

Antioxidant and Antihypertensive Activities of Grains Grown in South Korea in Relation to Phenolic Compound and Amino Acid Contents

Narae Han, Koan Sik Woo*, Jin Young Lee**, Jiho Chu***, Mihyang Kim**,
Yu-Young Lee****, Moon Seok Kang**** and †Hyun-Joo Kim**

Post-Doctor, Dept. of Central Area Crop Science, National Institute of Crop Science, Rural Development Administration, Suwon 16613, Korea

**Senior Researcher, Bioenergy Crop Research Institute, National Institute of Crop Science, Rural Development Administration, Muan 58545, Korea*

***Researcher, Dept. of Central Area Crop Science, National Institute of Crop Science, Rural Development Administration, Suwon 16613, Korea*

****Associate Researcher, Dept. of Southern Area Crop Science, National Institute of Crop Science, Rural Development Administration, Miryang 50424, Korea*

*****Senior Researcher, Dept. of Central Area Crop Science, National Institute of Crop Science, Rural Development Administration, Suwon 16613, Korea*

Abstract

Hypertension is characterized by excessive renin-angiotensin system activity, leading to blood vessel constriction. Several synthetic compounds have been developed to inhibit renin and angiotensin-converting enzyme (ACE). These drugs often have adverse side effects, driving the exploration of plant protein-derived peptides as alternative or supplementary treatments. This study assessed the phenolic compound and amino acid content and the antioxidant and antihypertensive activity of 5 South Korean staple crops. Sorghum had the highest phenolic compound content and exhibited the highest antioxidant activity. Millet grains, particularly finger millet (38.86%), showed higher antihypertensive activity than red beans (14.42%) and sorghum (17.16%). Finger millet was found to contain a large proportion of branched-chain, aromatic, and sulfur-containing amino acids, which are associated with ACE inhibition. In particular, cysteine content was positively correlated with ACE inhibition in the crops tested ($r=0.696$, $p<0.01$). This study confirmed that the amino acid composition was more correlated with the antihypertensive activity of grains than the phenolic compound content. Finger millet mainly contained amino acids, which have higher ACE inhibitory activity, resulting in the strongest antihypertensive activity. These findings underscore the antihypertensive potential of select crops as plant-based food ingredients, offering insight into their biological functions.

Key words: angiotensin-converting enzyme, biological activity, finger millet, hypertension, phenolic compounds, sorghum

Introduction

Grains (including barley, sorghum, millet, and beans) are considered staple foods and sources of nutraceutical nutrients. The essential components of grains are proteins, starch, dietary fiber, non-starch polysaccharides, and phytochemicals (Girard & Awika 2018; Kim et al. 2018). *In vitro* and *in vivo* studies have shown that phytochemicals from grains, such as phenolic acids, flavonoids, carotenoids, amino acids, phytic acids, and lignin, have therapeutic effects against metabolic disorders such as diabetes, hypertension, cancer, and cardiovascular diseases (Masisi et al. 2016). Concerns about metabolic diseases have

influenced the demand for low-calorie foods, thereby increasing human consumption of grains (Queiroz et al. 2018).

Millet is a major source of protein and energy, and finger, Italian, and proso millet are common millet species (Chandra et al. 2016). Millets are gluten-free, have a low glycemic index, and contain numerous phytochemicals, particularly polyphenols (e.g., hydroxybenzoic acid, hydroxycinnamic acid, and flavonoids), that provide nutritional benefits (Singh & Adedeji 2017; Bangar et al. 2021). Sorghum is ranked as the world's 5th largest grain crop for human food, animal feed, and biomass production (Dahlberg J 2019). Functional compounds in sorghum, including phenolic acids, flavonoids, policosanols, phytosterols, stilbenes,

† Corresponding author: Hyun-Joo Kim, Researcher, Dept. of Central Area Crop Science, National Institute of Crop Science, Rural Development Administration, Suwon 16613, Korea. Tel: +82-31-698-0614, Fax: +82-31-695-0609, E-mail: tlrtod@Korea.kr

and tannins, may reduce the incidence of inflammation, cancer, obesity, and chronic diseases (Khalid et al. 2022). Red beans are cultivated internationally as food and for medicines (Wang et al. 2022), and red bean-derived polyphenols exhibit antihypertensive, anti-obesity, antioxidant, and immune-regulatory effects (Kitano-Okada et al. 2012; Shi et al. 2017).

