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Assessment and comparison of the antioxidant activities and nitrite scavenging activity of commonly consumed beverages in Korea

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ABSTRACT

In this study, the antioxidant potential of commercial beverages against a variety of radicals was determined using various antioxidant activity analytical methods. The physicochemical properties (pH value and °Brix), total phenolic content and antioxidant activities were assessed. Our results showed that the pH value and sugar content (°Brix) of commonly consumed beverages ranged from 2.4 to 3.9 and from 3.8 to 18.5, respectively. The DPPH radical scavenging activity and nitrite scavenging ability were highest in No. 45 vitamin beverage (87.5% and 86.0%, respectively). However, no clear correlation appeared between the total phenolic content and the DPPH radical scavenging activity ($R = 0.2565$). The total phenolic content and oxygen radical absorbance capacity (ORAC) values were highest in No. 55 pomegranate beverage (183.3 mg GAE/100 mL and 1824.4 μM TE/mL, respectively). In particular, a high correlation was shown between the total phenolic content and the ORAC value ($R = 0.7954$). Based on the results of various antioxidant activity methods, the greatest preventative antioxidant capacity of consumed beverages in Korea was found in No. 55 pomegranate. These results will enable further research on the daily phenolic compound intake as well as on the development of healthy beverages.

1. Introduction

Excessive reactive oxygen species (ROS) produced by the biological combustion process within the body can damage proteins, lipids and nucleic acids (Apel & Hirt, 2004; Finkel & Holbrook, 2000). Considerable evidence has shown oxidative stress to be a major cause of chronic and degenerative diseases, such as cancer (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006), heart disease, diabetes (Maritim, Sanders, & Watkins, 2003), cardiovascular disease and Alzheimer's disease. In previous studies, various antioxidant compounds (e.g., phenolic compounds, anthocyanins, carotenoids and vitamin C) in fruits (Fan et al., 2012) and vegetables protect the cells and organ systems of the body against ROS damage (Paganga, Miller, & Rice-Evans, 1999; Scalzo, Politi, Pellegrini, Mezzetti, & Battino, 2005).

Modern society has developed a heightened attention to healthy living. Thus, consumption of fruit and vegetable-related products, including healthy beverages, is increasing (Wu et al., 2004). For instance, citrus and berry fruits are well-known natural antioxidant bioresources (Kähkönen, Hopia, & Heinonen, 2001; Nagy, 1980), and they have been used extensively in many antioxidant food commodities (e.g., juice, jam, chocolate, etc.). However, many investigators have made no attempt to study the functionality of various beverages.

Several chemical assays including the total phenolic content (TPC), oxygen radical absorbance capacity (ORAC) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) level have been used to evaluate the antioxidant capacity of various commercial commodities and foodstuffs. Because the phenolic compounds act as antioxidants dur to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators (Kaur & Kapoor, 2002), the determination of TPC is very important for evaluating antioxidant activities. The ORAC assay evaluation is based on the reduction of a fluorescence probe by peroxyl radicals through the inhibition of autoxidation reactions (Yamashita et al., 1998). The DPPH assay represents a typical electron transfer reaction. When antioxidants react with this radical, they lighten the color of DPPH by donating electrons (Sánchez-Moreno, 2002).

The aim of this study was to provide basic health benefit data to consumers regarding the physicochemical properties and antioxidant and nitrite-scavenging activities of commonly consumed

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Korean beverages. Additionally, we examined the correlation between antioxidant activity and phenolic compounds.

2. Materials and methods

2.1. Sample preparation

The 62 kinds of commonly consumed beverages were purchased from a local market in Chuncheon, Korea. Sixty-two kinds of commonly consumed beverages were selected randomly based on their contents of vitamin and fruit which is known to contain abundant antioxidant compounds. These beverages were accurately weighed into 50 g portions. The samples were centrifuged at 3000 rpm for 10 min (416G, Gyrogen, Incheon, Korea). Each supernatant was analysed for physical properties and antioxidant activities.

2.2. Chemicals

Potassium phosphate monobasic and fluorescein (sodium salt) were purchased from Junsei Chemical (Tokyo, Japan). Sodium carbonate, Folin–Ciocalteu's phenol reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium phosphate dibasic, 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH), acetic acid, Griess reagent, 6-hydrolxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid (Trolox) and sodium nitrite were obtained from Sigma (Sigma–Aldrich Co., St. Louis, MO, USA).

2.3. pH value and °Brix (%)

The pH values of the beverages were measured at 25 °C using a pH meter (pH510, EUTECH, Co., Anyang, Korea), and the °Brix was measured by a hand-held reflectometer (PAL-α Brix 0–85%, Atago, Tokyo, Japan).

