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Comparative investigation on variations of nutritional components in whole seeds and seed coats of Korean black soybeans for different crop years and screening of their antioxidant and anti-aging properties

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ABSTRACT

This research was conducted to demonstrate the comparisons of nutritional constituents (isoflavone; anthocyanin; protein; fatty acid; oil) and biological properties (antioxidant, anti-aging) in whole seeds and seed coats of black soybeans for crop years. Isoflavones and anthocyanins showed considerable differences in cultivars and growth years with the ranges of 794.9–4195.3 μg/g and 2.3–14.4 mg/g, while other components exhibited slight variations. In particular, malonylgenistin and cyanidin-3-*O*-glucoside were observed the most abundant phenolics, comprising approximately 35.5 (778.0 μg/g) and 76.7% (4.6 mg/g) of total average contents (isoflavone: 2197.8 μg/g; anthocyanin: 6.0 mg/g). Moreover, the whole seeds and seed coats displayed excellent activities in antioxidant (radical; DNA protectant), tyrosinase inhibition, and elastase inhibition. Their effects significantly occurred with dose-dependent patterns as follows: elastase (150 μg/mL) > tyrosinase (600 μg/mL) > ABTS (1500 μg/mL) > DPPH (1500 μg/mL) with higher abilities of seed coats than whole seeds. The DNA protection exhibited higher rates in seed coats with > 90% at 200 μg/mL. Natably, Socheong (isoflavone; 4182.4 μg/g) and Geomjeong 2 (anthocyanin: 10.3 mg/g) cultivars may be recommended as potential sources to the development of functional agents and new cultivars owing to their high average phenolic contents.

Introduction

Leguminous plants are known as be important crops for nutritive sources in sevearal countries (Desta et al., 2022; Holden et al., 2018; Pueppke, 1996; Waqas et al., 2015). Among diverse legume species, soybean (*Glycine* max (L.) Merill.) has been considered one of the most excellent edible crops, providing significant nutrients, including protein, fatty acid, amino acid, and beneficial secondary metabolite (Bai et al., 2017; Charron et al., 2005; Desta et al., 2022; Ito et al., 2013; Yamabe et al., 2007; Yu et al., 2021). Soybeans and soy-based substrates are increasingly of interest in food and medicinal applications due to their high nutritional values and strong health promoting properties for many years (Choi et al., 2008; Varnosfadera et al., 2019; Waqas et al., 2015; Yu et al., 2021). This crop has diverse seed coat colors such as black, yellow, green, and brown (Cho et al., 2013; Messina, 1999), and their seed coats play an important role in supplying protection on insert or fungal pathogen and controlling inhibition (Moise et al., 2005). Moreover, it is commonly well-known that the phenolic profiles and concentrations in soybeans were observed considerable differences according to the colors of seed coat (Cho et al., 2013; Desta et al., 2022). In particular, many

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, D3G, delphinidine-3-*O*-glucoside; C3G, cyanidin-3-*O*-glucoside; P3G, petunidin-3-*O*-glucoside.

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studies have reported that the dark seed coat of black soybeans are correlated with the presence of anthocyanins and proanthocyanins (Ito et al., 2013; Lee et al., 2009; Todd and Vodkin, 1993). Black soybean has been a widely used crop material due to its human health benefit and folk medicine by comparing other seed coated soybeans (Cho et al., 2013; Desta et al., 2022; Lee et al., 2009). Interestingly, this source was used to prepare traditional fermented products, namely, cook (*Cheonggukjang*), paste (*Doenjang*), cake (*Meju*), and sauce (*Kanjang*) in Korea during the past decades and was associated with a wide range of various biological properties including antioxidant, anti-mutagenic, anti-aging, antidiabetic, and anti-inflammatory activities (Choi et al., 2008; Waqas et al., 2015; Yu et al., 2021). In these regards, the attention for evaluating and characterizing nutritional ingredients has increased tremendously with functional aspect in this crop (Bai et al., 2017; Waqas et al., 2015; Yamabe et al., 2007; Yu et al., 2021). Several literatures have shown that black soybeans contain abundant valuable nutrients (isoflavone, anthocyanin, saponin, protein, and oil) and these constituents possess high biological effects (Brummer et al., 1997; Charron et al., 2005; Desta et al., 2022; Ito et al., 2013; Lee and Cho, 2012). Specifically, isoflavones and anthocyanins have been categorized as the flavonoid phenolic group of beneficial dietary and nutritive constituents, which were related to the prevention of various human diseases (Bai et al., 2017; Fujimaki et al., 2018; Hemachandran et al., 2017; Kim et al., 2018; Liyanaarachchi et al., 2018; Messina, 1999). Some studiers are absolutely required to evaluate the investigations and comparisons of metabolite components and their potential bio availabilities in crops and natural plants (Bai et al., 2017; Boneza & Niemeyer, 2018; Cheng et al., 2022; Farooqi, 2021; Jiang et al., 2020; Li et al., 2021). In particular, many kinds of black soybean-based food products have been developed and their daily consumption ratios are consistently increasing in the world. Recent our researches have also revealed that the variations of isoflavones and digestive enzyme inhibitory capacities in fermented soybean sources (Hwang et al., 2021). In addition, plant breeding researchers have focused on the development of new or excellent black soybean cultivars containing abundant nutritional compositions and strong health beneficial properties (Yamabe et al., 2007; Yu et al., 2021). Although previous articles have revealed the phenolic contents and pharmaceutical abilities in soybeans (Cho et al., 2013; Bai et al., 2017), as far as we know, only a few literatures reported the verification and examination of isoflavones and anthocyanins from various black soybeans at growth years. Little information has also investigated and compared to nutritional components including protein, oil, and fatty acid for evaluating the black soybean quality. Furthermore, there is little intelligence available about the comparisons of antioxidant and anti-aging activities in whole seeds and seed coats of this source. Therefore, we investigated the beneficial information concern to suitable metabolite factors and functional properties as well as development of excellent cultivars for futher applications of food and nutraceutical industrious.

The key objectives of the present work was to determine the valuable cultivars have not only high metabolites but also strong biological effects from whole seeds and seed coats of various Korean black soybeans for different crop years. Herein, we demonstrated and compared the contents of twleve isoflavones and three anthocyanins in sixteen lines using HPLC. Specifically, three representative anthocyanins were isolated and elucidated using column chromatography and NMR spectroscopy from the acidic 50% methanol extract of seed coats. In addition, this study documented the protein, oil, and fatty acid levels as well as antioxidant properties on radical scavenging and DNA cleavage effects. Our research also accessed for the first time the comparison and evaluation of tyrosinase and elastase inhibition capacities.

Materials and methods

Crop sources and chemicals

Sixteen Korean black soybean cultivars (cv. Ilpumgeomjeong, Seonheuk, Geomjeong 3, Geomjeongol, Geomjeongsaeol, Tawon, Geomjeong 4, Cheongja, Geomjeong 1, Geomjeong 2, Cheongja 2, Cheongja 3, Ilpumgeomjeong 2, Mirang, Heugmi, and Socheong) were obtained from the National Institute of Crop Science, Rural Development Administration, Korea. These cultivars were harvested in the experimental field of the above institutution, Daegu, Gyeongbuk, during 2018 and 2019. All cultivars were grown in natural conditions. After harvesting, the soybean seeds were immediately dried under natural light at room temperature and maintained at − 10 °C prior to analysis. Analytical grade water and methanol were obtained from J.T. Baker (Philipsburg, NJ, USA) and CFCOOD-CD_3OD was purchased from Sigma Chemical Co. (St, Louis, USA). Moreover, TLC aluminum RP-18 F_{254} and Silica gel 60Rp-18 were supplied from Sigma-Aldrich. Isoflavone glucoside and aglycone standards were obtained by chromatographic techniques from soybean seeds, as the previous method (Cho et al., 2013; Lee and Cho, 2012) and acetyl- and malonyl glucosides were obtained from Sigma-Aldrich. 2,2-Diphenyl-1-pycrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), tyrosinase (EC 1.14.18.1), elastase (EC 3.4.21.36), *N*-succinyl-Ala-Ala-Ala-p-nitroanilide (SANA), ascorbic acid, ursolic acid, butylated hydroxytoluene (BHT), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), $FeCl_3$, dithiothreitol (DTT), L-tyrosine, *p*-nitrophenyl-α-D-glucopyranoside (PNP-G), *p*-nitrophenol (*p*-NP), and *p*-nitrophenol-β-D-glucopyranoside (*p*-NPG) were acquired from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other solvents and chemicals were also used analytical-grade.

Instruments

The ^1H and ^{13}C NMR data were measured by a Bruker AM 500 nuclear magnetic resonance (NMR) spectrometer (Karlsruhe, Germany) in CFCOOD-CD_3OD with TMS (tetramethyl silane). Biological activities including antioxidant, tyrosinase, and elastase assays were evaluated by UV–vis absorption spectra by Agilent BioTek microplate spectrophotometry (EPOCH 2, Winooski, VT, USA). Isoflavones and anthocyanins were qualitified using an Agilent 1200 system (Waldbronn, Germany) consisting of quaternary pump and diode array detector. The protein and oil contents were analyzed using a Buchi B-435 Kjeldahl digestion system (Buchi, Switzerland) and a Buchi B-811 extraction system (Buchi, Switzerland). The fatty acid compositions were estimated using an Agilent 7890A GC series (Boeblingen, Germany) equipped with flame ionization detector.

Preparation of samples and calibration curves for isoflavone and anthocyanin quantification

To determine the isoflavone contents, the black soybean seeds were ground (230 mesh) and extracted with 50% methanol in a shaking incubator at 25 °C for 48 h (pulverized seeds: 1 g, 50% methanol: 20 mL). The crude supernatants were filtered through a 0.45 μm membrane filter (Whatman Inc., Maidstone, USA) prior to HPLC analysis. To evaluate anthocyanins, the seed coats (0.5 g) were extracted with 1% HCl of 50% methanol (10 mL) for 3 days at 4 °C, in the dark, and then filtered through filter paper (Whatman No. 42). The mixture solution was filtered with a syringe filter (0.45 μm) before HPLC analysis. The preparation of calibration curves was conducted according to methods

of preivous literatures (Cho et al., 2013; Lee and Cho, 2012). In brief, the peak areas of the isoflavone and anthocyanin standards were integrated from the HPLC chromatograms at 254 nm (isoflavone) and 330 nm (anthocyanin), and plotted on the concentrations to create a linear curve. Each standard (2 mg) was prepared by dissolving in DMSO (isoflavone) and 0.1% TFA with DMSO (anthocyanin) to gain a 1 mg/mL concentration and their calibration curves were manufactured using eight concentrations (400, 200, 100, 50, 10, 5, 1, 0.5 μg/mL). All curves were obtained the high linearity ($r^2 > 0.998$).

Operation conditions of HPLC and NMR

The isoflavone analysis was examined as reported in earlier literature with minor modifications (Hwang et al., 2021). The HPLC operation was carried out using an Agilent 1200 series with a Lichrophore 100 RP-18e column (125 mm × 4 mm, LichroCART, 5 μm, Merck KGaA, Darmstadt, Germany) and the flow rate was 1.0 min/min at 254 nm with gradient elution using 0.1% acetic acid in H_2O (elution A) and CH_3CN (elution B) as the following procedures: 0–13 min, 13% B; in 13–25 min, 20% B; 25–40 min, 35% B; 40–60 min, 100% B. The sample injection volume was programmed as 20 μL and the column temperature was set at 25 °C. The anthocyanin contents were evaluated with reversed phase HPLC with a flow rate of 0.7 mL/min at 25 °C using isocratic elution (0.1 TFA in 50% methanol) (Lee and Cho, 2012). The remaining conditions were performed as follow: injection volume: 20 μL; column: Lichrophore 100 RP-18e; retention time: 20 min, detection: 530 nm. The isolated three anthocyanins were analyzed on spectra of ^{1}H NMR at 500 MHz and ^{13}C NMR at 125 MHz by CFCOOD-CD_3OD with tetramethylsilane as internal standard in NMR tubes from earlier study (Lee et al., 2009). The NMR data were determined as chemical shift values with coupling constant (*J*) on Hertz (Hz).