Hypertension is a chronic disease caused by several factors, including systemic diseases, diabetes, obesity, and kidney disease (Gharibzahedi & Smith 2021). Systemic hypertension involves excessive activity of the renin-angiotensin system, which leads to blood vessel contraction. Under normal conditions, angiotensinogen is converted to angiotensin I by renin, and angiotensin I is continuously transformed into angiotensin II by angiotensin-converting enzyme (ACE). However, in metabolic disorders, excessive activities of renin and ACE cause high blood pressure, leading to hypertension (Aluko RE 2015). In this respect, negatively modulating renin and ACE activity results in the maintenance of homeostatic angiotensin II levels, which is the primary approach for controlling hypertension and normalizing blood pressure. Several synthetic compounds have been used as renin- and ACE-inhibitory drugs (Chen et al. 2013); however, these drugs are often accompanied by adverse side effects (Abassi et al. 2009). Therefore, recent research has focused on producing plant protein-derived peptides to replace or complement synthetic medicines. Previous studies have reported that seed proteins, such as lentil, pea, and soybean hydrolysates, have ACE inhibitory activity, which is predominantly influenced by the structural properties of the peptide (Aluko RE 2015; Rezvankhah et al. 2022; Mirzaee et al. 2022). Generally, ACE inhibition is attributed to the presence of specific peptides such as proline and hydrophobic, aromatic, and branched-chain amino acids (Aluko RE 2015; Das et al. 2022). Therefore, determining the amino acid composition of various crops is crucial for estimating their antihypertensive activity.

In this study, the bioactive compound contents and biological activities of five crops grown in South Korea were evaluated. In addition, we hypothesized that ACE inhibition would be affected by the amino acid composition; therefore, the inhibitory effect of major individual amino acids detected in the crops on ACE was confirmed.

Material and Methods

1. Plant materials and reagents

Whole grains finger millet (*Eleusine coronana* L. cv. Finger

1 ho), Italian millet (*Panicum italicum* L. cv. Samdachal), proso millet (*P. meliaceous* L. cv. Geumsilchal), sorghum (*Sorghum bicolor* L. var. Sodamchal), and red beans (*Vigna angularis* L. cv. Arari) were obtained from the National Institute of Crop Science, Rural Development Administration (Miryang, Korea). The cultivars selected were those commonly grown in Korea. The grains were finely ground using a blender and sieved through 100 mesh to obtain uniform-sized particles. The lyophilized samples were stored at -20°C before experimentation.

All the chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). Distilled water was obtained using a Milli-Q Advantage A10 purification system (Merck Millipore, Billerica, MA, USA). Other reagents were of analytical grade and purchased from J.T. Baker Inc. (Phillipsburg, NJ, USA).

2. Extraction method

Samples were extracted as previously described by Han et al. (2022a). The ground sample was mixed with 80% ethanol (1:10 w/v) and stirred for 24 h at 25°C . The extract was centrifuged (CR22N, Eppendorf Himac Technologies Co., Ltd., Ibaraki, Japan) at $10,000\times g$ for 20 min, and the supernatant was collected and evaporated using a rotary evaporator (SB-1200, EYELA Co., Ltd., Tokyo, Japan). For further experiments, the concentrated extract was re-dissolved in dimethyl sulfoxide (DMSO) (1:100, w/v).

3. Determination of total phenolic compound and flavonoid contents

A modified Folin-Ciocalteu and aluminum chloride method was used for total phenolic and flavonoid content analysis, according to Han et al. (2022a). For this purpose, the extract was diluted in DMSO to 1 mg/mL, and the analysis was performed in triplicate.

For the total phenolic compound content analysis, the diluted extracts (10 μL) were mixed with 2% sodium carbonate (200 μL) and 50% Folin-Ciocalteu reagent (10 μL), and incubated at 25°C for 30 min. Absorbance was measured at 750 nm using a UV spectrophotometer (Elx 808; Bio-Tec Inc., Winooski, VT, USA). Gallic acid was used as a reference standard, and the content was expressed as mg gallic acid equivalent (GAE) per g of extract ($y=1.450x - 0.013$, $r^2=0.999$).

For the total flavonoid content analysis, the diluted extracts (75 μL) were mixed with deionized water (300 μL), 5% sodium

nitrite (22.5 μL), 10% aluminum chloride (45 μL), and 1 M sodium hydroxide (150 μL) and incubated at 25°C for 20 min. Absorbance was measured at 510 nm using a UV spectrophotometer. Catechin was used as a reference standard, and the content was expressed as mg of catechin equivalent (CE) per g of extract ($y=2.063x-0.002$, $r^2=0.998$).

4. Amino acid analysis

Amino acid composition was determined as described by Kim et al. (2022). Briefly, the samples were hydrolyzed using 6 M hydrochloric acid at 110°C for 12 h, digested, and filtered through a 0.22 μm polytetrafluoroethylene filter. The amino acid concentration was analyzed with ninhydrin reagent using an LA8900 amino acid automatic analyzer (Hitachi High-Tech Co., Tokyo, Japan). The ion-exchange column (Hitachi HPLC Packed column, 4.6 mm i.d., 60 mm length, 3 μm particle size) was used, and the analytical visible detector (Hitachi High-Tech Co.) set a 570 and 440 nm for proline. The analytical condition was followed as previously described by Shim et al. (2013). A standard solution comprising 17 amino acids was used as an external standard.

5. Determination of antioxidant capacity

The radical scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazolin-6-sulfonic acid) (ABTS) and ferric-reducing antioxidant power (FRAP) were analyzed as described in our previous study with slight modifications (Han et al. 2022b). The extract was diluted in DMSO to 1 mg/mL and analyzed in triplicate under low light.

