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ESTIMATION OF THE DPPH ANTIOXIDANT AND POLYPHENOL CONTENTS FROM CHEONGGUKJANG

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ABSTRACT

Cheonggukjang is a traditional fermented soybean food product manufactured using steamed soybeans. This study is planned to determine the antioxidant activity of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and total phenol content of Cheonggukjang. The inhibition percentage values of aqueous, ethanol, and methanol extracts at 1.0 mg/ml were 31.17%, 29.2%, and 27.4%, respectively. However, the all groups did not show a statistically significant difference (t = 0.108, df=2, p > 0.05. The values of IC_{50} were following: methanol extract (102.96 mg/mL) > ethanol extract (98.02 mg/mL) > aqueous extract (97.01 mg/mL). Total phenols as determined by the Folin-Ciocalteu method varied from 1.6 mg/L to 3.57 mg/L. There was no significant difference in total phenol content among extract solvents (p > 0.05). It is obvious that the fermentation of soybean broth could result in better antioxidant properties.

KEYWORDS: Antioxidant, Cheonggukjang, DPPH, total phenol content.

INTRODUCTION

Soybean is famous as a novel food with high contents of protein, amino acids, minerals, vitamins, free sugars, fatty acids, total polyphenols, and isoflavones.[1] Soybean can also serve as a substitute for meat protein.[2]

Cheonggukjang is a traditional fermented soybean food product manufactured using steamed soybeans and natural starter microorganisms originating from jip(dried rice straws).[3] It can be made in 2 to 3 days through fermentation of boiled soybeans, adding *Bacillus subtilis*, which is usually contained in the air or in the rice straw, at about 40°C without adding salt.

The strongly fermented paste contains many valuable and beneticial bacteria for the body. In addition, the paste has plenty of vegetable protin and little sola. Cheonggukjang is generally considered to be a healthy food (particularly in the winter), as it is rich in vitamins and other nutrients, though its very strong odor is not universally enjoyed. The most characteristic volatile flavor compounds in Cheonggukjang are thought to be pyrazine compounds.[4] Some people have commented that this soup gives off the aroma of wet socks.

The traditionally fermented soy paste has been reported to have several health benefits including antioxidant, antimicrobial, blood pressure lowering and antidiabetic activities, compared to the unprocessed soybeans and cooked soybeans.[5]

Numerous methods have been used to evaluate andtioxidant activities of natural compounds in foods or biological systems with varying results.

Among many antioxidants, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), and 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were commonly used to assess antioxidant activity in vitro. The DPPH method is widely used to determine antiradical or antioxidant activity of purified phenololic compounds as well as natural plant extracts.[6] DPPH is a stable free radical with an absorption band at 517 nm. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution.

Phenolic compounds such as flavonoids, phenolic acid and tannins are considered to be a major contributor to the antioxidant activity in plants. These compounds are among the most widely occurring secondary metabolites in the plant kingdom, acting mainly as phytoalexins, attractants for pollinators, contributors to plant pigmentation, antioxidants, and protective agents against UV light, among others.[7] These antioxidants also possess diverse biological activities such as anti-inflammatory, anti-carcinogenic and antiatherosclerotic effects. These activities may be related to their antioxidant activity.[8] Many studies have discussed the use of the Folin-Ciocalteau reagent to determine polyphenols, and the general or specific value of the

method, because some specific details may be modified.[9-10]

Cho and Joo[11] investigated total phenolic and isoflavone contents and antioxidant activities during Cheonggukjang fermentation with bitter melon powder (BMP). Generally, a fermented soybean broth is thought to be a healthy drink. However, the beneficial effects of this fermented soybean product are unknown, especially its antioxidant properties and scavenging effects on reactive oxygen.[12]

The purpose of the present study is to evaluate Cheonggukjang as sources of antioxidants for DPPH and total phenol content to examine whether they are significant antioxidant activity among three extractive solvents.