Measurement of protein, oil, and fatty acid contents

For evaluating protein content, the powdered seeds (0.2 g, 60 mesh) were mixed by a Buchi B-435 digestion system (20 mL H_2SO_4 and 3.0 g catalyst: K_2SO_4:$CuSO_4$ = 9:1) connected with a scrubber through the Kjeldahl nitrogen method in a distillation unit (AOAC, 1990). The oil quantification was measured by the Soxhlet skill (Buchi B-811 extraction system) (Lee and Cho, 2012). The pulverized seeds (2.0 g, 60 mesh) were added to hexane (200 mL) in an extraction thimble, and then boiled for 2 h at 105 °C. The extracted oil was weighted and represented as the dry matter of soybean seeds. The fatty acid contents were examined by methylation method (H_2O:toluene:methanol = 1:10:20) through the previous study of fatty acid methyl esters (Vinod et al., 2010). Briefly, the oil extract (100 μL) using hexane and methylation solution (5 mL) were added to methylesterfication. The mixture solution was heated on a water bath, and then cooled at 25 °C. The organic phase was injected into the gas chromatography and the individual fatty acid methylester was elucidated by comparing the retention time as those of standards (Lee and Cho, 2012; Vinod et al., 2010). Each composition was measured as a percentage of total fatty acids. The GC condition was analyzed according to the procedures reported by Lee and Cho (2012).

Extraction and isolation of anthocyanin standards

The extraction and isolation of anthocyanins in the anthocyanin-enriched source was conducted with slight modifications according to the previous reported methods (Lee and Cho, 2012; Lee et al., 2009). The seed coats (50 g) of black soybean (cv. Geomjeong 2) were extracted with 500 mL of 1% HCl (v/v) in 50% methanol for three days at 4 °C in the dark. The crude extract was concentrated with a rotary evaporator at 25 °C and purified by Amberlite XAD-7 column chromatography (CC) (4.5 × 60 cm) using water (500 mL) and methanol (1% TFA) (500 mL). The concentrated anthocyanin fraction was purified by silica gel 60 Rp-18 CC (4 × 50 cm, 40–63 μm) using MeOH-H_2O-TFA mixture elution of 10:89:1 → 20:79:1 → 25:74:1 → 35:64:1 → 45:54:1 → 60:39:1 → 70:29:1 (v/v, each 400 mL) to obtain 10 fractions (A–J). Fraction D (310 mg) was separated by CC on silica gel Rp-18 (1.5 × 40 cm) with MeOH-H_2O-TFA (5:94:1 → 50:49:1), and then chromatographed on Sephadex LH-20 using MeOH-H_2O-TFA (30:69:1) to yield delphinidin-3-*O*-glucoside (D3G) of compound **13** (16 mg). Fraction F (290 mg) was chromatographed on a silica gel Rp-18 column (2.0 × 40 cm) using a gradient of MeOH-H_2O-TFA (10:89:1 → 40:59:1) to obtain 13 subfractions, based on the TLC patterns, and then subfractions 7–9 (83 mg) were re-chromatgraphed on Sephadex LH-20 (1.5 × 40 cm) to produce cyanidin-3-*O*-glucoside (C3G) of compound **14** (27 mg). Fraction J (320 mg) was purified by a silica gel Rp-18 CC (2.0 × 40 cm) using gradient elution (MeOH-H_2O-TFA = 25:74:1 → 60:39:1) to afford 6 subfractions, and then petunidin-3-*O*-glucoside (P3G) (compound **15**, 11 mg) was separated by Sephadex LH-20 (1.0 × 30 cm) with MeOH-H_2O-TFA (40:59:1 → 55:44:1) in subfraction 4 (67 mg).

Measurement of antioxidant properties against radicals

The antioxidant properties on radical scavenging methods including DPPH and ABTS were demonstrated by the previous techniques (Kim et al., 2014; Lee and Cho, 2021). To evaluate DPPH assay, a solution of 1 mM DPPH was adjusted to 0.70 at 517 nm, and the 50% methanol extract (100 μL) of samples or BHT (positive control, 100 μL) with various concentrations were added to 1 mM DPPH solution (3.9 mL). The mixture solution was maintained for 30 min at 25 °C in darkness and the absorbance value was examined at 517 nm. The scavenging effect was determined as a percentage by the following formula:

$$\% = [(1 - At/Ao)] \times 100$$

At = absorbance of sample, Ao = absorbance of control.

The ABTS radical cation was produced by reacting the 7 mM $ABTS^{\bullet+}$ stock solution (abs 0.70 with ethanol at 734 nm) and 2.45 mM potassium persulphate (Kim et al., 2014). This mixture was retained in the dark for 12 h at 25 °C before use. To measure the ABTS radical scavenging ability, the ABTS solution (0.9 mL) was added with sample (0.1 mL), and the absorbance value at 734 nm was recorded. The sample or positive control (Trolox) concentrations were prepared as those of DPPH radical and their effects were confirmed as a percentage according to the equation:

$$\% = [(1 - At/Ao)] \times 100$$

At = absorbance of sample, Ao = absorbance of control.

DNA protection effect

The metal-catalyzed oxidation (MCO) DNA cleavage protection method was evaluated as described previously (Hu et al., 2010). In brief, the extract (10 μL) of various concentrations (500, 200, 100, 20, and 10 μg/mL) was added to both 5 μL of 15.4 μM $FeCl_3$ and 5 μL of 3.3 mM dithiothreitol (DTT). The reaction mixture was incubated for 2.5 h at 37 °C. The pUC18 super-coiled plasmid DNA (1 μg) was added to each

reaction mixture, and then incubated for 2.5 h at 37 °C. The crude solution was mixed with 2 μL loading dye including 0.5% bromophenol blue, 50% glycerol, and 0.5% xylene cyanol, and then the DNA protection was measured by 0.1% agarose gel electrophoresis containing ethidium bromide.

Inhibition activities against tyrosinase and elastase

The inhibitory ability on tyrosinase was demonstrated by a spectrophotometric technique using L-DOPA (substrate) as described by Liyanaarachchi et al., 2018 with a slight modification. The reaction solution was mixed with 0.1 M NaH_2PO_4 buffer (685 μL, pH 6.8), sample (20 μL, different concentrations), tyrosinase (15 μL, 250 unit/mL), and L-DOPA (5 mM, 100 μL). The mixture solution was maintained for 25 min at 37 °C, and then the optical density was measured at the wavelength of 475 nm using spectrophotometry by comparison with positive control (ascorbic acid). The percent inhibion ability was calculated using the following equation.

$$\text{Inhibition rate}(\%) = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}}\right) \times 100$$

A_{control} = the absorbance of buffer.

A_{sample} = the absorbance of the reaction solution containing sample extract.

Elastase inhibition activity was documented with the methods of Li et al (2021) and Liyanaarachchi et al. (2018). Elastase was dissolved to make a 3.33 mg/mL stock solution in sterile water and the substrate *N*-succinyl-Ala-Ala-Ala-*P*-nitroanilide (1.6 mM) was dissolved in 0.2 mM Tris-HCl buffer (pH 8.0). The reaction mixture contains 0.2 mM Tris-HCl buffer (100 μL), substrate (25 μL), sample extract (50 μL), and elastase (25 μL) in a 96-well plate. The crude solution was incubated at 25 °C for 20 min, and then the absorbance value was monitored at 410 nm. The inhibition rates of ursolic acid (positive control) and sample were expressed with percentage values using following equation:

$$\textit{Inhibition rate}(\%) = \left[\left(1 - A_{sample}\right)/A_{control}\right) \times 100$$

A_{control} = the absorbance of buffer.

A_{sample} = the absorbance of the reaction solution containing sample extract.

Statistical analysis

The isoflavone and anthocyanin contents were performed as the mean of triple measurements. The antioxidant and enzyme inhibition capacities were also measured as the mean values of three replicates. The results were expressed using statistical analysis software (SAS) 9.2 PC package (SAS Institute Inc. Cary, NC, USA) and the Duncan's multiple range tests were based on the 0.05 probability level.

Results and discussion

Identification of isolated anthocyanins

The chemical structures of three anthocyanins **13**–**15** are elucidated by spectroscopic data of ^{1}H and ^{13}C NMR, and their characteristics are reported as below:

*Compound **13** (D3G)*

Amorphous red powder; ^{1}H NMR (500 MHz, CFCOOD-CD_3OD) δ 3.50 (1H, dd, J = 9.3, 9.3 Hz, H-4 glc), 3.60 (2H, m, H-3, 5 glc), 3.73 (1H, dd, J = 8.6, 7.9 Hz, H-2 glc), 3.74 (1H, dd, J = 12.0, 5.5 Hz, H-6α glc), 3.94 (1H, dd, J = 11.9, 1.5 Hz, H-6 glc), 5.30 (1H, d, J = 7.5 Hz, H-1 glc), 6.61 (1H, s, H-6), 6.79 (1H, s, H-8), 7.67 (2H, d, J = 2.1 Hz, H-2′ and H-6′), 8.83 (1H, s, H-4). ^{13}C NMR (125 MHz, CFCOOD-CD_3OD) δ 169.2 (C-7), 162.9 (C-2), 159.0 (C-5), 157.0 (C-9), 147.0 (C-5′), 146.8 (C-3′), 145.7 (C-3), 144.5 (C-4′), 135.8 (C-4), 119.4 (C-1′), 112.8 (C-10), 112.1 (C-2′), 111.9 (C-6′), 103.1 (C-6), 103.0 (C-1 glc), 94.3 (C-8), 78.4 (C-5 glc), 77.8 (C-3 glc), 76.3 (C-2 glc), 70.3 (C-4 glc), 62.0 (C-6 glc).

*Compound **14** (C3G)*

Amorphous red powder; ^{1}H NMR (500 MHz, CFCOOD-CD_3OD) δ 3.42 (1H, dd, J = 9.1, 9.1 Hz, H-4 glc), 3.56 (2H, m, H-3, 5 glc), 3.66 (1H, dd, J = 8.4, 7.7 Hz, H-2 glc), 3.70 (1H, dd, J = 12.0, 5.8 Hz, H-6α glc), 3.89 (1H, dd, J = 12.0, 1.9 Hz, H-6β glc), 5.29 (1H, d, J = 7.4 Hz, H-1 glc), 6.60 (1H, s, H-6), 6.81 (1H, s, H-8), 6.92 (1H, d, J = 8.6 Hz, H-5′), 7.98 (1H, d, J = 2.0 Hz, H-2′), 8.10 (1H, dd, J = 8.5, 2.0 Hz, H-6′), 8.90 (1H, s, H-4). ^{13}C NMR (125 MHz, CFCOOD-CD_3OD) δ 170.2 (C-7), 164.0 (C-2), 159.0 (C-5), 157.2 (C-9), 155.6 (C-4′), 147.2 (C-3′), 145.4 (C-3), 136.8 (C-4), 128.1 (C-6′), 121.1 (C-1′), 117.9 (C-2′), 117.2 (C-5′), 113.2 (C-10), 103.9 (C-1 glc), 103.3 (C-6), 94.9 (C-8), 78.7 (C-5 glc), 78.2 (C-3 glc), 74.6 (C-2 glc), 70.9 (C-4 glc), 62.4 (C-6 glc).