For the DPPH assay, the diluted extracts (20 μL) were mixed with 0.2 mM DPPH solution (200 μL) and incubated at 25°C for 30 min. Absorbance was measured at 520 nm using a UV spectrophotometer. Trolox was used as the reference standard, and the activity was expressed as mg of trolox equivalent (TE) per g of extract ($y=-5.580x+1.155$, $r^2=0.999$).

For the ABTS assay, the diluted extract (20 μL) was mixed with 7.4 mM ABTS solution (200 μL) and incubated at 25°C for 30 min. Absorbance was measured at 734 nm using a UV spectrophotometer. Trolox was used as a reference standard, and the activity was expressed as mg TE per g of extract ($y=-6.801x+1.365$, $r^2=0.999$).

For the FRAP assay, the diluted extract (6 μL) was mixed with FRAP reagent (180 μL) and deionized water (18 μL) and incubated at 25°C for 10 min. Absorbance was measured at 593

nm using a UV spectrophotometer. Iron sulfate was used as the reference standard, and the activity was expressed in mmol per g of extract ($y=0.422x+0.0637$, $r^2=0.999$).

6. ACE inhibition assay

ACE inhibitory activity was analyzed according to the enzymatic methods of Kancabaş & Karakaya (2013) and Kim et al. (2019) to evaluate antihypertensive potential. The grain extracts and amino acid standards were prepared at concentrations of 10 and 5 mg/mL, respectively.

The test sample (10 μL) was mixed with 8.3 mM N-Hippuryl-His-Leu hydrate (substrate) dissolved in 0.1 M sodium borate buffer (pH 8.3; 30 μL) and incubated at 37°C for 10 min. Next, 1 unit/mL ACE from rabbit lung dissolved in 0.01 M potassium phosphate buffer (pH 7.3) containing 0.5 M NaCl (10 μL) was added to the mixture. After 30 min of incubation at 37°C, the reaction was terminated by adding 1 N HCl (50 μL). The mixture was combined with 300 μL of ethyl acetate and centrifuged at 1,000 \times g for 5 min. The supernatant (250 μL) was completely evaporated at 80°C and re-dissolved in deionized water (300 μL). Absorbance was measured at 228 nm using a UV spectrophotometer. Enalapril maleate salt (0.04 mg/mL) was the positive control. The inhibitory effects were calculated as follows:

$$\text{Inhibition rate (\%)} = \left(1 - \frac{A_{\text{sample}} - A_{\text{sample blank}}}{A_{\text{control}} - A_{\text{control blank}}} \right) \times 100,$$

where A_{sample} is the absorbance of a mixture consisting of a sample, enzyme, and substrate; $A_{\text{sample blank}}$ is the absorbance of a mixture without the enzyme; A_{control} is the absorbance of a mixture without the sample; and $A_{\text{control blank}}$ is the absorbance of a mixture without the sample and enzyme. Furthermore, the concentration of amino acid standards that inhibited 50% of ACE activity (IC_{50}) was determined using standard concentrations of 0.02–5 mg/mL.

7. Statistical analysis

All values are presented as the mean and standard deviation of triplicate measurements, calculated using SigmaPlot (version 14.0; Systat Software, San Jose, CA, USA). Samples were compared using Tukey's multiple range test at $p<0.05$ using SPSS statistical software version 18 (SPSS, Inc., Chicago, IL, USA). A heatmap and correlation analysis was applied using

MetaboAnalyst 5.0 (<https://www.metaboanalyst.ca>, accessed on 23 Jul 2023) to the normalized and log-transformed mean values of the relative content of the metabolites to clarify differences between the crops (Pang et al. 2021).

Result and Discussion

1. Functional compounds and antioxidant activities in grains

A representative image of five grains mainly grown in South Korea is shown in Fig. 1. Total phenolic compound and flavonoid contents ranged from 3.04 to 290.18 mg GAE/g and 0.19 to 110.55 mg CE/g, respectively (Table 1). The highest total phenolic and flavonoid contents were detected in sorghum, whereas Italian millet and proso millet contained significantly lower ($p < 0.05$) total phenolic and flavonoid contents than that in the other tested crops. Among the millet varieties, finger millet had, by far, the highest total phenolic (21.52 mg GAE/g) and flavonoid (14.76 mg CE/g) contents. Choi et al. (2007) reported that the total phenolic compound contents in sorghum,

foxtail millet (Italian millet), and proso millet were the equivalent of 138.30, 6.91, and 6.44 mg GAE/g, respectively. This is consistent with our results. In a previous study, the total phenolic compound content of red beans ranged from 2.61 to 15.53 mg GAE/g extract depending on the cultivars (Han et al. 2022b), which is less than that observed in this study. These differences may be due to the genotype, extraction methods, ecological region, cultivation environment, and climatic conditions (Ofosu et al. 2020; Ghimire et al. 2021). Ofosu et al. (2020) found that finger millet had the highest total phenolic compound and flavonoid contents among four varieties of millet grains (1.36 mg ferulic acid equivalent/g and 1.16 mg CE/g, respectively). This agrees with our finding that finger millet had a relatively higher phenolic compound content than Italian and proso millet. Biological activities are associated with antioxidant compounds such as phenolic compounds (Han et al. 2022a). These results indicate that grains that have abundant polyphenols can be used as rich sources of natural antioxidants.