MATERIALS AND METHODS

Sample extract

Cheonggukjang samples used in the study were purchased on Korean tea markets in April, 2021. Chenoggukjang sample was mixed in a ratio of ingredients as soybean 70%, water 28%, and salt 2%. The material (100 g) were smeared on each selective solvent (distilled water, 80% ethanol and 80% methanol) to grind materials using a Retsch GM 200 Mill (Fisher Bioblock, France). Then, ground plant materials with each selective solvent at 50°C under agitation. The ultrasound extraction was carried out using an ultrasonic bath (5510, Branson, USA). The mixture was shaken vigorously for one hour at room temperature and left in the dark at room temperature for 20 min. The extracts were filtered with a 0.45-µm Millipore PVDF filter (Schleicher & Schuell, GmbH, Dassel, Germany). After filtration, the water was removed in a rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan) at 70°C. The extracts were centrifuged 5 minutes at 7,500 rpm. To get dry powder, samples placed in a low temperature (-300°C) vacuum chamber. These powders were then used to determine antioxidant activities. All analyses were realized as much as possible in an area protected against light.

DPPH free radical

The antioxidant activity of the Cheonggukjang extracts was measured based on the scavenging activity of DPPH free radical according to the method described by Brand-Williams et al.[13] with slight modifications. This assay mixture contained 2 mL of 1.o mmol/L DPPH radical solution prepared in methanol and 1 mL of standard or extract solution of different concentrations (0.25, 0.5, and 1.0 mg/ml). The solution was rapidly mixed and incubated at 37ºC for 30 min. The 96-well microplate was shaken to ensure thorough mixing before being wrapped with aluminum foil and placed into the dark. The optical density (OD) of the solution was read using the Microplate Reader (VersaMax, Califonia, USA) at the wavelength 517 nm. Corresponding blank sample was prepared and L-Ascorbic acid (0.1, 0.5, and 1.0 mg/ml) was used as reference standard (positive control). Inhibition of free radical scavenging activity was calculated using the following equation.

The percentage of radical scavenging (%) was calculated by the following formula.

Free radical scavenging activity (%) = (Ac-As)/Ac×100

Where Ac is the absorbance of control at 517 nm and As is the absorbance of the sample. Ac and As were the values which were subtracted the average absorbance of the blank wells.

IC_{50} is defined as the concentration of inhibitor necessary for 50% inhibition of the enzyme reaction of a maximum scavenging capacity. To determine the IC_{50} value of the active component, the technique using 96-well microplates was employed. Regression analysis by a dose response curve was plotted to determine the IC_{50} values.

Total phenolic content

The concentration of phenolics in Cheonggukjang extracts was determined using spectrophotometric method.[14]

The total phenolic content of the plant extracts and the standard antioxidant materials was determined according to the Folin–Ciocalteu method.[15] 0.2 mL Folin–Ciocalteu reagent was added to 0.2 mL of the extract and butylated hydoxytoluene (BHT) solutions. After 5 min, 1 mL of 15% Na_2CO_3 was added and the mixture wasstored at room temperature for 2 h. The absorbance of the mixture was measured at 760 nm against water on a UV spectrophotometer. The concentration of the total phenolics was calculated as mg of gallic acid equivalent (GAE mg/g) by using an equation obtained from gallic acid calibration curve. The determination of total phenolic compounds in the fractions was carried out in triplicate and the results wereaveraged.

Statistical analysis

All experimental measurements were carried out in triplicate and were expressed as average of three analysis ± standard deviation. The magnitude of correlation between variables was done using Microsoft Excel and statistical software package (SPSS 21.0 for Windows, Chicago, IL, USA). A one-way and a two-way analysis of variance (ANOVA) followed by the Tukey post hoc test were used to analyze statistical significance ($p < 0.05$). Significance and confidence level were estimated at $p < 0.05$.

RESULTS AND DISCUSSION

The DPPH radical scavenging activity of the aqueous extract for Cheonggukjang at 0.25 mg/ml 11.3% and that of the ethanol extract was 9.5% at same concentration (Table 1). The methanol extract was 10.9% at same concentration. It was observed that inhibition percentage values go on increasing with enhancements in concentration of research Cheonggukjang extracts in the

assay mixture. The inhibition percentage values of aqueous, ethanol, and methanol extracts at 1.0 mg/ml were 31.17%, 29.2%, and 27.4%, respectively. The results that aqueous extract exhibited stronger DPPH radical scavenging ability than the ethanol and methanol extracts of Cheonggukjang. However, the all groups did not show a statistically significant difference (t = 0.108, df = 2, p > 0.05. The efficacies of as antioxidants were evaluated as the IC_{50} parameter. The values of IC_{50} were following: methanol extract (102.96 mg/mL) > ethanol extract (98.02 mg/mL) > aqueous extract (97.01 mg/mL). Figure 1 was shown the rate of DPPH inhibitory of L-ascorbic acid (positive control) and relative inhibitory rate for Cheonggukjang on 1.0 mg/ml. The values for aqueous, ethanol, and methanol extracts were 47.8%, 44.8%, and 42.0%, respectively.

