

DPPH, ABTS, AND APX RADICAL SCAVENGING CAPACITY OF GREEN TEA, OOLONG TEA, AND JEJU FERMENTED TEA BY PRODUCED PRODUCTSMan Kyu Huh^{1*} and Seong Yong Bae²¹Food Science & Technology Major, Dong-eui University, Busan 47340, Republic of Korea.²Jejulife B&F Co., Ltd., Jeju-do, Jungjeong-ro 123, Republic of Korea.***Corresponding Author: Man Kyu Huh**

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ABSTRACT

Tea is one of the most popular natural beverage ingredients in world community. This study was to evaluate and compare the antioxidant radical scavenging capacity of extracts from four types for commercial tea consumption, i.e. green tea, oolong tea, black tea, and the Jeju fermented tea. Three antioxidant properties were analyzed using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ascorbate peroxidase (APX) scavenging activity. The DPPH activity of the fermented tea (Jeju) was the highest among tested teas and there was significant difference at four concentration levels ($p < 0.01$). ABTS scavenging activity of the fermented tea was evaluated 92.6% at 1.0 μm of water extracts and those of green, oolong, and black teas at same concentration were 72.9, 68.9% and 67.8%, respectively. The three extracts of the produced product green tea, oolong tea, and black tea for APX scavenging activity were lower than that of the fermented product tea (Jeju). DPPH, ABTS, and APX scavenging activities were shown significant differences at all concentration levels between the Jeju fermented tea and other three teas ($p < 0.05$). The Jeju fermented tea was manufactured from a concentrate much thicker than other tea. It was judged that these strong concentration had a high antioxidant function.

KEYWORDS: ABTS, APX, DPPH, Jeju fermented tea, scavenging activity.**INTRODUCTION**

Tea (*Camellia* L.) is one of oldest, non-alcoholic and caffeine-containing beverage or medicine and is the most consumed beverage next to water. Tea has used for this purpose in China for nearly 3,000 years. The major tea-producing countries are China, India, Sri Lanka, Kenya and Korea. It is an important component of the warm temperate and subtropical forests of that region. 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.^[1]

This tea's oxidation for oolong tea by the quick application of heat after tea picking is stopped somewhere between the standards for green and black teas. In Chinese, semi-oxidized teas are collectively grouped as *blue tea* (literally: blue-green tea; also, celadon tea, for the pottery), while the term *oolong* is used specifically as a name for certain semi-oxidized teas.

Black tea is allowed to completely oxidize and is withered to induce protein breakdown and reduce water

content (68–77% of original).^[2] Fermented tea (post-fermented tea or dark tea) is a class of tea that has undergone microbial fermentation, from several months to many years. Fermented tea originates in China, where it is commonly known as *hei cha* or dark tea. Tteokcha in Korea is one of these fermented teas. Tteokcha (cake tea), also called Byeongcha, was the most commonly produced and consumed type of tea in pre-modern Korea.^[3]

The radical-scavenging and antioxidant properties of tea polyphenols are frequently cited as important contributors to some health-protection benefits.^[4] For example, green tea polyphenols have been shown to have anti-cancer activity in a number of laboratory studies, which could be mediated through antioxidant or pro-oxidant mechanisms.^[5] Green tea polyphenols such as (–)-epigallocatechin gallate (EGCG) inhibit cell viability and induce apoptosis in a number of cancer cell lines such as osteogenic sarcoma,^[6] lymphoblastoid cells,^[7] leukemia cells,^[8] melanoma cells,^[9] and T lymphocytes.^[10] The green tea phenolic compounds of highest concentration are gallic acid (GA), (–)-gallocatechin (GC), (+)-catechin (C), (–)-epicatechin

(EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG), p-coumaroylquinic acid (CA), and (-)-gallocatechin-3-gallate (GCG).

The antioxidant activity of polyphenolic compounds is mainly due to their properties, which allow them to act as reducing agents, hydrogen donors and metal chelators.^[19] The universally used parameters of free radicals' scavenging ability as well as the evaluation of antioxidant potency are reactions with the radical cation 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). Ascorbate peroxidase (APX) enzymes play a key role catalyzing the conversion of H₂O₂ into H₂O, using ascorbate as a specific electron donor.

