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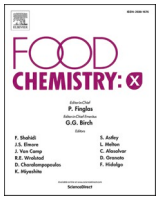

# Elucidation of phenolic metabolites in wheat seedlings (*Triticum aestivum* L.) by NMR and HPLC-Q-Orbitrap-MS/MS: Changes in isolated phenolics and antioxidant effects through diverse growth times

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ABSTRACT

The current research was characterized on phenolic metabolite profile including six chemical structures (phenolic acid, luteolin, orientin, apigenin, isoscoparin, and tricin) in wheat seedlings using HPLC-Q-Orbitrap-MS/MS and NMR techniques. Our study was also was the first to demonstrate fluctuations of isolated nine phenolic contents and antioxidant properties in various cultivars of this species with different growth times. The antioxidant abilities differed significantly in the 80 % methanol extracts (600 μg/mL) according to cultivar and growth time, with the highest average activities (DPPH: 82 %; ABTS: 87 %) observed after 7 days. The isolated nine compositions exhibited considerable differences in cultivars and growth times, specifically, isoorientin (**6**) and isochaftoside (**8**) were observed the most abundant average contents (99.3; 64.3 mg/100 g), representing approximately 28.3 and 18.3 % (total content: 350.8 mg/100 g). Their total phenolics showed the highest rates (420.8 mg/100 g) at 7 days, followed by 9 → 5 → 12 → 14 days with 374.6 → 366.7 → 350.7 → 241.1 mg/100 g, as the rank orders of antioxidant effects. These findings suggest that wheat seedlings may be a potent source of functional agents.

## Introduction

Natural metabolites have increased in popularity owing to their health functions and valuable nutrients during the past years (Deseo, Elkins, Rochfort, & Kitchen, 2020; Hwang et al., 2021; Ra et al., 2020; Sahu, Kundu, & Sethi, 2021; Zhang et al., 2015). In secondary metabolites, including phenolic compounds, terpenoids, anthocyanins, and alkaloids are commonly distributed in crops, fruits, vegetables, and food products (Celli, Pereira-Netto, & Beta, 2011; Dai, Hu, Li, Yan, & Zhang, 2015; del Baño et al., 2003; Ha et al., 2021). These metabolites are essential in protection against insects, microbes, and herbivores (Thakur, Bhattacharya, Khosla, & Puri, 2019), in addition to environmental factors (Chen et al., 2019; Ramakrishna & Ravishankar, 2011; Tsao, Papadopoulos, Yang, Young, & Mcrae, 2006), and secondary metabolites occur in plants that are subjected to elicitor, stress, and microbial factors (Hwang et al., 2021; Lee et al., 2015; Ramakrishna & Ravishankar, 2011). Phenolic compounds are one of the largest groups of secondary metabolites (Jaiswal, Műller, Műller, Karar, & Kuhnert, 2014; Kowalska, Pecio, Ciesla, Oleszek, & Stochmal, 2014; Li, Xiong, Ying, Cui, Zhu, & Li, 2006; Piasecka, Sawikowska, Krajewski, & Kachlicki, 2015; Wojakowska, Perkowski, Góral, & Stobiecki, 2013), and these substances are increasingly of interest in food and pharmaceutical applications (Teixeira et al., 2020; Xie et al., 2019; Zhang et al., 2015). These functional nutrients, which are chemically defined as phytochemicals containing at least one hydroxylated aromatic ring (Celli, Pereira-Netto, & Beta, 2011; Nørbæk, Brandt, & Kondo, 2000; Ha et al., 2021), are mainly classified as flavonoids (flavones, flavanones, and flavonols) and non-flavonoids (phenolic acid, stilbenes, phenolic alcohols) (Bai et al., 2017; Deseo, Elkins, Rochfort, & Kitchen, 2020; Jaiswal et al., 2014; Kim et al., 2018; Kim, Yang, Cho, & Lee, 2020). Moreover, these phytochemicals display human health-promoting properties such as antioxidant, anti-aging, antidiabetic, anticancer,

*Abbreviations:* DPPH, 2,2-diphenyl-1-pycrylhydrazyl;; ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); NMR, nuclear magnetic resonance; HPLC, high performance liquid chromatography; HPLC-Q-Orbitrap-MS, high performance liquid chromatography coupled with quadrupole Orbitrap mass spectrometry.

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antiviral, and cholesterol-lowering effects. (Sahu et al., 2021; Shewry, & Hey, 2015; Teixeira et al., 2020; Xie et al., 2019; Zhang et al., 2015). In addition, the profiles and concentrations of phenolic metabolites in metabolite-rich foods and their products exhibited remarkable differences according to climate, genetics, soil, farming method, processing skill, and microorganism (Escudero-López et al., 2014; Lee et al., 2015; Lee et al., 2016; Ramakrishna, & Ravishankar, 2011; Tian, Chen, Tilley, & Li, 2021). Based on these considerations, numerous researchers recently focused on the appropriate factors influencing the strong functional abilities and high phenolic contents of crops. Among diverse materials, wheat (*Triticum aestivum* L.) is one of the most valuable crops due to its effects in the human diet, including the reduction of diabetes, cardiovascular disease, cancer, and obesity, in addition to a preventive effect against chronic inflammation (Chen et al., 2019; Gawlik-Dziki et al., 2016; Ra et al., 2020; Riaz et al., 2017; Sahu et al., 2021). This crop is also of considerable interest in the food industry and applications concerning the human diet because of its high protein and carbohydrate concentrations (Shewry & Hey, 2015). Remarkably, it is consumed as a staple component of bakery products owing to its characteristic proteins formed via the complexation of glutenin and gliadin protein subunits (Goesaert et al., 2005). The phenolic metabolites (flavonoids, phenolic acids, etc.) of wheat may display various pharmacological activities (Chen et al., 2019; Riaz et al., 2017; Sahu et al., 2021; Tian, Chen, Tilley, & Li, 2021), and the flavonoids, as the exhibiting are vital in preventing chronic human diseases, anticancer, antioxidant, antimicrobial, and enzyme inhibition capacities (Gawlik-Dziki et al., 2016; Goesaert et al., 2005; Kowalska, Pecio, Ciesla, Oleszek, & Stochmal, 2014). Although numerous studies have documented the excellent functional values of the metabolite compositions within this crop (Chen et al., 2019; Kowalska, Pecio, Ciesla, Oleszek, & Stochmal, 2014; Wojakowska, Perkowski, Góral, & Stobiecki, 2013), the exact phenolic profiles of wheat sprouts have still not been fully characterized. Unfortunately, to the best of our knowledge, the variations in the phenolic metabolite contents according to environmental factors, such as growth period are not previously reported. Little data are also available regarding the antioxidant ability as a function of the growth time of this crop. The germination processes of crop seeds are critical in increasing the phenolic concentrations (Chen et al., 2019; Jaiswal et al., 2014; Lee et al., 2016; Lee et al., 2021) and the benefits of wheat seedlings are attributed to their high policosanol contents (Ra et al., 2020). In our continuing investigation of metabolites and the benefits of wheat, the 80 % methanol extract exhibited high phenolic contents and strong radical scavenging abilities. Therefore, this study was designed to evaluate the phenolic contents related to the antioxidant capacities of wheat seedlings at different growth times.

The main objective of this study is to identify the phenolic profiles in wheat seedlings as well as compare their contents in developing times of diverse cultivars. Herein, we characterized phenolic metabolites in this source using high performance liquid chromatography coupled with quadrupole Orbitrap mass spectrometry (HPLC-Q-Orbitrap-MS) and nuclear magnetic resonance (NMR) spectroscopy. In addition, our work is the first to compare the profiles of nine isolated phenolics via chromatography, using various cultivars after different growth times to identify the potential functional source. This research also compares the degree of antioxidant properties against 2,2-diphenyl-1-pycrylhydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radicals using 80 % methanol extracts of this material following different growth times.

## Materials and methods

### *Plant source and chemical reagents*

Seventeen Korean wheat cultivars, namely Keumkang, Jokyoung, Shinmichal1, Baekjoong, Suan, Goso, Joah, Hojoong, Baekgang, Saekeumkang, Dabun, Jeokjoong, Sugang, Hanbaek, Dajoong, Jojoong, and Johan were used in this research. All wheat cultivars were planted in 2018 with artificial soil under a growth chamber. The growth conditions were performed out as follows: illumination intensity, 3000–5000 lx; humidity, 60–70 %; temperature, 16–20 °C; light → dark, 9 h → 15 h. The seedlings of all cultivars were harvested at five growth times according to their lengths in the same time as the following order after sowing: 1st harvest, 5 days (3–5 cm); 2nd harvest, 7 days (6–8 cm); 3rd harvest, 9 days (9–11 cm); 4th harvest, 12 days (12–14 cm); 5th harvest, 14 days (15–18 cm). The collected seedlings (Fig. 1A, 14 days after sowing) were air-dried under natural light temperature for 3 days at room temperature, and then freeze-dried at − 78 °C until analysis. The chemical reagents used in this research are described in the supplementary information (section 1).

### *Instruments*

The antioxidant properties were conducted using UV/Vis spectrophotometer (UV-1800 240 V, Shimadzu, Japan). The NMR data ($^{1}$H NMR at 600 MHz, $^{13}$C NMR at 150 MHz) were obtained on a JNM-ECZ600R spectrometer (JEOL, Tokyo, Japan) using deuterated solvents ($CD_3OD$ and DMSO-$d_6$). The HPLC system was performed by an Agilent 1200 series (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, an autosampler, a vaccum degasser, a thermal chamber controller, and a UV detector. The mass spectrometry was performed using an Ultimate 3000 LC system with a Q Exactive Focus quadrupole-Orbitrap mass spectrometer system (Thermo Fisher Scientific Inc., Waltham, MA, USA).

### *Extraction and isolation of phenolic metabolites*

The collected wheat seedlings were dried under natural light for three days and then ground using a milling machine (J World Tech. Seoul, Korea). The pulverized materials (2.0 kg, cv. Jeokjoong, 14 days after sowing) (Fig. 1A) were extracted with 80 % methanol (4L × 3) for 7 days. The combined extract was evaporated at 40 °C and the concentrated mixture (dark green gum, 180 g) was separated via column chromatography (CC) on silica gel (14 × 45 cm, 230–400 mesh, 980 g) using hexane–acetone [20:1 (1.5 L), 15:1 (1.5 L), 10:1 (1.5 L), 7:1 (1.5 L), 5:1 (1.5 L), 2:1 (1.5 L), and 1:1 (1.5 L)] and $CHCl_3$-$CH_3OH$ [10:1 (1.2 L), 8:1 (1.2 L), 5:1 (1.2 L), 3:1 (1.2 L), and 1:1 (1.2 L)] mixtures as well as methanol (2 L), to give 20 fractions (A–T). Fractions L–N (2.3 g) were fractionated using silica gel flash CC (3.5 × 50 cm, 230–400 mesh, 55 g), with a gradient of $CHCl_3$ to $CH_3OH$, resulting in 25 subfractions (Fr. LN 1–25). Subfractions Fr. LN 14–18, which were enriched with phenolic metabolites, were combined (520 mg) and further purified by silica gel CC (2.5 × 45 cm, 230–400 mesh, 15 g), eluting with $CHCl_3$-$CH_3OH$ (15:1 → 2:1) to gain phenolic **6** (27 mg, 5.2 %). Moreover, subfraction Fr. LN 7 (390 mg) was separated by silica gel CC (2.0 × 40 cm, 230–400 mesh, 13 g) with $CHCl_3$-$CH_3OH$ mixtures of increasing polarity (10:1 → 1:1) to afford phenolic **9** (20 mg, 5.1 %), based on a comparison with the thin layer chromatography (TLC) profile. Fraction K (1.6 g) was subjected to flash CC (3.0 × 40 cm, 230–400 mesh, 32 g), employing a gradient of $CHCl_3$ to $CH_3OH$, giving 16 subfractions (Fr. K 1–16). Subfractions Fr. K 6–8 (220 mg) were purified via further flash silica gel CC (1.5 × 30 cm, 230–400 mesh, 8 g) using a gradient of $CHCl_3$-$CH_3OH$ mixtures [10:1 (100 mL) → 7:1 (100 mL) → 5:1 (100 mL) → 3:1 (100 mL) → 1:1 (100 mL)] to yield phenolic **8** (41 mg, 18.6 %). Subfractions Fr. K 12–13 (125 mg) were purified using Sephadex LH-20 CC, eluting with 80 % $CH_3OH$ to afford phenolic **10** (26 mg, 20.8 %). Fraction I (945 mg) was repeatedly chromatographed over silica gel (3.0 × 35 cm, 230–400 mesh, 26 g) using a stepwise gradient of $CHCl_3$-acetone [20:1 (350 mL) → 16:1 (350 mL) → 12:1 (350 mL) → 8:1 (350 mL) → 5:1 (350 mL) → 3:1 (350 mL) → 1:1 (300 mL)], and then the UV active mixtures were evaporated. The combined phenolic source (435 mg) was purified by second flash silica gel column (2.0 × 30 cm, 230–400 mesh, 18 g) using $CHCl_3$-acetone mixtures [10:1 (150 mL) → 7:1 (150 mL) → 5:1

