29,171.9 ± 1,007.0, 1,199.8 ± 217.9, and 4,263.6 ± 181.8 mg/kg DW, respectively. The decrease in flavonoid content after 14 DAF is related to the expression of genes involved in flavonoid synthesis, such as phenylalanine ammonium lyase, chalocone synthase, chalcone isomerase, and flavonol synthase, which are known to be associated with the changes in rutin content during different growth stages (Li et al., 2010; Li et al., 2012). Notably, rutin and chlorogenic acid contents in both flowers and seeds increased after 35 DAF and then decreased. The increase in flavonoid content just before full maturation of seeds is indicative of its nutritional and medicinal value, as reported in the literature (Song et al., 2016). Seeds consist of embryos and endosperms, and as seeds mature, flavonoids accumulate, with embryos containing more flavonoids than other parts (Steadman et al., 2001). Rutin, in particular, constitutes about 10% of the total nutrients in mature buckwheat seeds and plays an important role in reducing UV-B damage by absorbing ultraviolet radiation (Bieza and Lois, 2001).

In livestock, rutin and quercetin exhibit slightly different yet positive effects. The supplementation of rutin in dairy cow feed tends to increase milk production and enhance feed digestibility (Cui et al., 2015). On the other hand, quercetin plays a role in inhibiting the growth of parasites and bacteria (Dupuy et al., 2003). Furthermore, the antimicrobial activity of quercetin is potentiated in the presence of rutin (Arima et al., 2002). From a nutraceutical perspective, De Feo et al. (2006) demonstrated that rutin sourced from forage plants is partially excreted in goat milk. This suggests an opportunity to utilize buckwheat-based feed to introduce flavonoids into the concentrate production chain, benefiting foods that would not otherwise

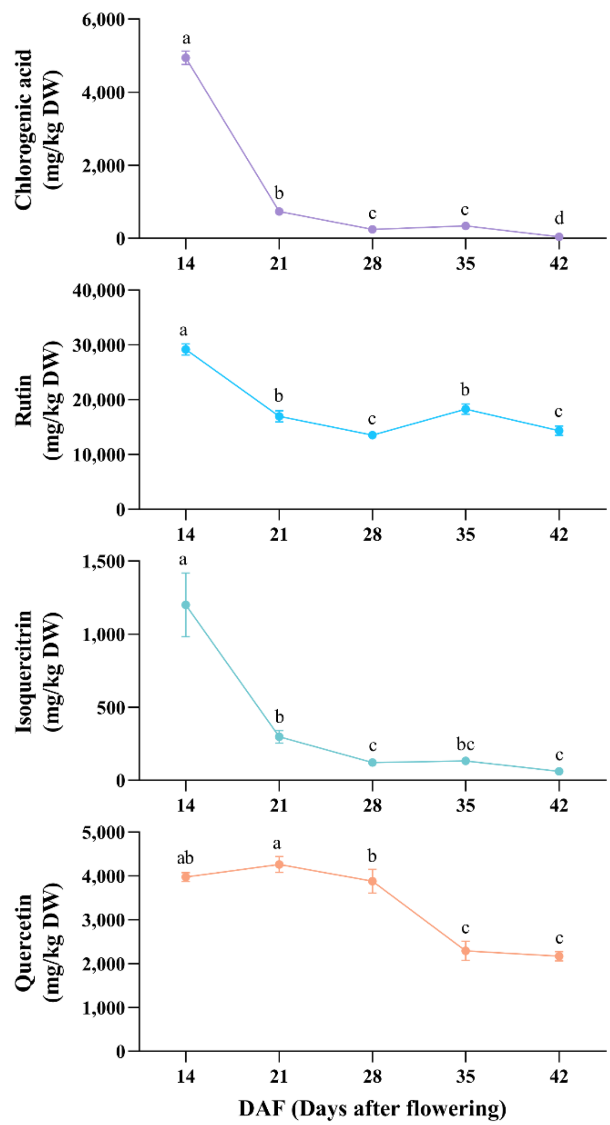


Fig. 5. Comparison of flavonoid content in tartary buckwheat seeds during the post-flowering growth. Flavonoid content was analyzed in triplicate, and the values are expressed as mean±standard deviation. Differences in flavonoid content between seed ripening stages were analyzed by one-way ANOVA, and post hoc testing was conducted using Tukey's honestly significant difference. Different letters indicate significant differences at $p < 0.05$.

contain them.

The process of extracting flavonoids, such as rutin, from various parts of the plant reveals an intriguing interplay between natural composition and production efficiency. In this study, we discovered that flavonoids, particularly rutin, are significantly more abundant in flowers compared to seeds. This disparity serves as the foundation for selecting the most suitable source for flavonoid extraction. However, the narrative becomes more intricate when we consider the practical aspects of extracting flavonoids from harvested flowers. Efficiency and productivity become paramount considerations. While flowers captivate with their rich flavonoid content, the task of separating and trimming them from the plant can be exceptionally inefficient, particularly when the yield of flavonoid compounds from a large quantity of flowers may not be substantial. From an efficiency and productivity perspective, compounds derived from seeds may outperform the initial allure of flavonoids derived from flowers. However, if tartary buckwheat is to be utilized as a feed additive, the most efficient approach may involve harvesting the entire plant at 14 DAF when the flavonoid content, including rutin, reaches its peak.

## Ⅳ. CONCLUSIONS

The various flavonoids in buckwheat not only have a positive impact on humans but also render it a valuable feed ingredient for ruminant cattle (Kälber et al., 2011; Mariotti et al., 2015). Overall in this study, it was observed that the flavonoid content in flowers and seeds of tartary buckwheat varied significantly. In flowers, chlorogenic acid, rutin, isoquercitrin, and quercetin were found to be 0.2 to 2.9 times more abundant than in seeds. Regarding the distribution of flavonoids by component, rutin constituted the highest percentage in flowers at 80.2%, followed by quercetin, chlorogenic acid, and isoquercitrin. In seeds, rutin accounted for the highest percentage at 78.9%, followed by quercetin, chlorogenic acid, and isoquercitrin. Flavonoid content in different parts of tartary buckwheat showed variations depending on the growth stage, with the highest content observed in flowers at 7 DAF and in seeds at 14 DAF.

Generally, tartary buckwheat seeds are commonly used for food processing. However, this research confirmed the presence of various flavonoids, including rutin, in high amounts not only in seeds but also in flowers. The findings suggest that tartar buckwheat, particularly the flowers and seeds of the Korean variety known as Hwanggeummiso, hold great potential for use as ingredients in food and medicine, as well as additives for feed crops.

## Ⅴ. ACKNOWLEDGEMENTS

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