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Comparison of antioxidant activities expressed as equivalents of standard antioxidant

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Abstract

The quantitative antioxidant activity of foods is expressed as the equivalent weight of the authentic standard compounds with antioxidant function. Even in the same food sample, antioxidant activity is defined differently depending on the standard antioxidant compounds, making it difficult to compare them. Antioxidant activity was measured by DPPH radical and ABTS radical scavenging assay using Trolox, ascorbic acid, catechin, and gallic acid as standard antioxidant compounds. The relationship between the antioxidant activity and the standard was used to convert the antioxidant activity expressed as the equivalent of a specific antioxidant standard to the equivalent of another antioxidant standard. To verify the converted antioxidant activity, the antioxidant activity of apples was analyzed with several antioxidant standards and compared with the antioxidant activity converted with other standard compounds. As a result of comparing the antioxidant activity measured using various antioxidant standards and the converted antioxidant activity of apples, it was confirmed that there was no difference. The results of this study show that antioxidant activity expressed as a specific antioxidant standard compound can be accurately converted into the equivalent of another standard compound.

Keywords: antioxidant activity; DPPH; ABTS; standard; comparison.

Practical Application: Through the fast and accurate conversion of antioxidant activity results with different antioxidant standards, it can facilitate the comparison of antioxidant activities reported by different researchers and expand antioxidant research.

1 Introduction

Antioxidant activity refers to scavenging harmful reactive oxygen species (ROS) generated by natural physiological action or aging, excessive exercise, infection, and toxic substances in the body (Wang et al., 2022; Nunes-Silva & Freitas-Lima, 2015; Marschall & Tudzynski, 2016). In vivo ROS includes lipid radicals (R[•]), alkoxyl (RO[•]), peroxyl (ROO[•]), and hydroxyl (OH) (Shahidi & Zhong, 2015). The ROS produced within tunable concentrations by intracellular antioxidant systems contribute to vasodilation or modulate primary immune function (Sareila et al., 2011; Goto et al., 2007). However, excessive intracellular production of ROS free radicals particularly damage cells in aging or unhealthy conditions, and cause various diseases such as inflammation, arteriosclerosis, arthritis, cancer, and aging (Tafani et al., 2016). Therefore, the intake of food or substances with function of scavenging free radicals plays a vital role in maintaining and promoting health.

Two researchers (Researchers A and B) analyzed the antioxidant activity of apples (Figure 1). Although both researchers measured the antioxidant activity of apples using the same DPPH radical scavenging method, the standard chemicals used for quantitative antioxidant analysis were ascorbic acid and Trolox. The antioxidant activity of apple by Researcher A and B was 100 mg ascorbic acid equivalent (AAE)/g dry weight (DW) and 85 mg Trolox equivalent (TE)/g DW, respectively. How do you compare the antioxidant results of apples by different antioxidant standard compounds?

The diversity of free radicals produced in the body necessitates multiple antioxidant assays to measure radical scavenging ability (Liang & Kitts, 2014). Various antioxidant activity assays, including DPPH radical scavenging activity and ABTS radical scavenging activity, are used to analyze antioxidant activity of food and food materials (Jang et al., 2022). In addition, the antioxidant activity of the sample can be expressed in various ways. The first is to express the ability of a food substance to remove ROS from a sample as a percentage of ROS present after the reactive oxygen species scavenging reaction (Akinmoladun et al., 2007; Hsu, 2006). The antioxidant activity expressed as a percentage represents only the free radical removal rate at the specific concentration used for the analysis. However, it is difficult to compare antioxidant activity expressed as a percentage because researchers can set different concentrations of the initial free radicals used in the assay. To compensate for this, the antioxidant activity of the sample can be quantitatively expressed as the equivalent of the antioxidant standard. However, it is difficult to compare antioxidant activity results even in the same food sample because antioxidant standards are not the same among researchers (Cho et al., 2014; Ballesteros et al., 2017).

In this study, the relationship between DPPH radical and ABTS radical scavenging activity was investigated to derive a conversion formula between antioxidant activity. The analyzed antioxidant activity was converted to the equivalent of another

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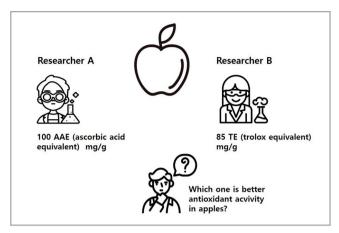


Figure 1. Antioxidant activity expressed with different standard compounds.

standard compound and were verified. Precise conversion of antioxidant activity results to other standard substances may facilitate comparing antioxidant activity results in foods and can be expanded and developed in the antioxidant research field.