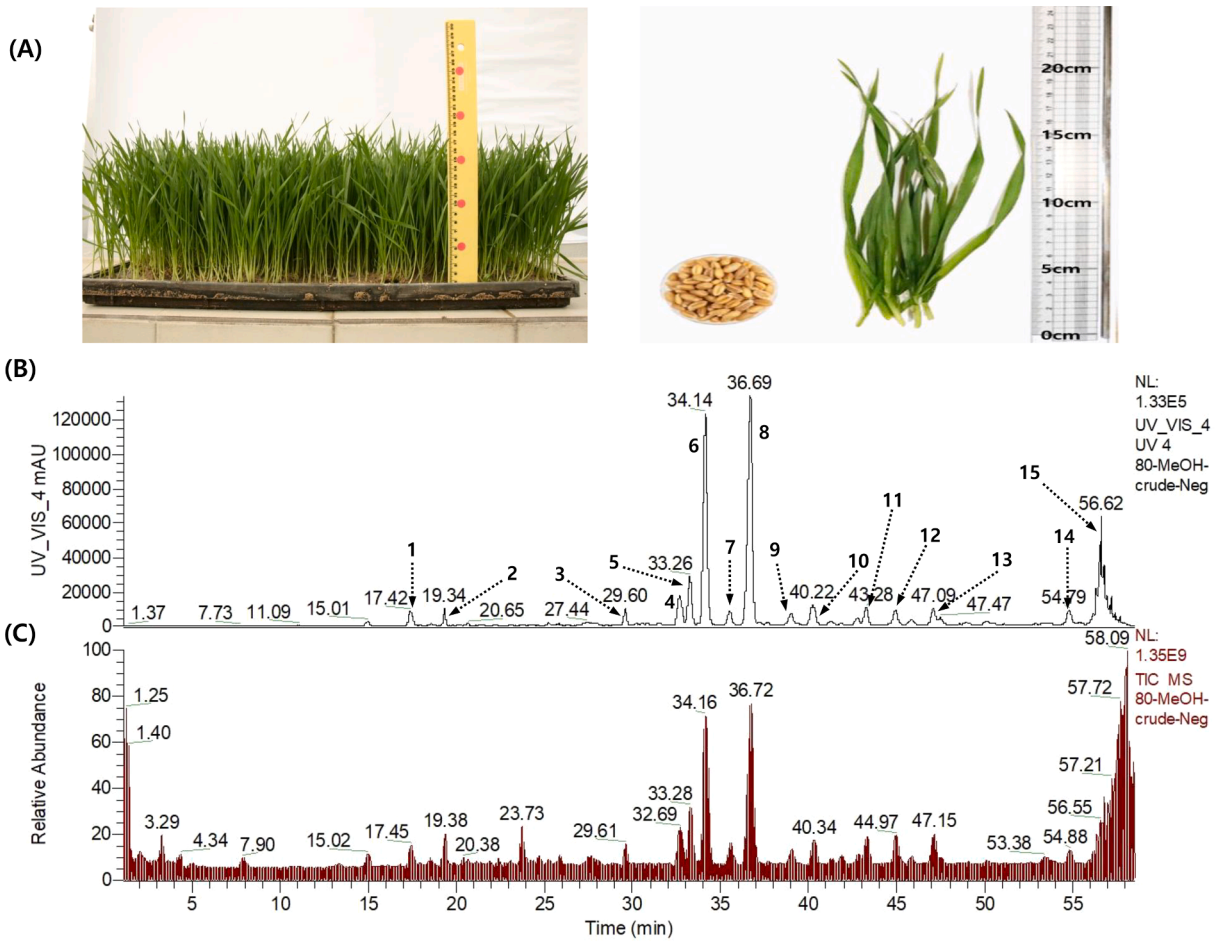


**Fig. 1.** Appearances of the wheat grains and seedlings (cv. Jeokjoong) through growth times of 14 days after sowing (A); (B) Typical chromatogram detected at 235 nm and (C) Total ion chromatogram of 15 phenolic metabolite compositions in the 80 % methanol extract of wheat seedlings by negative ion mode of HPLC-Q-Orbitrap-MS/MS.

(150 mL) → 3:1 (150 mL) → 1:1 (150 mL) → 1:2 (150 mL) → 1:1 (300 mL)] to yield phenolic metabolites **11** (21 mg, 4.8 %) and **12** (18 mg, 4.1 %). Fraction G (650 mg) was separated by silica gel CC (3.0 × 35 cm, 230–400 mesh, 25 g) with hexane–acetone mixtures of increasing polarity (12:1 → 8:1 → 5:1 → 3:1 → 1:1 → 1:2, each 250 mL) to afford 14 subfractions. Repeated chromatography of subfractions 9–13 (180 mg) was conducted with a silica gel column (1.8 × 35 cm, 230–400 mesh, 10 g) using hexane–acetone with a gradient of 7:1 → 1:2 (100 mL for each elution) to yield phenolic **15** (27 mg, 15.0 %). Fraction Q (890 mg) was subjected to silica gel CC (3.0 × 40 cm, 230–400 mesh, 23 g) using $CHCl_3$-$CH_3OH$ (16:1 → 1:2) to give 20 subfractions (1–20). Subfractions 17–20 (125 mg) were grouped and rechromatographed using a silica gel column (1.5 × 30 cm, 230–400 mesh, 10 g) using $CHCl_3$-$CH_3OH$ (8:1 → 1:2) to afford phenolics **2** (9 mg, 7.2 %) and **4** (11 mg, 8.8 %). HPLC yielded isolated phenolics with purities of > 95 %, and the individual chemical structure were elucidated by NMR spectroscopy.

### *NMR spectroscopy and HPLC-Q-Orbitrap-MS/MS condition for phenolic metabolites*

The nine isolated phenolic constituents were analyzed in $CD_3OD$ and DMSO-$d_6$ solvents with tetramethylsilane as internal standard using $^1H$ (600 MHz) and $^{13}C$ (150 MHz) NMR spectroscopy. Their data were documented as chemical shift values with coupling constants in Hertz. Moreover, the molecular weight using HPLC-MS/MS were examined by the HPLC coupled on-line with Q Exactive Focus quadrupole-Orbitrap mass spectrometer system. Phenolic separation was realized using a reverse phase column (C18, 100 × 2.1 mm, 2.7 μm, Halo, Advanced Materials Technology), and the mass spectra were measured between *m/z* 0 and *m/z* 1000 in the negative ion mode at a scan rate of 0.25 s/cycle. The other mass parameters are presented in the supplementary information (section 4).

### *Preparations of sample and calibration curve as well as HPLC conditions for quantification of phenolic phytochemicals.*

To investigate the phenolic metabolites contents, the dried wheat seedlings were ground using the milling machine for 5 min. The powdered sample (1.0 g) was extracted with 80 % methanol (20 mL) at 25 °C for 24 h in a shaking incubator, and the extract was then centrifuged for 5 min at 2000*g*. The crude extract was percolated with a syringe filter (0.45 μm, Whatman Inc., Maidstone, UK) prior to HPLC analysis, and then calibration curves was prepared using a previously reported method (Lee et al., 2015; Lee et al., 2016). The stock solutions (1000 μg/mL) of the isolated phenolics (standards) were made with DMSO and their curves were derived by comparing the peak areas obtained using nine different concentrations, namely 0.5, 1, 5, 10, 20, 50, 100, 200, and 500 μg/mL at 325 nm. The correlation coefficients ($r^2$) of all calibration curves exceeded 0.997 and the phenolic contents were calculated based on the integrated peak area of each sample according to the calibration curves. Nine phenolic metabolites were determined in milligrams per 100 g of dried wheat seedlings, and the detailed curves were presented in supplementary information (section 3), along with the conditions of HPLC used in quantifying the phenolic metabolites. The

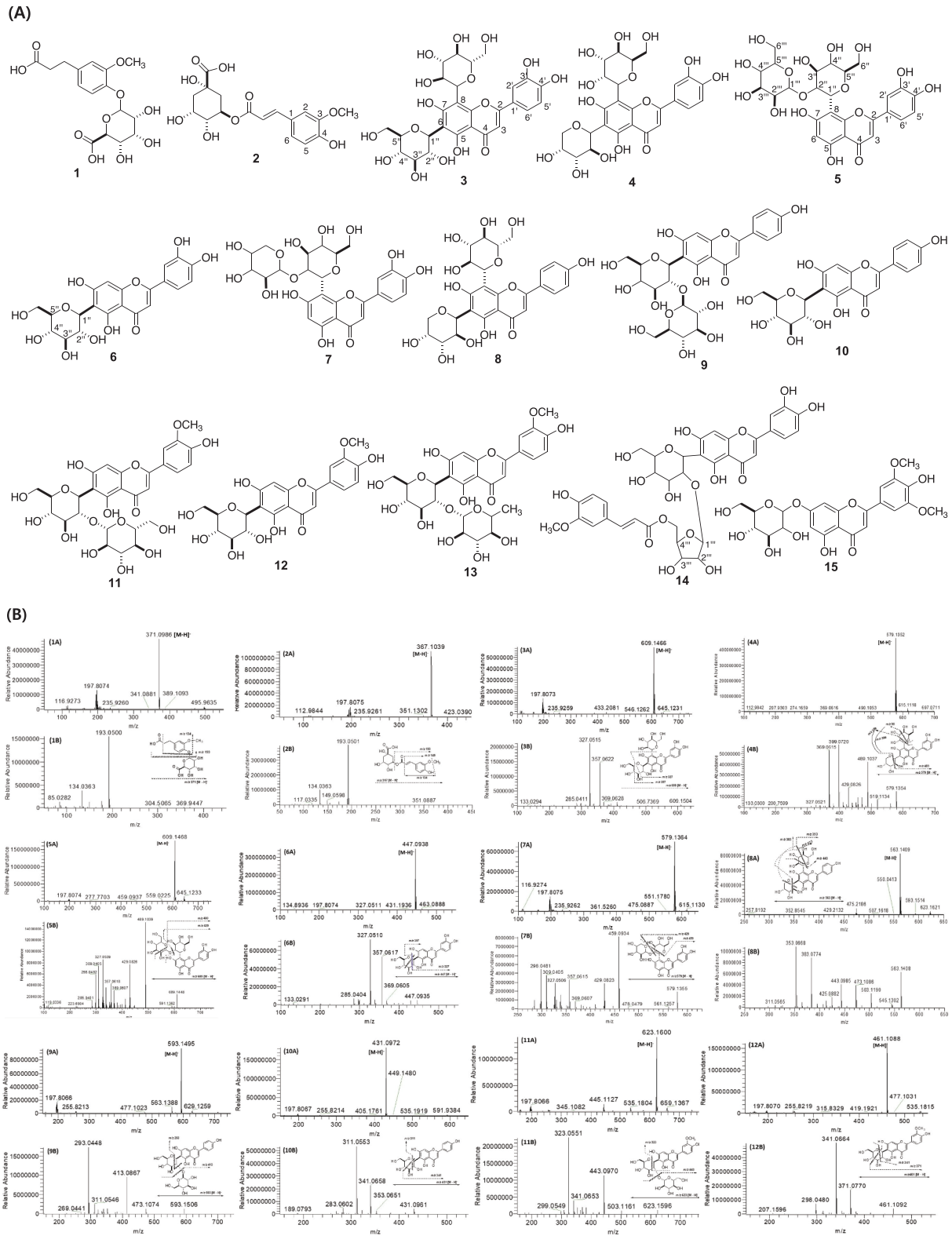


**Fig. 2.** Chemical structures (A) and fragmentation patterns (B) of 15 phenolic metabolites in negative ion mode using HPLC-Q-Orbitrap-MS/MS. (1A) MS and (1B) $MS^2$ data of dihydroferulic acid-4-*O*-glucuronide (**1**); (2A) MS and (2B) $MS^2$ data of 3-*O*-feruloylqunic acid (**2**); (3A) MS and (3B) $MS^2$ data of luteolin-6,8-di-*C*-glucoside (**3**); (4A) MS and (4B) $MS^2$ data of luteolin-6-*C*-arabinoside-8-*C*-glucoside (**4**); (5A) MS and (5B) $MS^2$ data of orientin-2″-*O*-glactopyranoside (**5**); (6A) MS and (6B) $MS^2$ data of luteolin-6-*C*-glucoside (isoorientin) (**6**); (7A) MS and (7B) $MS^2$ data of orientin-2″-*O*-arabinopyranoside (**7**); (8A) MS and (8B) $MS^2$ data of apigenin-6-*C*-arabinoside-8-*C*-glucoside (isochaftoside) (**8**); (9A) MS and (9B) $MS^2$ data of isovitexin-2″-*O*-glucoside (**9**); (10A) MS and (10B) $MS^2$ data of apigenin-6-*C*-glucoside (isovitexin) (**10**); (11A) MS and (11B) $MS^2$ data of isoscoparin-2″-*O*-glucoside (**11**); (12A) MS and (12B) $MS^2$ data of chrysoeriol-6-*C*-glucoside (isoscoparin) (**12**); (13A) MS and (13B) $MS^2$ data of isoscoparin-2″-*O*-rhamnopyranoside (**13**); (14A) MS and (14B) $MS^2$ data of luteolin-6-*C*-[2″-*O*-(5‴-*O*-(feruoyl)-ribofuranosyl)-glucopyranoside] (**14**); (15A) MS and (15B) $MS^2$ data of tricin-7-*O*-glucoside (**15**).

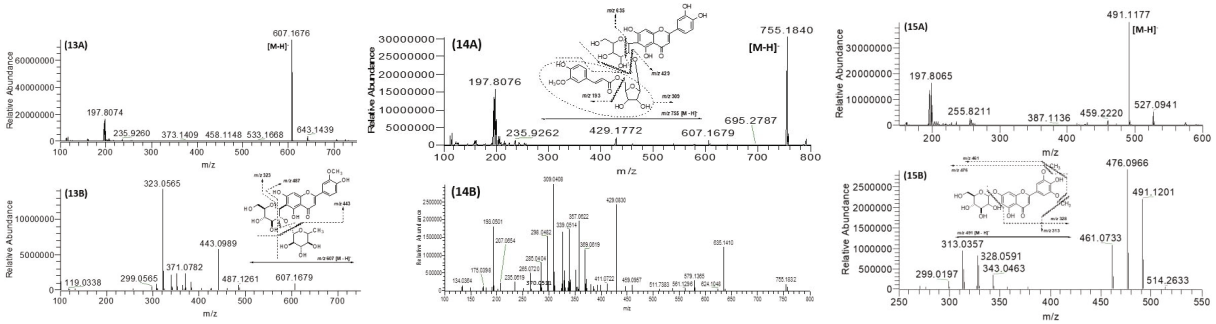


**Fig. 2.** (*continued*).

isolated phenolic contents are measured as microgram per 100 g of dried wheat seedlings.

