

## Comparison of Peroxyl Radical Scavenging Capacity of Commonly Consumed Beverages

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The antioxidant potential of commercial beverages against peroxyl radical was determined using the Total Oxyradical Scavenging Capacity (TOSC) assay. Peroxyl radicals generated from thermal homolysis of 2,2'-azobis-amidinopropane oxidize  $\alpha$ -keto- $\gamma$ -methiolbutyric acid to ethylene, which is monitored by gas chromatography. The TOSC of each beverage is quantified from its ability to inhibit ethylene generation relative to a control reaction. Nine different beverages (green tea, jasmine tea, black tea, instant coffee, brewed coffee, cocoa mix, oolong tea, prune juice, and grape juice) were selected for this study. Their antioxidant capacities per a cup-serving (125 mL) were measured and compared to peroxyl radical scavenging capacity provided by a recommended daily dose of ascorbic acid (90 mg) dissolved in the same volume of water. The greatest antioxidant capacity was found in brewed coffee, which was followed, in decreasing order, by prune juice, instant coffee, green tea, cocoa mix, grape juice, jasmine tea, black tea, oolong tea, and ascorbic acid. There was an almost 7-fold difference in the TOSC between brewed coffee and ascorbic acid. The data suggest a potential role for commonly consumed beverages in lowering the risk of pathophysiological events associated with peroxyl radical-mediated events.

**Key words:** TOSC, Antioxidant, Beverages, Oxidative stress, Peroxyl radical

### INTRODUCTION

There is a great interest in biological reactivity of reactive oxygen species (ROS) and their role in oxidative stress. Under normal metabolism, the levels of oxidants and antioxidants in humans are maintained in balance, which is important for sustaining optimal physiology. Overproduction of oxidants and/or reduction of antioxidant defenses in certain conditions can cause an imbalance in redox state leading to oxidative damage on the critical sites in tissues and cells. Oxidative stress has been implicated in various cellular toxicity processes, such as damage to proteins, enzyme inactivation, peroxidation of lipid membranes, DNA alteration (Cohen and d'Arcy Doherty, 1987), and numerous pathological states including carcinogenesis, heart disease, reperfusion injuries, rheumatoid arthritis, inflammation, and aging (Gey et al.,

1991; Cutler, 1991).

Recently there has been a growing interest in dietary components for their antioxidant activity in expectation of supplementing the body defense against various oxidant challenges. Especially the antioxidant potential of beverages attracts much attention due to the convenience of their consumption and the excellence in efficacy reported by some authors as well as via the mass media. In fact numerous studies have shown antioxidant capacity of both green tea and black tea (Bravo, 1998; Lakenbrink et al., 2000), which appears to account for their beneficial effects against certain chronic human diseases related to oxidative stress in epidemiological studies (McKay and Blumberg, 2002). However, studies examining antioxidant potentials of other cold or hot beverages are relatively scarce. In this study we selected nine different beverages widely consumed in East Asia and also worldwide, and determined their antioxidant capacity against peroxyl radical in comparison with that of ascorbic acid using the total oxyradical scavenging capacity (TOSC) assay. The TOSC assay has been shown to be a reliable tool for quantitative assessment of the antioxidant potency of

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chemical substances and biological samples against oxygen radicals (Regoli et al., 1999; Winston et al., 1998). Therefore, it was of interest to determine the peroxy radical scavenging capacity of the commercial beverages in this assay system and to assign quantifiable values to their antioxidant activities.

## MATERIALS AND METHODS

### Chemicals

Nine kinds of commercial beverages including black tea (Lipton Yellow Label Tea<sup>®</sup>, Lipton Co., London, U.K.), brewed coffee (Maxwell House<sup>®</sup>, Dongsuh Food Co., Seoul, Korea), cocoa mix (Mite Hot Choco<sup>®</sup>, Dongsuh Food Co., Seoul, Korea), grape juice (Welch's 100% Grape Juice<sup>®</sup>, Welch's Co., Concord, MA, U.S.A.), prune juice (Del Monte Prune Juice<sup>®</sup>, Del Monte Foods Co., San Francisco, CA, U.S.A.), green tea (Sulloc Cha<sup>®</sup>, Amorepacific Co., Seoul, Korea), instant coffee (Maxim Original<sup>®</sup>, Dongsuh Food Co., Seoul, Korea), jasmine tea (Sulloc Jasmine Cha<sup>®</sup>, Amorepacific Co., Seoul, Korea), and oolong tea (Dongsuh Oolong Tea<sup>®</sup>, Dongsuh Food Co., Seoul, Korea) were purchased from local markets. Ascorbic acid and  $\alpha$ -keto- $\gamma$ -(methylthio)butyric acid (KMBA) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.); 2,2'-azobis-amidinopropane (ABAP) from Wako Pure Chemical Co. (Osaka, Japan). Except for the cold beverages (prune juice, grape juice, and oolong tea) all beverages were prepared according to package instructions suggested by the manufacturers. Typically, one tea or coffee bag was steeped, with gentle stirring, in 125 mL of distilled water at 90°C for 3 min. The cold beverages were used as such in the assay. Ascorbic acid was dissolved in cold distilled water in such a way that one cup (125 mL) contained the recommended daily allowance (90 mg/day) for male adults established by the U.S. Food and Nutrition Board, Institute of Medicine, National Academy of Sciences.