2 Materials and methods

2.1 Materials and chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt cationic free radicals (ABTS⁺⁺), potassium persulfate, Trolox, ascorbic acid, catechin, and ascorbic acid were purchased from Sigma-Aldrich (Sigma-Aldrich Inc., St. Louis, MO, USA). Apples (Fuji, *Malus pumila* Mill.) were used as food samples to compare antioxidant activity results converted by different standard compound. Apples were cut into slices, dried and ground, and then extracted with 70% ethanol for 24 h. After filtration with Whatman No. 1, the solvent was removed by the speed-vac (EYELA Co., CVE-2200, Tokyo, Japan). Apple extract was prepared by dissolving in DMSO at 100 mg/mL concentration.

2.2 DPPH radical scavenging analysis

The DPPH radical scavenging activity of the sample was analyzed by Lee et al. (2009). The DPPH radical had a maximum absorbance at 517 nm, and the DPPH radical scavenging activity of the sample was evaluated as a decrease in the absorbance. Briefly, the sample (100 μ L), methanol (4.4 mL), and DPPH radical methanol solution (0.5 mL, 1 mmol/L) were mixed for 15 s and reacted at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm using a spectrophotometer (V-1100D, Labinno Co., Japan). A standard curve for the concentration of the standard compound and DPPH radical scavenging rate was plotted using Trolox, ascorbic acid, catechin, and gallic acid. The DPPH radical scavenging activity of the sample was expressed as mg standard compound equivalents/g DW.

2.3 ABTS radical scavenging analysis

The scavenging activity of ABTS⁺⁺ cationic free radicals was determined using the decolorization reaction of the ABTS⁺⁺

cation mixture (Thaipong et al., 2006; Li et al., 2022) with slight modification. First, to generate ABTS+ radicals, ABTS (7 mM) and potassium persulfate (2.45 mM) were dissolved in phosphate-buffered saline (pH 7.4) and reacted at room temperature in the dark for 24 h. For the measurement of the ABTS⁺⁺ scavenging activity, the dark blue-green ABTS⁺⁺ radical solution was diluted with PBS to obtain 0.8-0.9 of absorbance at 732 nm using a spectrophotometer (V-1100D, Labinno Co., Japan). To measure the scavenging ability of ABTS ^{+•} in sample, the diluted ABTS⁺⁺ solution (190 μ L) and the sample (10 μ L) were mixed and reacted in the dark for 30 min. The ABTS+. scavenging activity was assessed by decreasing the black-blue absorbance of the ABTS mixture at 734 nm. A standard curve was prepared using Trolox, ascorbic acid, catechin, and gallic acid, and the ABTS radical scavenging ability was expressed as mg equivalent/g DW.

2.4 Statistical analysis

Experimental results were statistically analyzed using SPSS 24.0 (IBM SPSS Inc., Chicago, IL, USA). All samples were analyzed at least 3 times, and the antioxidant results are expressed as mean \pm standard deviation (SD). Student's *t*-test was used to evaluate the significance between the antioxidant activity measurement results and the antioxidant activity results converted using other standard substances. The analysis result was considered non-significant when the *p*-value was 0.05 or more.

3 Results and discussion

The antioxidant activity of food samples is expressed as an antioxidant standard equivalent and has different values according to the antioxidant standard (Figure 1) (Cho et al., 2014; Ballesteros et al., 2017). Therefore, the antioxidant activity expressed in the specific standard compound needs to be converted into antioxidant activity with other standard compound for comparison. Plotting standard curves using several standard substances enables comparison between the antioxidant activities by using a conversion formula to convert between the standard substances.

3.1 Standard curves for DPPH and ABTS radical scavenging activity

Standard curves for DPPH radical (100 μ M) and ABTS radical scavenging activity based on the relationship between the concentration of standard substances and the amount of radicals were prepared using ascorbic acid, Trolox, catechin, and gallic acid (Figure 2) (Vikas et al., 2010; Jacobo-Velázquez & Cisneros-Zevallos, 2009). Gallic acid exhibited the strongest antioxidant activity against DPPH radicals by scavenging DPPH radicals (100 μ M) in the lowest concentration range (0-100 μ g/mL) (Figure 2A). In addition, DPPH radicals (100 μ M) were scavenged in the concentration range of 0-200 μ g/mL for ascorbic acid and 0-250 μ g/mL for catechin and Trolox. For ABTS radical scavenging activity, gallic acid and Trolox exhibited the concentration ranges of 0-50 μ g/mL and 0-250 μ g/mL, respectively (Figure 2B).

