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FRAP RADICAL SCAVENGING CAPACITY OF GREEN TEA, OOLONG TEA, AND JEJU FERMENTED TEA BY PRODUCED PRODUCTS

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ABSTRACT

Tea (*Camellia* L.) is one of oldest, non-alcoholic and caffeine-containing beverage or medicine in world community. This study determined antioxidant activity in terms of the ferric reducing antioxidant power (FRAP) of commercial tea products, i.e., green tea, oolong tea, black tea, and Jeju fermented tea. Jeju fermented tea was showed the highest inhibition activity of FRAP among four groups. There was a significant difference among all concentration levels ($p < 0.05$ and 0.001). When the BHA used as a control, relative FRAP scavenging activities of green tea, Oolong tea, black tea, and fermented tea were 77.4%, 83.7%, 88.7%, and 104.2, respectively. The antiradical activity analysis permitted classifying tea extracts in medium activity group. The sample concentration providing 0.5 of absorbance (IC_{50}) of green tea, Oolong tea, black tea, and fermented tea were 61.4 µg/ml, 52.1 µg/ml, 57.9 µg/ml, and 48.5 µg/ml, respectively. The mode of inhibition of the tea extracts against FRAP was competitively inhibited FRAP by Lineweaver–Burk plots. In the processing of tea, fermentation plays an important role in determining the quality of Jeju tea.

KEYWORDS: Ferric reducing antioxidant power (FRAP), Jeju fermented tea, Lineweaver–Burk plots.

INTRODUCTION

Tea (*Camellia* L.) is native to China, later spread to India, Korea and Japan, then to Europe and Russia, arriving in the New World in the late 17th century.[1] The major tea-producing countries are China, India, Sri Lanka, Kenya and Korea. Black, green and oolong tea differ in their appearance, taste, chemical content as well as favor due to the difference in the fermentation process involved in making three main tea types.[2] Today tea is the most widely used beverage next to water.

The chemical components of tea leaves include polyphenols (catechins and flavonoids), alkaloids (caffeine, theobromine, theophylline, etc.), volatile oils, polysaccharides, amino acids, lipids, vitamins (e.g., vitamin C), inorganic elements (e.g., aluminum, fluorine and manganese), etc. However, the polyphenols are primarily responsible for the beneficial healthful properties of tea.[1]

Green tea is a type of tea that is made from mostly *Camellia sinensis* L. leaves and buds that have not undergone the same withering and oxidation process used to make oolong teas and black teas. Several varieties of green tea exist, which differ substantially based on the variety of *C. sinensis* used, growing conditions, horticultural methods, production processing, and time of harvest. Although there has been considerable research on the possible health effects of consuming green tea regularly, there is little evidence that drinking green tea has any effects on health. Polyphenols found in green tea include epigallocatechin gallate (EGCG), epicatechin gallate, epicatechins and flavanols, which are under laboratory research for their potential effects in vivo.[3] Black tea undergoes fermentation which transforms its color and flavor, whereas green tea remains unprocessed and retains its color. Green tea is grown in higher altitudes, more specifically the mountainous regions of East Asia. Oolong tea is a partially fermented tea and has the flavor and health characteristics of both green and black teas. It contains a high number of antioxidants, which protects healthy skin cells and the aging process slows down.

Black tea accounts for approximately 72% of the world's total tea production.[4] While most of the EGCG antioxidants are oxidized during the fermenting process, black tea retains a high number of the antioxidant polyphenols such as flavonoids. These antioxidants help rid the body of harmful toxins.

Antioxidant activity significantly changes according to the substrate, the parameter adopted to compare the substances in the same method, and the form used to express antioxidant concentration. The ferric reducing antioxidant power (FRAP) mechanism is based on electron transfer rather than hydrogen atom transfer.[5] The FRAP assay is based on the ability of PH to reduce Fe^{3+} to Fe^{2+}. The FRAP reaction is conducted at acidic pH 3.6 to maintain iron solubility, so the reaction at low pH decreases the ionization potential that drives hydrogen atom transfer and increases the redox potential, which is the dominant reaction mechanism. FRAP was developed to evaluate the antioxidant effect of nonenzymatic defense in biological fluids, in which the response could provide a measure of antioxidant ability.[6]

Although the antioxidant properties of green, oolong, and black teas have been well studied, antioxidant activity has not been examined in personal fermented tea such as Jeju green tea in Korea. Therefore, in the current studies, I investigated the antioxidant activity of fermented tea in comparison with those of green, oolong, and black teas.