### Assays

A slight modification of the method developed by Winston et al. (1998) was employed to determine the TOSC of the beverages. Peroxyl radicals generated by the thermal homolysis of 2,2'-azobis-amidinopropane (ABAP) react with  $\alpha$ -keto- $\gamma$ -methylthiolbutyric acid (KMBA) to generate ethylene. The reaction mixture included 0.2 mM KMBA and 20 mM ABAP in 100 mM phosphate buffer, pH 7.4, in a final volume of 1.0 mL. Incubation was conducted at 37°C in a sealed vial. An aliquot (400  $\mu$ L) taken from the headspace of a reaction vial was injected into a Varian 3300 gas chromatograph (Varian Instrument Division, Palo Alto, CA, U.S.A.) equipped with a flame ionization detector and Porapak Q column (Supelco,

Bellefonte, PA, U.S.A.). The oven, injector and detector were maintained at 60°C, 180°C and 180°C, respectively. Helium was used as the carrier gas at a flow rate of 30 mL/min. Formation of ethylene was monitored for 60 min. The TOSC values were quantified from the equation  $TOSC = 100 - (\int SA / \int CA \times 100)$ , where  $\int SA$  and  $\int CA$  were the integrated ethylene peak areas obtained from the sample and control reactions, respectively. Because the TOSC is calculated from the relative inhibition of ethylene generation, this value is unitless. From the linear portion of TOSC versus volume of the beverage used, a specific TOSC (sTOSC) was obtained by extrapolation to a final equivalent volume of 1 mL. Relative TOSC (rTOSC) values were then calculated by dividing the sTOSC of each beverage with the sTOSC of ascorbic acid.

