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Antioxidant and Angiotensin-Converting Enzyme (ACE) Inhibitory Activity of Cereal Grain

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Abstract

Phenolic compounds are important products from secondary metabolism in plants. These cannot be synthesized in the human body and are mainly taken from plant source. Cereal grains are important sources of dietary polyphenols. Phenolic compounds are the main source of antioxidant that reduces the incidence of chronic diseases including heart disease, blood cholesterol, blood pressure, diabetic etc. Angiotensin-converting enzyme (ACE) inhibitors are medications that help to relax the veins and arteries to lower blood pressure. The aim of this experiment was to study the phenolic compounds identified in eight cereal grain (white rice, viscosity rice, brown rice, black rice, sticky rice, oatmeal, millet and sorghum) with their antioxidant and angiotensin-converting enzyme (ACE) inhibitory activity level. The phenolic content determined according to the Folin Ciocalteu's method. Black rice contains maximum phenolic compound (94.90 mg Tannic acid equivalent/g of extract) that was statistically different from other cereal grains ($p<0.05$) but followed by oatmeal and sorghum (58.82 and 58.57 mg Tannic acid equivalent/g of extract respectively). Antioxidant activities were comparatively assessed by ABTS (2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) free radical decolorization assay method. It was observed that maximum cereal grain has no antioxidant activity. Among eight cereal grain, only black rice, oatmeal and sorghum showed considerable free radical scavenging capacity by ABTS radical. Angiotensin-converting enzyme (ACE) inhibition rate was determined by using L-hippuryl-histidyl-leucine (HHL). Maximum angiotensin-converting enzyme (ACE) inhibitory activity was also found in black rice (66.80%) followed by oatmeal (48.62%) and sorghum (37.45%). It might be concluded that polyphenol content was closely related to antioxidant activity and angiotensin-converting enzyme (ACE) inhibitor activity. Black rice, oatmeal and sorghum can be used as functional food for their high phenolic content, antioxidant activity as well as angiotensin-converting enzyme (ACE) inhibitory activity against chronic diseases.

Keywords: Polyphenol; Antioxidant; Angiotensin-converting enzyme; Cereal grain

Introduction

Cereals are the most important primary sources of human diet throughout the world [1,2] that possesses great nutritional and bioactive properties such as phenolic acids-antioxidants, carbohydrate, protein, free amino acid, fibers, vitamins and minerals. Some cereals have been used as staple food both directly for human consumption and indirectly via livestock feed since the beginning of civilization [3]. It is the major source of energy for the world population. Based on the World Health Organization report for 2012–2016 [4], consumption of cereal grains may decrease the risk of non-communicable diseases (type 2 diabetes, cardiovascular disease, hypertension, and obesity). Instead, they comprise most of the micronutrients, fiber, and phytochemicals of the grain that could significantly impact on the nutritional quality of human food if integrated in flours or used as food ingredients [5].

Now a days, there has been a renewed interest in polyphenols as "life span essentials" due to their role in maintaining body functions and health throughout the adult and later phases of life [6]. Polyphenols are a large and diverse class of compounds, many of which occur naturally in a range of food plants. Natural polyphenols are mostly found in plants which are a kind of compounds with phenolic hydroxyl structure widely existing in nature [7]. Polyphenols not only have a strong antioxidation characteristic [8] but also have anticancer [9], bacteriostatic [10], liver protecting [11], anti-infection [12], cholesterol lowering [13] and immunity enhancing [14] properties. Moreover, these can prevent various biological activities such as type 2 diabetes [15,16]. Although, these compounds are not well known for direct role in nutrition (non-nutrients), many of them have antioxidant [17], anti-mutagenic, anti-osteogenic, anti-carcinogenic and anti-inflammatory, antiviral and platelet aggregation inhibitory activity that might potentially be beneficial in preventing or minimizing the incidence of diseases [18].

Angiotensin converting enzyme (ACE) is a multifunctional enzyme present in the rennin-angiotensin system that elevates blood pressure by generating the vasoconstrictor, angiotensin II [19]. Inhibition of ACE activity leads to a decrease in the concentration of angiotensin II, which decreases the tension in blood vessels and consequently reduces blood pressure [20]. The influence of ACE on blood pressure has made it an ideal target, and various synthetic medications such as captopril, enalapril, and lisinopril that inhibit angiotensin converting enzyme (ACE) are widely prescribed in the treatment and prevention of cardiovascular disease. Although, these drugs are often accompanied by undesirable side effects, no such side effects have been observed for ACE inhibitors derived from food peptides [21]. In this respect, the search for diet-related preventive agents for hypertension is obviously of interest within the scope of functional foods. Food-derived ACE inhibitory peptides are just the ideal candidates for such products; offering many advantages including safety of the natural product, low cost, and the additional nutritional benefits of the peptides as source of essential amino acids.