2.4. Total phenolic content (TPC)

The total phenolic content was determined using Folin–Ciocalteu's colorimetric method (Tawaha, Alali, Gharaibeh, Mohammad, & El-Elimat, 2007). The sample was appropriately diluted with distilled water. One milliliter of the sample or standard (gallic acid) was mixed with 1 mL of 10% sodium carbonate and 1 mL of 2% Folin–Ciocalteu's phenol reagent. After 90 min, the absorbance was measured at 750 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA). The results were expressed in gallic acid equivalents (GAE) using a gallic acid (0–0.2 mg/mL) standard curve.

2.5. Determination of antioxidant activity

2.5.1. Oxygen radical absorbance capacity (ORAC)

The ORAC assay was done according to the method of Ou, Hampsch-Woodill, and Prior (2001) with some modifications. Samples were diluted with a potassium sodium phosphate buffer (75 mM, pH 7.4). Twenty-five microliters of either the diluted sample containing Trolox (0–10 μM) or the potassium sodium phosphate buffer alone (blank), together with 150 μL of fluorescein (40 nM), were added into black-walled 96-well plates. Finally, 25 μL of AAPH (18 mM) was pre-incubated at 37 °C for 15 min and transferred to each well, and the plate was immediately carried to the fluorescence microplate reader (Spectramax GEMINI EM, Molecular Devices, Sunnyvale, CA, USA) to measure the fluorescence. The analyser was set to an excitation wavelength of 485 nm and an emission wavelength of 530 nm, and readings were recorded every 3 for 90 min at 37 °C. The ORAC value results were calculated using a Trolox calibration curve and the area under the fluorescence decay curve. The ORAC value was expressed as Trolox equivalents in micromoles per milliliter.

$$\text{Area under the curve (AUC)} = 1 + f1/f0 + f2/f0 + f3/f0 + f4/f0 + \ldots f/31/f0. \quad (1)$$

2.5.2. DPPH radical-scavenging activity (DPPH)

DPPH radical scavenging activity was determined according to the method of Molyneux (2004) with some modifications. Two hundred microliters of sample was added to 800 μL of 0.4 mM DPPH solution (yielding an absorbance of 1.0 ± .0.2 at 490 nm) and mixed thoroughly. Next, the mixture was allowed to react for 10 min at room temperature in the dark. The absorbance was then measured at 490 nm using a microplate reader. The results were calculated using the following formula (2):

$$\text{DPPH radical scavenging activity (\%)} = (1 - A_{\text{Experiment}}/A_{\text{control}}) \times 100. \quad (2)$$

2.5.3. Nitrite scavenging activity (NSA)

The nitrite scavenging activity was determined according to a method using Griess reagent (Choi et al., 2008) with some modifications. One milliliter of sample was added to 1 mL of 1 mM $NaNO_2$ and mixed. The mixture was then adjusted to pH 1.2 with 0.1 N HCl and adjusted to a volume of 10 mL with distilled water. The reaction solution was incubated at 37 °C for 1 h. Five milliliters of 2% acetic acid and 400 μL of Griess reagent were added to the reaction solution. After vigorous mixing with a vortex, the mixture was placed at room temperature for 15 min, and absorbance was measured at 520 nm by a microplate reader. The results were calculated using the following formula (3):

$$\text{Nitrite scavenging activity (\%)} = (1 - A_{\text{Experiment}}/A_{\text{control}}) \times 100. \quad (3)$$

2.6. Statistical analysis

All the data are presented as the means ± SD of triplicate samples. Correlations were calculated using Pearson's correlation coefficient (r).

3. Results and discussion

3.1. pH value and °Brix (%)

The pH values and °Brix of commonly consumed beverages were shown in Table 1. The results ranged from pH 2.4 to 3.9 and from 3.8 to 18.5 °Brix. The lowest pH value belonged to No. 60 lime beverage (pH 2.4). In addition, No. 54 lemon beverage also presented a low pH value (pH 2.5). Penniston, Nakada, Holmes, and Assimos (2008) reported that the juices of lemons and limes contained the most citric acid (48 and 46 g/L, respectively). Citric acid was believed to affect the pH value. The highest levels of °Brix came from Nos. 51 and 52 kiwi beverages (18.5 and 16.9 °Brix).

3.2. Total phenolic content

Phenolic compounds and flavonoids, such as rutin, catechin and naringenin, are generally found in groceries derived from plant sources, and these compounds have been known to possess important antioxidant activities (Van Acker et al., 1996).

Table 2 showed the total phenolic content in 62 kinds of commonly consumed beverages. The total phenolic content was