Food Sci. Biotechnol. Vol. 18, No. 5, pp. 1258 ~ 1262 (2009)

Food Science and Biotechnology

In vitro Antioxidant Properties and Phenolic Composition of Korean Commercial Vinegars

Chang-Ho Jeong, Gwi Nam Choi, Ji Hye Kim, Ji Hyun Kwak, Su-Tae Kang[1], Sung-Gil Choi, and Ho Jin Heo*

Division of Agriculture and Life Sciences, and Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, Gyeongnam 660-701, Korea

[1]*Department of Food Science and Biotechnology, Pukyong National University, Busan 608-737, Korea*

Abstract Total phenolics and antioxidant properties of various Korean commercial vinegars (apple vinegar, AV; blueberry vinegar, BV; grape vinegar, GV; lemon vinegar, LV; *Opuntia ficus* vinegar, OFV; persimmon vinegar, PV; *Prunus mume* vinegar, PMV; rice vinegar, RV) were investigated. The total phenolic contents of 8 vinegars were within the range of 54.18-491.02 μg/mL. The vinegars were also capable of scavenging 1,1-dipehnyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radicals in a manner dependent on concentration. The greatest reducing power was observed in PV relative to the other vinegars. The ferric reducing ability of plasma (FRAP) of PV, PMV, GV, and BV were 1.012, 0.969, 0.931, and 0.856 at a dose of 1 mL, respectively. Therefore, our study verified that the GV, PV, and PMV have powerful antioxidant activities which are correlated with its high level of phenolics, particularly gallic acid, and epigallocatechin.

Keywords: vinegar, total phenolic, antioxidant activity, gallic acid, epigallocatechin

Introduction

Polyphenols are found in food (vegetables, fruits, chocolate, tea, coffee, wine, grape juice, and vinegar) at different concentrations (1). Polyphenols are bioactive compounds believed to be involved in the defense process against deleterious oxidative damage, at least in part, due to their antioxidant properties (2). Phenolic acids have been widely investigated as potential models for the development of new primary antioxidants, which can prevent and delay *in vitro* or *in vivo* oxidation processes (3). These phenolic compounds are powerful antioxidants and act in a structure-dependent manner, since they can scavenge reactive oxygen species (ROS), and chelate transition metals which play a vital role in the initiation of deleterious free radical reactions (2). Because purified phenolic compounds are difficult to obtain and because extract sometimes has better antioxidant activities than those of pure molecules, there is a growing interest for the use of plant extracts (4). Natural antioxidant phenolic acids, and their derivatives, either present in the diet or synthetically prepared, were shown to have promising chemopreventive properties being identified as promising agent for future development (5). In addition, the consumption of this material has increased recently with release of findings that Korean fermented foods such as *kochujang*, *doenjang*, *cheonggukjang*, and *kimchi* may have some beneficial effects to health like anti-obesity, antimutagenic, anticancer, fibrinolytic, immunostimulating, and antimicrobial activities (6-9).

To find new natural food sources of active compounds, we studied the antioxidant potential of commercial vinegar.

*Corresponding author: Tel: +82-55-751-5476; Fax: +82-55-753-4630
E-mail: hjher@gnu.ac.kr
Received May 24, 2009; Revised July 14, 2009;
Accepted July 21, 2009

Vinegar, which can be made from rice, apples, wine, and various other materials, is a widely used acidic seasoning (10). Vinegar also has medicinal uses by virtue of its physiological effects, such as aiding digestion, stimulating the appetite, and promoting recovery from exhaustion (11). Vinegar has been used for various foods for preservation and often used for flavoring food and for pickling. Moreover, diluted unpolished rice vinegar has been drunken as a health food in Japan and its antioxidant activity has been reported (12,13). Vinegar has been reported to have antibacterial effects (14,15), to have prophylactic effects on hypertension (16), to accelerate glycogen repletion and calcium absorption (17,18), to decrease serum cholesterol (19), and to have antioxidant effects (20). Although it has already been demonstrated that Japanese unpolished rice vinegar (*kurosu*) contains phenolic compounds (13), little is known about their antioxidant potential and active compounds of commercial vinegars. Sakanaka and Ishihara (21) recently reported that markedly high phenolic contents and radical scavenging activities were found for vinegar made from persimmon (*saijo* varieties). However, the characterization of antioxidant compounds in the persimmon vinegar was not discovered. Therefore, establishing the connection between antioxidant activity and chemical composition is very important. In addition, the antioxidant activities and active compounds of Korean commercial vinegars have not previously been reported. The objectives of this study were to investigate the antioxidant activities and active compounds of Korean commercial vinegars.