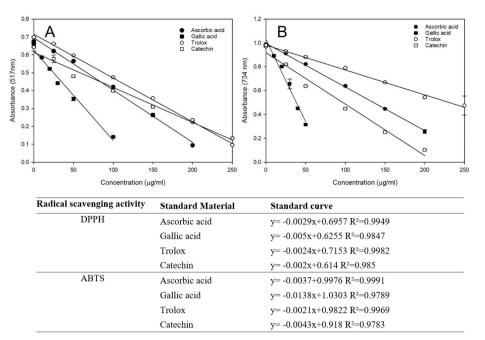


Figure 2. Standard curves of ascorbic acid, gallic acid, trolox, catechin by DPPH and ABTS assay. (A) DPPH radical scavenging activity; (B) ABTS radical scavenging activity.

Standard curve equations for various standard compounds for DPPH and ABTS radical scavenging capacity were shown in Figure 2. Standard curves for DPPH and ABTS radical scavenging activities had straight lines with R² values ranging from 0.978-0.999. Among the antioxidant standards, the slope of the gallic acid standard curve for DPPH radical scavenging activity (100 μ M) was approximately -0.0138, which was the most sensitive. However, gallic acid had a narrow concentration range for scavenging DPPH radicals (100 µM) that it was difficult to describe the radical scavenging activity of the foods in detail. On the contrary, ascorbic acid, catechin, and Trolox exhibited slopes of -0.0037, -0.0043, and -0.0021 for DPPH radical scavenging activity (100 µM), respectively. The slope of the standard curve determines the concentration range of the standard that scavenges DPPH radicals (100 μ M). If the slope of the standard curve is gentle, the concentration range of the standard compound that scavenging DPPH and ABTS radicals is wide, and it is possible to analyze the antioxidant activity of the sample more sensitively.

Antioxidant standard compounds showed differences in DPPH and ABTS radical scavenging kinetics (Figure 2). Correspondingly, antioxidant activity for food samples is displayed differently depending on the antioxidant standard (Tables 1 and 2). The most ideal compound for antioxidant standard curve should be able to detect activity in a wide concentration range and have a gentle slope, so that the amount of radicals measured by absorbance can be more accurately expressed as an equivalent. As a result of considering Figure 2, the suitability of standard materials for DPPH radicals was in the order of catechin > Trolox > ascorbic acid > gallic acid. As a standard for ABTS radical scavenging activity, Trolox > ascorbic acid > catechin > gallic acid was appropriate. Indeed, many researchers have frequently used Trolox and catechins as standards for quantitative indications of DPPH and ABTS radical scavenging activity (Saini et al., 2014; Liu et al., 2009; Sánchez-García et al., 2022; Pinto et al., 2021).

3.2 Conversion formula of DPPH and ABTS radical scavenging activity by standard compounds

The formulas for converting the DPPH and ABTS radical scavenging abilities expressed by other standard compounds using the formula of the standard curve (Figure 2) are shown in Table 1. DPPH and ABTS radical scavenging activities expressed as specific standard substance equivalents can be converted into antioxidant activity by other standard substance equivalents using the conversion formula (Table 1), enabling the comparison between the antioxidant activity results by different standards.

3.3 Validation for the conversion of antioxidant activity in apples

DPPH and ABTS radical scavenging ability of apples using ascorbic acid, gallic acid, Trolox, and catechin as standard compounds are shown in Table 2. The DPPH radical scavenging activity of apples was 49.08 mg ascorbic acid equivalent (AAE)/g DW. The ascorbic acid equivalent-antioxidant activity was converted to gallic acid equivalent and was 14.43 mg GAE/g DW, consistent with the DPPH radical scavenging activity measured directly using gallic acid as a standard material. In addition, the ascorbic acid equivalent-antioxidant activity of apple was converted to 67.47 mg TE/g DW and 30.32 mg CE/g DW in Trolox and catechin equivalents, respectively. The converted antioxidant activity was almost consistent to the antioxidant activity measured with each standard (Table 2). As such, it is expected that antioxidant activity using different standard substances can be compared indirectly through conversion using standard substance equivalents.