Phenolic compounds present in various grains are secondary metabolites directly associated with antioxidant activity (Ofosu et



Fig. 1. Typical phenotypic characteristic of ‘Finger 1 ho’, ‘Samdachal’, ‘Geumsilchal’, ‘Arari’, and ‘Sodamchal’.

Table 1. Bioactive compounds contents and antioxidant activities of grains

Crops	Total phenolic compounds (mg GAE ² /g)	Total flavonoids (mg CE/g)	DPPH (mg TE/g)	ABTS (mg TE/g)	FRAP (mM)
Finger millet	21.52±0.31 ^{bc1)}	14.76±0.15 ^b	14.06±0.55 ^c	37.68±0.73 ^b	117.28±5.48 ^{bc}
Italian millet	5.05±0.07 ^c	0.43±0.12 ^c	0.43±0.12 ^d	4.33±0.12 ^c	30.28±1.97 ^c
Proso millet	3.04±0.14 ^c	0.15±0.11 ^c	0.97±0.02 ^e	2.33±0.03 ^c	34.72±3.98 ^c
Red bean	28.74±1.43 ^b	16.16±0.33 ^b	19.21±0.28 ^b	36.19±0.34 ^b	202.47±4.08 ^b
Sorghum	290.18±17.95 ^a	110.55±5.41 ^a	110.55±5.41 ^a	407.99±10.95 ^a	1,404.25±90.90 ^a

¹⁾ All data are presented as the mean±standard deviation of three replicates. Data marked with different letters in the same column indicate significant differences at $p < 0.05$.

²⁾ GAE, gallic acid equivalent; CE: catechin equivalent; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS, 2,2'-azino-bis (3-ethylbenzothiazolin-6-sulfonic acid); FRAP, ferric-reducing antioxidant power; TE, trolox equivalent.

al. 2020). DPPH and ABTS radical scavenging activity and ferric reducing ability assay are a wide range of spectrophotometric assay to measure antioxidant capacity of food. In detail, DPPH and ABTS assay is based on the reduction of purple DPPH and blue/green ABTS^{•+} by antioxidants, respectively. In contrast, FRAP assay is monitored the reduction of Fe³⁺ to Fe²⁺ with no free radical involved (Floegel et al. 2011). The antioxidant activity measurements of the grains may be different in accordance with the assay used. Thus, free radical scavenging activity (DPPH and ABTS) and reductive potential (FRAP) assays were performed to compare the antioxidant activity of the grains (Table 1). As with the functional compound content assay, sorghum exhibited the highest DPPH and ABTS radical scavenging activities (162.74 and 407.99 mg TE/g, respectively). In addition, the free radical scavenging activities of Italian and proso millet were significantly lower than that of finger millet ($p < 0.05$). Consistent with the free-radical scavenging activity, sorghum had the highest FRAP (1,404.25 mM), followed by red bean (202.47 mM), finger millet (117.28 mM), proso millet (34.72 mM), and Italian millet (30.28 mM). Our results are consistent with those of a previous study describing phenolic compounds that significantly contributed to antioxidant activity (Ghimire et al. 2021).

2. Amino acid composition and antihypertensive property of grains

The distribution of amino acids in the grains is shown in Fig. 2 and Table 2. Red beans contained a relatively high proportion of positively charged amino acids, including lysine (1,286.62 mg/100 g dw), histidine (519.81 mg/100 g dw), and arginine (1,019.50 mg/100 g dw). Branched-chain amino acids including valine (401.03 mg/100 g dw) and isoleucine (267.99 mg/100 g dw) were more prevalent in finger millet than aromatic amino acids such as phenylalanine (377.54 mg/100 g dw) and tyrosine (134.37 mg/100 g dw). Methionine and cysteine, which are sulfur-containing amino acids, were also detected as major constituents of finger millet. Proso millet, Italian millet and sorghum showed relatively higher proportions of alanine, proline, and leucine and lower proportions of valine, isoleucine, aspartic acid, lysine, histidine, and arginine than those in red bean and finger millet.