MATERIALS AND METHODS

Sample extract

The four types of tea (green tea, oolong tea, black tea, and fermented tea) were collected from Korean tea markets. Each of them is sold as its main ingredient, but the exact content and information for the product including tea weight or concentration is not indicated. Green tea, oolong tea and black tea are dried leaves, while Jeju fermented tea is concentrated in liquid. Daily method of tea making (household preparation) from the stock of teas has been used to prepare aqueous extracts. The aqueous extract is prepared in ratio 10 g (tea) per 500 ml (3rd distilled water). Samples were added to hot water (92-95℃). Then, the tea cooled down to room temperature. After filtration, the water was removed in a rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan) at 70℃. To get dry powder, samples placed in a low temperature vacuum chamber. These powders diluted to different concentration and were then used to determine antioxidant activities.

Ferricon Reducing Antioxidant power (FRAP)

The ferric reducing capacity of extracts was investigated by using the potassium ferricyanide-ferric chloride method.[6] Briefly, 0.2 mL of each of the extracts at different concentrations, 2.5 mL of phosphate buffer (0.2 M, pH 6.6), and 2.5 mL of potassium ferricyanide $K_3Fe(CN)_6$ (1%) were mixed and incubated at 50°C for 20 min, to reduce ferricyanide into ferrocyanide. The reaction was stopped by adding 2.5 mL of 10% (w/v) trichloroacetic acid followed by centrifugation at 1000 rpm for 10 min. Finally, 2.5 mL of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%) and the absorbance was measured at a wavelength of 700 nm with a 3-tertbutyl-4-hydroxyanisole (BHA) solution as positive control. The sample concentration providing 0.5 of absorbance (IC_{50}) was calculated by plotting absorbance against the corresponding sample concentration. The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents per gram of sample (DW).

Inhibition types were then determined by Lineweaver–Burk plot (1/v versus 1/[S]) where [S] analysis of data is according to Michaelis–Menten kinetics.[7]

Statistical analysis

All determinations were conducted in triplicate, and the results were calculated as mean value ± standard deviation (SD). Significance and confidence level were estimated at $p < 0.05$. The antioxidant activity was calculated as the decolourization percentage of the test sample using the following formula:

Activity (%) = (IA-As)/IA×100

Where IA is the absorbance of the 100% initial and As is the absorbance of the sample. IA 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. The time needed to reach the steady state to IC_{50} concentration (T_{IC50}) was calculated graphically.[8] Lower IC_{50} valueproves the higher antioxidant ability of studied substra time.

RESULTS

Antioxidant capacity of extracts was determined using FRAP assay (Table 1). Jeju fermented tea was showed the highest inhibition activity of FRAP among four groups. Antioxidants increased with the fermentation of green tea. Namely, the unfermented tea (green tea) was showed low inhibition activity. FRAP of green tea was evaluated at 1.0 mg/ml was 69.6 and that Oolong tea scavenging activity 70.8% at same concentration, and that of black tea was 75.2%. Green tea very similar value to Oolong tea. The fermented tea was evaluated 93.6% at 1.0 mg/ml. There was a significant difference among all concentration levels ($p < 0.05$ and 0.001). When the BHA used as a control, relative FRAP scavenging activities of green tea, Oolong tea, black tea, and fermented tea were 77.4%, 83.7%, 88.7%, and 104.2, respectively (Fig. 1). The IC_{50} values of green tea, Oolong tea, black tea, and fermented tea were 61.4 μg/ml, 52.1 μg/ml, 57.9 μg/ml, and 48.5 μg/ml, respectively (Table 2). The dependence curves of remaining FRAP radicalin the reaction system and four tea extract's concentration in precise time was calurated. The antiradical activity analysis permitted classifying tea extracts in medium activity group (Table 2). Jeju fermented tea extract showed the highest ability of scavenging studiedradicals ($3.93 \cdot 10^{-3}$), the lowest showed green tea aqueous extract ($1.22 \cdot 10^{-3}$).

The Lineweaver–Burk plot was used to determine important terms in enzyme kinetics, such as K_m and V_{max}. The mode of inhibition of the tea extracts against FRAP was confirmed by Lineweaver–Burk plots (Figure 2). Crude water extracts competitively inhibited FRAP.