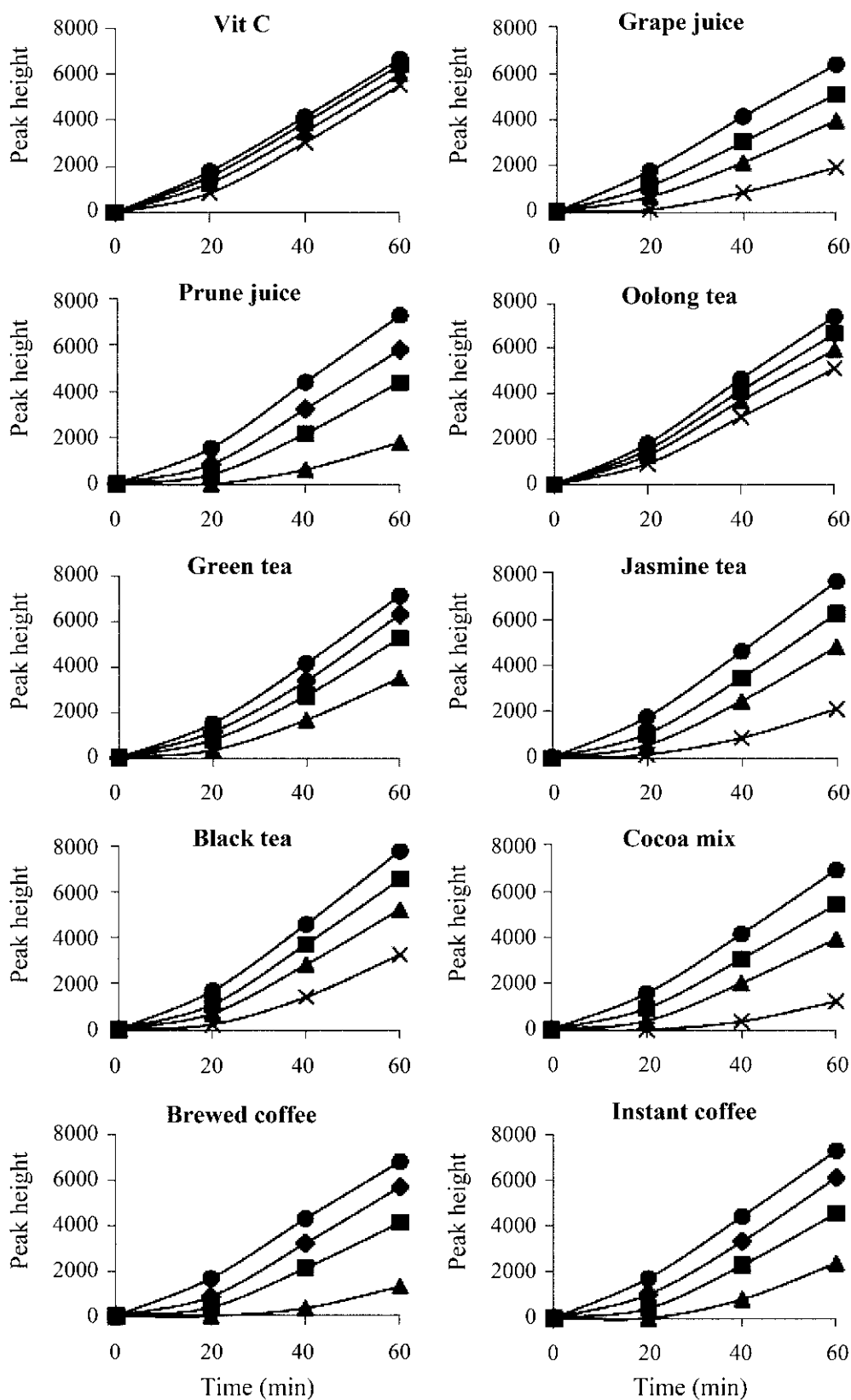
### Statistical analysis

For the statistical comparison of sTOSC of different beverages, results were analyzed using one-way ANOVA followed by Newman-Keuls multiple range test. The acceptable level of significance was established at  $P < 0.01$ .

## RESULTS AND DISCUSSION

Animals are constantly exposed to ROS, which are formed in several metabolic pathways. These include reduction of molecular oxygen coupled with oxidative phosphorylation in mitochondria, microsomal electron transport chain, active phagocytosis, and metabolic reactions mediated by several enzymes which produce ROS as intermediates. The main ROS produced in these cellular processes comprise superoxide anion, hydrogen peroxide, hypochlorous acid, hydroxyl radical, peroxy radical, alkoxy radical, and peroxynitrite. All of these species are strong oxidants, however, their reactivity towards biological macromolecules varies greatly; hydroxyl radicals and superoxide anion are regarded as the most potent and weakest oxidants, respectively (Halliwell and Aruoma, 1991). Peroxyl radical formed via the reaction of carbon-centered radical with oxygen is a biologically relevant active species because of its likelihood to damage cellular constituents. Moreover, formation of peroxy radical is the key chain-propagation step in lipid peroxidation; thus, prompting the study of various substances with respect to their ability to scavenge this radical (Wayner et al., 1985; DeLange and Glazer, 1989; Dugas et al., 2000).

In this study the antioxidant capacities of frequently consumed beverages against peroxy radical were determined using the TOSC assay. This method was shown to be effective in the determination of the peroxy radical scavenging capacity of bioflavonoids (Dugas et al., 2000; Winston et al., 2001), apple extracts (Eberhardt et al.,



**Fig. 1.** Time-course of ethylene generation from KMBA oxidation upon thermal homolysis of ABAP for reactions with different volumes of the beverages. Each point represents the mean of four separate measurements. Standard errors were omitted for the sake of brevity [ ● ; control, ◆ ; 0.25 μL, ■ ; 0.5 μL, ▲ ; 1.0 μL, × ; 2.0 μL].

2000), and various endogenous antioxidants including glutathione, ascorbic acid, melatonin and uric acid (Winston et al., 1998). All the beverages used in this study exhibited considerable antioxidant capacities against peroxy radical in a dose-dependent manner (Fig. 1). The slopes and intercepts of the regression lines were calculated from the linear portion of TOSC versus volume of the beverage used (Table I). The sTOSC and rTOSC values for the beverages are listed in Table II. Among the beverages examined, brewed coffee was shown to have the greatest antioxidant capacity, which was followed by prune juice, instant coffee, green tea, cocoa mix, grape juice, jasmine tea, black tea, and oolong tea. All of the beverages revealed a significantly greater antioxidant capacity than that of ascorbic acid, one of the major antioxidants in biological systems. A cup of brewed coffee demonstrated a peroxy radical scavenging capacity as high as almost seven-fold of the capacity provided by a recommended daily dose of ascorbic acid.