Previous studies have indicated that grains may reduce oxidative damage and chronic diseases, including obesity, diabetes, and hypertension (Masisi et al. 2016). Our study focused

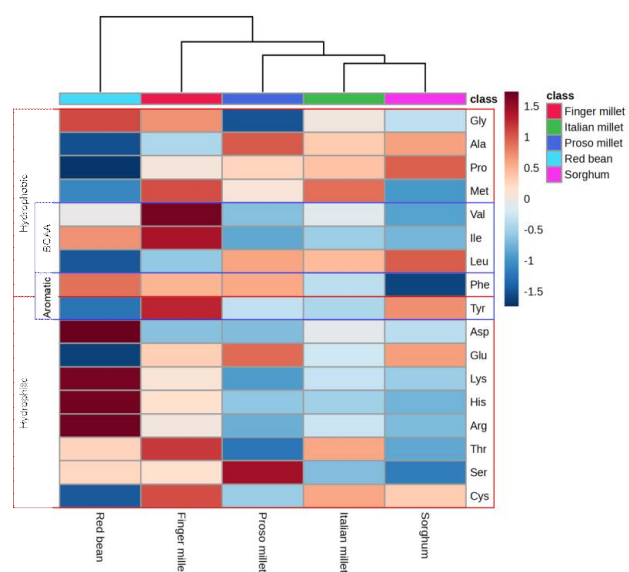


Fig. 2. Hierarchical clustering and heatmap of the normalized response of the amino acids in grains. The levels of amino acid contents correspond to the color scale. A color gradient from red to blue represents a high level (1.901) to a low level (-1.747) of normalized response. BCAA, branched-chain amino acid.

on the antihypertensive potential of grains by investigating the inhibition of ACE by grain extracts (Fig. 3). Antihypertensive activity varied significantly between the crops assessed ($p < 0.05$), which ranked as follows (strongest to weakest): finger millet (38.86%), proso millet (31.39%), Italian millet (28.89%), sorghum (17.16%), and red beans (14.42%). Therefore, millet grains, especially finger millet, had a higher ACE inhibitory activity than sorghum and red beans. Aluko RE (2015) reported that branched chain, aromatic, and hydrophobic amino acids may potentiate ACE inhibition with antihypertensive effects. In our study, we assumed that the improved antihypertensive effect of finger millet resulted from a higher ratio of branched-chain and aromatic amino acids.

3. Antihypertensive property of amino acid standard compounds

Pearson's correlation was used to determine the relative contribution of the tested constituents to biological activity. As shown in Fig. 4, the levels of functional compounds were positively correlated with antioxidant activity ($p < 0.001$) and negatively correlated with antihypertensive activity ($p < 0.05$). The ACE inhibitory effect was positively correlated with most

Table 2. Amino acid contents in grains

Crops	Finger millet	Italian millet	Proso millet	Red bean	Sorghum
Asp	379.42±7.88 ^{d1)}	561.72±10.53 ^d	668.40±16.20 ^f	1,900.02±4.44 ^b	518.23±17.74 ^c
Thr	278.73±6.31 ^f	318.31±6.13 ^h	332.78±10.20 ⁱ	604.23±4.19 ^g	251.58±9.10 ⁱ
Ser	339.82±8.52 ^e	382.78±10.15 ^g	713.26±25.63 ^e	863.07±4.33 ^e	358.08±9.66 ^g
Glu	1,396.30±31.68 ^a	1,670.75±30.64 ^a	2,612.67±58.10 ^a	2,960.04±4.21 ^a	1,805.09±66.02 ^a
Gly	230.74±4.85 ^g	245.26±4.94 ^j	239.16±6.44 ^j	638.66±4.17 ^{fg}	224.56±7.01 ⁱ
Ala	412.24±7.99 ^c	685.01±13.25 ^c	1,215.84±30.22 ^c	710.86±4.29 ^f	751.14±24.39 ^c
Cys	165.55±1.10 ⁱ	179.83±9.95 ^l	178.50±2.35 ^k	192.03±4.06 ^j	165.91±0.16 ^j
Val	401.03±7.30 ^c	416.07±9.19 ^f	553.12±6.91 ^g	837.34±3.90 ^e	382.50±18.30 ^g
Met	194.71±7.60 ^h	225.96±5.27 ^k	222.72±14.66 ^j	183.60±2.71 ^j	98.16±6.59 ^k
Ile	267.99±3.42 ^f	306.32±9.36 ^h	426.37±4.63 ^h	650.10±4.13 ^{fg}	303.56±14.09 ^h
Leu	615.50±14.60 ^b	980.32±19.38 ^b	1,430.30±35.24 ^b	1,269.62±4.53 ^c	1,105.31±42.12 ^b
Tyr	134.37±7.29 ^j	147.36±2.74 ^m	210.62±18.81 ^{jk}	276.59±5.52 ⁱ	161.32±5.05 ^j
Phe	377.54±21.30 ^d	463.91±4.87 ^e	671.04±12.78 ^f	956.34±3.78 ^d	449.72±17.21 ^f
Lys	187.06±0.76 ^h	182.22±1.98 ^l	173.33±2.20 ^k	1,286.62±3.069 ^c	154.62±6.76 ^j
His	160.07±0.63 ⁱ	179.45±5.77 ^l	249.39±3.39 ⁱ	519.81±3.40 ^h	172.46±12.52 ^j
Arg	242.10±4.36 ^g	277.36±7.49 ⁱ	333.38±10.53 ⁱ	1,019.50±4.71 ^d	242.77±14.51 ⁱ
Pro	405.72±3.36 ^c	552.13±0.28 ^d	756.21±26.53 ^d	672.04±4.79 ^{fg}	632.96±13.12 ^d

¹⁾ All data are presented as the mean±standard deviation of three replicates. Data marked with different letters in the same column indicate significant differences at $p<0.05$.