In the present study, antioxidant capacity of four types of tea (green, oolong, and fermented teas) prepared from *Camellia sinensis* in Korea were determined.

MATERIALS AND METHODS

Sample extract

The samples for analysis were prepared four products, green, oolong, black, and fermented teas. They were purchased on Korean tea markets. Each of them is sold as its main ingredient, but the exact content is not indicated. Green, oolong, and black teas are dried leaves, while the Jeju fermented tea is concentrated in liquid. Jeju green tea was diluted in the process of setting the standard for absorbance. Dilution of Jeju green tea solution is needed to be able to analyse it with optical density. Daily method of tea making (household preparation) from the stock of teas has been used to prepare aqueous extracts. 1.5~2 g of tea is usually put in 100 ml of hot water. The aqueous extract is prepared in ratio 10 g (tea) per 500 ml (3rd distilled water). The time to boil tea was doubled to five minutes, compared with 2.5 minutes. Tea is put into tea pottery and boiled for times (5.0 min.). 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°C. To get dry powder, samples placed in a low temperature vacuum chamber. These powders were then used to determine antioxidant activities.

DPPH free radical

The DPPH scavenging activity was measured by the method by Blois^[12] with slight modifications. Each sample stock solution (1.0 mg/ml) was diluted to final concentrations of 0.25, 0.50, 0.75, and 1.0 mg/ml. A solution of DPPH was prepared by dissolving 5 mg DPPH in 2 ml of 80% ethanol. A stock solution of the compounds was prepared at 1 mg/ml in DMSO. The stock solution was diluted to varying concentrations in 96-well microplates. DPPH was added to the solutions prepared with sample extracts and standard antioxidant substances. The radical scavenging reaction was carried out at 37°C in dark for 30 min. The optical density (OD) of the solution was read using the Microplate Reader

(VersaMax, California, USA) at the wavelength 515 nm. Corresponding blank samples (water, DMSO, and DPPH solution) and L-Ascorbic acid (0.25, 0.50, 0.75, and 1.0 mg/ml) as positive control were used as reference standard.

ABTS radical scavenging activity

The antioxidant activity of three ginseng extracts was measured on the basis of the scavenging activity of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) free radical according to the method described by Brand-Williams et al.^[13] with slight modifications. ABTS was dissolved in water to a 7 mM concentration, and 2.45 mM potassium persulfate was prepared. Two stock solutions were mixed and kept in the dark at room temperature for 16 h before use. After the addition of 200 µl of the diluted ABTS solution to 40 µl of the sample extracts, the decrease in absorbance was measured for 1 min after mixing the solution, and the final absorbance reading was monitored for 33 min by absorbance at 734 nm using the Microplate Reader. Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a positive control inhibiting the formation of the radical cation in a dose dependent manner.

Ascorbate peroxidase (APX) scavenging activity

The activity of APX (EC 1.11.1.1) was determined according to Nakano and Asada.^[14] The reaction medium was incubated at 28°C. The assay mixture contained 50 mM potassium buffer (pH 7.0), 0.1 mM EDTA, 1.25 mM H₂O₂, 0.5 mM ascorbic acid and 100 µl enzyme extract. The reaction was initiated by adding H₂O₂. Reaction mixture contained 50 mM potassium buffer (pH 7.0), 25 mM guaiacol, 10 mM H₂O₂ and 100 µl enzyme extract. Activity was measured by the increase in absorbance at 470 nm for 70s.

Statistical analysis

Data was conducted using Microsoft Excel and SPSS 21.0 for Windows (Chicago, IL, USA). A one-way and a two-way analysis of variance (ANOVA) followed by the Tukey post hoc test were used to analyze statistical significance ($p < 0.05$). The analysis was carried out at least in triplicate. The results were expressed as the mean ± SD. Significance and confidence level were estimated at $p < 0.05$. The percent inhibition was calculated as the decolorization percentage of the test sample using the following formula:

$$\text{Inhibition (\%)} = (IA - As) / IA \times 100$$

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.