Most studies for deriving ACE-inhibitory peptides from enzymatic hydrolysis of food proteins have focused on milk proteins [21-23] and fish proteins [24-26] while a few have explored the potential of alternatives such as soybean, mushroom garlic and pea [27-30]. With the growing demand for natural health products that can help to maintain healthy blood pressure, there is a need to consider other possible sources that can tailor to the wide spectrum of consumers with different dietary restrictions. Plant seeds, especially cereals are one of the most important sources of proteins worldwide and cereal proteins have been found to be potential precursors of antihypertensive peptides [31]. Therefore, it is necessary to proceed more research on cereal grain to obtain proper functional foods with high content of bioactive compounds and ACE-inhibitory peptides with strong antioxidant activity.

Materials and Methods

Plant materials

Eight cereal grain samples were selected for the study namely white rice, viscosity rice, brown rice, black rice, sticky rice, Oatmeal, Millet and sorghum. All seed samples were collected from Republic of Korea seed market.

Chemicals and other materials

Tannic acid, Folin & Ciocalteu's phenol, 2,2'-azinobis-(3-ethylbenzothiazoline-6- sulfonic acid) (ABTS) were obtained from Sigma-Aldrich (Saint Louis, MO, USA). HHL (L-hippuryl-histidyl-leucine), HA (Hippuric acid), ACE enzyme extract and captopril were also purchased from Sigma- Aldrich (Saint Louis, MO, USA).

Preparation of sample extraction

Dried cereal grain samples were ground with a laboratory grinder to make flour and sieved with a 100-mesh sieve. One gram of sample was extracted in 10 mL 70% ethanol by shaking for three hours (h) at 180 rpm, and then centrifuged 10 min on 3000 rpm. Extracted supernatant sample was prepared after passing through syringe-filter and preserved in refrigerator.

Polyphenol content analysis

Total phenolic content (TPC) of cereal grain sample extract was determined following the reported method [32] with slight modification. Firstly, 100 µL of each sample was mixed with 100 µL of Folin-Denis reagent, after 3 minutes 1 mL of 0.7 M Sodium carbonate was added in the mixture and then incubated at room temperature for 1 h. Absorbance of sample mixture was measured at 750 nm. Total phenolic compounds in the cereal grain extracts were determined using an equation obtained from a standard curve of tannic acid (0–500 µg/mL, $Y = 0.0028x - 0.0351$, $R^2 = 0.9968$) where concentrations of tannic acid on the X-axis and their corresponding absorbance values on the Y-axis was given. The results are expressed as mg Tannic acid equivalents (TA eq) per g of extract of cereal grain sample. All determinations were carried out in triplicate.

Determination of antioxidant activity using free radical-scavenging ability by the use of ABTS radical

Antioxidant activity was determined by the ABTS (2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) free radical decolorization assay method developed by Re, et al. [33]. The ABTS positive (+) radical cation was progenerated by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and incubating for 12-16 h in the dark at room temperature until the reaction was complete and the absorbance was stable. The absorbance of the ABTS positive (+) solution was equilibrated to 0.70 (± 0.02) by diluting with water at room temperature, then 100µl was mixed with 50 µl of the test sample and the absorbance was measured at 734 nm after 6 min. All experiments were repeated three times. The radical scavenging activities of cereal grain sample were calculated by the following equation:

$$ABTS\ radical\ scavenging\ activity\,(\%) = \left(1 - \frac{sample\ absorbance}{Control\ absorbance}\right) \times 100$$

Then, curves were constructed by plotting percentage of inhibition against concentration in µg/mL. The equation of this curve allowed to calculate the IC_{50} corresponding to the sample concentration that reduced the initial ABTS absorbance of 50 %. A smaller IC_{50} value corresponds to a higher antioxidant activity. All test analyses were realized in triplicate.

In Vitro Colorimetric Assay of ACE

ACE activity was assayed following the method of Jimsheena and Lalitha [34] by monitoring the release of HA (Hippuric acid) from the hydrolysis of the substrate HHL (L-hippuryl-histidyl-leucine). The assay mixture contained 0.125 mL of 0.05 M sodium borate buffer pH 8.2 containing 0.3 M NaCl, 0.05 mL of 5 mM HHL, and 0.025 mL of ACE enzyme extract. The reaction was arrested after incubation at 37°C for 30 min by the addition of 0.2 mL of 1 M HCl. After stopping the reaction, 0.4 mL of pyridine was added followed by 0.2 mL of BSC, and the solution was mixed by inversion for 1 min and cooled on ice. After developing yellow color, absorbance was measured at 410 nm. One unit of ACE activity is defined as the amount of enzyme that releases 1 μmol of HA per min at 37°C and pH 8.2. For control group captopril was used.