*Antioxidant capacities on radical scavenging methods.*

The antioxidant properties of the wheat seedlings were evaluated by scavenging assays against DPPH and ABTS radicals, as described in previous studies (Ha et al., 2021; Kim, Yang, Cho & Lee, 2020). The blended seedlings (1 g, 100 mesh) were extracted with 80 % methanol (20 mL) at room temperature for 7 days, and then filtered using a Whatman No. 42 filter paper. The crude supernatant was immediately examined for scavenging activities, and the detailed experimental methods were presented in the supplementary information (section 2).

*Statistical analysis.*

The antioxidant activities and phenolic contents were expressed as the mean ± SD (standard derivation) values of three replicates. All results were undertaken using statistical analysis software (9.2 PC package, Cary, NC, USA), and Duncan's multiple range tests were analyzed at the 0.05 probability level.

## Results and discussion.

*2.1. Structural elucidation of phenolic metabolites in wheat seedlings and NMR spectroscopic data of the isolated phenolics.*

The phenolic metabolites in the wheat seedlings (cv. Jeokjoong) were characterized using HPLC-Q-Orbitrap-MS analysis (Fig. 1B and C), based on the 1D NMR and 2D NMR spectroscopic data of the isolated compounds. Additionally, their chemical structures were verified by comparison of various data, including the mass data reported in previous literatures and those of the confirmed phenolics shown in the supplementary information (section 5). The isolated phenolic metabolites **2**, **4**, **6**, **8**, **9**–**12**, and **15** were documented by NMR spectroscopy and their structures were identified as phenolic derivatives: 3-*O*-feruloylquinic acid (**2**), luteolin-6-*C*-arabinoside-8-*C*-glucoside (**4**), luteolin-6-*C*-glucoside (**6**), apigenin-6-*C*-arabinoside-8-*C*-glucoside (**8**), isovitexin-2″-*O*-glucoside (**9**), apigenin-6-*C*-glucoside (**10**), isoscoparin-2″-*O*-glucoside (**11**), isoscoparin (**12**), and tricin-7-*O*-glucoside (**15**) (Fig. 2A). Their structural characteristics including $^{1}H$ and $^{13}C$ NMR data are detailed in the supplementary information (section 5).

*Characterization of phenolic metabolites in wheat seedlings by HPLC-Q-Orbitrap-MS/MS analysis.*

The on-line coupling of HPLC with mass by electrospray ionization provided to be powerful technique owing to its high resolution and suitability for use in characterizing in a wide range of phenolic metabolites (Dai, Hu, Li, Yan, & Zhang, 2015; Ferreres, Andrade, Valentão, & Gil-Izquierdo, 2008; Lee et al., 2016; Li et al., 2006; Wojakowska, Perkowski, Góral, & Stobiecki, 2013). It is also well-known that the Orbitrap-MS method may provide an excellent mass accuracy because of the highly complex matrix system for elucidating target metabolites (Deseo, Elkins, Rochfort, & Kitchen, 2020; Ha et al., 2021). Hence, the phenolic profiles in the 80 % methanol extract of wheat seedlings were processed using HPLC-Orbitrap-MS/MS, and 15 phenolic metabolites, including major and minor peaks, were identified by the negative mass mode (Fig. 1B, C and Table 1). The MS analysis of peak 1 ($t_R$ = 17.4 min, mass error −3.5840 ppm) exhibited the molecular ion $[M-H]^-$ at *m/z* 371.0986 (Fig. 2B-1A), with the fragment ions in the MS/MS spectrum observed at *m/z* 193.0500 and 134.0363 (Fig. 2B-1B). The major fragment at *m/z* 193.0500 (−178 amu) corresponded to the loss of a glucuronide moiety from an *O*-glycosyl on the phenolic hydroxyl and the minor ion at *m/z* 134.0363 was formed by the loss of *m/z* 59 (*m/z* 193 → 134) (Escudero-López, et al., 2014). This ion may be formed by the losses of the acid [(*m/z* 44, COOH − H)$^-$] and methyl (*m/z* 15) moieties of the feruloyl group (*m/z* 193). Based on these mass data, it was tentatively assigned as dihydroferulic acid-4-*O*-glucuronide (**1**) (Escudero-López, et al., 2014). Peak 2 ($t_R$ = 19.3 min, mass error −4.1950 ppm) possessed an identical molecular ion $[M-H]^-$ at *m/z* 367.1039 (Fig. 2B-2A) and two fragment ions at *m/z* 193.0501 and *m/z* 134.0363 (Fig. 2B-2B). The major fragment at *m/z* 193.0501 (–174 amu) was characterized as the feruloyl moiety due to the loss of quinic acid (192 amu) followed by the addition of $H_2O$ (18 amu) (Jaiswal et al., 2014). The minor fragment at *m/z* 134.0363 was attributed to the loss of *m/z* 59 (*m/z* 193 → 134). The fragment may be resulted from the losses of the methyl (*m/z* 193 → 15) and acid groups (*m/z* 193 → 49, $[COOH-H]^-$) of the feruloyl moiety (*m/z* 193). These fragment ions were observed similar patterns to those observed in the mass spectrum of peak 1. Furthermore, these ionic tendencies were in agreement with previously reported research (Piasecka et al., 2015), and the exact chemical structure was evaluated by interpretation of NMR spectroscopic data. According to the above considerations, peak 2 was assumed to be 3-*O*-feruloylquinic acid (**2**) (Kowalska, Jedrejek, Jonczyk, & Stochmal, 2019; Lee et al., 2016; Piasecka et al., 2015). The HPLC-Orbitrap-MS/MS in the negative ion mode of peak 3 ($t_R$ = 29.6 min, mass error −2.6102 ppm) showed the molecular ion $[M-H]^-$ at *m/z* 609.1466 (Fig. 2B-3A) and two fragment ions at *m/z* 357.0622 and *m/z* 327.0515 (Fig. 2B-3B). The fragment ions at *m/z* 357.0622 $[(M-H)-(90+162)]^-$ and *m/z* 327.0515 $[(M-H)-(90+30+162)]^-$ were typically characterized of the *C*-glycosylflavone group, based on a previous literature (Ferreres, Andrade, Valentão, & Gil-Izquierdo, 2008). Moreover, these two ions (*m/z* 357.0622 and *m/z* 327.0515) were formed by mono glycosylflavone with the losses of − 90 and − 120 amu. According to the published fragment ion patterns, the two peaks were characteristic of luteolin + 71 and luteolin + 41 (Kim et al., 2018; Lee et al., 2016). Therefore, this peak was elucidated as luteolin-6,8-di-*C*-glucoside (lutonarin) (**3**) (Ferreres, Andrade, Valentão, & Gil-Izquierdo, 2008; Lee et al., 2016). Peak 4 at *m/z* 579.1352 ($[M-H]^-$) (mass error −1.1224 ppm) was detected at a retention time of 32.7 min, its mass spectrum exhibited pseudomolecular ions at *m/z* 579.1352 ($[M-H]^-$) (Fig. 2B-

**Table 1**
List of identified phenolic compounds in wheat seedlings by HPLC-Q-Orbitrap-MS/MS analysis.

| Peak | RT (min) | Elemental Composition | Calculated ions [M] (*m/z*) | Calculated ions $[M-H]^-$ (*m/z*) | Observed ions $[M-H]^-$ (*m/z*) | Mass Error (ppm) | Product ions $[M-H]^-$ (*m/z*) | Identification | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 17.4 | $C_{16}H_{20}O_{10}$ | 372.1056 | 371.0978 | 371.0986 | −3.5840 | 193, 134 | Dihydroferulic acid-4-*O*-glucuronide | Escudero-López et al., 2014 |
| 2 | 19.3 | $C_{17}H_{20}O_9$ | 368.1107 | 367.1029 | 367.1039 | −4.1950 | 193, 134 | 3-*O*-Feruloylqunic acid | Kowalska et al., 201 Lee et al., 2016;Piasecka et al., 2015 |
| 3 | 29.6 | $C_{27}H_{30}O_{16}$ | 610.1533 | 609.1455 | 609.1466 | −2.6102 | 369, 357, 327 | Luteolin-6,8-di-*C*-glucoside (Lutonarin) | Ferreres et al., 2008;Lee et al., 2016 |
| 4 | 32.7 | $C_{26}H_{28}O_{15}$ | 580.1428 | 579.1350 | 579.1352 | −1.1224 | 519, 489, 459, 429, 399, 369 | Luteolin-6-*C*-arabinoside-8-*C*- glucoside (Isocarlinoside) | Ferreres et al., 2008;Piasecka et al., 2015 |
| 5 | 33.3 | $C_{27}H_{30}O_{16}$ | 610.1533 | 609.1455 | 609.1468 | −2.9385 | 489, 429, 357, 327, 309, 298 | Orientin-2″-*O*-glactopyranoside | Li et al., 2006 |
| 6 | 34.1 | $C_{21}H_{20}O_{11}$ | 448.1005 | 447.0927 | 447.0938 | −3.6010 | 357, 327, 285 | Luteolin-6-*C*-glucoside (Isoorientin) | Attip et al., 2021; Kim et al., 2018; Kowalska et al., 2019 |
| 7 | 35.6 | $C_{26}H_{28}O_{15}$ | 580.1428 | 579.1350 | 579.1364 | −3.3671 | 459, 429, 357, 327, 309, 297 | Orientin-2″-*O*-arabinopyranoside | Li et al., 2006 |
| 8 | 36.7 | $C_{26}H_{28}O_{14}$ | 564.1479 | 563.1400 | 563.1409 | −2.4328 | 503, 473, 443, 425, 383, 353 | Apigenin-6-*C*-arabinoside-8-*C*-glucoside (Isochaftoside) | Kim et al., 2018;Kowalska et al., 2019;Xie et al., 2019 |
| 9 | 39.0 | $C_{27}H_{30}O_{15}$ | 594.1584 | 593.1506 | 593.1495 | 1.0115 | 413, 293 | Isovitexin-2″-*O*-glucoside | Ferreres et al., 2008; Teixeira et al., 2020 |
| 10 | 40.2 | $C_{21}H_{20}O_{10}$ | 432.1056 | 431.0978 | 431.0972 | 0.1624 | 341, 311 | Apigenin-6-*C*-glucoside (Isovitexin) | Ferreres et al., 2008;Kim et al., 2018 |
| 11 | 43.3 | $C_{28}H_{32}O_{16}$ | 624.1690 | 623.1612 | 623.1600 | 1.0591 | 443, 323 | Isoscoparin-2″-*O*-glucoside | Ferreres et al., 2008;Kim et al., 2018; Piasecka et al., 2015 |
| 12 | 45.0 | $C_{22}H_{22}O_{11}$ | 462.1162 | 461.1083 | 461.1088 | −2.0819 | 371, 341 | Chrysoeriol-6-*C*-glucoside (Isoscoparin) | Ferreres et al., 2008;Kim et al., 2018; Piasecka et al., 2015 |
| 13 | 47.1 | $C_{28}H_{32}O_{15}$ | 624.1690 | 623.1612 | 607.1676 | −3.0469 | 443, 323 | Isoscoparin-2″-*O*-rhamnopyranoside | Kowalska et al., 2019 |
| 14 | 54.8 | $C_{36}H_{36}O_{18}$ | 758.2058 | 757.1979 | 755.1840 | −2.9264 | 635, 579, 429, 309, 193 | Luteolin-6-*C*-[2″-*O*-(5‴-*O*-(feruoyl)-ribofuranosyl)-glucopyranoside] | Kowalska et al., 2014Kowalska et al., 2019 |
| 15 | 56.6 | $C_{23}H_{24}O_{12}$ | 492.1267 | 491.1189 | 491.1177 | 1.4253 | 476, 461, 343, 329, 313, 299 | Tricin-7-*O*-glucoside | Dai et al., 2015;Deseo et al., 2020; Piasecka et al., 2015 |