*Compound **15** (P3G)*

Amorphous red powder; ^{1}H NMR (500 MHz, CFCOOD-CD_3OD) δ 3.33 (1H, dd, J = 9.1, 9.1 Hz, H-4 glc), 3.40 (2H, m, H-3, 5 glc), 3.58 (1H, dd, J = 8.6, 8.0 Hz, H-2 glc), 3.66 (1H, dd, J = 12.0, 2.1 Hz, H-6α glc), 3.79 (1H, dd, J = 12.0, 2.1 Hz, H-6β glc), 4.01 (3H, s, OCH_3), 5.37 (1H, d, J = 7.6 Hz, H-1 glc), 6.66 (1H, s, H-6), 6.96 (1H, s, H-8), 7.94 (1H, d, J = 2.2 Hz, H-6′), 7.95 (1H, d, J = 2.2 Hz, H-2′), 8.93 (1H, s, H-4). ^{13}C NMR (125 MHz, CFCOOD-CD_3OD) δ 170.1 (C-7), 164.6 (C-2), 163.6 (C-4′), 159.1 (C-5), 156.8 (C-9), 145.3 (C-3), 137.2 (C-4), 135.1 (C-2′), 134.7 (C-6′), 119.0 (C-1′), 117.1 (C-3′), 117.0 (C-5′), 112.9 (C-10), 103.1 (C-1 glc), 103.0 (C-6), 95.0 (C-8), 77.6 (C-3 glc), 77.4 (C-5 glc), 73.2 (C-2 glc), 70.2 (C-4 glc), 61.3 (C-6 glc), 56.5 (OCH_3).

Comparison of isoflavone contents in whole seeds of black soybeans at different crop years

Several studies have previously revealed on the quantification and variation of isoflavones and other phytochemicals in soybean seeds and their process products (Desta et al., 2022; Ito et al., 2013; Lee and Cho, 2012; Sakthivelu et al., 2008). However, to the best of our knowledge, there are only few reports on excellent information concern to comparison of isoflavone profiles in various black soybeans at growth years. The evaluation of nutritional constituents for the development of new cultivars possessing high isoflavone contents has not been also extensively investigated. For these reasons, we examined isoflavone concentrations in the 50% methanol extracts of various cultivars for different crop years. In other words, this work was to compare and investigate the isoflavone compositions in sixteen black soybeans for two years. The representative HPLC chromatogram and chemical structures of soybean isoflavones are exhibited in Fig. 1A and 1B. Their retention times are as follows: peak 1 (daidzin, t_R = 9.3 min), peak 2 (glycitin, t_R = 10.7 min), peak 3 (genistin, t_R = 15.2 min), peak 4 (malonyldaidzin, t_R = 16.3 min), peak 5 (malonylglycitin, t_R = 17.4 min), peak 6 (acetyldaidzin, t_R = 21.2 min), peak 7 (acetylglycitin, t_R = 21.9 min), peak 8 (malonylgenistin, t_R = 22.5 min), peak 9 (daidzein, t_R = 24.8 min), peak 10 (glycitein, t_R = 26.9 min), peak 11 (acetylgenistin, t_R = 28.7 min), and peak 12 (genistein, t_R = 34.3 min).

The individual and total isoflavone contents are presented in Table 1. Among four isoflavone types, the most abundant component was

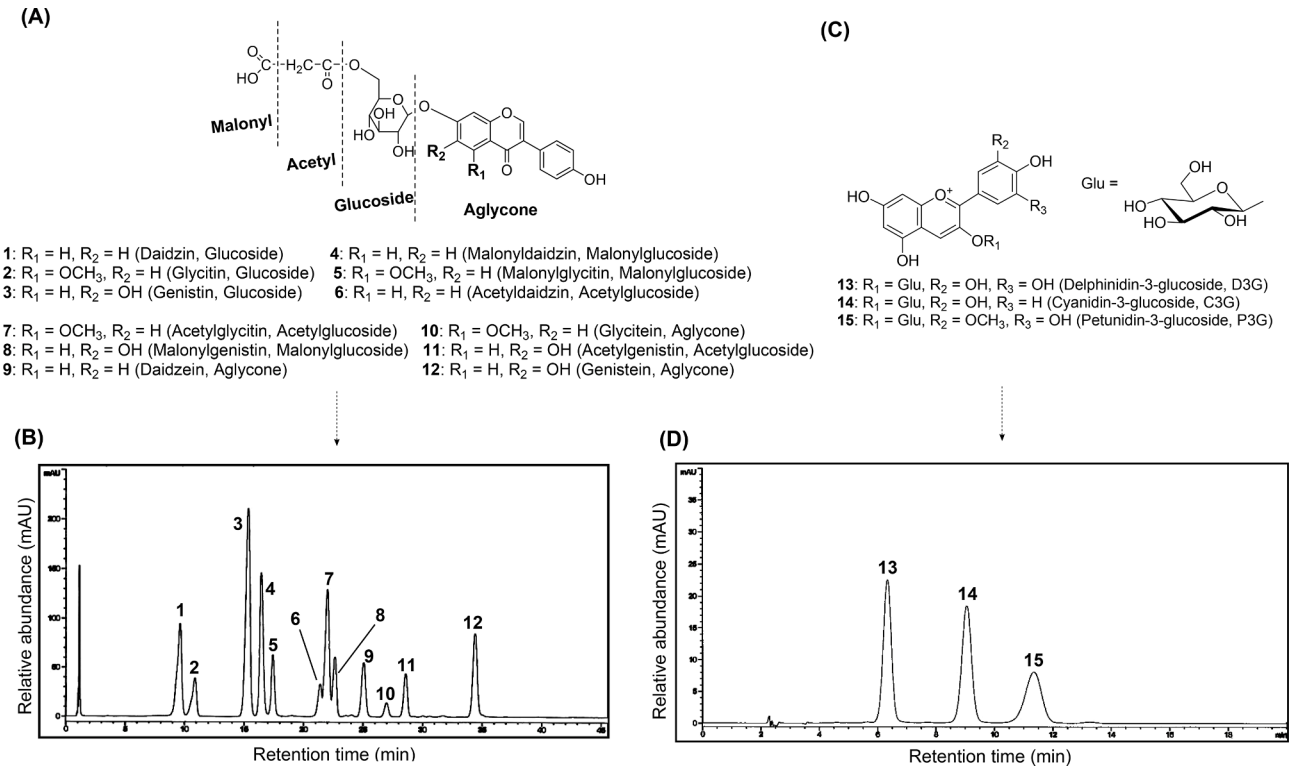

Fig. 1. Chemical structures and HPLC chromatograms of representative 12 isoflavones and 3 anthocyanins in black soybean: (A) Chemical structures regarding malonyl, acetyl, glucoside, and aglycone isoflavone groups, (B) HPLC chromatogram of isoflavone standards, (C) Chemical structures regarding anthocyanins, (D) HPLC chromatogram of anthocyanin standards.

Table 1
Comparison of isoflavone contents in whole seeds of sixteen Korean black soybeans for two crop years.

Cultivar	Crop year	Isoflavone content (μg/g)[a] Glucoside			Malonylglucoside			Acetylglucoside			Aglycone			
		Di (**1**)	Gly (**2**)	Gi (**3**)	MDi (**4**)	MGly (**5**)	MGi (**8**)	AcDi (**6**)	AcGly (**7**)	AcGi (**11**)	De (**9**)	Gle (**10**)	Ge (**12**)	Total
Ilpumgeomjeong	2018	317.9	234.1	443.4	379.4	38.7	433.6	nd[b]	nd	nd	9.5	nd	8.4	1865.0
	2019	143.3	110.4	145.6	988.3	77.8	963.8	nd	tr[c]	nd	29.6	tr	44.0	2502.8
Seonheuk	2018	84.9	12.9	247.1	67.4	20.1	251.5	nd	nd	nd	nd	nd	nd	683.9
	2019	80.8	68.8	116.8	332.2	60.4	557.1	nd	13.2	nd	4.7	nd	16.9	1250.9
Geomjeong 3	2018	339.3	183.4	643.3	455.8	28.0	729.6	nd	nd	nd	9.7	nd	11.0	2400.1
	2019	152.1	151.	144.8	975.3	109. 8	1038.9	nd	tr	nd	28.9	tr	49.4	2650.2
Geomjeongol	2018	416.7	236.4	525.1	381.8	27.3	410.1	nd	nd	nd	10.5	nd	8.2	2016.1
	2019	102.9	96.7	49.1	424.7	46.7	336.9	tr	tr	nd	14.1	nd	12.7	1083.8
Geomjeongsaeol	2018	281.4	207.4	342.2	263.5	20.6	300.2	nd	nd	nd	1.9	nd	0.7	1417.9
	2019	141.5	86.5	131.7	627.5	32.7	577.3	nd	tr	nd	18.8	nd	26.7	1642.7
Tawon	2018	329.2	221.3	574.8	382.9	32.6	522.0	nd	nd	35.7	1.5	nd	3.7	2103.7
	2019	77.0	114.6	87.1	357.7	29.2	579.8	nd	nd	32.9	1.5	nd	18.2	1298.0
Geomjeong 4	2018	287.1	329.0	404.4	255.9	37.5	347.9	nd	nd	nd	7.4	tr	6.4	1675.6
	2019	236.9	105.9	238.2	1717.8	73.3	1430.1	tr	tr	nd	43.5	tr	56.1	3901.8
Cheongja	2018	292.5	149.6	517.0	270.2	12.6	414.7	nd	nd	nd	5.8	nd	8.9	1671.3
	2019	122.7	86.4	158.5	740.9	30.9	962.4	nd	tr	nd	11.7	nd	25.9	2139.4
Geomjeong 1	2018	218.8	209.7	238.2	172.0	23.4	194.5	nd	nd	21.5	3.8	nd	3.2	1085.1
	2019	92.0	89.3	92.9	558.6	74.0	618.9	nd	tr	10.0	7.7	tr	16.1	1559.5
Geomjeong 2	2018	379.6	211.5	475.0	337.5	21.9	347.7	nd	nd	nd	14.8	tr	3.5	1791.5
	2019	180.0	90.9	249.8	1234.8	57.4	1509.5	nd	tr	nd	23.5	tr	49.2	3395.1
Cheongja 2	2018	339.4	229.8	616.6	332.1	22.2	494.5	nd	nd	nd	2.8	nd	4.8	2042.2
	2019	129.7	184.1	201.6	831.1	83.5	1175.0	nd	nd	nd	14.4	tr	39.7	2659.1
Cheongja 3	2018	332.5	165.1	498.5	380.2	15.8	538.8	nd	nd	nd	28.2	nd	31.2	1990.3
	2019	197.2	95.4	294.8	1200.9	36.0	1521.1	nd	tr	nd	25.3	nd	57.4	3428.1
Ilpumgeomjeong 2	2018	165.1	164.0	297.4	576.8	62.7	1144.8	tr	tr	0.9	72.6	2.5	188.2	2675.0
	2019	97.4	100.8	146.3	877.7	83.6	1428.8	nd	nd	nd	nd	nd	tr	2734.6
Mirang	2018	82.3	116.7	88.9	267.1	25.6	519.0	nd	nd	nd	2.7	nd	23.4	1125.7
	2019	83.0	nd	75.3	434.2	27.1	684.5	nd	nd	nd	nd	nd	nd	1304.1
Heugmi	2018	218.7	238.2	220.9	701.4	58.0	808.4	nd	nd	4.5	87.0	2.3	116.8	2456.2
	2019	171.2	166.4	165.7	1344.2	63.2	1322.3	nd	nd	nd	29.8	tr	43.4	3306.2

(*continued on next page*)

Table 1 (*continued*)

Cultivar	Crop year	Isoflavone content (μg/g)[a] Glucoside			Malonylglucoside			Acetylglucoside			Aglycone			
		Di (**1**)	Gly (**2**)	Gi (**3**)	MDi (**4**)	MGly (**5**)	MGi (**8**)	AcDi (**6**)	AcGly (**7**)	AcGi (**11**)	De (**9**)	Gle (**10**)	Ge (**12**)	Total
Socheong	2018	363.6	291.0	337.6	1403.6	118.2	1316.5	tr	nd	5.5	159.0	7.5	192.8	4195.3
	2019	193.2	179.3	127.4	2143.6	109.3	1416.1	nd	nd	nd	0.5	nd	nd	4169.4

[a]Data are expressed as means of triplicate experiments on dry weight basis. Different letters correspond to the significant differences relating to the processing steps using.