**Table I.** Linear regression slopes and intercepts of TOSC curves of the beverages

Beverage	Y-intercept	Slope	R <sup>2</sup>
Vitamin C	0.131	14.27	0.991
Oolong tea	1.728	17.69	0.986
Black tea	2.541	33.66	0.990
Jasmine tea	3.467	39.25	0.988
Welch's grape juice	4.288	38.79	0.983
Cocoa mix	3.452	44.39	0.989
Green tea	1.492	58.89	0.995
Instant coffee	3.637	78.94	0.983
Prune juice	4.024	83.35	0.986
Brewed coffee	2.898	88.86	0.992

The slopes and intercepts of the regression lines were calculated from the linear portion of TOSC, each was mean of four measurements, versus volume of the beverage used.

**Table II.** sTOSC (per mL) and rTOSC of the beverages tested

Beverage	sTOSC	rTOSC
Vitamin C	14.28 ± 0.63 <sup>a</sup>	1
Oolong tea	20.81 ± 0.89 <sup>b</sup>	1.46
Black tea	38.19 ± 0.56 <sup>c</sup>	2.67
Jasmine tea	45.53 ± 0.81 <sup>d</sup>	3.19
Welch's grape juice	46.61 ± 1.26 <sup>d</sup>	3.26
Cocoa mix	50.51 ± 0.75 <sup>d</sup>	3.54
Green tea	64.13 ± 2.89 <sup>e</sup>	4.49
Instant coffee	91.55 ± 1.27 <sup>f</sup>	6.41
Prune juice	97.94 ± 2.13 <sup>g</sup>	6.86
Brewed coffee	99.08 ± 1.27 <sup>g</sup>	6.94

Values with different superscripts (a, b, c, d, e, f, g) are significantly different one from another ( $P < 0.01$ ). Values are means ± SE for four separate measurements.

It has been known that various drinking products, including coffee, cocoa, and tea, are rich in flavonoids and phenolic compounds, which appear to account for their antioxidant activity (Bravo, 1998; Lakenbrink et al., 2000). Black, green and oolong teas made from *Camellia sinensis* contain high levels of catechins (flavonols and flavonol gallates). Catechins, especially epigallocatechin gallate, in green tea are rapidly extracted in hot water infusions, and transformed by enzymatic oxidation during the black tea manufacture into more complex compounds such as theaflavins and thearubigins. The present results suggest that the antioxidant compounds in tea may be depreciated in the manufacturing process for black tea and oolong tea. Unfermented cocoa beans are rich in polyphenols, which comprise 12-18% of the whole beans' dry weight. The polyphenols in cocoa, such as catechins, procyanidins and anthocyanidins, exhibit antioxidant properties in vivo (Hammerstone et al., 1999; Richelle et al., 2001). Coffee contains several polyphenolic components. Among the phenolic compounds identified in coffee are chlorogenic acids, a family of esters formed between quinic acid and several cinnamic acids such as caffeic, ferulic and *p*-coumaric acids, with caffeoylquinic acid being the most abundant (Clifford & Wight, 1976). It has been shown that coffee drinking influences the plasma antioxidant capacity in humans (Natella et al., 2002). Purple grape juice is rich in phenolic compounds among which flavan-3-ols, anthocyanins, and hydroxycinnamates are the main components (Mullen et al., 2007). Prunes contain large amounts of phenolic compounds, mainly as neochlorogenic and chlorogenic acids (Stacewicz-Sapuntzakis et al., 2001). These molecules in beverages could have synergistic or antagonistic effects when present in complex mixtures.

In this study we determined the relative antioxidant capacity of commonly consumed beverages on a cup-serving basis. Coffee, fruit juices, and green tea products examined were all shown to possess significant peroxy radical scavenging capacities, at least several times greater than the capacity provided by a recommended daily dose of ascorbic acid. In light of the potential role of peroxy radical as a mediator of numerous pathophysiological states, the antioxidant capacity of these products against peroxy radical suggests their possible beneficial role in the prevention of various chronic diseases that are linked with oxidative stress. It should be also noted that the antioxidant capacities of the beverages measured in vitro are not necessarily consistent with their effects in vivo. An understanding of the protective role of drinking products in vivo requires a better characterization of antioxidant component(s) in the beverages as well as quantitative data on their bioavailability, tissue distribution, metabolism, and effects on endogenous antioxidant substances and enzyme systems.

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