4A), and its MS/MS spectrum revealed molecular ions at *m/z* 489.1037, *m/z* 399.0720, and *m/z* 369.0615 (Fig. 2B-4B). These fragments were attributed to the losses of *m/z* 90 (579–90 = 489), *m/z* 90 (489–90 = 399), and *m/z* 120 (489–120 = 369) fragments from a flavone structure, as observed for phenolic metabolite **3**, with glycosyl and arabinosyl moieties attached at the *C*-6 and *C*-8 positions (Beelders, Sigge, Joubert, de Beer, & de Villiers, 2012). In other words, these fragments of *m/z* 489.1037 and *m/z* 369.0615 were considered as the mono glycosyl-flavones formed via the losses of − 90 and − 120 amu (Ferreres, Andrade, Valentão, & Gil-Izquierdo, 2008). In accordance with these ions and earlier evidences, this peak was confimed as luteolin-6-*C*-arabinoside-8-*C*-glucoside (isocarlinoside) (**4**), with the chemical formula $C_{26}H_{28}O_{15}$ (Ferreres, Andrade, Valentão, & Gil-Izquierdo, 2008; Piasecka et al., 2015). In addition, the exact sugar sites of this peak were identified by the 2D NMR spectroscopic data. The Orbitrap-MS and MS/MS spectra of peak 5 ($t_R$ = 33.3 min, mass error −2.9385 ppm) displayed a precursor ion at *m/z* 609.1468 $(M - H)^-$ (Fig. 2B-5A), with fragment ions at *m/z* 489.1039 and *m/z* 429.0826 (Fig. 2B-5B). The minor fragment at *m/z* 429.0826, was formed by the loss of an *m/z* 180 fragment (*m/z* 609.1468 → 429.0826; $[(M - H)^-$−180 amu]. This ion was characteistic of an orientin moiety and the lost fragment (−180 amu) corresponded to a hexose residue (Li et al., 2006). The major fragment at *m/z* 489.1039 was characterized as a mono-glucosyl flavone, with the loss of the *m/z* 120 fragment. The mono-glucosyl flavone ion was coincident with orientin + 41, as previously reported (Nørbæk et al., 2000). On the basis of the described fragmentation ions, peak 5 was assumed to beorientin-2″-*O*-glactopyranoside (**5**) (Li et al., 2006). The Orbitrap mass spectrum of peak 6 ($t_R$ = 34.1 min, mass error −3.6010 ppm) displayed a deprotonated molecular ion at *m/z* 447.0938 (Fig. 2B-6A) and MS/MS fragmentation possessed two main ions at *m/z* 357.0617 and *m/z* 327.0510 (Fig. 2B-6B). These fragment ions were assigned as mono-glycosyl flavone, with the losses of the *m/z* 90 and *m/z* 120, and were in agreement with those of luteolin + 71 and luteolin + 41, acccording to the data published in earlier studies (Ferreres, Andrade, Valentão, & Gil-Izquierdo, 2008; Kowalska et al., 2019). The above ion patterns were also similar to those of peak 3 and the structural characteristics were identified by NMR spectroscopic data (Attip, Jalil, Husain, Mohamad, & Ahmad, 2021). As a result, peak 6 was tentatively elucidated as luteolin-6-*C*-glucoside (isoorientin) (**6**) (Kim et al., 2018; Kowalska et al., 2019). Subsequently, the HPLC-Q-Orbitrap-MS spectrum of peak 7 ($t_R$ = 35.6 min, mass error −3.3671 ppm) obtained in the negative ion mode revealed molecular ion at *m/z* 579.1364 (Fig. 2B-7A) and two fragment ions at *m/z* 459.0934 and *m/z* 429.0823 (Fig. 2B-7B). The minor fragment at *m/z* 429.0823 was attributed to the loss of *m/z* 150 (pentose group) (Li et al., 2006), and the remaining major fragment ion at *m/z* 459.0934 was characteristic of a clevage residue $[(M - H)^-$−120 amu] in 8-*C*-pentoside moiety (Wojakowska, Perkowski, Góral, & Stobiecki, 2013). Additionally, these fragmentation patterns were similar to those of peak 4. Based on the above evidences, it was tentatively confirmed with orientin-2″-*O*-arabinopyranoside (**7**) (Li et al., 2006). Peak 8 (mass error −2.4328 ppm) was detected with a retention time at 36.7 min in HPLC-Q-Orbitrap-MS chromatogram, with a deprotonated molecular ion at *m/z* 563.1409 (Fig. 2B-8A). The fragmentation pattern of the MS/MS spectrum exhibited four main fragment ions at *m/z* 473.1086, *m/z* 443.0985, *m/z* 383.0774, and *m/z* 353.0668, as shown in Fig. 2B-8B. Two fragment ions at *m/z* 473.1086 and *m/z* 443.0985, which were formed by the losses of *m/z* 90 (*m/z* 563 → *m/z* 473; $[(M - H)^-$−90 amu]) and *m/z* 120 (*m/z* 563 → *m/z* 443; $[(M - H)^-$−120 amu]). These ions had characteristic of a 6,8-di-*C*-glycoside of apigenin, with glycosyl and arabinosyl moieties attached at the *C*-6 and *C*-8 positions (Ferreres, Andrade, Valentão, & Gil-Izquierdo, 2008). Furthermore, the above described fragmentation patterns were similar to those of peak 4, and thus, this peak was assigned as apigenin-6-*C*-arabinoside-8-*C*-glucoside (isochaftoside) (**8**) (Kim et al., 2018; Kowalska et al., 2019; Xie et al., 2019). The Orbitrap-MS spectrum of peak 9 ($t_R$ = 39.0 min, mass error 1.0115 ppm) presented a major $[M - H]^-$ ion at *m/z* 593.1495 (Fig. 2B-9A) and the MS/MS spectrum exhibited three signals (*m/z* 473.1074, *m/z* 413.0867, and *m/z* 293.0448) (Fig. 2B-9B), corresponding to fragmentations of the molecular ion with losses of 120, 180, and 120 + 180 amu, respectively. The fragment ion at *m/z* 413.0867 was observed typical characteristics of the *C*-glycosylflavones, indicating the presence of *O*,*C*-diglycosylation (Ferreres, Gil-Izquierdo, Andrade, Valentão, & Tomás-Barberán, 2007). The major ion at *m/z* 293.0448 was attributed to the loss of *m/z* 300 ($[(M - H)^-$−(180 + 120)], hexosyl unit loss via glycosidic cleavage and cleavage of the glycosyl moiety), as observed in the earlier researches of other plants (Teixeira et al., 2020). In addition, the exact sugar site of this peak was determined by the 2D NMR spectroscopy, and thus, peak 9 was tentatively assigned as isovitexin-2″-*O*-glucoside (**9**) (Ferreres, Andrade, Valentão, & Gil-Izquierdo, 2008; Teixeira et al., 2020). Full Orbitrap mass analysis of peak 10 ($t_R$ = 40.2 min, mass error 0.1624 ppm) exhibited the molecular ion $[M - H]^-$ at *m/z* 431.0972 (Fig. 2B-10A) and the MS/MS spectrum was observed three fragment ions at *m/z* 341.0658, *m/z* 311.0553, and *m/z* 283.0602 (Fig. 2B-10B). Two major fragment ions (*m/z* 341.0658 and *m/z* 311.0553) were due to losses of 90 and 120 amu, as the previously reported for the intraglycosidic cleavage of a *C*-hexosyl moiety (Ferreres, Andrade, Valentão, & Gil-Izquierdo, 2008; Teixeira et al., 2020). Their fragrments were also in agreement with the patterns of phenolic compounds in barley seedlings (Lee et al., 2016). Thus, this peak was tentatively identified as isovitexin (apigenin-6-*C*-glucoside) (**10**) (Ferreres, Andrade, Valentão, & Gil-Izquierdo, 2008; Kim et al., 2018). The Orbitrap-MS and MS/MS profiles of peak 11 ($t_R$ = 43.3 min, mass error 1.0591 ppm) displayed the molecular ion $[M - H]^-$ at *m/z* 623.1600 (Fig. 2B-11A) with three fragment ions at *m/z* 443.0970, *m/z* 341.0653, and *m/z* 323.0551 (Fig. 2B-11B). Peak 12 ($t_R$ = 45.0 min, mass error −2.0819 ppm) possessed an identical molecular ion $[M - H]^-$ at *m/z* 461.1088 (Fig. 2B-12A) and three fragment ions at *m/z* 371.0770, *m/z* 341.0664, and *m/z* 298.0480 (Fig. 2B-12B). The fragmentation patterns of peaks 11 and 12 were similarly characteristic of isoscoparin (chrysoeriol-6-*C*-glucoside) derivatives (Kim et al., 2018; Piasecka et al., 2015). Their ions may be considered to contain chrysoeriol skeleton (aglycone) frameworks based on the residues of − 180 amu ($[M - H]^-$−180; *m/z* 443), chrysoeriol + 41; *m/z* 341, andchrysoeriol+(41–18); *m/z* 323 in peak 11, and − 90 amu ($[M - H]^-$−90; *m/z* 371; chrysoeriol + 71) and chrysoeriol + 41; *m/z* 341 of peak 12 (Ferreres, Andrade, Valentão, & Gil-Izquierdo, 2008; Piasecka et al., 2015). Additionally, the exact glucose sites in the chemical structures assigned to peaks 11 and 12 were identified by NMR spectroscopy and comparison with previously reported. Based on the mentioned evidences, peaks 11 and 12 were assumed to be isoscoparin-2″-*O*-glucoside (**11**) and chrysoeriol-6-*C*-glucoside (isoscoparin) (**12**) (Ferreres, Andrade, Valentão, & Gil-Izquierdo, 2008; Kim et al., 2018; Piasecka et al., 2015).

The product ion mass spectrum of peak 13 ($t_R$ = 47.1 min, mass error −3.0469 ppm) exhibited as deprotonated ion $[M - H]^-$ at *m/z* 607.1676 (Fig. 2B-13A), and the MS/MS profile were observed with signals at *m/z* 443.0989 ($[M - H]^-$−164 amu) and *m/z* 323.0565 ($[M - H]^-$−(164 + 120) amu) (Fig. 2B-13B). Especially, two fragment ions were consistent with the patterns of peak 11. Furthermore, the fragment ion (*m/z* 443.0989) would correspond to the molecular ion with *O*-glycosylation at the 2″ position of *C*-glycosylated sugar, as comparison with literature data (Ferreres, Gil-Izquierdo, Andrade, Valentão, & Tomás-Barberán, 2007; Teixeira et al., 2020). By comparing the above described ions and the previous data regarding wheat metablites, this peak was identified as isoscoparin-2″-*O*-rhamnopyranoside (**13**), with the formula $C_{28}H_{32}O_{15}$ (Kowalska et al., 2019). The Orbitrap-MS and MS/MS spectra of peak 14 ($t_R$ = 54.8 min, mass error −2.9264 ppm) showed the molecular ion $[M - H]^-$ at *m/z* 755.1840 (Fig. 2B-14A) with four fragment ions at *m/z* 635.1410, *m/z* 429.0830, *m/z* 309.0408, and *m/z* 193.0501 (Fig. 2B-14B). Among them, a major fragment ion was observed at *m/z* 429.0830, which was formed by the loss of *m/z* 326 fragment (*m/z* 755 → *m/z* 429: ($[M - H]^-$−(feruoylribofuranosy moiety, *m/z* 309 + hydroxyl (*m/z* 17))

(Fig. 2B-14A) and the other major fragment ion at *m/z* 309.0408 corresponded to the deprotonated feruoylribofuranosy residue (Kowalska, Pecio, Ciesla, Oleszek, & Stochmal, 2014). The fragment ion at *m/z* 309.0408 was also formed by the loss of 120 amu of the luteolin glucoside group ([M − H]$^-$−120; 429–120 amu), based on the earlier research concern to intraglucosidic cleavage of the *C*-hexosyl moiety (Ferreres, Andrade, Valentão, & Gil-Izquierdo, 2008; Teixeira et al., 2020). This pattern, which was similar to those of peaks 10–13, and was in agreement with a previous work (Kowalska et al., 2019). Thus, peak 14 was tentatively assigned as luteolin-6-*C*-[2″-*O*-(5‴-*O*-(feruoyl)-ribofuranosyl)-glucopyranoside] (**14**) ($C_{36}H_{36}O_{18}$) (Kowalska et al., 2019; Kowalska, Pecio, Ciesla, Oleszek, & Stochmal, 2014). The mass spectrum of peak 15 ($t_R$ = 56.6 min, mass error 1.4253 ppm) exhibited the molecular ion [M − H]$^-$ at *m/z* 491.1177 (Fig. 2B-15A) and four fragment ions at *m/z* 476.0966, *m/z* 461.0733, *m/z* 328.0591, and *m/z* 313.0357 (Fig. 2B-15B). The fragment ions at *m/z* 476.0966 and *m/z* 461.0733 may be due to the losses of the one methyl (*m/z* 15, [M − H]$^-$−15; 476) and two methyl (*m/z* 30, [M − H]$^-$−(15 + 15); 461) groups. Two minor fragment ions at *m/z* 328.0591 and *m/z* 313.0357 were characterized as the losses of a 7-glucoside ([M − H]$^-$−162 amu; 328), and methyl and 7-glucoside ([M − H]$^-$−(15 + 162) amu; 313) moieties. These above data concern to the molecular weight and fragmentation patterns of this peak were in agreement with a previous study (Dai, Hu, Li, Yan, & Zhang, 2015). Also, the position of the glucoside moiety was demonstated using $^1$H–$^{13}$C HMBC NMR data. From the above findings, peak 15 was tentatively confirmed as tricin-7-*O*-glucoside (**15**) ($C_{23}H_{24}O_{12}$) (Dai, Hu, Li, Yan, & Zhang, 2015; Deseo, Elkins, Rochfort, & Kitchen, 2020; Piasecka et al., 2015). Table 1 exhibits the peak number, retention time, and elemental composition as well as mass spectral data, including molecular ion and fragmentation ion through negative ion mode of the identified phenolic metabolites by comparison with those relevant literature references. To the best of our knowledge, fifteen compositions extracted from wheat seedlings were elucidated for the first time as phenolic derivatives of phenolic acid, luteolin, orientin, apigenin, isoscoparin, and