Materials and Methods

Chemicals Eight kinds of vinegars (AV, BV, GV, LV, OFV, PV, PMV, and RV) were obtained from local market in Jinju, Korea. Acetic acid contents of these vinegars were 6.01, 2.36, 5.19, 7.15, 2.19, 2.74, 2.70, and 6.06%, and

solid contents of them were 6.01, 35.1, 5.57, 5.12, 36.84, 6.51, 7.02, and 4.50%, respectively. Folin-Ciocalteu's reagent, 1,1-dipehnyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), potassium persulfate, potassium ferricyanide, trichloroacetic acid, ferric chloride, 2,4,6-tripyridyl-S-triazine (TPTZ), and all solvents used were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

Determination of total phenolics Total phenolics were determined by the spectrophotometric analysis (22). In brief, a 1 mL portion of appropriately diluted vinegars was added to a 25-mL volumetric flask containing 9 mL of deionized distilled water. Reagent blank using deionized distilled water was prepared. One mL of Folin-Ciocalteu's phenol reagent was added to the mixture and then shaken. After 5 min, 10 mL of a 7% Na_2CO_3 solution was added with mixing. The mixed solution was then immediately diluted to volume (25 mL) deionized distilled water and mixed thoroughly. After 90 min at 23°C, the absorbance was read at 750 nm (UV-1201; Shimadzu, Tokyo, Japan). The standard curve for total phenolics was made using gallic acid standard solution (0-100 mg/L) under the same procedure as above. Total phenolics in vinegars were expressed as mg of gallic acid equivalents (GAE)/1 mL of sample.

DPPH free radical scavenging activity This was carried according to Blois method with a slight modification (23). Briefly, a 1 mM solution of DPPH radical solution in ethanol was prepared, and then 4 mL of this solution was mixed with 1 mL of vinegars; finally, after 30 min, the absorbance was measured at 517 nm (UV-1201; Shimadzu Co.). This activity is given as percent DPPH scavenging that is calculated as;

$$\% \text{ DPPH scavenging} = [(\text{control absorbance} - \text{sample absorbance})/(\text{control absorbance})] \times 100$$

ABTS radical scavenging activity ABTS was dissolved in water to make a concentration of 7 mmol/L. ABTS was produced by reacting the ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 hr before use. For the study of samples, the ABTS stock solution was diluted with phosphate buffered saline (PBS) 5 mmol/L, pH 7.4 to an absorbance of 0.70 at 734 nm. After the addition of 980 μL of diluted ABTS to 20 μL of sample, the absorbance reading was taken 5 min after the initial mixing (24). This activity is given as percent ABTS scavenging that is calculated as;

$$\% \text{ ABTS scavenging activity} = [(\text{control absorbance} - \text{sample absorbance})/(\text{control absorbance})] \times 100$$

Reducing power This was carried by Oyaizu (25). Briefly, 0.1 mL of vinegars were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%), and then the mixture was incubated at 50°C for 30 min. Afterward, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 2,090×g for 10 min. Finally, 2.5 mL of the upper layer solution was mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm (UV-1201; Shimadzu Co.).