Tukey's multiple test ($p < 0.05$).[b] ND, not detected. [c] tr, trace. Din, daidzin; Gly, glycitin; Gin, genistin; MDin, malonyldaidzin; MGly, malonylglycitin; MGin, malonylgenitin; AcDin, acetyldaidzin; AcGly, acetylglycitin; AcGin, acetylgenistin; Dein, dadzein; Glein, glycitein; Gein, genistein.

observed in malonylglucoside structures, followed by glucosides and aglycone groups, whereas the acetylglucoside type showed the lowest contents (Fig. 2A–F). Specifically, the malonylglucoside isoflavones were the predominant average contents with 1496.1 μg/g in all cultivars, representing approximately 68.2% of the total isoflavones for two years (2194.4 μg/g). In each isoflavone, malonylgenistin was found the highest average content (778.0 μg/g), and other malonyl derivatives were in increasing order: malonyldaidzin (669.3 μg/g) > malonylglycitin (48.8 μg/g). The second major group, the isoflavone glucoside showed average contents as the following order: genistin (278.0 μg/g) > daidzin (207.8 μg/g) > glycitin (154.0 μg/g) (Table 1). Namely, three main soybean isoflavones have accumulated in the order of genistein, daidzein, and glyciten. The remaining groups (agycone and acetylglucoside) were also observed similar patterns as those of malonyl- and glucoside- isoflavone types, and their average contents occurred as the following order with slight variations: aglycone (genistein: 33.3 μg/g > daidzein: 21.0 μg/g > glycitein: 0.4 μg/g) > acetylglucoside (acetylgenistin: 3.5 μg/g > acetyldaidzin: 0.4 μg/g > acetylglycitin: tr or ND) (Table 1). To summarize, the highest average type was obtained as malonylglucosides with 1496.1 μg/g (68.2%) and the second main content was detected in isoflavone glucosides (639.8 μg/g, 29.2%), followed by aglycone (54.7 μg/g, 0.03%) > acetylglucoside (3.9 μg/g, < 0.05%). It is confirmed that significant differences in isoflavone types may be affected by the concerted rates of isoflavone synthase and chalcone synthase through phenylpropanoid pathway in soy plant (Dixon and Pacia, 1995) as well as the intense cellular division and biosynthesis in soybean growth (Bai et al., 2017). Although soybean isoflavones commonly exist the four chemical groups with three aglycone compositions (daidzein, glycitein, and genistein) as the following order: malonylglucosides (70%) > glucosides (25%) > acetylglucosides (3%) > aglycones (2%) (Kim and Chung, 2007; Yamabe et al., 2007), the present our data were detected conflicting results (Table 1). We believe that isoflavone accumulation may be connected with the genetics, environmental factors, and cultivation skills, as previously reported data (Sakthivelu et al., 2008; Veremeichik et al., 2021; Yamabe et al., 2007). As illustrated in Table 1, the total isoflavone contents had exhibited by the wide range of 683.9–4195.3 (2018) and 1083.9–4169.4 (2019) according to the crop years and cultivars. In the case of 2018 year, the highest isoflavone was detected in Socheong with 4195.3 μg/g (Fig. 2C) and the Ilpumgeomjong 2 cultivar made up the second main content (2675.0 μg/g) with similar patterns by comparison with those of Heugmi (2456.2 μg/g) and Geomjeong 3 (2400.1 μg/g). The Seonheuk cultivar

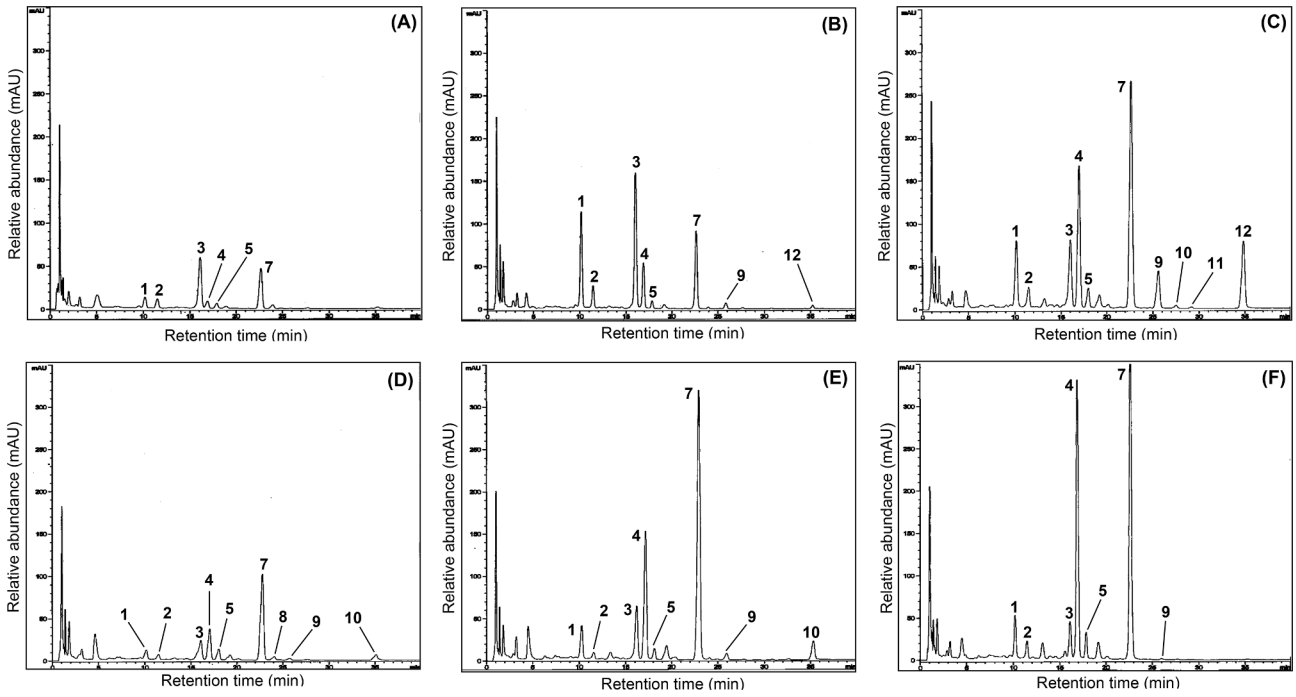

Fig. 2. Comparisons of representative HPLC chromatograms concern to isoflavones (A–F) and anthocyanins (G–J) in whole seeds (50% methanol extract) and seed coats (acidic 50% methanol extract) of black soybeans during two crop years: (A) Seonheuk (2018); (B) Geomjeong 2 (2018); (C) Socheong (2018); (D) Seonheuk (2019); (E) Geomjeong 2 (2019); (F) Socheong (2019); (G) Geomjeong 2 (2018); (H) Mirang (2018); (I) Geomjeong 2 (2019); (J) Mirang (2019); **1**. Daidzin, **2**. Glycitin, **3**. Genistin, **4**. Malonyldaidzin, **5**. Malonylglycitin, **6**. Acetyldaidzin, **7**. Acetylglycitin, **8**. Malonylgenistin, **9**. Daidzein, **10**. Glycitein, **11**. Acetylgenistin, **12**. Genistein, **13**. Delphinidin-3-*O*-glucoside, **14**. Cyanidin-3-*O*-glucoside, **15**. Petunidin-3-*O*-glucoside.

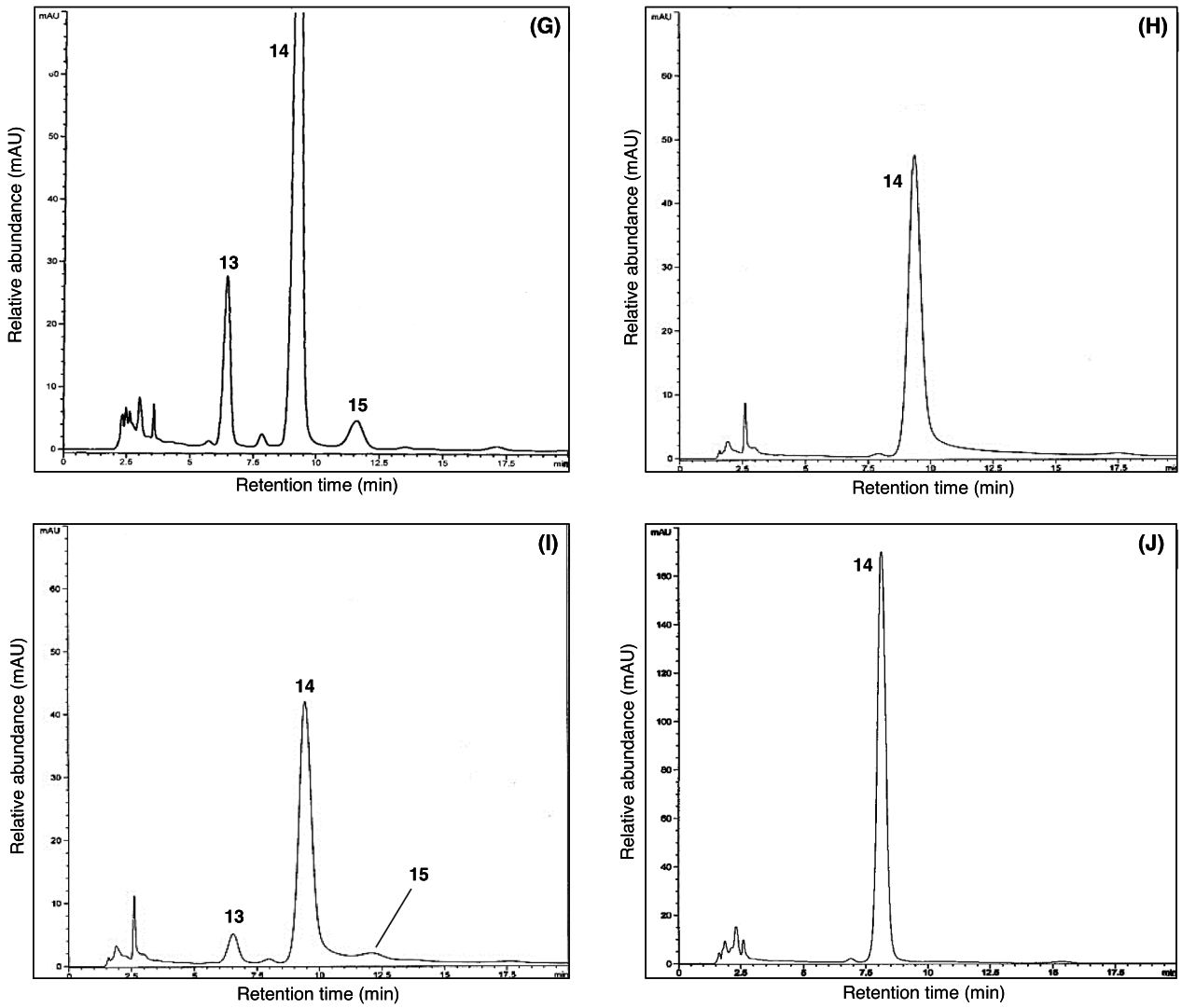

Fig. 2. (*continued*).