Table 1: The degree of inhibition (%) of FRAP by green tea, oolong tea, black tea, and fermented tea at different concentrations.

Concentration (㎛)	Green tea	Oolong tea	Black tea	Fermented tea (Jeju)	*t*-test
0.25	35.07±3.04	39.39±2.06	43.60±2.49	49.35±2.51	6.902*
0.50	49.97±2.94	53.56±2.57	58.17±3.12	74.84±2.87	28.648***
0.75	60.25±3.53	63.55±3.94	70.20±1.19	86.55±1.59	33.776***
1.00	69.58±2.92	70.76±3.17	75.22±2.32	93.64±0.65	40.212***
t-test	0.055	0.064	0.024	0.013	1.101

*: $p<0.05$, ***: $p<0.001$.

Table 2: The 50% inhibition (IC_{50}) of FRAP for green tea, oolong tea, black tea, and fermented tea.

Sample	IC_{50}	T_{IC50}	AE (10^{-3})	Antiradical efficiency classification
Green tea	57.9	14.15	1.22	Medium antiradical activity
Oolong tea	61.4	22.50	1.30	Medium antiradical activity
Black tea	52.1	8.25	2.33	Medium antiradical activity
Fermented tea	48.5	15.25	3.93	Medium antiradical activity

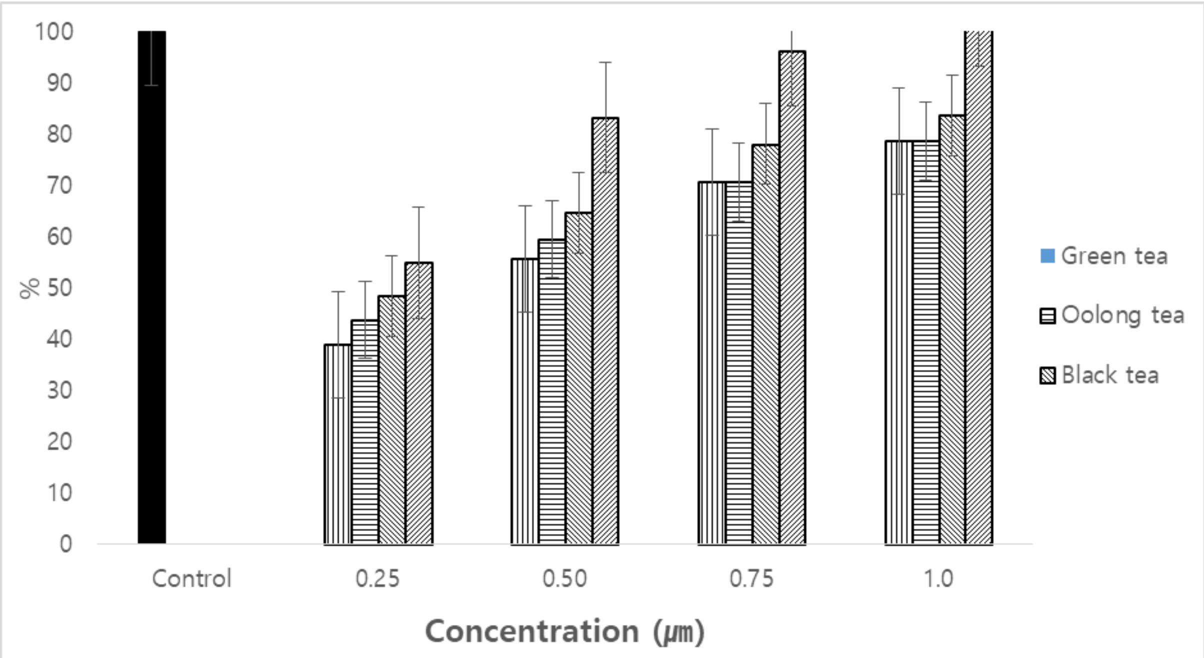

Fig. 1. The rate of FRAP inhibitory of BHA (positive control) and relative inhibitory rate for green tea, oolong tea, black tea, and fermented tea at different concentrations.