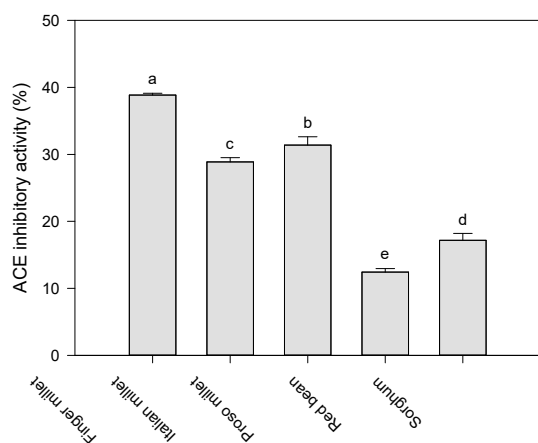


Fig. 3. Angiotensin-converting enzyme (ACE) inhibitory activity (%) in grains. Extracts were tested at 10 mg/mL, and the values are presented as the mean ± standard deviation of three replicates. The data marked with different letters are significantly different at $p<0.05$.

individual amino acids, except for aspartic acid, lysine, histidine, arginine, and glycine. In particular, the methionine ($r=0.916$, $p<0.001$), cysteine ($r=0.696$, $p<0.01$), and glutamic acid ($r=0.637$,

$p<0.05$) levels were highly correlated with ACE inhibition.

Based on previous findings, we hypothesized that the amino acid type would influence the antihypertensive activity. Thus, the inhibition of ACE was evaluated using amino acid standard chemicals (Fig. 5). Among the 17 amino acids detected in the grains, phenylalanine (99.83%), aspartic acid (99.35%), and cysteine (99.67%) exhibited the highest ACE inhibitory activity ($p<0.05$), followed by glutamic acid (87.91%) and histidine (72.87%). The least inhibitory were glycine (1.47%), proline (2.221%), methionine (1.18%), isoleucine (2.49%), leucine (0.00%), and serine (0.69%) (Fig. 5A). As illustrated in Fig. 5B, the IC_{50} values for phenylalanine, glutamic acid, aspartic acid, histidine, and cysteine were 0.72, 2.09, 2.41, 3.43, and 0.07 mg/mL, respectively. In fact, the IC_{50} value for cysteine did not significantly differ from that of the positive control ($p>0.05$). To our knowledge, no study has extensively investigated the antihypertensive effects of isolated or purified peptides from millet grains with strong ACE inhibitory activity. Further studies are required to investigate the antihypertensive effects of plant-derived protein hydrolysates and peptides isolated from millet grains.

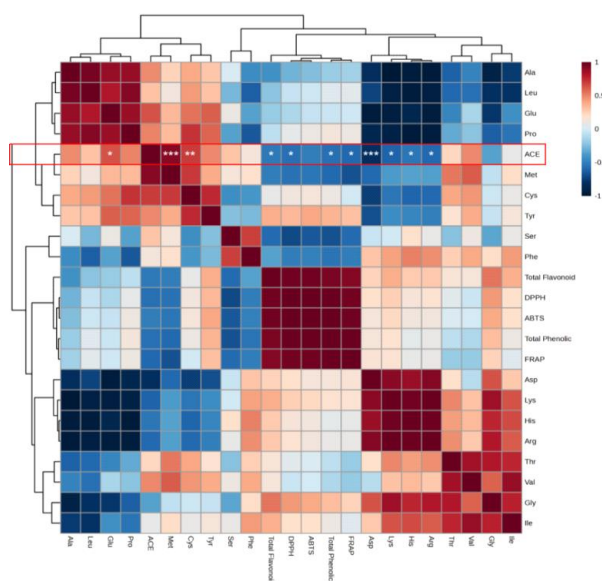


Fig. 4. Pearson's correlation coefficient matrices from functional compounds contents and biological activities in grains. Red and blue colors indicate positive and negative correlations, respectively. *, ** and *** indicate significant correlations between ACE and individual parameters at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Conclusion

This study provides a new perspective on the amino acid composition and antihypertensive activity of grains. Finger millet contains a higher ratio of branched-chain, aromatic, and sulfur-containing amino acids than the other crops, resulting in the strongest antihypertensive activity. Furthermore, sorghum shows potent antioxidant activity with abundant total phenolic compounds. To the best of our knowledge, this is the first study to attempt an antihypertensive activity analysis of amino acid standards, although several studies are available on extracted peptides and protein hydrolysates. Among the 17 amino acids detected in the cereal grains and beans, cysteine, phenylalanine, glutamic acid, aspartic acid, and histidine showed the strongest inhibitory activity against ACE. Our findings provide information on the biological activities of grains grown in South Korea and highlight their potential as plant-based food ingredients with antihypertensive activity.