EC₅₀ is defined as the concentration of inhibitor necessary for 50% inhibition of the enzyme reaction of a maximum scavenging capacity. To determine the EC₅₀ 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 EC₅₀ values.

RESULTS

DPPH scavenging assay

Table 1 was shown the antioxidant activity for DPPH radical of four teas. It was observed that inhibition percentage values go on increasing with enhancements in concentration of research tea extracts in the assay mixture. The DPPH activity of the fermented tea (Jeju) was the highest among tested teas. DPPH scavenging activity of the fermented tea was evaluated 81.8% on 1.0 mg/ml and that of the green tea was 64.6% at same concentration. Both oolong tea and black tea did not show high radical scavenging effects at all concentration. There was no significant difference between oolong tea and black tea ($p > 0.01$ and 0.001). However, there was significant difference at four concentration levels ($p < 0.01$).

Figure 1 was shown the rate of DPPH inhibitory of L-ascorbic acid (positive control) and relative inhibitory rate for four teas on 1.0 M. The values for the fermented tea, green tea, oolong tea and black tea were 95.4%, 68.1%, 65.1%, and 63.3%, respectively. An EC₅₀ value is the concentration of the sample required to scavenge 50% of the free radicals present in the system. EC₅₀ value was inversely related to the antioxidant activity of crude extracts. The values of EC₅₀ for the fermented tea, green tea, oolong tea and black tea were 35.3, 40.5, 40.8, and 40.9 $\mu\text{g/ml}$, respectively (Table 4).

ABTS scavenging assay

Using ABTS radical scavenging method, for the determination of antioxidant activity, the obtained results recorded in Table 2. The rates of antioxidant activities of

the water extracts for teas were also dependent on concentrations. ABTS scavenging activities for various concentrations of the fermented tea were higher than those of the green, oolong, and black teas. ABTS scavenging activity of the fermented tea was evaluated on 1.0 μm of water extracts was 92.6%, those of green tea, oolong tea and black tea at same concentration were 72.9%, 68.9% and 67.8%, respectively. All concentration groups were shown statistically significant differences ($p > 0.05$, 0.01 , and 0.001). Figure 2 was shown the rate of ABTS inhibitory of Trolox (positive control) and relative inhibitory rate for teas on 1.0 M. The values for green, oolong, black, and fermented teas were 60.9%, 69.5%, 89.4%, and 95.8%, respectively. The values of EC₅₀ for green tea, oolong tea, black tea, and fermented tea were 61.3, 48.4, 63.4, and 55.9 $\mu\text{g/ml}$, respectively (Table 4).

APX scavenging assay

APX scavenging activity of green tea was evaluated was 50.5% on 1.0 mg/ml, those of oolong tea, black tea, and fermented tea were 57.8%, 56.8%, and 64.8% at same concentration, respectively. The three extracts of the produced product green, oolong, and black teas for APX scavenging activity were lower than that of the fermented product tea. All groups did not show a statistically significant difference ($p > 0.05$). However, there was a significant difference among four concentration levels ($p < 0.05$ and 0.001). Figure 3 was shown the rate of APX inhibitory of H₂O₂ (positive control) and relative inhibitory rate for teas on 1.0 M. The values for green, oolong, black, and fermented teas were 32.2%, 50.8%, 60.5%, and 69.7%, respectively. The values of EC₅₀ for green, oolong, black, and fermented teas were 71.3, 71.9, 72.1, and 66.1 $\mu\text{g/ml}$, respectively (Table 4).

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

Concentration (μm)	Green tea	Oolong tea	Black tea	Fermented tea (Jeju)	<i>t</i> -test
0.25	32.97±2.19	27.70±2.24	25.99±2.26	46.94±3.37	16.360**
0.50	45.02±1.58	36.28±1.54	33.30±1.28	63.78±1.39	175.735***
0.75	58.40±0.60	46.20±0.65	43.88±0.71	71.85±1.56	350.115***
1.00	64.61±1.25	55.85±2.27	54.33±2.37	81.81±0.20	101.692***
<i>t</i> -test	0.003	0.015	0.014	0.001	2.257

Data represented the mean \pm SD from three replicates. **: $p < 0.01$, ***: $p < 0.001$.