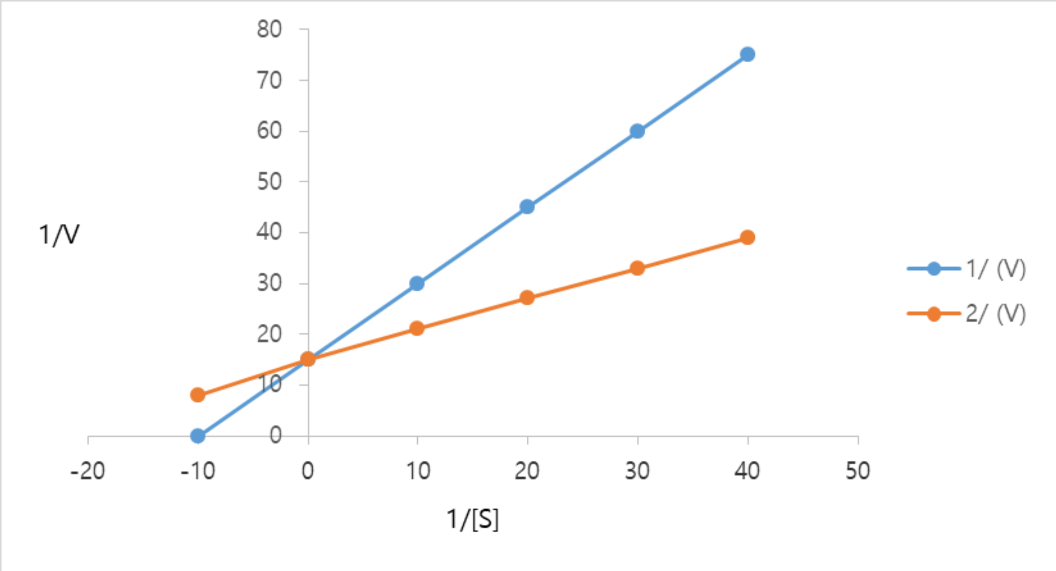

Fig. 2. Lineweaver-Burk plot for the activity of FRAP in the presence of concentration (1 ug/ml) of the fermented tea extract and inhibitor.

DISCUSSION

Antioxidants are those substances that prevent oxidation. Urban living has created an above-normal range of free radicals that the body is able to combat. So, foods rich in antioxidants are necessary to make up for this deficit. Fruits and vegetables are a common source of antioxidants. But, tea is thought to have more antioxidants than fruits and vegetables. Tea beverages are produced on the basis of tea extracts or tea infusions prepared as a part of beverage manufacturing process and available on the market bottled or canned.[9] Generally, very low content of catechins was noted for most tea beverages studied in comparison to tea-cup- or tea-pot-prepared tea.[10]

The range of extract concentrations and measurement frequencies were established experimentally. Considering IC_{50} and T_{IC50}, affect the antiradical capacity, antiradical efficiency was defined: AE = 1/ IC_{50}· T_{IC50}.[8] Antiradical efficiency parameter allowed dividing the extracts into different antiradical activity groups: AE = 1 · 10-3 low antiradical activity. 1· 10-3 <AE < 5· 10-3 : medium antiradical activity, 5· 10-3 <AE < 10 · 10-3 : high antiradical activity, AE > 10 · 10-3 : very high antiradical activity. According to this standard, although the results showed that the four teas were medium antiradical activity (Table 2), antioxidant increased with moderate fermentation of green tea (Table 1). Some research also investigated that black tea, oolong tea and green tea possess the antioxidant activity.[11]

There is assumption to be found in the steaming of fermented tea leaf processing that steaming makes the increased production of phenolic and some compounds in tea leaf.[12] Therefore, depending on the type of tea, the final products contain highly distinct profiles of antioxidant compounds.[10]

The many researches have examined the different culinary methods used in the domestic preparation of tea infusions, taking into account factors such as water temperature, infusion time, stirring and dosage form, i.e., loose-leaf tea versus tea bag.[13] The extraction temperature, extraction time, water quality and water-to-tea ratio, tea particle size, extraction pH, and the number of extractions are all important factors which directly affected the efficiency of the extraction of antioxidants.[14] The number of extraction stages depends on the efficiency of the equipment used.[15]