showed the lowest isoflavone content (683.9 μg/g) (Fig. 2A) and the remaining cultivars were found to display isoflavone rates ranging of 1000–2000 μg/g (Fig. 2B and Table 1). These phenomenon suggest that the isoflavone contents in black soybeans may be remarkably influenced by genetic differences of each cultivar, and were consistent with the earlier studies regard to variations of secondary metabolites in other crops (Boneza & Niemeyer, 2018; Kim et al., 2014). Our current results were believed that Socheong cultivar (4195.3 μg/g) may be considered as a beneficial material in human health aspect and the development of new cultivars in breeding aspect because of the most abundant isoflavone contents. Many cultivars of 2019 crop year also exhibited considerable differences in individual and total isoflavone contents, as evidenced by the results of 2018. Interestingly, the total average isoflavone content (2439.0 μg/g) in sixteen cultivars at 2019 year was higher than those observed in 2018 samples (1949.7 μg/g) (Table 1). Our results were in agreement with previous literatures that the soybean isoflavones can be affected by multiple environmental stresses such as temperature, light, moisture, soil, and cultivation skill (Sakthivelu et al., 2008; Veremeichik et al., 2021; Yamabe et al., 2007). The absolutely dominant isoflavones were observed in Socheong with 4169.4 μg/g (Fig. 2F), and other major contents were ranked order as follows with the increase rates: Geomjeong 4 (3901.7 μg/g) > Cheongja 3 (3428.0 μg/g) > Geomjeong 2 (3395.1 μg/g) > Heugmi (3306.1 μg/g). The lowest small amounts exhibited Geomjeongol (1083.8 μg/g) and other cultivars were detected with the range of 1100–2800 μg/g (Fig. 2D and 2E). The isoflavone levels at 2019 crop year were also increased in the order malonylglucoside > glucoside > aglycone > acetylglucoside as those of 2018, and specially, the malonylglucosides have significant influences on total isoflavone contents (Fig. 2A–F). Even though many researches have demonstrated that the most abundant individual isoflavone was detected in malonylgenistin (Cho et al., 2013; Yu et al., 2021), Socheong (MDi: 1717.8; MGi: 1430.1 μg/g at 2019) and Gemjeong 4 (MDi: 2143.6; MGi: 1430.1 μg/g at 2019) cultivars of the predominant total isoflavones have higher ratios of manolyldaidzin compared to the malonylgenistin (Table 1). Most black soybeans showed remarkable differences in isoflavone contents for two crop years, but Socheong cultivar of the highest isoflavones did not considerably vary (2018: 4195.3 μg/g; 2019: 4169.4 μg/g). Therefore, this cultivar may be recommended as excellent source for functional uses and development of new cultivars. Our findings are believed that the isoflavone contents and profiles in black soybeans may be primarily influenced with the molecular figuration or transformation in chemical structures (Wang et al., 2015) and nodulation of soy plant by the gene expression through rhizobial bacteria (Pueppke, 1996) in various environmental factors during growth periods. Overall, the individual and total isoflavone contents were considerably different in black soybeans for two years (Fig. 2A–F). These data are similar to the earlier literatures concern to secondary metabolites such as lignans and phenolic acids in sesame and

perilla plants (Kang and Lee, 2011; Kim et al., 2014). We confirmed that soybean isoflavones can be markedly altered by growth conditions of year, agronomic factor, and cultivation. The current research demonstrated for the first time the quantification and comparison of the isoflavone contents in diverse black soybeans at growth years.

Comparison of anthocyanin contents in seed coats of black soybeans at different crop years

Anthocyanins are known to show various human health benefits (Hemachandran et al., 217; Jiang et al., 2020; Leal et al., 2020; Moise et al., 2005), especially, their contents and profiles exhibited significant differences in crops and natural plants (Cheng et al., 2022; Ito et al., 2013; Kang and Lee, 2011). However, as far as we are aware, there is little intelligence available information on the demonstration of anthocyanin contents in black soybeans through cultivars and growth years. For this consideration, we examined three anthocyanins in the acidic 50% methanol extracts from the seed coats of various cultivars. The anthocyanin structures and their complete chromatographic separations are shown in Fig. 1C and 1D. Three anthocyanins were achieved in 12 min at 530 nm and their retention times were as follows: peak 13 (delphinidin-3-*O*-glucoside; D3G, $t_R = 6.3$ min), peak 14 (cyanidin-3-*O*-glucoside; C3G, $t_R = 9.1$ min), peak 15 (petunidin-3-*O*-glucoside; P3G, $t_R = 11.4$ min). The anthocyanin contents in sixteen black soybean seed coats during two crop years are presented in Table 2, and exhibited considerable differences between cultivars and growth years (Fig. 2G–J). Interestingly, their contents were found to be remarkably different by comparing the isoflavone contents. Although the earlier literature has reported that black soybeans are renowned as nine compositions including three main and six minor anthocyanins (Lee et al., 2009), the present results were detected only three anthocyanins in various cultivars. These observations may be affected by environmental factors such as genetics, climate changes, and growth states (Cheng et al., 2022; Ito et al., 2013; Lee and Cho, 2012; Todd and Vodkin, 1993). In all cultivars for two crop years, the C3G component displayed the predominant anthocyanin, followed by D3G, and P3G (Fig. 2G–J). The C3G contents exhibited the highest rates with the range of 1.8–13.0 mg/g compared to D3G (ND–3.7 mg/g) and P3G (ND–1.0 mg/g) (Table 2). Moreover, the total average anthocyanin contents (6.0 mg/g) showed the following order in sixteen cultivars of two crop years: C3G (4.6 mg/g, 77%) > D3G (1.2 mg/g, 20%) > P3G (0.2 mg/g, 3%) (Fig. 2G–J). Our data were similar patterns the earlier study reporting that C3G was found in large contents from black soybeans (Cho et al., 2013; Lee and Cho, 2012). Our experimental results are also consistent with the prior researches that the glucosidic linkage regarding cyanidine type and glucose form of monosaccharaides in anthocyanin derivatives have been widely distributed in crops, vegetables, and food sources (Hemachandran et al., 2017; Jiang et al., 2020). In sixteen cultivars harvested on 2018 crop year, the most abundant anthocyanins were observed in Geomjeong 2 with 14.4 mg/g (C3G: 10.6, D3G: 3.0, and P3G: 0.8 mg/g) (Fig. 2G). The second main anthocyanins were obtained from Cheongja 3 cultivar (12.7 mg/g; C3G: 8.1, D3G: 3.7, and P3G: 0.9 mg/g), followed by Geomjeong 3 (10.4 mg/g; C3G: 7.0, D3G: 3.1, and P3G: 0.3 mg/g) > Cheongja 2 (7.5 mg/g) > Tawon (6.6 mg/g) > Mirang (6.0 mg/g) (Fig. 2H), and the remaining cultivars were detected mildly contents (< 6.0 mg/g). All tested cultivars at 2019 growth year displayed significant differences when compared to the anthocyanin contents of 2018 (Fig. 2G–J and Table 2). The highest content was 13.0 mg/g in Mirang with C3G: 13.0, D3G: ND, and P3G: ND mg/g (Fig. 2J), while the lowest contents were 2.9 mg/g in Geomjeong 1 (C3G: 2.9 mg/g, D3G: ND, and

Table 2
Comparisons of anthocyanin, protein, oil, and fatty acid contents in seed coats and whole seeds of sixteen Korean black soybeans for two years.

Cultivar	Crop year	Nutritional composition content[a]										
		Anthocyanin (mg/g)				Protein (%)	Oil (%)	Fatty acid composition (%)				
		D3G (**13**)	C3G (**14**)	P3G (**15**)	Total			C16:0	C18:0	C18:1	C18:2	C18:3
Ilpumgeomjeong	2018	2.0	3.6	0.3	5.9	42.1	20.3	11.7	3.2	25.4	50.1	7.5
	2019	0.9	4.3	nd	5.2	43.9	19.5	12.6	3.7	23.7	49.4	8.9
Seonheuk	2018	nd	4.9	nd	4.9	41.7	21.6	10.9	4.0	22.9	48.7	8.4
	2019	nd	4.5	nd	4.5	41.3	20.9	11.3	3.5	22.0	49.3	7.0
Geomjeong 3	2018	3.1	7.0	0.3	10.4	44.2	19.8	12.4	2.9	23.5	52.6	10.0
	2019	1.0	4.4	nd	5.4	44.9	21.0	12.0	3.4	23.6	51.1	9.4
Geomjeongol	2018	1.2	2.1	0.2	3.5	42.6	20.7	11.9	3.7	21.9	49.5	11.3
	2019	1.5	2.2	0.2	3.9	41.5	20.2	12.8	3.0	21.3	50.7	10.1
Geomjeongsaeol	2018	1.4	2.9	0.2	4.5	43.7	19.8	12.1	2.6	22.0	48.4	9.8
	2019	1.2	5.8	nd	7.0	42.0	18.5	12.3	3.1	24.8	49.1	9.0
Tawon	2018	2.0	3.7	0.9	6.6	41.9	19.6	11.6	3.3	25.0	52.9	8.6
	2019	0.9	1.9	0.4	3.2	41.2	21.1	11.8	2.4	23.4	50.3	9.5
Geomjeong 4	2018	1.8	3.8	0.3	5.9	43.9	18.5	12.0	3.8	22.7	48.9	9.1
	2019	0.9	4.1	0.2	5.2	40.5	19.1	12.5	3.5	22.3	47.0	9.6
Cheongja	2018	1.7	3.0	0.2	4.9	44.3	20.4	10.9	4.1	24.0	51.4	9.0
	2019	1.3	3.8	nd	5.1	43.8	21.3	10.6	4.0	23.1	52.1	10.2
Geomjeong 1	2018	nd	2.3	nd	2.3	42.6	19.5	11.4	3.2	21.5	49.6	10.7
	2019	nd	2.9	nd	2.9	41.4	19.0	11.0	3.6	24.9	50.3	10.3
Geomjeong 2	2018	3.0	10.6	0.8	14.4	42.9	19.2	12.7	2.9	22.7	52.0	9.1
	2019	0.8	5.2	0.2	6.2	44.1	20.4	11.6	3.1	23.0	50.7	10.4
Cheongja 2	2018	2.4	4.7	0.4	7.5	40.8	21.7	11.7	3.4	23.6	53.5	8.9
	2019	2.5	7.5	1.0	11.0	42.3	20.6	11.5	3.9	23.2	51.4	9.4
Cheongja 3	2018	3.7	8.1	0.9	12.7	41.9	19.8	12.6	4.3	24.7	54.9	10.6
	2019	1.0	4.7	0.3	6.0	40.0	19.1	11.3	4.0	22.9	53.2	9.8
Ilpumgeomjeong 2	2018	1.5	4.0	0.3	5.8	44.1	20.3	11.5	2.9	23.5	51.2	11.2
	2019	0.8	4.3	nd	5.1	42.6	21.4	11.1	3.6	24.1	50.8	10.3
Mirang	2018	nd	6.0	nd	6.0	41.5	20.8	12.4	3.2	22.3	49.6	9.4
	2019	nd	13.0	nd	13.0	43.9	21.6	12.8	3.2	23.8	50.7	9.2
Heugmi	2018	0.8	1.8	0.2	2.8	42.0	20.0	11.7	4.1	23.1	52.1	9.0
	2019	0.5	2.4	nd	2.9	41.2	19.7	11.2	3.4	24.6	51.0	8.8
Socheong	2018	nd	3.3	nd	3.3	45.2	20.4	12.6	2.7	22.5	49.3	7.5
	2019	nd	4.9	nd	4.9	43.6	21.0	11.3	3.0	24.0	51.2	9.6

[a]Data are expressed as means of triplicate experiments on dry weight basis. Different letters correspond to the significant differences relating to the processing. steps using Tukey's multiple test ($p < 0.05$). D3G, delphinidine-3-*O*-glucoside; C3G, cyanidin-3-*O*-glucoside; P3G, petunidin-3-*O*-glucoside.