Statistical Analysis

All assay data for continuous variables were conducted in triplicates. The values were expressed as the mean ± standard deviation (SD) calculated using Microsoft Excel 2010 and Sigma plot 12.5. All assay data were subjected to one-way analysis of variance (ANOVA) using PROC GLM in SAS program [35]. Mean values were compared with Duncan's Multiple Range Test at 0.05 level of Type I error.

Results and Discussion

Polyphenol content of cereal grain

In humans, polyphenols in the diet can enhance the immune defense ability of the body, reduce the incidence of chronic diseases, and have significant effects such as antiallergy, anti-arterial atherosclerosis, anti-inflammation, antioxidation, antibacterial, antithrombotic, and protecting heart and blood vessels [36]. The health benefits of polyphenols on the human body are mainly due to their oxidation resistance. Polyphenols in grains have a stronger antioxidant effect in the body through the synergistic effect of multiple bioactive compounds than the single active ingredient and can eliminate too many oxidations free radicals in the body as anti-oxidants or after the intestinal digestion. These are known to have antioxidant activity and it is likely that the activity of these extracts is due to this phenolic compound [37]. The result of phenolic content of cereal grain was presented in Table 1. The results showed that black rice contained maximum polyphenol content (94.90 mg/g extract) that was statistically different from other cereal grain *($p<0.05$).* Oatmeal and sorghum contain 58.75 and 58.82 mg/g extract of polyphenol content that was statistically similar. While sticky rice contained minimum polyphenol content (0.11 mg/g extract) that was statistically different from other cereal grains. Similar result was described by Dykes and Rooney [38] where black rice had the height level of polyphenol content and antioxidant activity whereas non pigmented cereals i.e. white rice, wheat and waxy barley had the lowest levels. Pedro, et al. [39]

reported that black rice contains 52.02 mg/g of polyphenol. Bolea, et al. [40] observed that total polyphenol content of black rice is 48.3 mg/g fresh weight. The phenolic content values of red rice varieties reported by Sompong, et al. [41] were between 0.14 and 0.34 mg GA/g, whereas the black rice varieties displayed 4-fold higher values (0.74-0.105 mg GA/g). The analysis of polyphenols from black rice assessed by Murdifin, et al. [42] presented a value of 11.97 ± 9.00 mg GAE/g. The deviation in results can be due to the difference in extraction technique, unit and assay method. Black rice has a higher content of phenolic compounds as compared to white rice and sticky rice [43]. Mira, et al. [44] found that total phenolic compound was four times higher in pigmented rice than in non- pigmented rice.

Antioxidant activity of cereal grain

The concentration of total polyphenol content in cereal grain has been positively associated with the antioxidant activity [45-47] with potential beneficial effects on health, such as reduction of oxidative stress [48,49], aid in the prevention of cancer [50,51], in the control of blood lipids and related diseases, which may help in the prevention of cardiovascular problems [48], and in the prevention of the complications of diabetes [43,52,53]. Nam, et al. [54] reported that cereal grains with red and black pericarp presented higher antioxidant activity than those with light brown pericarp color. ABTS radical is widely used to evaluate the free-radical scavenging capacity of antioxidants level according to their hydrogen donating ability [55]. In addition to that, reactions involved in these methods are fully unaffected by side reactions [56]. The antioxidant proprieties of extracts were measured in terms of their efficient IC_{50} concentration corresponding to the sample concentration that reduced the initial ABTS radical absorbance of 50%. These IC_{50} values for ABTS is given in Table 1.

Sample	Polyphenol content (mg/g extract)	Radical scavenging activity (RC_{50}) ABTs (µg/mL)
White Rice	11.29 ± 1.25^{d}	ND
Viscosity Rice	3.11 ± 1.07^{f}	ND
Brown Rice	32.89 ± 1.79^{c}	ND
Black Rice	94.90 ± 1.16^{a}	211.25
Sticky rice	0.11 ± 0.19^{g}	ND
Oatmeal	58.82 ± 0.44^{b}	282.67
Millet	4.12 ± 0.96^{e}	ND
Sorghum	58.57 ± 1.60^{b}	291.24

Each value represents the mean ± SD (n = 3). Means having different letters are significantly different (DMRT, $p < 0.05$). IC_{50}: Inhibition capacity for 50% reduction of ABTS radical. ND means not detected.