Phenolic compounds are important antioxidant ability which is responsible for deactivating free radical based on their ability to donate hydrogen atoms to free radicals. Table 3 showed the total phenol contents (mg/L) of Cheonggukjang. Total phenols as determined by the Folin-Ciocalteu method varied from 1.6 mg/L to 3.57 mg/L. It was similar to previous results (Ali et al 2018). Cheonggukjang with 3 and 5% *Bacillus subtilis* had total phenol content of 4.56 ± 0.3 and 3.39 ± 0.15 mg/g, respectively.[1] Figure 2 was shown the rate of total phenol content of gallic acid (positive control) and relative inhibitory rate for Cheonggukjang on 1.0 mg/ml. The values for aqueous, ethanol, and methanol extracts were 76.8%, 81.8%, and 84.4%, respectively.

In raw soybeans, isoflavones are present in four chemical forms: malonylglycosides (70-80%), acetylglycosides (5%), glycosides (25%), and aglycones (2%).[16-17] It was found that fermentation enhanced the total phenolic and isoflavone-aglycone and -malonylglycoside contents, corresponding to antioxidant activities increased.[18] It is obvious that the fermentation of soybean broth could result in better antioxidant properties.[12] In addition, fermented soybean broth was superior to soybean broth in most antioxidant properties. Kim and Yun[19] reported a significant increase in the total polyphenol extract content.

Fermented soybean extract not only has antioxidant activity but also has an effect on the activity of antioxidant enzymes in liver.[20]

The fermentation of Cheonggukjang for 5 h resulted in a significant increase in the antioxidant capacity as evaluated by radical scavenging assay (DPPH), total phenolic contents and flavonoid contents.[5] Germinated (12-h) soybeancheonggukjang produced the highest amount of viscous substance (13.22%) after 48 h of fermentation, and the contents were inversely proportional to the germination time of the soybeans.[21] Thus, the results of this study may be that the antioxidant capacity is not high using non- germinated soybeans.

Table 1: The degree of inhibition (%) of DPPH by Cheonggukjang at different concentrations.

Concentration (mg/ml)	Solvent			*t*-test
	Distilled water	Ethanol	Methanol	
0.25	11.53±3.48	9.49±2.16	10.93±1.21	0.108
0.50	22.84±3.90	16.14±2.17	15.53±1.88	
0.75	27.28±3.47	24.42±3.18	22.92±2.36	
1.00	31.14±4.34	29.15±2.04	27.36±1.62	
t-test	0.298	0.105	0.081	

Data represented the mean ± SD from three replicates.

Table 2: The 50% inhibition (IC_{50}) of DPPH by Cheonggukjang.

Solvent		
Distilled water	Ethanol	Methanol
97.01	98.02	102.96

Table 3: Total phenolic content (as gallic acid equivalent) of Cheonggukjang extracts.

Solvents	Phenolics (mg/L)
Water extract	3.57±0.23
Ethanol extract	1.64±0.16
Methanol extract	2.02±0.06

Data represented the mean ± SD from three replicates.