P3G: ND) and Heugmi (C3G: 2.4 mg/g, D3G: 0.5 mg/g, and P3G: ND) cultivars. The Cheongja 2 cultivar was the second major anthocyanin contents with 11.0 mg/g as observed amounts of 2.5 (D3G), 7.5 (C3G), and 1.0 (P3G) mg/g, respectively, and other all cultivars were < 7.0 mg/g (Fig. I). These distribution rates have documented that the anthocyanin concentrations and types may be positively correlated with genetics of cultivar and environmental stresses of growth state (Cheng et al., 2022; Ito et al., 2013; Todd and Vodkin, 1993). As evidenced by the above data, the Geomjeong 2, Mirang, Cheongja 3, and Cheongja 2 cultivars may be considered as excellent materials to develop better black soybeans and dietary foods owing to their high average anthocyanins for two years. Overall, the individual and totalt anthocyanin contents in each cultivar varied remakaly by comparing isoflavones, and the C3G component showed the highest variations between cultivars and crop years. Our work indicates that the anthocyanin contents may be considered as more excellent factor than isoflavones in determining the black soybean quality because of their high concentrations. We demonstrated for the first time the quantification and comparion of main anthocyanins in various black soybeans at different growth years.

Comparisons of fatty acid, oil, and protein contents in whole seeds of black soybeans at different crop years

Previous literatures have reported that soybean protein, oil, and fatty acid play an important role in human beneficial properties (Charron et al., 2005; Messina, 1999; Mujoo et al., 2003; Hwang et al., 2021). Moreover, this crop is one of the most excellent sources in food and feed industries owing to high primary metabolite contents (protein: 40–50%, oil: 20–30%, and carbohydrate: 26–30%) (Brummer et al., 1997; Varnosfadera et al., 2019). Unfortunately, there are few reports for the evaluations of protein, oil, and fatty acid contents in diverse black soybeans at crop years. As illustrated in Table 2, the protein contents varied from 40.8 to 45.2 (2018) or from 40.0 to 44.9% (2019) in sixteen cultivars and their average contents showed 42.8 (2018) and 42.4% (2019). To be specific, this composition was observed only slight variations, demonstrating no considerable differences between crop years and cultivars. Also, Geomjeong 3 (44.6%), Socheng (44.4%), and Cheongja (44.1%) exhibited slightly high average contents by comparing other cultivars with a range of 41.0–43.5% during two years (Table 2). Our results were similar to the earlier researches (Charron et al., 2005; Lee and Cho, 2012), and this constituent may not depend on genetics and environmental factors (crop year, growth state, temperature, light, and soil nutrition). The oil contents also exhibited no remarkable differences between growth year and cultivars (Table 2). Even though soybean oil can be dependent to much degree on various factors such as process, storage, and other environmental conditions (Cosio et al., 2007; Mujoo et al., 2003), the current work did not show remarkable differences in genetics, growth years, and other factors, as reported in previous studies (Lee & Cho, 2012; Charron et al., 2005). In the 2018 and 2019 crop years, the average oil contents exhibited 20.2% and 20.3%, and were measured with ranges of 18.5–21.7% and 18.5–21.6% (Table 2). This constituent was not detected high variations and contents, compared with the results of isoflavone and anthocyanin levels. Thus, soybean oil may not be connected with crop years and cultivars as well as their interior relationships. Our results suggest that the protein and oil may be not essential components in determining the nutritional characteristic, quality, and breeding factors of black soybeans. The fatty acid compositions also displayed slight variations in cultivars and crop years as those of protein and oil (Table 2). In other words, the individual and total fatty acid contents were not detected considerable differneces in all samples. The abundant compositions, linoleic acid (C18:2) (2018: 48.4–54.9%; 2019: 47.0–53.2%) and oleic acid (C18:1) (2018: 21.5–25.4%; 2019: 21.3–24.9%) displayed mildly variations during two years. Moreover, the C18:2 composition showed the predominant average content with 50.7%, followed by C18:1 (23.3%) > palmitic acid (C16:0) (11.8%) > linolenic acid (C18:3) (9.4%), and stearic acid (C18:0) exhibited the lowest content (3.4%). Especially, the total average unsaturated fatty acid contents (83.4%) were considerably higher than those of saturated fatty acids (15.2%) (Table 2). These findings are in agreement with a previous work reporting the fatty acid profiles (Lee and Cho, 2012). As a result, our work did not show remarkable differences in fatty acid, oil, and protein contens of black soybeans between cultivars and crop years (Brummer et al., 1997; Cho et al., 2013; Kim et al., 2014). It is confirmed that protein, oil, and fatty acid may not be the main factors for development of better black soybean lines by comparing the isoflavones and anthocyanins.

Measurement of antioxidant properties on radical scavenging activities in whole seeds and seed coats of black soybeans at different crop years

Up to now, the phenolic phytochemicals are known to have a range of biological health-promoting properties including antioxidant abilities (Cho et al., 2013; Choi et al., 2008; Desta et al., 2022; Farooqi et al., 2022; Messina et al., 1999; Sakthivelu et al., 2008; Varnosfadera et al., 2019; Waqas et al., 2015). However, many literatures have not shown the relationship between phenolic contents and antioxidant activities in black soybeans. To explore more excellent information on human health aspects, we measured the potential scavenging effects against DPPH and ABTS radicals through spectrophotometric assays owing to their simple controls and reproducibilities (Bai et al., 2017; Cho et al., 2013; Floegel et al., 2011). Their capacities were evaluated by comparing the percentage inhibitions concerning the formation of DPPH and ABTS radicals by the 50% methanol extracts of whole seeds and acidic 50% methanol extracts of seed coats as well as positive controls (BHT and Trolox) (Kim et al., 2014; Lee and Cho, 2021). In the preliminary experiments, remarkable differences of antioxidant activities were found in each extract through different concentrations of black soybean (cv. Geomjeong 3, 2018 crop year). Briefly, the DPPH radical scavenging abilities were observed significant differences in whole seeds and seed coats of various cultivars for crop years, and their activities increased with increasing concentrtions (50, 100, 200, 500, 1000, 1500, and 2000 μg/mL). The potential scavenging abilities of two extracts were in the following order at different concentrations: whole seeds: 2000 μg/mL (93%), 1500 μg/mL (81%), 1000 μg/mL (64%), 500 μg/mL (41%), 200 μg/mL (28%), 100 or 50 μg/mL (< 10%); seed coats: 2000–1500 μg/mL (100%), 1000 μg/mL (94%), 500 μg/mL (90%), 200 μg/mL (87%), 100 μg/mL (69%), 50 μg/mL (45%). As support to these above results, the antioxidant capacities against the radical scavenger of whole seeds and seed coats were measured at 1500 and 200 μg/mL because of the dose-dependent changes through activity ratios. First, the DPPH radical scavenging effects varied considerably in whole seeds of diverse cultivars for two crop years. The predominant average DPPH radical scavenging activity was displayed with 90% in Socheong cultivar (average isoflavone: 4182.4 μg/g) at a concentration of 1500 μg/mL, while Seonheuk (average isoflavone: 1022.9 μg/g) and Mirang (average isoflavone: 1215.0 μg/g) showed the lowest effects with approximately 42%. Furthermore, the Heugmi, Cheongja 3, Ilpumgemjeong 2, Geomjeong 4 cultivars exhibited high scavenging abilities (> 70%, 1500 μg/mL) by comparison with those of other samples (< 70%). Although all extracts of whole seeds showed lower DPPH radical scavenging capacities than BHT (89%) at 1000 μg/mL, the Socheong cultivar may be used as a potential source of natural antioxidant agent. These observations suggest that the isoflavone contents in the 50% methanol extracts of whole seeds may be contributed to the main portion of the scavenging activities against DPPH radical (Bai et al., 2017; Cho et al., 2013; Sakthivelu et al., 2008). Our work was similar to those of the previous studies, which the phenolic compounds of soybeans have potential beneficial abilities on various diseases such as antioxidative agents (Desta et al., 2022; Sakthivelu et al., 2008; Yu et al., 2021). Interestingly, our data exhibited lower antioxidant abilities by comparing the results reported in the earlier research (80% methanol extract at 1000 μg/g) (Cho et al., 2013). This phenomenon suggests that the DPPH radical scavenging abilities in whole seeds may be important considered by

other phenolic contents including isoflavones in different cultivars through extraction conditions (Desta et al., 2022; Kim et al., 2014; Floegel et al., 2011; Jiang et al., 2020). The environmental factors under growth conditions may be also connected with the radical scavenging capacities (Dixon and Pacia, 1995; Todd and Vodkin, 1993; Veremeichik et al., 2021; Wang et al., 2015). Overall, our research reveals that the high isoflavone contents of whole seeds can contribute remarkably to their strong antioxidant properties on DPPH radical. In the ABTS radical method, the antioxidant abilities of the whole seeds were measured at 1500 μg/mL owing to the dose-dependent variations under different concentrations of samples. The acidic 50% methanol extracts of all samples were observed higher scavenging effects than those of DPPH radical. In addition, Socheong cultivar exhibited the highest ABTS radical scavenging abilities with 97% at a concentration of 1500 μg/mL, whereas the lowest capacity was detected in Mirang (54%). The predominant scavenging effect of Socheong exhibited similar property by comparing positive control (Trolox, 95%) at 1500 μg/mL, and other cultivars displayed similar patterns as those of DPPH radical. These data indicate that ABTS radical may be attributed to the scavenging capacities regarding hydrogen donating and chain breaking properties of isoflavones and other phenolics in the 50% methanol extract of whole seeds when compared to the hydrogen donating abilities of DPPH radical (Cho et al., 2013; Floegel et al., 2011; Lee and Cho, 2021). As a result, the antioxidant activities against ABTS radical of whole seeds were detected approximately 1.5-fold higher scavenging levels than those of DPPH radial, and Socheong may be recommended as potential source to develop better black soybean cultivars for health food agents. Our findings indicate that the black soybean seeds may be considered as excellent natural scavenger agents against ABTS radical by comparing the DPPH inhibitions. Secondary, the antioxidant activities in seed coats of black soybeans exhibited strong scavenger when compared to the whole seeds. The acidic 50% methanol extracts of all samples had higher antioxidant properties towords radical scavengers compared with whole seeds. From our preliminary experiments, the acidic extract (cv. Mirang, 2019 crop year) were detected 100% radical scavenging abilities at 1500 μg/mL. According to the results of the inhibition rates and dose-dependent variations in seed coats, the antioxidant activities on the radical scavengers were measured at 1000 μg/mL. The antioxidant patterns were observed significant differences in cultivars and crop years, and exhibited remarkable variations in comparison with those of observed from whole seeds. Although our data were similar patterns as the results obtained by whole seeds, the acidic 50% methanol extracts of seed coats had higher effects. The basic results of our work indicate that the anthocyanin contents are key factor influencing the antioxidant properties against radical scavengers than isoflavone concentrations (Cho et al., 2013; Lee and Cho, 2012). Therefore, the accumulation rates and contents of anthocyanin in seed coat extracts may be attributed to the antioxidant capacities on radicals (Fujimaki et al., 2018; Desta et al., 2022). The ABTS radical scavenging abilities in seed coats of all cultivars were detected with having higher properties when compared to the DPPH radical as the data obtained by whole seeds. These results indicate that the anthocyanin contents of seed coats were highly correlated with ABTS radical due to the lipophilic, hydrophilic, and pigmented antioxidant systems in the acidic 50% methanol extracts than antioxidant activities through hydrophobic system detected by DPPH radical (Floegel et al., 2011; Jiang et al., 2020; Moise et al., 2005). The average DPPH and ABTS scavenging capacities in seed coats of various cultivars occurred in the following order: Geomjeong 2 > Mirang > Cheongja 2 at 1000 μg/mL. In particular, Geomjeong 2 was detected the predominant antioxidant activities (DPPH: 89% and ABTS: 94%), and Mirang and Cheongja 2 cultivars also showed high average radical scavenging abilities of > 80%. Also, the Geomjeong 2 (94%), Mirang (91%), Cheongja 2 (88%), and Cheongja 3 (87%) cultivars were higher ABTS radical scavenging effects than Trolox (positive control, 85% at 1000 μg/mL). Interestingly, the Heugmi and Socheong cultivars of high isoflavone contents and strong radical scavenging effects in whole seeds exhibited low anthocyanins and weak antioxidant capacities in seed coats. As mentioned above, we can confirm that the antioxidant properties in the anthocyanin extracts of seed coats may be considered as higher scavenging agent than isoflavones of whole seeds (Cho et al., 2013; Desta et al., 2022). In addition, it is believed that the antioxidant ratios of seed coats can be considerably influenced by anthocyanin contents. And the Geomjeong 2 cultivar may be recommended as potential source to develop better black soybeans regarding human beneficial food agents because of high isoflavone and anthocyanin contents.