Table 1: Polyphenol content and radical scavenging activity of different cereal grain by using ABTS radical.

In this study, it was reported that all cereal grain did not show antioxidant activity except black rice, Oatmeal and sorghum. Maximum ABTS radical scavenging ability was shown in black rice that was 50% inhibition at 211.25 µg/ml concentration. In same way, oatmeal and sorghum also showed 50% radical inhibition at 282.67 and 291.24 µg/ml concentrations respectively. The antioxidant properties of black rice are more effective than red rice varieties [41]. Black rice and sorghum showed a stronger scavenging activity than other cereal grain. Black rice might be a potential material for antioxidants. Yildirim, et al. [57] reported that some potent antioxidants applied in food might cause serious side effects. Therefore, it is important to find more natural anti-oxidative ingredients for food industries. A study of Saenkod *et al.* [58] on Chinese black rice (Brown Himi variety) showed a high antioxidant activity of 70.82%. Antioxidant activity of Korean black rice is 40.39 to 55.20% obtained by Park, et al. [59]. The genetic and environmental factors, the processing conditions [60,61], as well as the extraction procedures may all affect the antioxidant levels of cereal grain sample.

In recent years, pigmented rice has been classified as one of the most consumed products among functional foods, due to the fact that it contains a high amount of phenolic compounds that have antioxidant properties [62,63]. Besides, grains with darker pericarp colour, such as red and black rice, contain higher amounts of polyphenols [64]. The concentration of total phenolics in the grain has been positively associated with the antioxidant activity [46,47], with potential beneficial effects on health.

ACE inhibition ability

Angiotensin converting enzyme (ACE) is a proteolytic enzyme that regulates blood pressure (BP) by its hydrolytic actions. ACE converts angiotensin-I (that has no direct effect on BP) to angiotensin-II, which constricts blood vessels (vasoconstrictor) and thereby elevates BP. Since ACE is an enzyme, it can be inactivated by blocking its active site with selective enzyme inhibitors. Synthetic ACE-inhibitors are widely used in the pharmacological treatment of hypertension. Certain peptides with suitable structures are also able to inhibit the activity of ACE by binding to its active site. In this experiment maximum ACE inhabitation was observed in Black rice (66.80%) followed by oatmeal (48.62%) that was statistically different from other cereal grain. Minimum ACE inhibition ability was observed in millet (8.50%).

Sample	ACE inhibition Rate (%) (Conc. 1,000 µg/mL)
White Rice	32.31±1.15[d]
Viscosity Rice	26.16±2.82[e]
Brown Rice	32.30±1.63[d]
Black Rice	66.80±2.45[a]
Sticky rice	25.00±2.36[e]
Oatmeal	48.62±1.97[b]
Millet	8.50±2.04[f]
Sorghum	37.45±2.71[c]

Each value represents the mean ± SD (n = 3). Means having different letters are significantly different (DMRT, $p < 0.05$).

Table 2: Angiotensin converting enzyme (ACE) inhabitation ability of different cereal grain.

In a literature data base survey, twenty-two potential ACE-inhibitory peptides in cereal proteins have been reported [65]. In a recent study, *in silico* digestion of oat and barely by thermoslysin resulted in generating 6 and 3 potent peptides from their parent proteins, respectively [66]. Furthermore, several cereal food-derived peptides have been shown to have *in vitro* ACE inhibitory activity that included corn, wheat, oat and rice [67-72].

Conclusion

Black rice contained maximum polyphenol content (94.90 mg Tannic acid equivalent/g of extract) that are responsible for maximum antioxidant activity as well as maximum ACE inhibition ability in black rice. Maximum angiotensin converting enzyme (ACE) inhibitory activity was also shown in black rice 66.80% following by oatmeal (48.62%) and sorghum (37.45%). It can be concluded that polyphenol content was closely related with antioxidant activity and angiotensin-converting enzyme (ACE) inhibitory activity. Black rice, oatmeal and sorghum can be used as functional ingredient with their high phenolic content, antioxidant activity as well as angiotensin-converting enzyme (ACE) inhibitory activity.

Author Contribution

Soyema Khatun: Methodology, Investigation, Experiment conduction, writing original draft, Visualization.
Hyunhhwa Lee : Help to analysis ACE by using colorimetric method.
Md. Mahi Imam Mollah: Data analysis, Review of Manuscript

Conflict of Interest

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

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