Green tea has been consumed in China and other Asian countries since ancient times in order to maintain and improve health.[16] The differences among green, oolong, black, fermented teas were resulted from the manufacturing process. The FRAP values of different categories of teas were in a decreasing order: green tea (3663.32 ± 535.63 μmol Fe^{2+}/g DW), yellow tea (3582.93 ± 433.94 μmol Fe^{2+}/g DW), oolong tea (1539.13 ± 351.86 μmol Fe^{2+}/g DW), dark tea (1472.27 ± 691.91 μmol Fe^{2+}/g DW), black tea (1283.47 ± 858.62 μmol Fe^{2+}/g DW) and white tea (1160.80 ± 190.32 μmol Fe^{2+}/g DW).[17] In this study, IC_{50} values of green tea, Oolong tea, black tea, and fermented tea were 61.4 μg/ml, 52.1 μg/ml, 57.9 μg/ml, and 48.5 μg/ml, respectively (Table 2). Jeju green tea is fermented tea. Green, white and yellow teas are subject to very little oxidation because they are heated soon after picking. Jeju tea did not treat with high temperature heat. It is allowed to oxidize for a short period before being exposed to heat, and therefore it retains a higher polyphenol content than green tea, oolong tea and black tea. Fermentation involves enzymatic oxidation. In the processing of tea, fermentation plays an important role in determining the quality of Jeju tea.

REFERENCES

1. Sharangi AB. Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.) – A review. Food Research International, 2009; 42: 529-35.
2. Kottawa-Arachchi JD, Gunasekare MTK, Ranatunga MAB, et al. Biochemical characteristics of tea (*Camellia* L. spp.) germplasm accessions in Sri Lanka: Correlation between black tea quality parameters and organoleptic evaluation. Int J Tea Sci, 2014; 10: 3-13.
3. Khan N, Mukhtar H. Tea and health: studies in humans. Current Pharmaceutical Design (Literature Review), 2013; 19: 6141–7.
4. Katiyar SK, Mukhtar H. Tea in chemoprevention of cancer: epidemiological and experimental studies. Int J Oncol, 1996; 8: 221-38.
5. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem, 2005; 53: 4290-302.
6. Tundis R, Menichini F, Bonesi M, et al. Antioxidant and hypoglycaemic activities and their relationship to phytochemicals in *Capsicum annuum* cultivars during fruit development. LWT-Food Science and Technology, 2013; 53: 370-7.
7. Lineweaver H, Burk D. The determination of enzyme dissociation constant. J Am Chem Soc, 1934; 56: 658-66.
8. Gramza A, Pawlak-Lemanska K, Korczak J, et al. Tea extracts as free radical scavengers. Polish J Environ. Studies, 2005; 14**:** 861-7.
9. Cordero C, Canale F, Del Rio, et al. Identification, quantitation, and method validation for flavan-3-ols in fermented ready-to-drink teas from the Italian market using HPLC-UV/DAD and LC-MS/MS. J Sep Sci, 2009; 32: 3643-51.
10. Kosińska A, Andlauer W. Chapter 12 -Antioxidant capacity of tea: effect of processing and storage. In: Preedy, V. (Ed.), Processing and impact on antioxidants in beverages. Academic Press, San Diego, 2014, pp. 109-20.
11. Xie B, Shi H, Chen Q. Antioxidant properties of fractions and polyphenol constituents from green, oolong, and black tea. Proc Nat Sci Repub China, 1993; 17: 77-84.
12. Graham HN. Green tea composition, consumption, and polyphenol chemistry. Preventive Medicine, 1992; 21: 334-50.
13. Jin Y, Zhao J, Kim EM, et al. Comprehensive investigation of the effects of brewing conditions in sample preparation of green tea infusions. Molecules, 2019; 24: 1735; doi:10.3390/molecules24091735.
14. Vuong QV, Golding JB, Stathopoulos CE, et al. Optimizing conditions for the extraction of catechins from green tea using hot water. J Sep Sci. 2011; 34: 3099-106.
15. Bharadwaz A, Bhattacharjee C. Extraction of polyphenols from dried tea leaves. Int J Sci Eng Res, 2012; 3: 1-5.
16. Chen Y, Huang JY, Lin Y, et al. Antioxidative and antimelanoma effects of various tea extracts via a green extraction method. J Food Qual, 2018; Article ID 5156073.
17. Zhao CN, Tang GY, Cao SH, et al. Phenolic profiles and antioxidant activities of 30 tea infusions from green, black, oolong, white, yellow and dark teas. Antioxidants (Basel), 2019; 8: 215. doi: 10.3390/antiox8070215