Measurement of DNA protection in whole seeds and seed coats of black soybeans at different crop years

To gather more information on antioxidant capacities of black soybeans, we investigated the super-coiled DNA protection effects of recombinant samples. A nicked DNA technique with super-coiled plasmid DNA pUC18 was examined in a MCO system (Baiseitova et al., 2021; Hu et al., 2010). According to the radical scavenging experiments, we

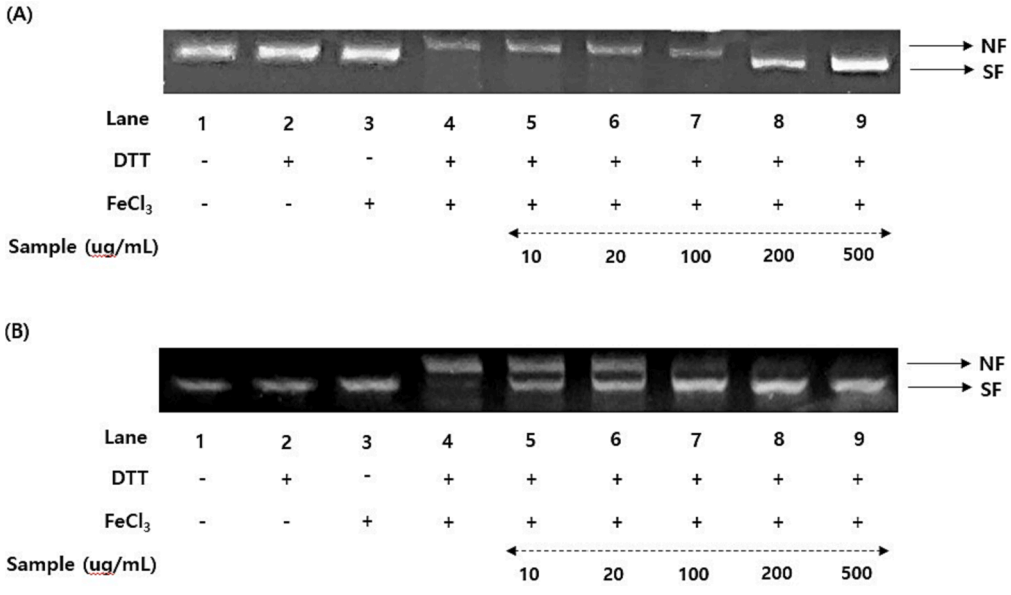

Fig. 3. Comparisons of DNA protectant properties in whole seeds and seed coats of black soybeans: (A) DNA protectant effects of the 50% methanol extracts in whole seeds (cv. Socheong, 2018) of the predominant isoflavone contents. Lane 1, pUC18 only; lane 2, pUC18 with DDT only; lane 3, pUC18 with $FeCl_3$ only; lane 4, pUC18 with MCO system; lane 5–9, pUC18 with combinant extracts in the MCO system (lane 5: 10 μg/mL, lane 6: 20 μg/mL, lane 7: 100 μg/mL, lane 8: 200 μg/mL, and lane 9: 500 μg/mL). (B) DNA protectant effects of the acidic 50% methanol extracts in seed coats (cv. Geomjeong 2, 2018) of the predominant anthocyanin contents. Lane 1, pUC18 only; lane 2, pUC18 with DDT only; lane 3, pUC18 with $FeCl_3$ only; lane 4, pUC18 with MCO system; lane 5–9, pUC18 with combinant extracts in the MCO system (lane 5: 10 μg/mL, lane 6: 20 μg/mL, lane 7: 100 μg/mL, lane 8: 200 μg/mL, and lane 9: 500 μg/mL). Nicked form (NF) and super-coiled form (SF) of the plasmid DNA are indicated by arrows.

evaluated the DNA protection assay by comparing the different concentrations (500, 200, 100, 20, and 10 μg/mL) with the percentage protections of control in whole seeds and seed coats of black soybeans. In the absence of recombinant extracts of black soybeans, the hydroxyl radicals produced with the nicked DNA pUC18 into a linear form through based on gel mobility from the MCO system (Fig. 3). Especially, we characterized the comparisons and variations of DNA protection in the Socheong and Geomjeong 2 cultivars of the most abundant isoflavone and anthocyain contents for two crop years. The defense rates on DNA oxidation exhibited considerable differences according to the concentration-dependent manner of sample extracts (Fig. 3). As illustrated in Fig. 3A, the 50% methanol extracts of Socheong whole seeds (2018 crop year) were not protected at 10, 20, and 100 μg/mL, respectively, but when each of the above extract was treated at 200 and 500 μg/mL, the DNA protection bands were observed in electrophoretic analysis. In more details, the DNA bands were protected with 49.9 and 97.7% after incubating of the 50% methanol extracts at 200 and 500 μg/mL, compared to that of control (1 kb DNA marker pUC18 only) (Fig. 3A). Thus, a nicked DNA skill by an MCO system revealed that the 50% methanol extract of black soybean seeds protected on DNA nicking by the hydroxyl radical and this source may be considered as excellent material owing to the DNA protection against ROS, as the previous evidences using natural plants (Wan et al., 2014; Hu et al., 2010). Secondly, the DNA protective effects in the acidic 50% methanol extracts of seed coats (cv. Geomjeong 2, 2018 crop year) were also carried out at a dose-dependent concentration, as based on gel mobility in a MCO system (Fig. 3B), and their DNA protection rates exhibited significant differences. The acidic 50% methanol extracts protected the mobility of DNA fragment up to the concentration of 10 μg/mL with 68.0% by comparing the control. Other concentrations were observed as follows, in decreasing order: 500 μg/mL (92.7%) > 200 μg/mL (90.8%) > 100 μg/mL (88.7%) > 20 μg/mL (72.0%) (Fig. 3B). Overall, the antioxidant abilities in the acidic 50% methanol extracts of seed coats displayed high DNA protection rates (68.0% at 10 μg/mL) with differences of approximately 10 times when compared with whole seeds (49.9% in 50% methanol extract at 200 μg/mL) of black soybeans. These findings may be considerably influenced by the phenolic profiles and contents as well as their chemical structure types, as shown in earlier studies (Baiseitova et al., 2021; Wan et al., 2014). The current results confirmed that the acidic 50% methanol extracts of black soybean seed coats can be excellent source for the development of human health beneficial agents and strong potential candidate against oxidative DNA damage. The evaluation and comparison of radical scavenging and DNA protection assays concern to isoflavone and anthocyanin extracts were firstly examined from black soybeans.

Measurement of enzymatic inhibitions against tyrosinase in whole seeds and seed coats of black soybeans at different crop years

It is well-known that tyrosinase plays an important role in pigmentation of fruits and melanin formation of mammals (Chiocchio et al., 2018; Choi et al., 2008; Hemachandran et al., 2008). And the inhibition agents of this enzyme have been reported to reduce the melanin production and achieve skin whitening regard to effectively solving pigmentation (Chiocchio et al., 2018; Leal et al., 2020). At present, numerous researchers are seeking new tyrosinase inhibition materials with a better safety properties from natural and edible sources owing to adverse side-effects of synthetic drugs (Li et al., 2021; Liyanaarachchi et al., 2018). Especially, many literatures have demonstrated that the potential anti-tyrosinase and anti-elastase properties are positively correlated with the phenolic contents in crops, foods, and natural plants (Chiocchio et al. 2018; Deniz et al., 2020; Fujimaki et al., 2018; Kim et al., 2018; Li et al., 2021; Liyanaarachchi et al., 2018). Although black soybean possessed the abundant phenolics and various biological capacities, unfortunately, the evaluation and comparison of tyrosinase inhibitions in isoflavone and anthocyanin extracts of diverse cultivars has not been extensively documented. We investigated the tyrosinase inhibitory activities by comparison of the percentage ratios from the different extracts in whole seeds and seed coats of black soybeans. From the antioxidant results on radicals and the preliminary tests of tyrosinase inhibition, the 50% methanol extracts (600 μg/mL) of whole seeds exhibited good inhibitory effects with dose dependent variations. Their capacities were detected remarkable differences in various cultivars of crop years, and the rank order of inhibition abilities exhibited similar patterns as the results gained from antioxidant properties. At a concentration of 600 μg/mL, the average tyrosinase inhibitory capacities in whole seeds of black soybeans for two years were as follows, in decreasing order: Socheong (66.1%) > Geomjeong 4 (59.3%) > Heugmi (59.0%) > Cheongja 3 (56.7%) > Ilpumgeomjeong 2 (53.4%) > Geomjeong 2 (52.8%) > Geomjeong 3 (51.7%), and other cultivars were observed low inhibition with < 50%. Our investigation have shown that the 50% methanol extracts of abundant isoflavone contents displayed high tyrosinase inhibitory abilities, and differed between cultivars and crop years. The present results are similar to the earlier studies reporting that the tyrosinase inhibitions of black soybeans were observed high rates according to the increase of isoflavone contents (Choi et al., 2008; Waqas et al., 2015). Even though the 50% methanol extract of Socheong was detected lower inhibitory capacities than positive control (ascorbic acid at 50 μg/mL: 89.2%), we may be recommended as important source to develop excellent cultivars and functional foods because of its high isoflavone contents. To facilitate further information on tyrosinase inhibitions, we examined the acidic 50% methanol extracts of seed coats including anthocyanin mixtures. It is well established that the anthocyanin extracts have been reported to have strong tyrosinase inhibitory capacities (Fujimaki et al., 2018; Zhang et al., 2021). However, as far as we know, the tyroninase inhibitions in anthocyanin extracts of black soybeans have yet to be evaluated. At present data, the acidic 50% methanol extracts of seed coats possessed significantly higher tyrosinase inhibition activities than those of the 50% methanol extracts in whole seeds of all black soybeans. Among various cultivars, Geomjeong 2 had the predominant average inhibition effect with 87.6% at 600 μg/mL and other cultivars, namely, Cheongja 2, Cheongja 3, and Mirang exhibited higher tyrosinase inhibitions (> 80%) with approximately 20% increase rates than whole seeds. The remaining cultivars showed mildly inhibitory abilities (< 60%), and Heumi and Geomjeong 1 were detected the lowest effects (< 40%). These observations may be considered that the anthocyanin ratios are attribute to the tyrosinase inhibition capacities. In particular, the inhibition patterns exhibited clearly differences according to the anthocyanin concentrations in Geomjeong 3 (2018: 10.4 and 2019: 5.4 mg/g), Geomjeong 2 (2018: 14.4 and 2019: 6.2 mg/g), and Mirang (2018: 6.0 and 2019: 13.0 mg/g) cultivars. The above inhibition effects are similar pattern as the results by previous literatures regarding tyrosinase inhibitions in the anthocyanin extracts of *Akebia trifoliate* flowers and red wine (Fujimaki et al., 2018; Jiang et al., 2020). Our current results confirmed that the tyrosinase inhibition rates may be significantly influenced by the extracted anthocyanins. Moreover, the presence of hydroxyl group in anthocyanin structures may be contributed to determine the tyrosinase inhibition properties, as similar to those of previously reported (Hemachandran et al., 2017; Hemachandran et al., 2008). For gathering exact information of black soybean extracts, Socheong (2018) of the highest isoflavones and Geomjeong 2 (2018) of the most abundant anthocyanins were examined for variations of tyrosinase inhibitory capacities through reaction times at different concentrations (600, 100, 50, and 10 μg/mL) by comparing with ascorbic acid (positive control) (Fig. 4A and B). As presented in Fig. 4A, ascorbic acid exhibited a dose-dependent inhibition activities (89.2 → 59.4% for 30 min) and the 50% methanol extract (600 μg/mL) of Socheong cultivar slightly decreased with tyrosinase inhibitions (66.1 → 50.2% for 30 min) according to the increases of reaction times. Other concentrations (100, 50, and 10 μg/mL) were observed considerable differences (approximately 62.9 → 27.4% for 30 min) by increasing reaction times and concentrations. In brief, the enzyme inhibition effects