Ferric reducing ability of plasma (FRAP) assay The FRAP assay developed by Benzie and Strain (26). In short, 1.5 mL of working, pre-warmed 37°C FRAP reagent (10 volumes 300 mmol/L acetate buffer, pH 3.6+1 vol. of 10 mmol/L 2,4,6-tripyridyl-*S*-triazine in 40 mmol/L HCl+1 vol. of 20 mmol/L $FeCl_3$) was mixed with 50 μL of test sample and standards. This was vortex mixed and absorbance at 593 nm was read against a reagent blank at a predetermined time after sample-reagent mixing. The test was performed at 37°C and the 0-4 min reaction time window was used.

Determination of phenolic composition Phenolic compounds were measured at 280 nm using an Agilent HPLC 1100 series (Agilent Technologies Inc., Santa Clara, CA, USA). Separation was achieved with a LiChrospher RP-18 column (250×4 mm i.d., 5 μm, E. Merck Co., Darmstadt, Germany). The mobile phase consisted of acetonitrile:acetic acid:methanol:water (113:5:20:862, v/v/v/v). The flow rate was 1.0 mL/min and the injection volume was 20 μL. Compounds were detected by monitoring the elution at 280 nm. Identification of the phenolic compounds was carried out by comparing their retention times and absorption spectra to those of standards. Content of phenolic compounds was expressed in mg/100 mL extract.

Statistical analysis Data were analyzed statistically by analysis of variance (ANOVA) and Duncan's multiple range tests using SPSS software program (SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered significant.

Results and Discussion

Total phenolics in vinegars The Folin-Ciocalteu's assay is fast and simple methods rapidly determine a content of phenolics in vinegars. Phenolics or polyphenols are secondary plant metabolites that are present in every plants and plant products. Many of the phenolics have been shown to contain high levels of antioxidant activities. The total phenolic contents of 8 kinds of vinegars were determined (Fig. 1). The total phenolic content of various vinegars was within the range of 54.18-491.02 μg/mL. Of the vinegars, the total phenolic content of the PV was highest at 491.02±3.69 μg of gallic acid equivalents/mL, followed by 471.28±1.27 μg/mL for GV, and 452.72±1.95 μg/mL for PMV. Total phenolic contents of PV, GV, and PMV were significant higher ($p<0.05$) than other vinegars.

Scavenging effect on DPPH radical The DPPH radical scavenging activities of the vinegars were estimated. It was found that the radical scavenging activities of vinegars increased with increasing concentration (Fig. 2). The abilities to scavenge DPPH radicals of vinegars were in the order of PV (93.16%)>GV (90.91%)>PMV (87.86%) at 1 fold dilution of vinegars, respectively. An almost linear correlation between DPPH radical scavenging activity and concentrations

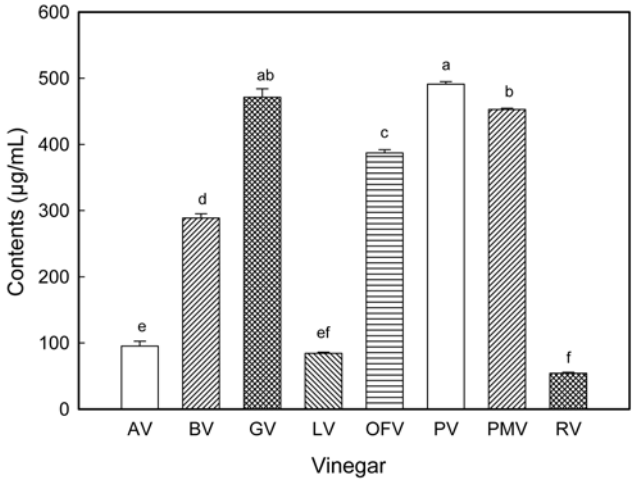

Fig. 1. Total phenolics of vinegars. AV, apple vinegar; BV, blueberry vinegar; GV, grape vinegar; LV, lemon vinegar; OFV, *Opuntia ficus* vinegar; PV, persimmon vinegar; PMV, *Prunus mume* vinegar; RV, rice vinegar. Values indicate the mean's 3 replications. A p value <0.05 was considered significant.