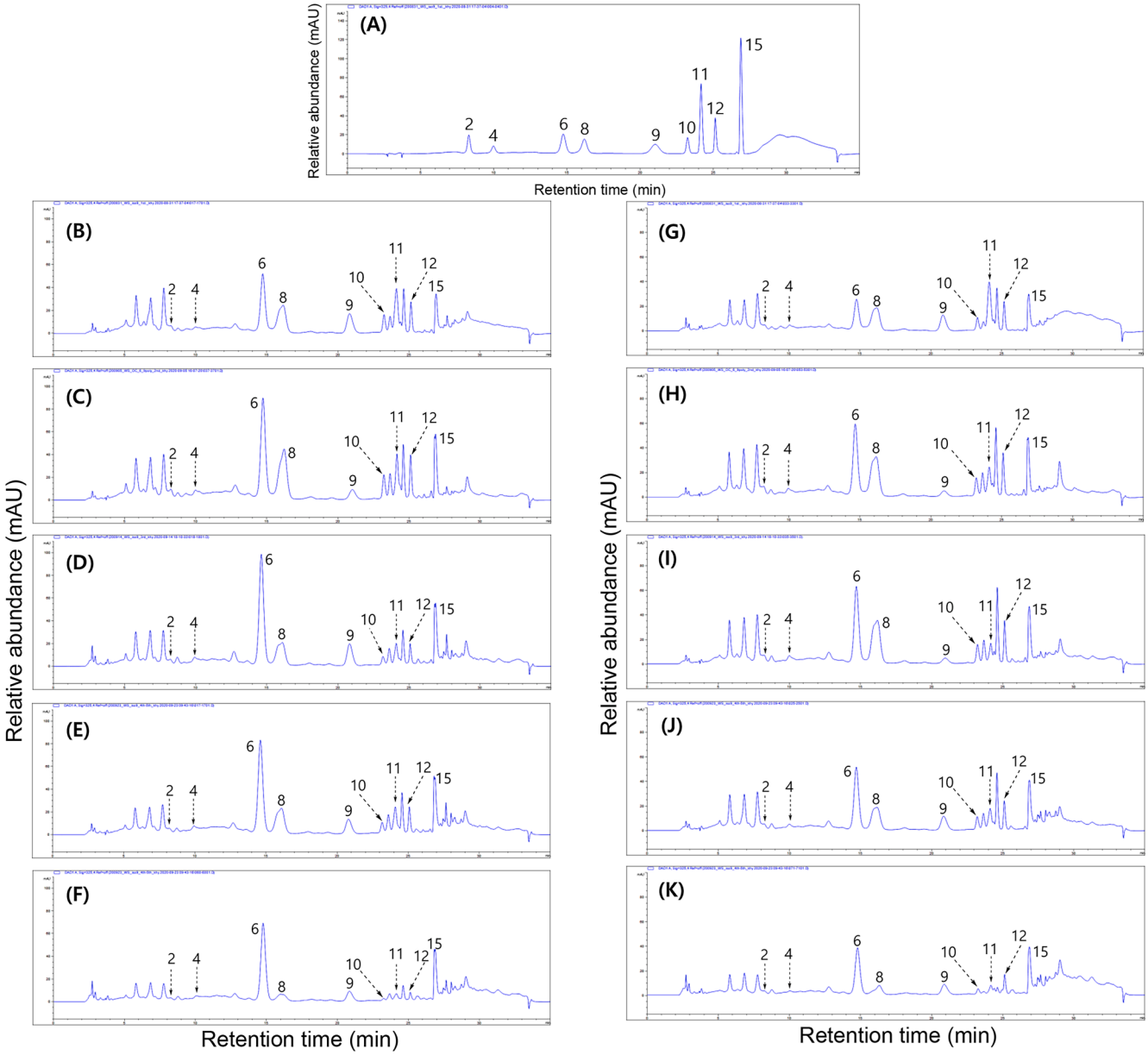


**Fig. 3.** HPLC chromatograms regarding the 9 isolated phenolic metabolites in the 80 % methanol extracts of wheat seedlings (cv. Shinmichal 1; B-F and Saekeumkang; G-K) at different growth times: (A) phenolic phytochemical standard mixture, (B) 5 days, (C) 7 days, (D) 9 days, (E) 12 days, (F) 14 days, (G) 5 days, (H) 7 days, (I) 9 days, (J) 12 days, (K) 14 days.

tricin structures using HPLC-Q-Orbitrap-MS/MS and NMR spectroscopy. Our results may be excellent factors in evaluating the qualities of wheat seedlings.

*Comparison of phenolic metabolites in various wheat seedlings at different growth times.*

The secondary metabolite contents in crops and natural plants differ considerably according to the environmental parameters, such as degree of maturity, genetics, and processing skills (Bai et al., 2017; Celli, Pereira-Netto, & Beta, 2011; Hwang et al., 2021; Kim, Yang, Cho, & Lee, 2020; Lee et al., 2021). Moreover, numerous studies report phenolic compositions to enhance the pharmaceutical and nutritional values of wheat (Chen et al., 2019; Tian, Chen, Tilley, & Li, 2021; Sahu et al., 2021). Unfortunately, few results are published regarding the variations of phenolic metabolites in wheat seedlings with growth time. The present research was designed to use HPLC to evaluate the variations in the phenolic contents of various cultivars of wheat seedlings over five different growth times between 5 days and 14 days after sowing. The nine isolated compositions were examined based on the peak areas in the HPLC chromatograms, and their contents were summarized in Tables 2 and 3. The representative HPLC chromatograms of the isolated phenolic standards and the 80 % methanol extracts of the wheat seedlings are shown in Fig. 3. Although many peaks were observed, the nine phenolic metabolites in the 80 % methanol extracts were examined by comparing thier retention times to those observed in the HPLC chromatograms of the isolated standard materials, as described previously (Hwang et al., 2021; Kim, Yang, Cho, & Lee, 2020; Lee et al., 2015; Zhang et al., 2015). Their retention times are as follows: peak 2 (8.3 min), peak 4 (9.8 min), peak 6 (14.7 min), peak 8 (16.2 min), peak 9 (21.1 min), peak 10 (23.3 min), peak 11 (24.2 min), peak 12 (25.2 min), and peak 15 (26.9 min) (Fig. 3A). The isolated 9 phenolic contents extracted from the seedlings of 17 wheat cultivars exhibit wide concentration ranges according to the growth times: 316.9–461.6 (5 days), 340.1–605.0 (7 days), 212.3–494.7 (9 days), 231.3–503.1 (12 days), and 157.8–294.8 mg/100 g (14 days). The individual and total phenolic contents differ considerably between growth times and cultivars, and their average contents were 366.7 (5 days), 420.8 (7 days), 374.6 (9 days), 350.7 (12 days), and 241.1 mg/100 g (14 days), respectively (Tables 2, 3, and Fig. 3B-K). Over the five growth times, among the various phenolics, isoorientin (luteolin-6-*C*-glucoside) (**6**) and isochaftoside (apigenin-6-*C*-arabinoside-8-*C*-glucoside) (**8**) showed the most abundant average contents with 99.3 and 64.3 mg/100 g, comprising 28.3 and 18.3 % of the total content (average 350.8 mg/100 g), followed by isovitexin-2″-*O*-glucoside (**9**) (45.2 mg/100 g, 12.9 %) > apigenin-6-*C*-glucoside (**10**) (27.3 mg/100 g, 7.8 %) > isoscoparin (**12**) (26.2 mg/100 g, 7.5 %) > luteolin-6-*C*-arabinoside-8-*C*-glucoside (isocarlinoside) (**4**) (25.3 mg/100 g, 7.2 %) > 3-*O*-feruloylqunic acid (**2**) (24.0 mg/100 g, 6.8 %) > tricin-7-*O*-glucoside (**15**) (20.0 mg/100 g, 5.7 %), and the isoscoparin-2″-*O*-glucoside (**11**) exhibited the lowest content with 19.0 mg/100 g (5.4 %) (Table 2 and 3). The highest average phenolic contents of 465.3 mg/100 g was detected in Shinmichal 1, in particular, the Dajoong cultivar showed the second highest content (402.0 mg/100 g), with a similar profile compared with those of Joah (399.4 mg/100 g) and Goso (394.0 mg/100 g). Most of the remaining cultivars were found to display mild average contents in the range 301.7–383.3 mg/100 g over growth times of 5 → 14 days, and the Keumkang and Hanbaek cultivars showed the lowest average contents of 266.2 and 260.2 mg/100 g, respectively. Therefore, Shinmichal 1 may be utilized as an excellent source in developing functional foods and new cultivars, based on the quality of the wheat seedlings due to the high phenolic contents (Celli, Pereira-Netto, & Beta, 2011; Ha et al., 2021; Teixeira et al., 2020; Zhang et al., 2015), e.g., the HPLC chromatograms of phenolic metabolites at different growth times of wheat seedlings (cv. Shinmichal 1: Fig. 3B − F and Saekeumkang: Fig. 3G − K) are shown in Fig. 3, and their phenolic patterns were similar to the results obtained by other cultivars. When the seedlings of this crop are grown for longer times in the range 5 → 14 days, the phenolic contents decreased as follows: 425.2 → 605.0 → 494.7 → 503.1 → 294.8 mg/100 g (cv. Shinmichal 1) and 327.9 → 469.8 → 484.0→ 386.0 → 248.9 mg/100 g (cv. Saekeumkang) with variations at each growth time (Table 2 and 3, and Fig. 3B − K). However, the individual and total contents in the diverse cultivars were observed remarkable differences at different growth times. Our results were similar to the previously reported data regarding changes in phenolic contents of crops and natural sources due to environmental factors (growth time, plant organ, climate, soil, moisture, temperature, etc.) and genetics (Bai et al., 2017; Celli, Pereira-Netto, & Beta, 2011; Kim, Yang, Cho, & Lee, 2020; Lee et al., 2015).

The wheat seedlings with growth times of 5 days displayed significant differences in phenolic phytochemical contents (Table 2), Especially, isochaftoside (**8**) showed the predomiant phenolic (average content of 72.4 mg/100 g) in all cultivars, representing approximately 19.7 % of the total average content (366.7 mg/100 g), and the second major content was detected with a concentration of 61.8 mg/100 g (16.9 %) in isoorientin (**6**). The concentrations of the other phenolics exhibited as following order: isovitexin-2″-*O*-glucoside (**9**) (54.7 mg/100 g, 14.9 %) > isoscoparin-2″-*O*-glucoside (**11**) (37.6 mg/100 g, 10.3 %) > apigenin-6-*C*-glucoside (**10**) (36.1 mg/100 g, 9.8 %) > chrysoeriol-6-*C*-glucoside (**12**) (33.5 mg/100 g, 9.1 %), and the remaining phenolics were observed low contents (<30.0 mg/100 g). Extending the growth periods from 5 to 7 days increased the average phenolic contents. The wheat seedlings with this growth time contain higher total phenolics than those of the wheat seedlings with growth time of 5 days and their average contents vary considerably between 366.7 and 420.8 mg/100 g. Luteolin-6-*C*-glucoside (**6**) was the highest average increase (5 days: 61.8 → 7 days: 121.1 mg/100 g) compared to those of the other phenolic metabolites, and the remaining phenolics decreased slightly as the growth time increases from 5 to 7 days, except those of 3-*O*-feruloylqunic acid (**2**) (24.7 → 24.7 mg/100 g), luteolin-6-*C*-arabinoside-8-*C*-glucoside (**4**) (27.4 → 31.8 mg/100 g), apigenin-6-*C*-arabinoside-8-*C*-glucoside (**8**) (72.4 → 78.8 mg/100 g), and isovitexin-2″-*O*-glucoside (**9**) (54.7 → 63.7 mg/100 g). At this growth time, abundant phenolic contents were observed in Shinmichal 1 (605.0 mg/100 g), while the Hanbaek cultivar exhibited the lowest content of 340.1 mg/100 g (Table 2). Our data suggest that the Shinmichal 1 cultivar may be recommended as a potential material for use in developing agents beneficial to human health. This cultivar, in particular, exhibited the the most abundant content (990.6 mg/100 g) of policosanol, which is a health promoting aliphatic alcohol metabolite, at a growth time of 6 days (Ra et al., 2020).

When the wheat seedlings are grown for longer times in the range 7 → 14 days, the total average phenolic metabolites decreased (approximately 1.5 times) with the decrease rates as follows: 374.6 → 350.7 → 241.1 mg/100 g in the order: 7 → 9 days > 9 → 12 days > 12 → 14 days. At 12 → 14 days of growth, the total average phenolics decreased strongly (350.7 → 241.1 mg/100 g), and this rate of decrease was higher than that observed from 7 to 12 days (420.8 → 374.6 → 350.7 mg/100 g) (Table 2). This phenomenon may be primarily influenced by apigenin-6-*C*-arabinoside-8-*C*-glucoside (**8**), which exhibits variations of 71.8 → 26.8 mg/100 g compared to the rates of decrease (1–20 mg/100 g) in the other phenolics during growth times of 7 → 14 days. Remarkably, the various wheat seedlings with growth times between 12 and 14 days displayed considerable variations in their phenolic metabolites, with rapid decrease (average content: 350.7 → 241.1 mg/100 g) compared with those of other growth times (5 → 7 → 9 days: 366.7 → 420.8 → 374.6 mg/100 g) (Table 2). The above findings implies that the phenolic contents may be determined by diverse parameters, such as an intense cellular rate, conversion, degradation, and biosynthesis during the growth of the plant (Bai et al., 2017; Kim, Yang, Cho, & Lee, 2020; Tsao et al., 2006). Furthermore, the accumulation and profiles of phenolic metabolites in wheat seedlings may be associated with the growth time and cultivar, as previously reported regarding the variations in

**Table 2**
Changes in individual and total phenolic compounds in the seedlings of wheat cultivars under five growth times.