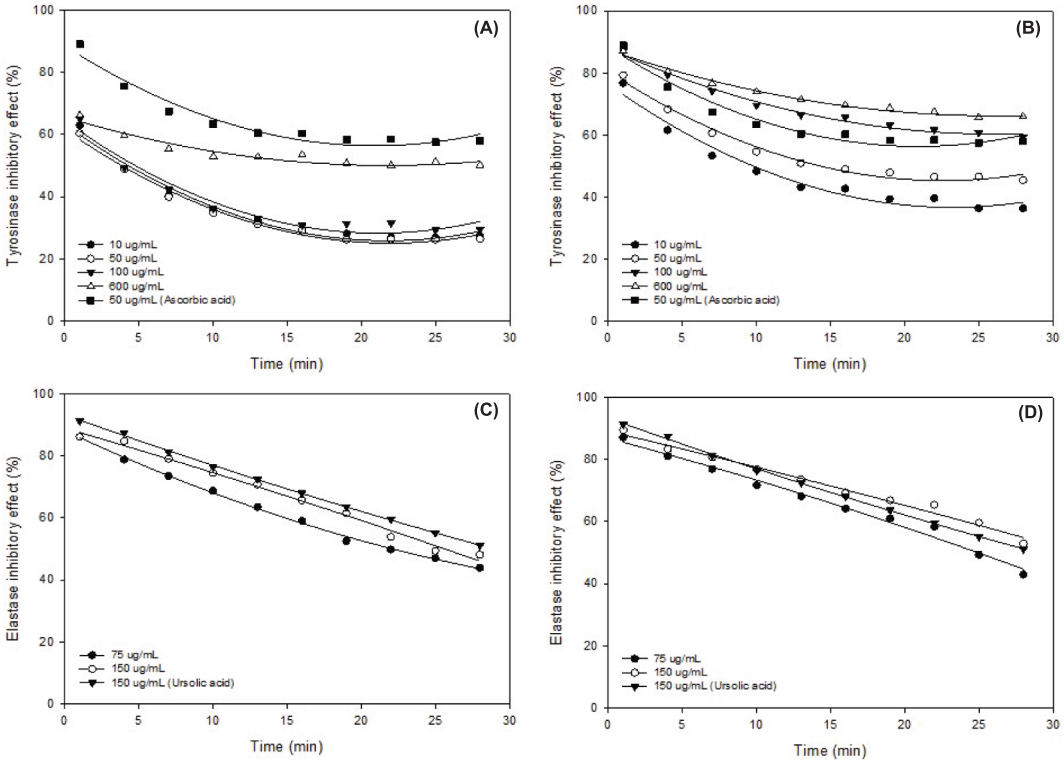

Fig. 4. Comparisons of tyrosinase and elastase inhibitory effects in whole seeds and seed coats of black soybeans at different reaction times: (A) Tyrosinase inhibitory activities of the 50% methanol extracts in whole seeds (cv. Socheong, 2018) of the predominant isoflavone contents; (B) Tyrosinase inhibitory activities of the acidic 50% methanol extracts in seed coats (cv. Geomjeong 2, 2018) of the predominant anthocyanin contents; (C) Elastase inhibitory activities of the 50% methanol extracts in whole seeds (cv. Socheong, 2018) of the predominant isoflavone contents; (D) Elastase inhibitory activities of the acidic 50% methanol extracts in seed coats (cv. Geomjeong 2, 2018) of the predominant anthocyanin contents.

have a great impact up to 10 min with a dose-dependent manner of remarkable differences, and the retention times between 10 min and 30 min showed slightly decreases with completely suppressed (Fig. 4A). The tyrosinase inhibitions in the acidic 50% methanol extract of seed coats (cv. Geomjeong 2) were rapidly decreased with dose-dependent capacities under the reaction times (Fig. 4B), compared to those of whole seeds. Their activities were observed higher rates about 20% than whole seeds with increasing concentrations. As mentioned above, the tyrosinase inhibition patterns of seed coats showed high abilities with considerable differences during 30 min as follows: 600 μg/mL (87.1 → 65.9%) > 100 μg/mL (85.0 → 59.4%) > 50 μg/mL (78.5 → 45.5%) > 10 μg/mL (73.0 → 36.5%) (Fig. 4B). These findings indicate that secondary metabolites, such as isoflavone and anthocyanin derivatives may be contributed to the tyrosinase inhibitory capacities of black soybeans. In addition, the Socheong and Geomjeong 2 cultivars may be recommended as potential materials for the black soybean values concerning development of new human health and breeding uses. We documented for the first time the comparison of tyrosinase inhibitory abilities from the whole seeds and seed coats of black soybeans for different crop years.

Measurement of enzymatic inhibitions against elastase in whole seeds and seed coats of black soybeans at different crop years

To gain information on another method of anti-aging assessment (Kim et al., 2018; Leal et al., 2020; Liyanaarachchi et al., 2018), we observed the elastase inhibition abilities by comparing the percentage ratios from the whole seeds and seed coats of black soybeans. The inhibiton properties of two extracts and positive control (ursolic acid) were remarkably enhanced with the increasing concentrations, specifically, their activities exhibited high proportions with dose-dependent patterns at a concentration of 150 μg/mL. The elastase inhibitions displayed higher effects approximately 4 times compared with the tyrosinase results (600 μg/mL) and exhibited significant differences in cultivars and crop years. In the 50% methanol extracts of whole seeds, the predominant inhibition activity was observed in Socheong cultivar (87.9% at 150 μg/mL, 2019 crop year). The abundant isoflavone cultivars showed high elastase inhibition rates as the rank order of Socheong (87.1%, 2018) > Cheomjeong 4 (79.7% 2019) > Cheongja 3 (75.8%, 2019) > Geomjeong 2 (75.1%, 2019) > Heugmi (73.6%, 2019), and other cultivars showed mild inhibition activities with < 70%. This enzyme inhibition pattern was similar to those of tyrosinase assay, and may be correlated with the isoflavone contents in extracts of whole seeds as described in earlier researches through nature plants (Deniz et al., 2020; Kim et al., 2018). The elastase inhibitory effects in the acidic extracts of seed coats were also in agreement with earlier data that the tyrosinase inhibition abilities increased considerably according to the increase of anthocyanin contents (Fujimaki et al., 2018; Hemachandran et al., 2008). The absolutely highest effect was observed in Geomjeong 2 (86.1%, 2018) at 150 μg/mL, followed by Mirang (85.9%, 2019) > Cheongja 3 (85.3%, 2018) > Cheongja 2 (83.6%, 2019) > Geomjeong 3 (81.7%, 2018), and other cultivars exhibited mild inhibition capacities (<80%). Also, the average inhibitions for two crop years showed high activities with approximately > 80% in Geomjeong 2, Cheongja 2, Cheongja 3 and Mirang cultivars and were similar patterns when compared to the positive control (88.7% at 150 μg/mL, urosolic acid). Therefore, the mentioned cultivars may be considered as potent anti-aging sources for cosmeceutical products. These phenomena assumed that the elastase inhibition abilities may be dependent to much degree on the anthocyanin contents (Chiocchio et al., 2018; Deniz et al., 2020; Liyanaarachchi et al., 2018; Waqas et al., 2015). In order to evaluate the influence rates under reaction times, based on the elastase inhibition

experimental data, we measured inhibition efficiency from the extracts of whole seeds (cv. Socheong, 2018) and seed coats (cv. Geomjeong 2, 2018) of the highest elastase capacities for 30 min. When the increase of reaction times, the inhibition patterns decreased with dose-dependent activities (Fig. 4C and D). In more details, the 50% methanol extract of whole seeds was reduced with 87.9 → 81.0 → 75.0 → 69.7 → 64.4 → 58.5 → < 50% (Fig. 4C) at 150 μg/mL and the acidic 50% methanol extract of seed coats was observed with ranges of 88.8 → 85.1 → 80.1 → 74.2 → 69.1 → 64.2 → 61.8 → < 60% (Fig. 4D). Although two extracts showed similar inhibition effects, the seed coats displayed higher elastase inhibitory activities than whole seeds. These differences suggest that the elastase inhibition rates were positively correlated with anthocyanin contents compared to isoflavones (Leal et al., 2020; Waqas et al., 2015). Our work provides available information that black soybean is considered as effective agent for pharmaceutical industry concern to anti-aging and skin-whitening properties. As far as we know, this research was the first to demonstrate that the whole seeds and seed coats of black soybeans have potent inhibition capacities against tyrosinase and elastase.

Conclusion

The present work is the first proved that the comparisons and variations of nutritional components and bioactive properties in whole seeds and seed coats of various black soybeans for two crop years. The isoflavone and anthocyanin contents displayed remarkable differences in the ranges of 794.9–4195.3 μg/g and 2.3–14.4 mg/g between cultivars and growth years, specifically, anthocyanins showed the absolutely dominant changes (2018: 2.3–14.4 mg/g; 2019: 2.9–13.0 mg/g), whereas other compositions exhibited only slight variations. The highest average isoflavone was observed in malonylgenistin (778.0 μg/g), comprising approximately 35.5% of the total isoflavones (2197.8 μg/g), and C3G was the predominant anthocyanin with 4.6 mg/g (76.7%) in average 6.0 mg/g. Moreover, Socheong cultivar was detected the most abundant average isoflavone content with 4182.4 μg/g, and the average anthocyanin contents were in the following order: Geomjeong 2 (10.3 mg/g) > Mirang (9.5 mg/g) > Cheongja 3 (9.4 mg/g) > Cheongja 2 (9.3 mg/g). In particular, their extracts showed excellent beneficial properties including antioxidant and DNA protectant as well as tyrosinase and elastase inhibitions, and occurred as follows: elastase > tyrosinase > ABTS > DPPH with higher capacities of seed coats than whole seeds. The DNA protection also displayed high rates with approximately 10 times in seed coats (68.0% at 10 μg/mL) when compared with whole seeds (49.9% at 200 μg/mL). From our findings, it is confirmed that the potential properties of natural antioxidants and cosmeceutical enzyme inhibitions can be positively correlated with isoflavone and anthocyanin contents in whole seeds and seed coats of black soybeans. In addtition, our research may be employed an excellent opportunities concern to the black soybean qualities for soybean breeders and producers. We believe that the black soybean extracts may be an important candidates for further studies in their uses for natural source-based product, functional food, nutraceutical, and cosmeceutical agents.

CRediT authorship contribution statement

Jin Hwan Lee: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Methodology, Data curation, Funding acquisition, Project administration. **Eun Young Seo:** Methodology, Data curation, Investigation, Validation. **Young Min Lee:** Data curation, Investigation.

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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