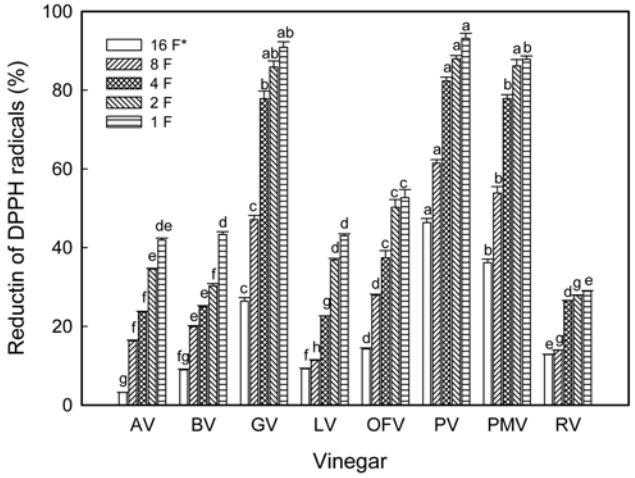

Fig. 2. DPPH radical scavenging activities of vinegars. AV, apple vinegar; BV, blueberry vinegar; GV, grape vinegar; LV, lemon vinegar; OFV, *Opuntia ficus* vinegar; PV, persimmon vinegar; PMV, *Prunus mume* vinegar; RV, rice vinegar. *Dilution folds of vinegars. Values indicate the mean's 3 replications. A p value <0.05 was considered significant.

of polyphenolic compounds in various vegetable and fruits have been reported (27,28). This indicated that DPPH radical scavenging activities of vinegars were related to the amount of antioxidant components from vinegars. These results revealed that GV, PV, and PMV were free radical scavengers, acting possibly as primary antioxidants.

Scavenging effect on ABTS radical The vinegars exhibited ABTS radical scavenging activities to different extents in a concentration-dependent manner. The GV and PV exhibited the highest radical scavenging activities when they reacted with the ABTS radicals (Fig. 3). In contrast, the AV and LV only showed low activities. Figure 3 demonstrated a steady increase in the percentage inhibition of the ABTS radicals by the BV, GV, OFV, PV, and PMV

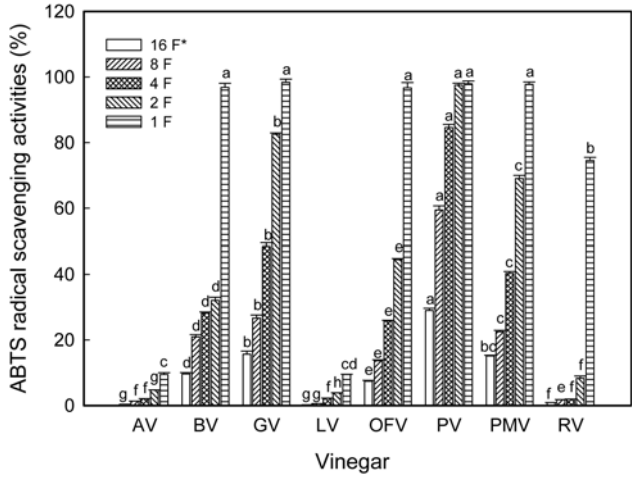

Fig. 3. ABTS radical scavenging activities of vinegars. AV, apple vinegar; BV, blueberry vinegar; GV, grape vinegar; LV, lemon vinegar; OFV, *Opuntia ficus* vinegar; PV, persimmon vinegar; PMV, *Prunus mume* vinegar; RV, rice vinegar. *Dilution folds of vinegars. Values indicate the mean's 3 replications. A p value <0.05 was considered significant.

and maximum inhibition was achieved above 1 fold dilution of vinegars. As seen in Fig. 3, the scavenging effect on ABTS radical by 1 fold dilution concentration of BV, GV, OFV, PV, and PMV were found as 96.81, 98.37, 96.67, 97.93, and 97.78%, respectively. Sakanaka and Ishihara (21) reported that tested vinegars showed hydroxyl radical scavenging activity (32.7-67.1%) and persimmon vinegar made from persimmon *saijo* showed the highest scavenging activity.