Acknowledgements

This research was funded by the Cooperative Research

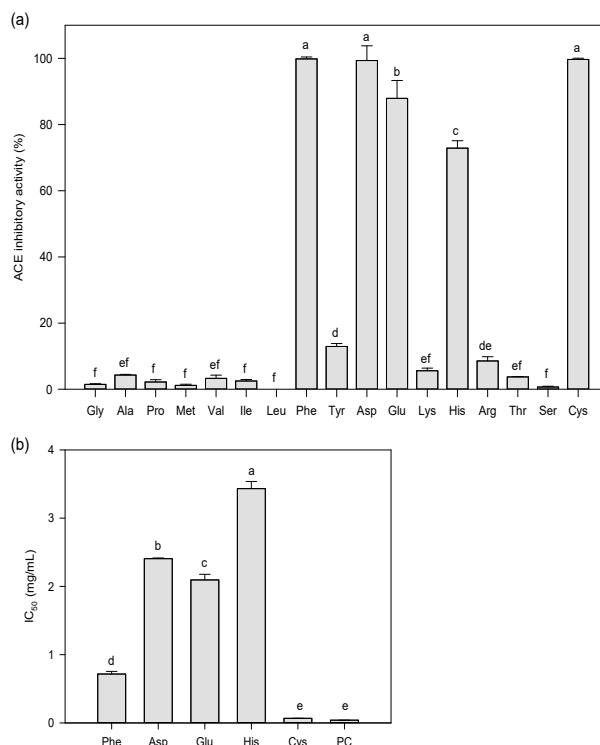


Fig. 5. Antihypertensive activity in amino acid standards. (A) Angiotensin-converting enzyme (ACE) inhibitory activity (%) in 17 amino acids treated at 5 mg/mL; (B) IC₅₀ values representing the concentration of phenylalanine, aspartic acid, glutamic acid, and histidine required to cause a 50% inhibition of ACE. Enalapril maleate salt was used as a positive control (PC). The values are presented as the mean \pm standard deviation of three replicates. The data marked with different letters are significantly different at $p < 0.05$.

Program for Agriculture Science and Technology Development (RS-2019-RD007816) of the Rural Development Administration, Korea and the 2023 RDA Fellowship Program of the Department of Central Area Crop Science, National Institute of Crop Science, Rural Development Administration, Republic of Korea.

References

- Abassi Z, Winaver J, Feuerstein GZ. 2009. The biochemical pharmacology of renin inhibitors: Implications for translational medicine in hypertension, diabetic nephropathy and heart failure: Expectations and reality. *Biochem Pharmacol* 78: 933-940
- Aluko RE. 2015. Structure and function of plant protein-derived