| Growth times and cultivars | | Phenolic content (mg/100 g of dry weight)[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3-*O*-FQA[b] (**2**) | Lu-6-*C*-ara-8-*C*-G[b] (**4**) | Isoorientin (**6**) | Isochaftoside (**8**) | Isovitexin-2″-*O*-G[b] (**9**) | Apigenin-6-*C*-G[b] (**10**) | Isoscoparin-2″-*O*-G[b] (**11**) | Isoscoparin (**12**) | Tricin-7-*O*-G[b] (**15**) | Total |
| 5 days | Keumkang | 24.8 ± 1.1bc | 19.1 ± 1.7a | 38.2 ± 3.8a | 65.9 ± 6.8efgh | 55.9 ± 0.4ef | 29.9 ± 2.9 g | 29.8 ± 0.1 g | 33.5 ± 1.7ef | 19.9 ± 0.4b | 316.9 ± 18.7i |
| | Jokyoung | 25.2 ± 0.5abc | 18.7 ± 1.2 g | 54.5 ± 3.8 h | 64.6 ± 3.9fgh | 45.6 ± 0.9i | 31.3 ± 2.0 fg | 25.7 ± 0.5 h | 33.1 ± 0.8ef | 18.1 ± 0.4d | 317.0 ± 13.9i |
| | Shinmichal1 | 24.5 ± 0.1bcd | 11.1 ± 0.0 g | 104.3 ± 0.8efg | 96.4 ± 0.0b | 59.8 ± 0.4c | 40.8 ± 0.2 cd | 34.3 ± 0.6f | 33.7 ± 0.1ef | 20.3 ± 0.1b | 425.2 ± 2.0bc |
| | Baekjoong | 26.2 ± 0.7abc | 29.5 ± 1.3 h | 54.7 ± 0.0efg | 70.3 ± 0.0efgh | 66.4 ± 0.1a | 31.5 ± 0.1 fg | 37.8 ± 1.2e | 28.7 ± 0.1 g | 20.3 ± 0.0b | 365.4 ± 0.9efg |
| | Suan | 25.5 ± 2.2abc | 22.3 ± 1.2de | 67.4 ± 4.4 cd | 60.8 ± 3.9gh | 55.1 ± 1.0 fg | 35.3 ± 2.2ef | 25.0 ± 0.3 h | 28.0 ± 0.7 g | 18.8 ± 0.2c | 338.3 ± 16.2ghi |
| | Goso | 22.4 ± 3.9cde | 19.6 ± 0.7 fg | 68.3 ± 5.2 cd | 83.0 ± 6.3c | 50.5 ± 1.5 h | 38.6 ± 2.8de | 61.3 ± 1.3a | 32.5 ± 1.2ef | 16.9 ± 0.4e | 393.1 ± 23.4cde |
| | Joah | 25.3 ± 0.7abc | 26.2 ± 1.1 fg | 86.5 ± 6.3b | 115.6 ± 9.3a | 60.4 ± 1.9c | 54.9 ± 4.3a | 36.a ± 1.1e | 40.7 ± 1.5bc | 15.8 ± 0.4f | 461.6 ± 26.5a |
| | Hojoong | 23.7 ± 0.4bcde | 19.6 ± 0.1ef | 71.3 ± 0.2c | 73.5 ± 0.3cdef | 65.6 ± 0.1ab | 40.9 ± 0.2 cd | 41.2 ± 2.0d | 39.4 ± 0.2c | 19.2 ± 0.0c | 394.5 ± 2.6cde |
| | Baekgang | 27.5 ± 0.6ab | 21.7 ± 1.9 fg | 61.9 ± 3.1de | 72.6 ± 5.0cdef | 42.3 ± 0.9j | 35.2 ± 2.4ef | 32.6 ± 0.8f | 36.2 ± 1.1d | 15.6 ± 0.2 fg | 345.5 ± 16.0ghi |
| | Saekeumkang | 25.1 ± 17.7bc | 22.7 ± 16.0 fg | 49.4 ± 34.9 fg | 74.9 ± 5.3cdef | 44.6 ± 31.5ij | 28.4 ± 20.1gh | 34.2 ± 24.1f | 29.8 ± 21.g | 18.5 ± 13.1d | 327.9 ± 14.0hi |
| | Dabun | 29.1 ± 1.4a | 22.1 ± 0.2 fg | 36.6 ± 0.1 h | 67.3 ± 0.3efgh | 49.7 ± 0.0 h | 39.0 ± 0.1cde | 32.8 ± 0.2f | 41.5 ± 0.0b | 18.2 ± 0.0d | 336.3 ± 0.8ghi |
| | Jeokjoong | 26.4 ± 0.8abc | 31.7 ± 9.5 fg | 55.9 ± 3.4ef | 76.1 ± 7.7cde | 56.4 ± 0.3ef | 29.5 ± 0.6gh | 28.7 ± 0.1 g | 32.3 ± 0.4f | 20.4 ± 0.2b | 357.3 ± 18.0fgh |
| | Sugang | 25.9 ± 0.3abc | 42.3 ± 0.1cde | 60.5 ± 2.4de | 67.5 ± 0.0defgh | 57.5 ± 0.0de | 49.9 ± 0.0b | 44.9 ± 0.1c | 49.8 ± 0.1a | 17.8 ± 0.1d | 416.0 ± 2.4 cd |
| | Hanbaek | 24.8 ± 5.2bc | 17.7 ± 0.3b | 47.1 ± 0.8 g | 34.8 ± 0.6i | 43.2 ± 0.3j | 22.1 ± 0.4i | 29.7 ± 0.4 g | 24.1 ± 0.3 h | 25.4 ± 0.3a | 268.7 ± 8.5j |
| | Dajoong | 22.3 ± 0.4cde | 70.6 ± 5.3 g | 72.8 ± 6.6c | 77.5 ± 6.0 cd | 64.2 ± 1.3b | 43.4 ± 3.2c | 55.4 ± 1.0b | 34.4 ± 1.1e | 16.4 ± 0.3e | 456.9 ± 25.1ab |
| | Jojoong | 20.2 ± 0.1e | 35.2 ± 1.6 cd | 66.9 ± 4.9 cd | 71.2 ± 5.0defg | 59.1 ± 1.6 cd | 37.1 ± 2.7de | 44.3 ± 0.9c | 34.2 ± 1.0ef | 16.4 ± 0.2e | 384.7 ± 18.0def |
| | Johan | 20.5 ± 0.1de | 36.2 ± 2.0c | 54.7 ± 4.5efg | 59.6 ± 3.7 h | 53.3 ± 0.9 g | 25.2 ± 1.6hi | 45.0 ± 0.6c | 18.2 ± 0.4i | 15.2 ± 0.1 g | 327.9 ± 14.0hi |
| 7 days | Keumkang | 21.4 ± 0.3i | 25.9 ± 1.9gh | 78.1 ± 6.2 k | 68.1 ± 5.1e | 84.1 ± 1.4d | 21.6 ± 1.5gh | 19.9 ± 0.4de | 25.7 ± 0.7 g | 18.7 ± 0.3efg | 363.4 ± 17.6hi |
| | Jokyoung | 33.8 ± 0.1b | 32.1 ± 0.1de | 124.1 ± 0.5def | 57.7 ± 0.2f | 69.3 ± 0.2 h | 18.6 ± 0.0hi | 18.4 ± 0.0efg | 21.1 ± 0.1j | 19.5 ± 0.1cdef | 394.5 ± 0.7efg |
| | Shinmichal 1 | 23.8 ± 0.0ef | 15.8 ± 0.2 L | 188.1 ± 0.3a | 170.6 ± 0.3a | 33.3 ± 0.1i | 64.1 ± 0.1a | 37.9 ± 0.0a | 48.6 ± 0.1b | 22.8 ± 0.0ab | 605.0 ± 0.7a |
| | Baekjoong | 23.1 ± 0.3 fg | 33.8 ± 2.0d | 115.7 ± 6.2efg | 60.7 ± 3.0ef | 82.9 ± 1.4d | 19.6 ± 0.6 h | 25.8 ± 0.4bc | 19.3 ± 0.4 k | 20.9 ± 0.4bcde | 401.8 ± 14.8efg |
| | Suan | 22.2 ± 0.1hi | 20.6 ± 0.1jk | 153.6 ± 0.1b | 46.8 ± 0.0 g | 99.1 ± 0.5a | 15.9 ± 0.0ij | 24.6 ± 0.1c | 16.5 ± 0.0 L | 19.9 ± 0.2bcdef | 419.2 ± 0.7de |
| | Goso | 22.1 ± 0.3hi | 30.1 ± 1.9ef | 126. ± 10.3de | 143.6 ± 10.0b | 20.4 ± 1.1j | 51.6 ± 3.4b | 16.3 ± 0.4 h | 45.2 ± 0.8 cd | 22.2 ± 0.1abcd | 477.9 ± 28.8b |
| | Joah | 21.9 ± 0.1i | 23.5 ± 0.3hi | 115.7 ± 0.9efg | 121.7 ± 1.1c | 20.9 ± 0.2j | 52.1 ± 0.5b | 17.8 ± 0.1fgh | 46.0 ± 0.3c | 17.7 ± 0.1efg | 437.3 ± 3.4 cd |
| | Hojoong | 26.3 ± 0.5c | 19.4 ± 0.9 k | 120.5 ± 7.8defg | 58.9 ± 3.2ef | 77.2 ± 2.0f | 26.7 ± 1.1ef | 18.1 ± 0.4 fg | 30.4 ± 0.5f | 17.6 ± 0.4 fg | 395.0 ± 15.8efg |
| | Baekgang | 35.5 ± 0.4a | 28.4 ± 0.4 fg | 109.8 ± 1.9gh | 29.2 ± 0.5 h | 92.7 ± 0.4c | 8.7 ± 0.1 k | 25.0 ± 0.1c | 13.3 ± 0.0 *m* | 20.2 ± 0.1bcdef | 362.7 ± 3.8hi |
| | Saekeumkang | 21.7 ± 0.3i | 31.1 ± 1.7def | 128.3 ± 10.1d | 142.2 ± 10.0b | 19.9 ± 1.0j | 42.1 ± 3.2d | 20.1 ± 0.6d | 44.3 ± 1.4d | 20.2 ± 0.5bcdef | 469.8 ± 29.6b |
| | Dabun | 22.0 ± 0.0hi | 31.2 ± 0.1def | 86.5 ± 0.5jk | 106.8 ± 0.3d | 14.1 ± 0.0 k | 48.8 ± 0.3c | 13.1 ± 0.0i | 52.0 ± 0.3a | 24.1 ± 5.5a | 398.5 ± 6.8efg |
| | Jeokjoong | 23.9 ± 0.0ef | 32.6 ± 1.0de | 101.2 ± 0.4hi | 64.0 ± 0.2ef | 73.7 ± 0.2 g | 21.5 ± 0.0gh | 24.5 ± 0.1c | 21.5 ± 0.1ij | 20.6 ± 0.4bcdef | 383.6 ± 0.4gh |
| | Sugang | 22.8 ± 1.1gh | 55.2 ± 0.6a | 119.7 ± 0.9defg | 57.9 ± 0.2f | 76.6 ± 0.8f | 29.4 ± 0.2e | 19.0 ± 0.0def | 36.1 ± 0.3e | 20.7 ± 0.2bcdef | 437.5 ± 4.3 cd |
| | Hanbaek | 24.6 ± 0.1de | 22.7 ± 0.2ij | 93.2 ± 1.2ij | 38.7 ± 0.5 g | 79.3 ± 0.5e | 14.7 ± 0.1j | 26.0 ± 0.2bc | 18.3 ± 0.1 k | 22.6 ± 0.1abc | 340.1 ± 2.8i |
| | Dajoong | 25.0 ± 0.1d | 48.9 ± 0.3b | 143.8 ± 0.6bc | 58.0 ± 0.2f | 95.6 ± 0.3b | 24.0 ± 0.2 fg | 27.1 ± 0.1b | 22.2 ± 0.0i | 18.4 ± 0.0efg | 463.0 ± 0.8bc |
| | Jojoong | 25.5 ± 0.7d | 39.8 ± 3.4c | 142.1 ± 10.2c | 56.5 ± 3.9f | 75.3 ± 1.6 fg | 18.8 ± 1.3hi | 17.0 ± 0.4gh | 23.9 ± 0.6 h | 19.2 ± 0.3def | 418.0 ± 21.2def |
| | Johan | | | | 59.0 ± 0.2ef | | | 20.5 ± 2.7d | 17.0 ± 0.0 L | | |

(*continued on next page*)

**Table 2** (*continued*)

| Growth times and cultivars | | Phenolic content (mg/100 g of dry weight)[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3-*O*-FQA[b] (**2**) | Lu-6-*C*-ara-8-*C*-G[b] (**4**) | Isoorientin (**6**) | Isochaftoside (**8**) | Isovitexin-2″-*O*-G[b] (**9**) | Apigenin-6-*C*-G[b] (**10**) | Isoscoparin-2″-*O*-G[b] (**11**) | Isoscoparin (**12**) | Tricin-7-*O*-G[b] (**15**) | Total |
| | | 24.0 ± 0.0ef | 49.0 ± 0.1b | 112.6 ± 0.6fgh | | 68.7 ± 0.1 h | 19.9 ± 1.7 h | | | 15.8 ± 0.0 g | 386.5 ± 3.3fgh |
| 9 days | Keumkang | 21.4 ± 0.6bcd | 13.7 ± 0.0i | 52.6 ± 0.1i | 27.2 ± 0.1i | 52.2 ± 0.0 cd | 8.7 ± 0.0 h | 7.7 ± 0.0c | 12.6 ± 0.0j | 16.2 ± 0.2ef | 212.3 ± 0.4j |
| | Jokyoung | 21.8 ± 0.0bcd | 24.4 ± 0.6f | 119.0 ± 0.6de | 52.8 ± 0.2 g | 42.8 ± 0.0e | 16.0 ± 0.2fgh | 11.6 ± 0.0abc | 19.2 ± 0.0hi | 18.5 ± 0.1cdef | 326.1 ± 1.5fgh |
| | Shinmichal 1 | 24.5 ± 0.2bc | 26.0 ± 0.6ef | 209.9 ± 1.6a | 89.4 ± 0.5d | 62.3 ± 0.2ab | 22.5 ± 0.2defg | 15.5 ± 0.1abc | 22.6 ± 0.0fgh | 21.9 ± 0.1abcde | 494.7 ± 1.9a |
| | Baekjoong | 29.6 ± 0.1a | 32.9 ± 0.0c | 104.7 ± 0.4 fg | 102.8 ± 0.5c | 30.1 ± 0.0f | 34.3 ± 0.1bcd | 23.7 ± 0.0a | 30.1 ± 0.0de | 25.8 ± 0.3ab | 414.0 ± 0.9 cd |
| | Suan | 24.5 ± 5.9bc | 16.3 ± 1.3hi | 116.7 ± 10.1de | 50.4 ± 3.9 g | 64.2 ± 0.4ab | 20.2 ± 1.4efgh | 12.5 ± 0.4abc | 17.7 ± 0.6hij | 21.9 ± 4.3abcde | 344.3 ± 7.7ef |
| | Goso | 21.1 ± 0.2 cd | 25.6 ± 0.7ef | 120.2 ± 4.3de | 126.1 ± 3.7b | 22.2 ± 0.4 fg | 42.1 ± 1.1ab | 15.5 ± 0.2abc | 38.3 ± 0.7bc | 24.6 ± 0.3abc | 435.7 ± 11.5c |
| | Joah | 19.1 ± 0.0d | 24.7 ± 0.0f | 127.5 ± 0.4 cd | 131.5 ± 0.5b | 24.5 ± 0.0 fg | 50.7 ± 0.3a | 17.7 ± 0.1abc | 42.1 ± 0.3b | 23.3 ± 0.1abcd | 461.2 ± 1.7b |
| | Hojoong | 28.7 ± 1.1a | 15.8 ± 0.1i | 93.4 ± 0.2gh | 73.7 ± 0.0e | 21.6 ± 0.0 fg | 36.5 ± 0.2bc | 17.2 ± 0.0abc | 50.9 ± 0.0a | 21.7 ± 0.1abcde | 359.5 ± 1.7e |
| | Baekgang | 23.1 ± 2.0bcd | 20.1 ± 1.6 g | 108.7 ± 8.3ef | 25.1 ± 1.8i | 51.7 ± 0.2 cd | 11.8 ± 6.9gh | 14.4 ± 14.7abc | 16.3 ± 2.5ij | 12.2 ± 9.2f | 283.3 ± 13.8i |
| | Saekeumkang | 28.9 ± 0.5a | 30.6 ± 0.0 cd | 132.4 ± 1.7bc | 150.7 ± 2.3a | 18.3 ± 0.2 g | 43.4 ± 0.9ab | 13.1 ± 0.2abc | 41.3 ± 0.6b | 25.4 ± 0.2abc | 484.0 ± 5.7ab |
| | Dabun | 22.9 ± 3.8bcd | 22.5 ± 0.3 fg | 98.9 ± 0.0 fg | 47.3 ± 0.4 g | 52.3 ± 0.0 cd | 26.0 ± 12.7cdef | 7.7 ± 1.9c | 18.4 ± 3.2hij | 20.4 ± 0.0abcde | 316.4 ± 10.7gh |
| | Jeokjoong | 21.2 ± 0.2 cd | 28.2 ± 2.5de | 100.9 ± 8.5 fg | 61.7 ± 4.8f | 50.3 ± 1.6de | 28.6 ± 9.6cde | 12.2 ± 7.0abc | 26.5 ± 7.1ef | 23.8 ± 4.1abc | 353.3 ± 4.0e |
| | Sugang | 21.2 ± 0.1 cd | 30.2 ± 0.2 cd | 117.0 ± 0.4de | 28.8 ± 12.0i | 49.3 ± 15.5de | 19.3 ± 0.0efgh | 21.5 ± 14.0ab | 25.5 ± 0.1efg | 22.9 ± 5.0abcde | 335.8 ± 7.9efg |
| | Hanbaek | 21.9 ± 0.3bcd | 19.6 ± 1.6gh | 87.2 ± 7.1 h | 37.9 ± 2.8 h | 56.2 ± 1.1bcd | 22.6 ± 11.8defg | 8.7 ± 3.6bc | 21.9 ± 6.4fghi | 27.0 ± 0.6a | 302.9 ± 28.0hi |
| | Dajoong | 25.7 ± 1.2ab | 37.7 ± 1.1b | 140.8 ± 2.7b | 51.7 ± 1.0 g | 65.4 ± 0.0a | 21.2 ± 0.3efg | 15.5 ± 0.1abc | 19.8 ± 0.0ghi | 19.1 ± 0.1bcde | 396.9 ± 6.2d |
| | Jojoong | 20.5 ± 0.2 cd | 37.2 ± 3.4b | 131.7 ± 10.1bc | 84.2 ± 6.3d | 50.3 ± 1.2de | 29.1 ± 2.0cde | 16.7 ± 0.4abc | 33.5 ± 0.8 cd | 16.6 ± 0.3def | 419.8 ± 24.8 cd |
| | Johan | 28.6 ± 0.4a | 56.6 ± 3.6a | 125.2 ± 0.5 cd | 80.8 ± 0.3de | 59.6 ± 0.4abc | 24.0 ± 1.5defg | 16.1 ± 2.3abc | 18.1 ± 0.1hij | 18.4 ± 0.2cdef | 427.3 ± 0.3c |