Standard compound	Target standard compound	Conversion formula for DPPH radical scavenging activity	Conversion formula for ABTS radical scavenging activity	
Ascorbic acid (mg AAE/g)	Gallic acid (mg GAE/g)	GAE=0.58×AAE-14.04	GAE=0.27×AAE+2.37	
	Trolox (mg TE/g)	TE=1.21×AAE+8.17	TE=1.76×AAE-7.33	
	Catechin (mg CE/g)	CE=1.45×AAE-40.85	CE=0.86×AAE-18.51	
Gallic acid (mg GAE/g)	Ascorbic acid (mg AAE/g)	AAE=1.72×GAE+24.21	AAE=3.73×GAE-8.84	
	Trolox (mg TE/g)	TE=2.08×GAE+37.42	TE=6.57×GAE-22.90	
	Catechin (mg CE/g)	CE=2.5×GAE-5.75	CE=3.21×GAE-26.12	
Trolox (mg TE/g)	Ascorbic acid (mg AAE/g)	AAE=0.83×TE-6.76	AAE=0.57×TE+4.16	
	Gallic acid (mg GAE/g)	GAE=0.48×TE-17.96	GAE=0.15×TE+3.49	
	Catechin (mg CE/g)	CE=1.2×TE-50.65	CE=0.49×TE-14.93	
Catechin (mg CE/g)	Ascorbic acid (mg AAE/g)	AAE=0.69×CE+28.17	AAE=1.16×CE+21.51	
	Gallic acid (mg GAE/g)	GAE=0.4×CE+2.3	GAE=0.31×CE+8.14	
	Trolox (mg TE/g)	TE=0.83×CE+42.21	TE=2.05×CE+30.57	

Table 1. Conversion formula for DPPH and ABTS radical scavenging activity according to various standard compounds.

Table 2. Verification of conversion for DPPH and ABTS radical scavenging activity of apple.

	Measured antioxidant activity		Converted antioxidant activity			
	Standard compound		Ascorbic acid (mg AAE/g)	Gallic acid (mg GAE/g)	Trolox (mg TE/g)	Catechin (mg CE/g)
DPPH	Ascorbic acid (mg AAE/g)	49.08 ± 6.08	49.08 ± 6.08	14.43 ± 3.53	67.47 ± 7.35	30.32 ± 8.82
	Gallic acid (mg GAE/g)	14.43 ± 3.53	49.09 ± 6.09 (0.013% ▲)	14.43 ± 3.53	67.48 ± 7.35 (0.014%▲)	30.33 ± 8.83 (0.016% ▲)
	Trolox (mg TE/g)	67.47 ± 7.35	49.08 ± 6.08	14.43 ± 3.53	67.47 ± 7.35	30.31 ± 8.82 (0.020% ♥)
	Catechin (mg CE/g)	30.32 ± 8.82	49.08 ± 6.09	14.43 ± 3.53	67.48 ± 7.35 (0.007%▲)	30.32 ± 8.82
ABTS	Ascorbic acid (mg AAE/g)	38.09 ± 1.31	38.09 ± 1.31	12.58 ± 0.35	59.78 ± 2.31	14.26 ± 1.13
	Gallic acid (mg GAE/g)	12.58 ± 0.35	38.08 ± 1.31 (0.021% ▼)	12.58 ± 0.35	59.76 ± 2.30 (0.027% ▼)	14.26 ± 1.12
	Trolox (mg TE/g)	59.78 ± 2.31	38.09 ± 1.31	12.58 ± 0.35	59.78 ± 2.31	14.26 ± 1.13
	Catechin (mg CE/g)	14.26 ± 1.13	38.09 ± 1.32	12.58 ± 0.35	59.77 ± 2.31 (0.016% ▼)	14.26 ± 1.13

All antioxidant activity was expressed as mean ± SD. Shaded values are directly measured antioxidant activity. The converted antioxidant results were statistically compared by Student's *t*-test to the measured antioxidant activity, and the *p*-value was not indicated when the value was 0.05 or more. A: Increase in antioxidant activity, ∇ : Decrease in antioxidant activity.

There are some limitations in converting the antioxidant activity by the equivalent of a specific standard compound to another standard equivalent. First, a standard curve for a standard compound is required to convert the antioxidant activity to the equivalent of another standard compounds. In addition, it is challenging to compare antioxidant activities because of the differences between the detailed analysis and sample preparation methods. The converted antioxidant activity may be slightly different depending on the time or temperature of the antioxidant activity assay, and the concentration of the assay reagent. In addition, differences in antioxidant assay sample preparation processes, i.e., extraction temperature, extraction solvent, degree of grinding of the sample, and extraction time, may make it more difficult to compare the converted antioxidant activity.

4 Conclusions

In this study, the antioxidant activity in the equivalent of a specific compound was converted to the equivalent of other standard compounds, and these values were compared with directly analyzed antioxidant activity. Despite various limitations, the results of this study are expected to further activate the evaluation of antioxidant activity between foods and food ingredients by making it possible to compare the antioxidant activities expressed by different standard substances.

Comparison of antioxidant activities using different standard compounds enables rapid and accurate selection of novel antioxidant

food materials, development and production of antioxidant functional foods in the antioxidant functional food industry. In addition, by quickly and accurately comparing the antioxidant activity of food materials, it is expected to contribute to the establishment of a food database that compares the antioxidant activity of various food materials and pharmaceutical materials.

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