Reducing power In the reducing power assay, the presence of reductants (antioxidants) in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Figure 4 shows the dose-response curves for the reducing powers of the vinegars. It was found that the reducing powers of all the vinegars also increased with the increase of their concentrations. The AV, BV, GV, LV, OFV, PV, PMV, and RV, exhibited a good reducing power of 0.31, 1.69, 3.73, 0.39, 2.68, 3.92, 3.57, and 0.16 at 1 fold dilution of vinegars, respectively. Chen *et al.* (29) reported that gallic acid is the major phenolic, out of these 6 kinds of phenolics, which contributes to the antioxidant activity of the *Mopan* persimmon. From these observations, it is suggested that PV has a remarkable potency to react with free radicals to convert them into more stable non-reactive species and to terminate radical chain reaction.

FRAP assay In this assay, samples are used in a redox-linked reaction whereby the antioxidants present in the sample act as the oxidants. Reduction of the ferric-tripyridyltriazine to the ferrous complex forms an intense blue color which can be measured at a wavelength of 593 nm. The intensity of the color is related to the amount of antioxidant reductants in the samples. In the present study, the trend for ferric ion reducing activities of vinegars was

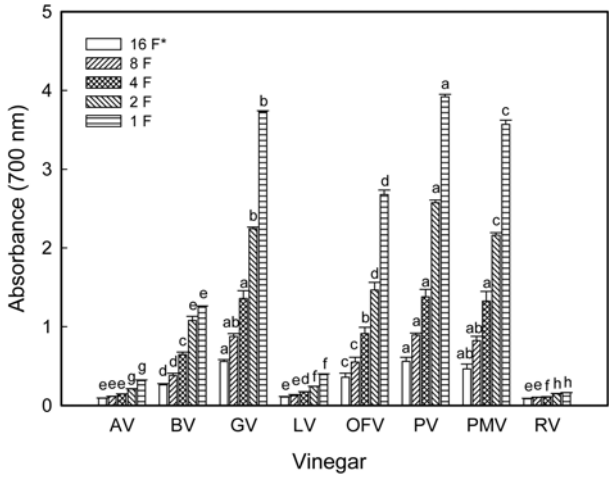

Fig. 4. Reducing power of vinegars. AV, apple vinegar; BV, blueberry vinegar; GV, grape vinegar; LV, lemon vinegar; OFV, *Opuntia ficus* vinegar; PV, persimmon vinegar; PMV, *Prunus mume* vinegar; RV, rice vinegar. *Dilution folds of vinegars. Values indicate the mean's 3 replications. A p value <0.05 was considered significant.

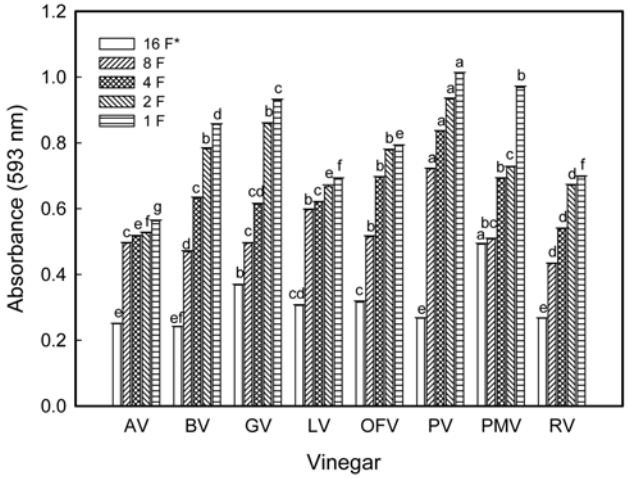

Fig. 5. Ferric reducing ability of plasma of vinegars. AV, apple vinegar; BV, blueberry vinegar; GV, grape vinegar; LV, lemon vinegar; OFV, *Opuntia ficus* vinegar; PV, persimmon vinegar; PMV, *Prunus mume* vinegar; RV, rice vinegar. *Dilution folds of vinegars. Values indicate the mean's 3 replications. A p value <0.05 was considered significant.