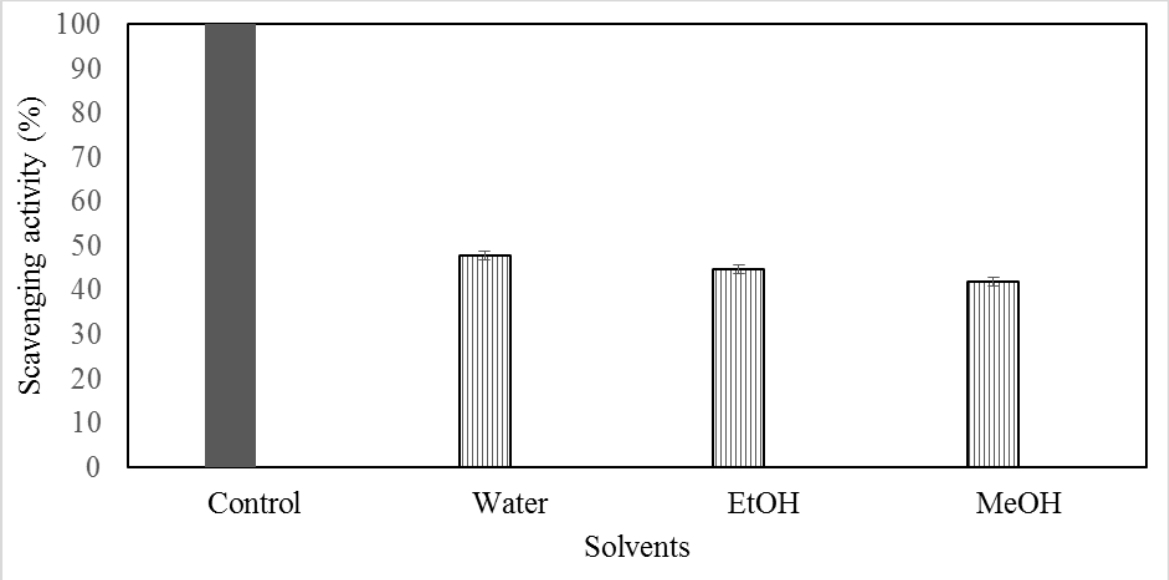

Figure 1: The rate of DPPH inhibitory of L-ascorbic acid (positive control) and relative inhibitory rate for Cheonggukjang on 1.0 mg/ml.

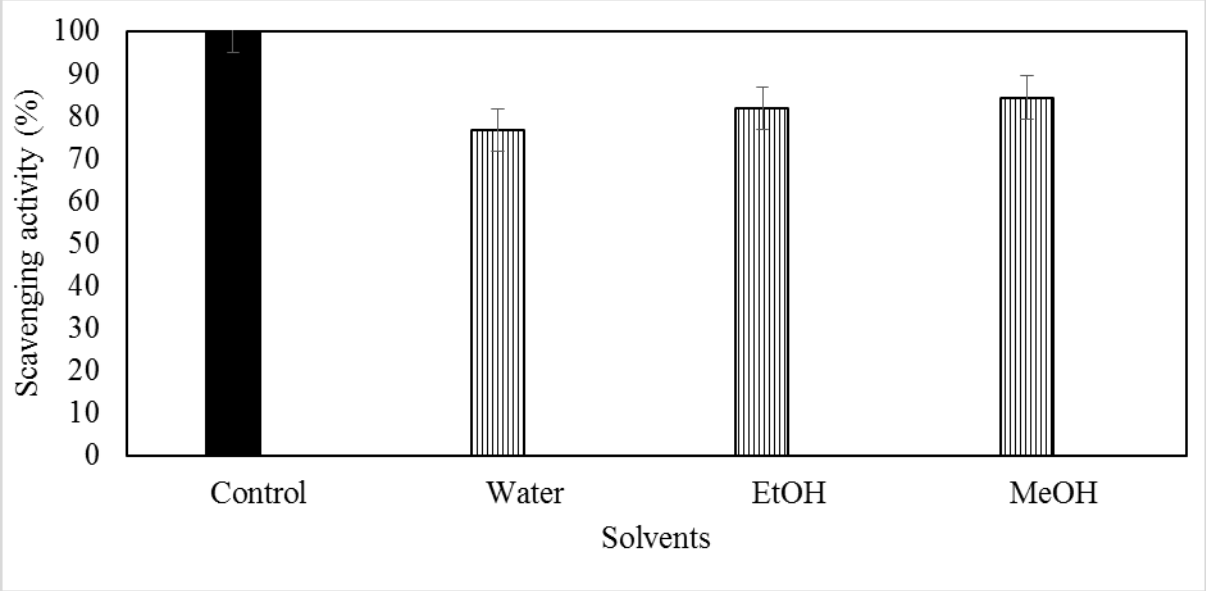

Figure 2: The rate of polyphenol contents of gallic acid (positive control) and relative values for Cheonggukjang on 1.0 mg/ml.

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