- antihypertensive peptides. *Curr Opin Food Sci* 4:44-50
- Bangar SP, Ashogbon AO, Dhull SB, Thirumdas R, Kumar M, Hasan M, Chaudhary V, Pathem S. 2021. Proso-millet starch: Properties, functionality, and applications. *Int J Biol Macromol* 190:960-968
- Chandra D, Chandra S, Pallavi, Sharma AK. 2016. Review of finger millet (*Eleusine coracana* (L.) Gaertn): A power house of health benefiting nutrients. *Food Sci Hum Wellness* 5:149-155
- Chen Y, Meng L, Shao H, Yu F. 2013. Aliskiren vs. other antihypertensive drugs in the treatment of hypertension: A meta-analysis. *Hypertens Res* 36:252-261
- Choi Y, Jeong HS, Lee J. 2007. Antioxidant activity of methanolic extracts from some grains consumed in Korea. *Food Chem* 103:130-138
- Dahlberg J. 2019. The role of sorghum in renewables and biofuels. In Zhao ZY, Dahlberg J (Eds.), *Sorghum: Methods and Protocols* (Methods in Molecular Biology, 1931). Humana Press
- Das D, Kabir ME, Sarkar S, Wann SB, Kalita J, Manna P. 2022. Antidiabetic potential of soy protein/peptide: A therapeutic insight. *Int J Biol Macromol* 194:276-288
- Floegel A, Kim DO, Chung SJ, Koo SI, Chun OK. 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *J Food Compos Anal* 24:1043-1048
- Gharibzahedi SMT, Smith B. 2021. Effects of high hydrostatic pressure on the quality and functionality of protein isolates, concentrates, and hydrolysates derived from pulse legumes: A review. *Trends Food Sci Technol* 107:466-479
- Ghimire BK, Seo JW, Yu CY, Kim SH, Chung IM. 2021. Comparative study on seed characteristics, antioxidant activity, and total phenolic and flavonoid contents in accessions of *Sorghum bicolor* (L.) Moench. *Molecules* 26: 3964
- Girard AL, Awika JM. 2018. Sorghum polyphenols and other bioactive components as functional and health promoting food ingredients. *J Cereal Sci* 84:112-124
- Han N, Kim J, Bae JH, Kim M, Lee JY, Lee YY, Kang MS, Han D, Park S, Kim HJ. 2022a. Effect of atmospheric-pressure plasma on functional compounds and physiological activities in peanut shells. *Antioxidants* 11:2214
- Han N, Woo KS, Lee JY, Song SB, Lee YY, Kim M, Kang MS, Kim HJ. 2022b. Comparison of physicochemical characteristics, functional compounds, and physiological activities in adzuki bean cultivars. *J Korean Soc Food Sci Nutr* 51:428-438
- Kancabaş A, Karakaya S. 2013. Angiotensin-converting enzyme (ACE)-inhibitory activity of boza, a traditional fermented beverage. *J Sci Food Agric* 93:641-645
- Khalid W, Ali A, Arshad MS, Afzal F, Akram R, Siddeeg A, Kousar S, Rahim MA, Aziz A, Maqbool Z, Saeed A. 2022. Nutrients and bioactive compounds of *Sorghum bicolor* L. used to prepare functional foods: A review on the efficacy against different chronic disorders. *Int J Food Prop* 25: 1045-1062
- Kim HJ, Lee JH, Lee BW, Lee YY, Lee BK, Jeon YH, Ko JY, Woo KS. 2018. Quality and physicochemical characteristics of rice cooked along with various mixed grains and by following different cooking methods. *Korean J Food Nutr* 31:653-667
- Kim HJ, Seo JH, Won DJ, Han N, Lee JY, Kim M, Lee YY, Kang MS. 2022. Comparison of physicochemical properties of seed protein in soybean cultivars. *J Korean Soc Food Sci Nutr* 51:1048-1055
- Kim MY, Kim HJ, Lee YY, Kim MH, Lee JY, Lee B, Lee BW. 2019. Enzyme inhibitory and anti-proliferation effects of peanut skin extracts depending on cultivar. *Korean J Food Nutr* 32:511-521
- Kitano-Okada T, Ito A, Koide A, Nakamura Y, Han KH, Shimada K, Sasaki K, Ohba K, Sibayama S, Fukushima M. 2012. Anti-obesity role of adzuki bean extract containing polyphenols: *In vivo* and *in vitro* effects. *J Sci Food Agric* 92:2644-2651
- Masisi K, Beta T, Moghadasian MH. 2016. Antioxidant properties of diverse cereal grains: A review on *in vitro* and *in vivo* studies. *Food Chem* 196:90-97
- Mirzaee H, Ahmadi Gavlighi H, Nikoo M, Udenigwe CC, Khodaiyan F. 2022. Relation of amino acid composition, hydrophobicity, and molecular weight with antidiabetic, antihypertensive, and antioxidant properties of mixtures of corn gluten and soy protein hydrolysates. *Food Sci Nutr* 11:1257-1271
- Ofori FK, Elahi F, Daliri EBM, Chelliah R, Ham HJ, Kim JH, Han SI, Hur JH, Oh DH. 2020. Phenolic profile, antioxidant, and antidiabetic potential exerted by millet grain varieties. *Antioxidants* 9:254
- Pang Z, Chong J, Zhou G, de Lima Morais DA, Chang L, Barrette M, Gauthier C, Jacques PÉ, Li S, Xia J. 2021.

- MetaboAnalyst 5.0: Narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res* 49:W388-W396
- Queiroz VAV, da Silva Aguiar A, de Menezes CB, de Carvalho CWP, Paiva CL, Fonseca PC, da Conceição RRP. 2018. A low calorie and nutritive sorghum powdered drink mix: Influence of tannin on the sensorial and functional properties. *J Cereal Sci* 79:43-49
- Rezvankhah A, Yarmand MS, Ghanbarzadeh B. 2022. The effects of combined enzymatic and physical modifications of lentil protein applying alcalase, flavourzyme, microbial transglutaminase, and ultrasound: Antioxidant, antihypertension, and antidiabetic activities. *J Food Meas Charact* 16:3743-3759
- Shi Z, Yao Y, Zhu Y, Ren G. 2017. Nutritional composition and biological activities of 17 Chinese adzuki bean (*Vigna angularis*) varieties. *Food Agric Immunol* 28:78-89
- Shim YS, Yoon WJ, Ha J, Seo D, Lee KW, Lee WY, Kwon KI, Kang TS, Lee JH, Kim HJ, Kwak HJ, Lee SP, Kim SJ, Yun WK, Lee J, Hwang JB. 2013. Method validation of 16 types of structural amino acids using an automated amino acid analyzer. *Food Sci Biotechnol* 22:1567-1571
- Singh M, Adedeji AA. 2017. Characterization of hydrothermal and acid modified proso millet starch. *LWT Food Sci Technol* 79:21-26
- Wang Y, Yao X, Shen H, Zhao R, Li Z, Shen X, Wang F, Chen K, Zhou Y, Li B, Zheng X, Lu S. 2022. Nutritional composition, efficacy, and processing of *Vigna angularis* (adzuki bean) for the human diet: An overview. *Molecules* 27:6079

Received 22 November, 2023
Revised 05 December, 2023
Accepted 08 December, 2023