[a] All values are presented as the mean ± SD of triplicate determinations, content expressed as mg of each phenolic equivalents per 100 g of dry weight.
[b] 3-*O*-FQA, 3-*O*-feruloylquinic acid; ara, arabinoside; G, glucoside.
[c]ND: not detected.

metabolites (del Baño et al., 2003; Kim, Yang, Cho, & Lee, 2020; Tsao et al., 2006; Zhang et al., 2015). Although numerous studies reported that the secondary metabolite contents increased with the maturation of crops and plants, the phenolic metabolites of wheat were not dependent on the growth time. In addition, the phenolic distributions revealed that the most abundant contents are observed at 7 days, followed by 9 days > 5 days > 12 days > 14 days (Table 2). Overall, the proper harvest time of wheat seedlings to yield the highest phenolic metabolites may be 7 days after sowing and their sources may be critical natural materials for use in human health agents. For the first time, our data provide excellent information regarding the comparison and fluctuations of phenolic distributions in wheat seedlings with various growth times.

*Variations of antioxidant properties on radical scavenging abilities in various wheat seedlings at different growth times.*

Several researches have focused on the antioxidant capacities of crops, vegetables, and natural plants, as determined using radical scavenging assays based on DPPH and ABTS because of their characteristics, including stability, simple control, and cost effectiveness (Celli, Pereira-Netto, & Beta, 2011; Ha et al., 2021; Lee et al., 2015; Schaich, Tian, & Xie, 2015). Furthermore, these radicals have been commonly used in measuring natural antioxidants, such as carotenoids, phenolic acids, flavonoids, and anthocyanins (Chen et al., 2019; Hwang et al., 2021; Lee et al., 2016; Zhang et al., 2015). Additionally, the antioxidant capacities of crops differ remarkably due to environmental factors, gentics, and biotransformations (Bai et al., 2017; del Baño et al., 2003; Hwang et al., 2021; Lee et al., 2021). However, to the best of our knowledge, fluctuations in the radical scavenging effects have never been observed in wheat seedlings with different growth times. Therefore, we investigated the antioxidant properties of the 80 % methanol extracts of various wheat seedlings with different growth times against radicals. Their capacities were investigated by comparing the percentage inhibitions of the two radicals in samples with those of positive controls (DPPH: BHT, ABTS: Trolox). In our preliminary test, the DPPH and ABTS scavenging activities of the sample (cv. Shinmichal 1) and positive controls increased with increasing concentrations (100, 200, 400, 600, 800, and 1000 μg/mL). Even though the 80 % methanol extract exhibited 100 % scavenging capacities at 800 and 1000 μg/mL, we subsequently used a concentration of 600 μg/mL to confirm the dose-dependent variation in the inhibition rate. The DPPH radical scavenging abilities exhibited remarkable differences according to the growth time of each cultivar. The wheat seedlings collected after 7 days displayed the highest average capacities (82 %), and those of the wheat seedlings collected at other growth times decreased as follows: 9 days (73 %) > 5 days (68 %) > 12 days (63 %) > 14 days (46 %) (Fig. 4A). These results

**Table 2**
(continued).

| Growth times and cultivars | | Phenolic content (mg/100 g of dry weight)[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3-*O*-FQA[b] (**2**) | Lu-6-*C*-ara-8-*C*-G[b] (**4**) | Isoorientin (**6**) | Isochaftoside (**8**) | Isovitexin-2″-*O*-G[b] (**9**) | Apigenin-6-*C*-G[b] (**10**) | Isoscoparin-2″-*O*-G[b] (**11**) | Isoscoparin (**12**) | Tricin-7-*O*-G[b] (**15**) | Total |
| 12 days | Keumkang | 24.5 ± 0.2 cd | 13.6 ± 0.8 fg | 47.8 ± 4.1 g | 55.9 ± 5.3jk | 11.9 ± 0.3f | 22.0 ± 1.8 cd | 8.9 ± 0.2ij | 28.4 ± 1.1cde | 19.4 ± 0.3cdef | 232.5 ± 14.1 h |
| | Jokyoung | 25.7 ± 0.1bc | 17.6 ± 1.1ef | 84.3 ± 6.7ef | 63.2 ± 4.8ij | 14.2 ± 0.5f | 27.0 ± 1.8 cd | 11.0 ± 0.2ghij | 29.9 ± 0.9bcde | 17.7 ± 4.8ef | 290.5 ± 20.8 g |
| | Shinmichal 1 | 23.8 ± 1.2 cd | 26.2 ± 3.5c | 193.7 ± 15.7a | 104.8 ± 8.2a | 46.5 ± 1.4ab | 33.0 ± 1.7bcd | 25.1 ± 0.4a | 30.2 ± 0.8bcde | 19.8 ± 0.6cdef | 503.1 ± 33.6a |
| | Baekjoong | 22.3 ± 1.1d | 24.6 ± 1.7 cd | 90.2 ± 7.4e | 72.4 ± 6.1fghi | 42.2 ± 1.7abc | 26.0 ± 1.5 cd | 20.4 ± 0.5b | 25.2 ± 0.5ef | 22.3 ± 5.5abcde | 345.7 ± 12.7ef |
| | Suan | 25.4 ± 0.2c | 13.9 ± 0.7 fg | 107.7 ± 6.0d | 50.2 ± 2.8 k | 40.5 ± 1.1abc | 24.5 ± 1.0 cd | 18.6 ± 0.3bc | 19.0 ± 0.4 fg | 17.0 ± 0.1ef | 316.7 ± 12.6 fg |
| | Goso | 25.4 ± 0.4c | 20.5 ± 0.9de | 109.6 ± 3.7 cd | 89.8 ± 3.0bc | 47.3 ± 0.7a | 28.2 ± 0.6 cd | 13.6 ± 0.0defgh | 28.3 ± 0.3cde | 21.5 ± 5.0bcde | 384.2 ± 14.6 cd |
| | Joah | 24.8 ± 1.7c | 17.2 ± 1.0ef | 107.4 ± 7.2d | 80.7 ± 5.0cdef | 40.2 ± 0.9abc | 28.2 ± 1.6 cd | 15.3 ± 0.5cdef | 29.0 ± 0.6cde | 23.8 ± 0.4abcd | 366.7 ± 15.4de |
| | Hojoong | 23.8 ± 1.1 cd | 12.4 ± 0.1 g | 84.4 ± 0.3ef | 54.7 ± 0.3jk | 45.4 ± 0.0ab | 28.6 ± 0.1 cd | 13.9 ± 0.1defg | 40.0 ± 0.0a | 20.8 ± 0.2bcdef | 324.0 ± 1.9 fg |
| | Baekgang | 24.0 ± 0.0 cd | 17.2 ± 0.5ef | 79.0 ± 2.3ef | 79.3 ± 2.0defg | 15.6 ± 0.2ef | 27.2 ± 0.6 cd | 11.4 ± 0.1fghij | 32.8 ± 0.3abcde | 15.2 ± 0.0f | 301.6 ± 5.8 g |
| | Saekeumkang | 25.9 ± 0.3bc | 19.9 ± 2.1e | 107.5 ± 7.6d | 94.7 ± 6.6b | 41.7 ± 1.0abc | 28.1 ± 2.1 cd | 12.4 ± 0.3efghi | 30.7 ± 0.7bcde | 25.0 ± 0.4abc | 386.0 ± 20.3 cd |
| | Dabun | 23.6 ± 0.8 cd | 19.5 ± 0.0e | 77.2 ± 0.5ef | 69.4 ± 0.5ghi | 35.3 ± 0.2abcd | 28.1 ± 0.3 cd | 9.8 ± 0.1hij | 33.9 ± 0.0abcd | 25.0 ± 0.2abc | 321.8 ± 0.3 fg |
| | Jeokjoong | 23.6 ± 0.5 cd | 24.4 ± 3.5 cd | 78.0 ± 0.2ef | 68.6 ± 0.1hi | 20.6 ± 0.2def | 25.6 ± 0.1 cd | 16.4 ± 0.1cde | 27.6 ± 0.2de | 27.5 ± 0.2a | 312.3 ± 2.3 fg |
| | Sugang | 28.5 ± 2.3a | 37.3 ± 2.8b | 106.5 ± 7.5d | 68.5 ± 4.4hi | 29.9 ± 13.2cde | 56.4 ± 32.2ab | 19.0 ± 4.2bc | 37.4 ± 8.4ab | 21.3 ± 4.8bcde | 404.7 ± 26.9 cd |
| | Hanbaek | 25.9 ± 0.7bc | 12.3 ± 1.2 g | 71.0 ± 4.0f | 23.3 ± 1.1 L | 31.3 ± 0.5bcd | 16.5 ± 9.4d | 7.8 ± 4.3j | 17.0 ± 4.8 g | 26.2 ± 0.4ab | 231.3 ± 16.2 h |
| | Dajoong | 27.9 ± 0.3ab | 37.6 ± 0.8b | 123.3 ± 1.4bc | 75.6 ± 1.0efgh | 16.4 ± 0.2ef | 48.9 ± 0.4bc | 16.7 ± 0.1bcd | 35.1 ± 0.0abcd | 25.9 ± 0.0ab | 407.4 ± 4.3bc |
| | Jojoong | 23.9 ± 0.4 cd | 34.2 ± 2.5b | 128.2 ± 8.4b | 83.3 ± 5.2cde | 33.8 ± 22.6abcd | 74.6 ± 31.9a | 12.0 ± 3.4fghi | 35.8 ± 1.0abc | 17.4 ± 0.2ef | 443.1 ± 30.4b |
| | Johan | 27.9 ± 1.5ab | 49.7 ± 3.7a | 110.1 ± 7.6 cd | 85.8 ± 6.0bcd | 22.6 ± 0.6def | 33.7 ± 1.0bcd | 9.1 ± 0.3ij | 32.2 ± 9.2bcde | 18.8 ± 0.4def | 390.0 ± 8.3 cd |
| 14 days | Keumkang | 22.3 ± 0.5bcde | 13.7 ± 0.1fgh | 55.6 ± 0.5 h | 32.3 ± 0.4 cd | 25.9 ± 0.3ij | 11.1 ± 0.2de | 6.5 ± 0.0e | 15.3 ± 0.0a | 23.3 ± 0.1ab | 206.0 ± 1.5f |
| | Jokyoung | 20.6 ± 0.0 g | 12.1 ± 0.1ghi | 76.8 ± 0.1 g | 11.4 ± 4.1j | 36.3 ± 0.1b | 7.2 ± 0.0j | 5.2 ± 0.0gh | 5.7 ± 0.0gh | 15.4 ± 0.3de | 190.7 ± 4.0f |
| | Shinmichal 1 | 21.3 ± 0.2 fg | 15.3 ± 4.4defg | 152.2 ± 1.4a | 28.6 ± 0.3e | 31.8 ± 0.1e | 8.7 ± 0.1hi | 8.7 ± 0.0c | 10.4 ± 0.0def | 17.8 ± 0.0 cd | 294.8 ± 2.9a |
| | Baekjoong | 20.8 ± 0.0 g | 21.6 ± 0.4bc | 95.8 ± 2.2e | 35.2 ± 0.7bc | 27.2 ± 0.3 h | 11.8 ± 0.4d | 9.5 ± 1.1b | 12.7 ± 0.5bc | 20.7 ± 0.1abc | 255.3 ± 1.5 cd |
| | Suan | 21.9 ± 0.1cdef | 11.2 ± 0.4hi | 113.1 ± 5.7d | 21.9 ± 0.8gh | 34.9 ± 1.0c | 10.0 ± 0.2 fg | 7.4 ± 0.4d | 10.9 ± 3.5cdef | 17.5 ± 0.1 cd | 248.8 ± 11.4de |
| | Goso | 23.7 ± 0.1a | 16.5 ± 0.1def | 98.7 ± 0.3e | 51.2 ± 0.2a | 24.9 ± 0.0 k | 16.3 ± 0.0b | 7.3 ± 0.0d | 16.7 ± 0.0a | 24.3 ± 0.0a | 279.6 ± 0.2ab |
| | Joah | 22.4 ± 0.2bcd | 15.4 ± 0.2defg | 123.3 ± 1.0c | 32.0 ± 0.7 cd | 33.4 ± 0.0d | 9.0 ± 0.0ghi | 6.2 ± 0.0ef | 10.8 ± 0.0cdef | 18.1 ± 0.3 cd | 270.6 ± 2.5bc |
| | Hojoong | 22.8 ± 0.3b | 11.4 ± 0.1hi | 84.8 ± 0.4f | 30.5 ± 0.3de | 31.4 ± 0.0ef | 36.5 ± 0.3a | 5.9 ± 0.0ef | 12.4 ± 0.1bcd | 22.5 ± 0.1abc | 258.2 ± 1.5 cd |
| | Baekgang | 21.9 ± 0.1cdef | 13.8 ± 0.3fgh | 83.1 ± 4.9 fg | 18.5 ± 1.0i | 41.2 ± 0.2a | 8.0 ± 1.8ij | 8.0 ± 0.3d | 8.9 ± 0.1f | 11.4 ± 8.7e | 214.8 ± 22.4e |
| | Saekeumkang | 22.5 ± 0.3bc | 15.4 ± 0.9defg | 99.5 ± 5.9e | 35.8 ± 2.0b | 30.8 ± 0.6 fg | 9.6 ± 0.4fgh | 5.6 ± 0.1 fg | 12.2 ± 0.2bcde | 17.5 ± 0.2 cd | 248.9 ± 10.1de |
| | Dabun | 21.9 ± 0.2cdef | 14.1 ± 0.3efgh | 78.2 ± 2.6 fg | 20.2 ± 0.6hi | 22.3 ± 0.3 L | 7.0 ± 0.1j | 4.6 ± 0.0 h | 9.4 ± 0.1f | 19.1 ± 0.3bcd | 196.8 ± 3.9f |
| | Jeokjoong | 21.7 ± 0.1def | 18.4 ± 1.3 cd | 82.4 ± 7.0 fg | 34.7 ± 2.7bc | 26.5 ± 0.8hi | 12.9 ± 0.7c | 10.4 ± 0.3a | 14.9 ± 0.4a | 23.4 ± 1.0ab | 245.4 ± 14.1de |