shown in Fig. 5. For vinegars, the absorbance clearly increased, due to the formation of the Fe^{2+}-TPTZ complex with increasing concentration. The highest reducing activity was for PV, compared to those of the other vinegars (Fig. 5). Similar to the results obtained from the DPPH and ABTS assay, vinegars showed relatively strong ferric ion-reducing activity. AV and LV showed lower ferric ion-reducing activities. A strong correlation between the mean values of the total polyphenol content and FRAP deserves detailed attention, as it implies that phenolics in vinegars, were capable of reducing ferric ions. Some authors have reported similar correlations between polyphenols and antioxidant activity measured by various methods (30,31).

Analysis of phenolics by high performance chromatography (HPLC) Since the GV, PV, and PMV exhibited the strong antioxidant activity, it was subjected to further analysis by HPLC. GV, PV, and PMV contained a variety of phenolic compounds. By comparing the retention time and absorbance spectra of these compounds with those of standards, 6 phenolic compounds were identified (Table 1). Furthermore, the HPLC results indicated that epigallocatechin (10.75 mg/100 mL) was the predominant compounds in GV, followed by epicatechin (0.85 mg/100 mL). The highest content in PV is gallic acid (14.24 mg/100 mL), which is higher than the gallic acid and epigallocatechin content in GV. The epigallocatechin was shown to have the highest content among all 6 kinds of phenolics determined in PMV.

Flavonoids and phenolic acids are important contributing factors to the antioxidant activity of the human diet. Based on the results for the phenolic composition of GV, PV, and PMV, we can conclude that these compounds including particularly gallic acid and epigallocatechin may contribute to the antioxidant activities of GV, PV, and PMV. The activity of GV, PV, and PMV is attributed to these phenolic compounds. From the results obtained above, the most effective antioxidant activity of the PV is considered to be mainly base on the additive effect of gallic acid and epigallocatechin. Heo *et al.* (32) suggested that total antioxidant capacities measured, of the phenolic mixture at known concentrations, were equal to the summation of antioxidant capacities of individual phenolics.

In conclusion, all of the tested vinegars were found to be effective antioxidants in different *in vitro* assay systems. The GV, PV, and PMV were effective on antioxidant activities in comparison with other tested vinegars. The results obtained in this work are noteworthy, not only with

Table 1. Phenolic compounds of grape vinegar (GV), persimmon vinegar (PV), and *Prunus mume* vinegar (PMV)

Compounds	Content (mg/100 mL)		
	GV	PV	PMV
Gallic acid	0.74±0.019$^{b1)}$	14.24± .930^{a}	0.03±0.002^{c}
Epigallocatechin	10.75±0.865^{ab}	11.98±0.962^{a}	6.04±0.096^{b}
Catechin	0.78±0.044^{b}	4.42±0.078^{a}	0.27±0.018^{c}
Epicatechin	0.82±0.021^{b}	2.23±0.024^{a}	0.29±0.009^{c}
Chlorogenic acid	0.20±0.031^{b}	1.01±0.037^{a}	0.21±0.006^{b}
Epicatechin gallate	0.02±0.002^{a}	0.02±0.001^{a}	0.03±0.001^{a}

[1)]Values indicate the mean's 3 replications; A p value <0.05 was considered significant.

respect to the antioxidant activities of GV, PV, and PMV but also with respect to its content of a variety of phenolic compounds. The activity of this GV, PV, and PMV is attributed to these phenolic compounds and in particular to gallic acid and epigallocatechin. Since gallic acid and epigallocatechin are the major phenolic compound detected in GV, PV, and PMV the safety of gallic acid for using as antioxidant agent is very important. Many of the phenolics have been shown to contain high levels of antioxidant activities. GV, PV, and PMV may be as useful in food processing as are other naturally occurring antioxidants, helping to prevent the formation of off-flavor in food and their products and to increase shelf life.

Acknowledgments

This work was supported by the Korean Research Foundation Grant funded by the Korean Government (KRF-2008-521-F00074).

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