(*continued on next page*)

**Table 2** (*continued*)

| Growth times and cultivars | | Phenolic content (mg/100 g of dry weight)[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3-*O*-FQA[b] (**2**) | Lu-6-*C*-ara-8-*C*-G[b] (**4**) | Isoorientin (**6**) | Isochaftoside (**8**) | Isovitexin-2″-*O*-G[b] (**9**) | Apigenin-6-*C*-G[b] (**10**) | Isoscoparin-2″-*O*-G[b] (**11**) | Isoscoparin (**12**) | Tricin-7-*O*-G[b] (**15**) | Total |
| | Sugang | 23.5 ± 0.4a | 21.8 ± 1.0bc | 98.9 ± 3.9e | 23.7 ± 0.7 fg | 31.8 ± 0.1e | 10.7 ± 0.2ef | 7.4 ± 0.0d | 15.1 ± 0.1a | 18.6 ± 0.3bcd | 251.6 ± 6.9de |
| | Hanbaek | 21.7 ± 0.4def | 9.8 ± 1.3i | 61.4 ± 3.1 h | 7.7 ± 1.1 k | 30.1 ± 0.4 g | ND[c] | 4.9 ± 0.2 h | 4.7 ± 0.0 h | 17.6 ± 0.3 cd | 157.8 ± 7.0 g |
| | Dajoong | 21.6 ± 0.6ef | 22.1 ± 0.3b | 132.3 ± 2.1b | 25.2 ± 0.4f | 37.0 ± 0.6b | 10.0 ± 0.1 fg | 6.6 ± 0.0e | 10.3 ± 0.1ef | 20.7 ± 0.9abc | 285.8 ± 4.4ab |
| | Jojoong | 20.8 ± 0.2 g | 17.6 ± 0.0de | 103.5 ± 0.8e | 28.2 ± 0.2e | 25.0 ± 0.2jk | 9.5 ± 0.1gh | 6.0 ± 0.1ef | 13.0 ± 0.1b | 17.9 ± 0.1 cd | 241.5 ± 1.2de |
| | Johan | 20.8 ± 0.6 g | 30.2 ± 4.0a | 99.5 ± 2.1e | 18.0 ± 0.4i | 26.4 ± 0.0hi | 7.1 ± 0.2j | 6.1 ± 0.1ef | 7.1 ± 0.0 g | 17.6 ± 0.0 cd | 232.9 ± 7.4e |

[a] All values are presented as the mean ± SD of triplicate determinations, content expressed as mg of each phenolic equivalents per 100 g of dry weight.
[b] 3-*O*-FQA, 3-*O*-feruloylquinic acid; ara, arabinoside; G, glucoside.
[c] ND: not detected.

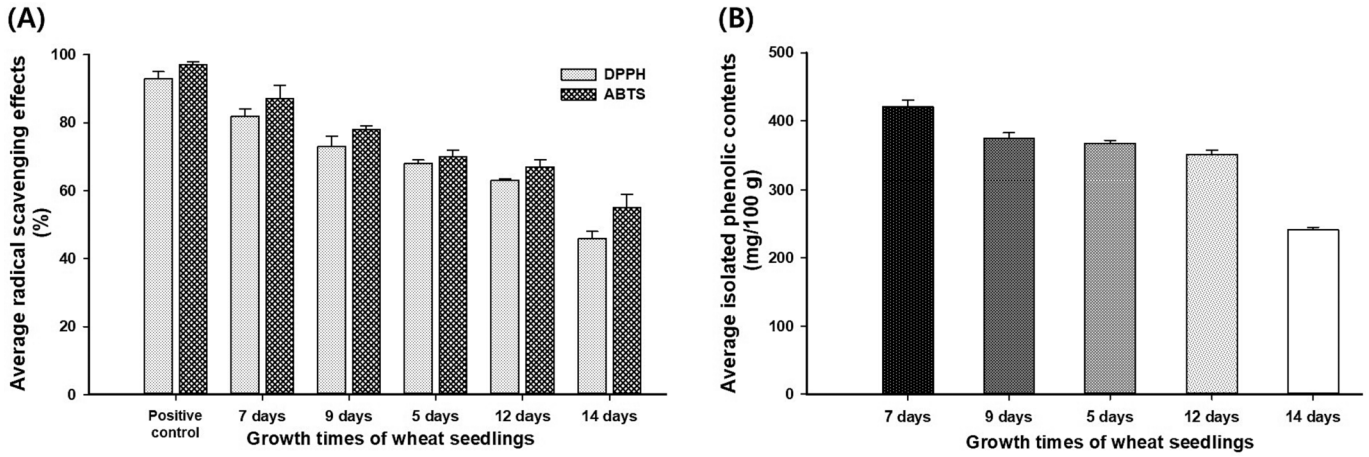


**Fig. 4.** Comparisons of average antioxidant properties and isolated 9 phenolic contents from 80% methanol extracts in wheat seedlings of 17 cultivars at 5 different growth times.

were consistent with the order of total average phenolic metabolites, as follows: 420.8 (7 days), 374.6 (9 days), 366.7 (5 days), 350.7 (12 days), and 241.1 mg/100 g (14 days) (Fig. 4B), and thus, the scavenging capacities are positively correlated with the nine phenolic contents, as supported by previous literatures (Bai et al., 2017; Celli, Pereira-Netto, & Beta, 2011; Chen et al., 2019; Lee et al., 2016; Tian, Chen, Tilley, & Li, 2021). Other metabolites may also be related to the DPPH radical scavenging abilities (Deseo, Elkins, Rochfort, & Kitchen, 2020; Floegel, Kim, Chung, Koo, & Chun, 2011; Sahu et al., 2021; Tian, Chen, Tilley, & Li, 2021). Among the various cultivars with growth times of 7 days, Shinmichal 1 exhibited the predominant DPPH radical inhibition rate of 87 %, whereas Hanbaek displayed the lowest DPPH radical scavenging effect (53 %). The remaining all cultivars displayed mild activities (<75 %) at growth times of 12 days except Shinmichal (80 %), and their scavenging abilities were similar patterns to those of the phenolic metabolite contents: Goso (74 %) > Saekeumkang; Dajoong (73 %) > Joah; Sugang (68 %) > Jokyoung (65 %) > Hojoong (62 %). As mentioned in the above data, the antioxidant properties on radical scavenging effects may be depenedent to much degree on the phenolic contents (Bai et al., 2017; Celli, Pereira-Netto, & Beta, 2011; Tian, Chen, Tilley, & Li, 2021). Their correlations regarding the phenolic concentration and distribution as well as antioxidant capacities may be also related to various environmetal conditions (cultivar, growth time, genetic, agronomic, moisture, etc.), as reported in earlier researches (Lee et al., 2016; Tsao et al., 2006; Zhang et al., 2015). In the ABTS radical method, all samples were observed significant differences according to growth time. The rank order of this radical inhibition was also similar to the results of the DPPH assay, and the average effects at 600 μg/ml were as follows: 7 days (87 %) > 9 days (78 %) > 5 days (70 %) > 12 days (67 %) > 14 days (55 %) (Fig. 4A). Interestingly, all cultivars were detected higher scavenging abilities for the ABTS radical compared to those for DPPH. This phenomenon may be affected by the reactions of the radical sources with the various metabolites, including the nine phenolics in the 80 % methanol extracts of wheat seedlings (del Baño et al., 2003; Ha et al., 2021; Schaich, Tian, & Xie, 2015). In other words, these above differences may be associated with the degrees of metabolite reactions with the hydrogen-donating (DPPH) or hydrogen-donating and chain cleaving (ABTS) components in this plant (Floegel, Kim, Chung, Koo, & Chun, 2011; Hwang et al., 2021; Lee et al., 2015). Although the 80 % methanol extract of this crop displayed low scavenging effects compared to those of the positive controls (DPPH: BHT, 93 %; ABTS: Trolox 97 % at 600 μg/mL), the wheat seedlings collected at 7 days may be utilized as potentially excellent sources of natural antioxidants because of high phenolic contents by comparing those of other growth times (Fig. 4). In particular, the Shinmichal 1 cultivar may be utilized in developing functional agents and excellent new cultivars. Furthermore, our study suggests that the appropriate harvest times of wheat seedlings may be 7 days after sowing. The growth times of this source may be recommended

as a potent parameter in developing health agents, such as antioxidant. For the first time, this research revealed the fluctuations in the antioxidant properties of wheat seedlings according to growth time.

*Conclusion*

This is the first to reveal the changes in the phenolic components from the seedlings of various Korean wheat cultivars according to growth time. Fifteen phenolic metabolites in the 80 % methanol extract were characterized using HPLC-Q-Orbitrap-MS/MS. Among them, nine phenolics were identified by silica gel column chromatography and NMR spectroscopy, and their contents were evaluated using HPLC analysis. The individual phenolics exhibited considerable differences according to growth time and cultivar, and the total average contents exhibited in the following order: 7 days (420.8 mg/100 g) > 9 days (374.6 mg/100 g) > 5 days (366.7 mg/100 g) > 12 days (350.7 mg/100 g) > 14 days (241.1 mg/100 g). Specifically, isoorientin (**6**) and isochaftoside (**8**) were observed high contents with average values of 99.3 and 64.3 mg/100 g, representing approximately 28.3 and 18.3 % of the total content (average 350.8 mg/100 g), respectively. Moreover, the wheat seedling extracts exhibited the highest scavenging activities against DPPH and ABTS radicals at growth times of 7 days (DPPH: 82 % and ABTS: 87 % at 600 μg/mL), followed by those at growth times of 9 days > 5 days > 12 days > 14 days. These findings may provide valuable evidence that the antioxidant activities of wheat seedlings be attributed to the phenolic contents. Also, Shinmichal 1 harvested after 7 days (phenolic content: 605.0 mg/100 g, antioxidant effects: DPPH 87 %, and ABTS 95 %) may be a potential source for use in developing functional foods and increasing wheat quality. Further research is necessary to evaluate the various biological effects and other metabolites of wheat seedlings.

## CRediT authorship contribution statement

**HanGyeol Lee:** Data curation, Investigation, Resources. **Ji Yeong Yang:** Methodology, Data curation, Investigation. **Ji Eun Ra:** Formal analysis, Data curation, Investigation. **Hyung-Jae Ahn:** . **Mi Ja Lee:** Software, Validation. **Hyun Young Kim:** Investigation, Software. **Seung-Yeob Song:** Software, Validation. **Du Hyun Kim:** Project administration, Supervision. **Jin Hwan Lee:** Conceptualization, Data curation, Investigation, Writing – original draft, Methodology. **Woo Duck Seo:** Conceptualization, Writing – review & editing, Project administration, Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100557.

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