Table 2: The degree of inhibition (%) of ABTS by green tea, oolong tea, black tea, and fermented teas at different concentrations.

Concentration (μm)	Green tea	Oolong tea	Black tea	Fermented tea (Jeju)	<i>t</i> -test
0.25	37.80±4.36	27.53±3.79	25.15±5.38	58.91±4.62	12.278*
0.50	57.92±4.68	47.27±2.43	44.52±3.30	67.19±1.88	20.161**
0.75	68.07±3.89	58.99±1.68	57.52±1.74	86.39±0.36	65.752***

1.00	72.88±1.99	68.87±0.94	67.76±0.99	92.61±0.74	177.078***
<i>t</i> -test	0.023	0.009	0.081	0.021	1.512

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Table 3: The degree of inhibition (%) of APX by green tea, oolong tea, black tea, and fermented teas at different concentrations.

Concentration (μM)	Green tea	Oolong tea	Black tea	Fermented tea (Jeju)	<i>t</i> -test
0.25	15.92±3.37	20.81±1.76	20.05±1.08	29.95±2.91	7.246*
0.50	26.21±2.79	31.75±1.64	30.78±2.45	47.21±1.65	34.234***
0.75	37.81±3.58	48.74±0.85	47.28±1.57	56.24±2.41	20.646***
1.00	50.50±2.23	57.81±3.09	56.80±2.87	64.79±1.21	11.139**
<i>t</i> -test	0.006	0.007	0.038	0.010	0.545

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Table 4: The 50% inhibition (EC_{50}) of DPPH, ABTS, and APX of green tea, oolong tea, black tea, and fermented tea.

Sample	DPPH	ABTS	APX
Green tea	40.5	61.3	73.1
Oolong tea	40.8	48.4	71.9
Black tea	40.9	63.4	72.1
Fermented tea	35.3	55.9	66.1

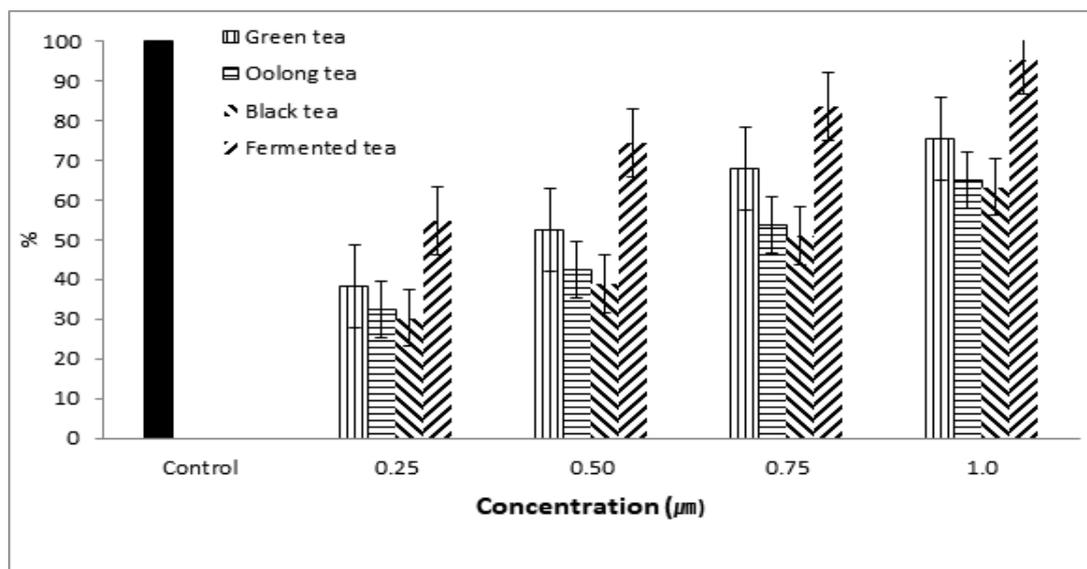


Figure 1. The rate of DPPH inhibitory of L-ascorbic acid (positive control) and relative inhibitory rate for green, oolong, black, and fermented teas at different concentrations.

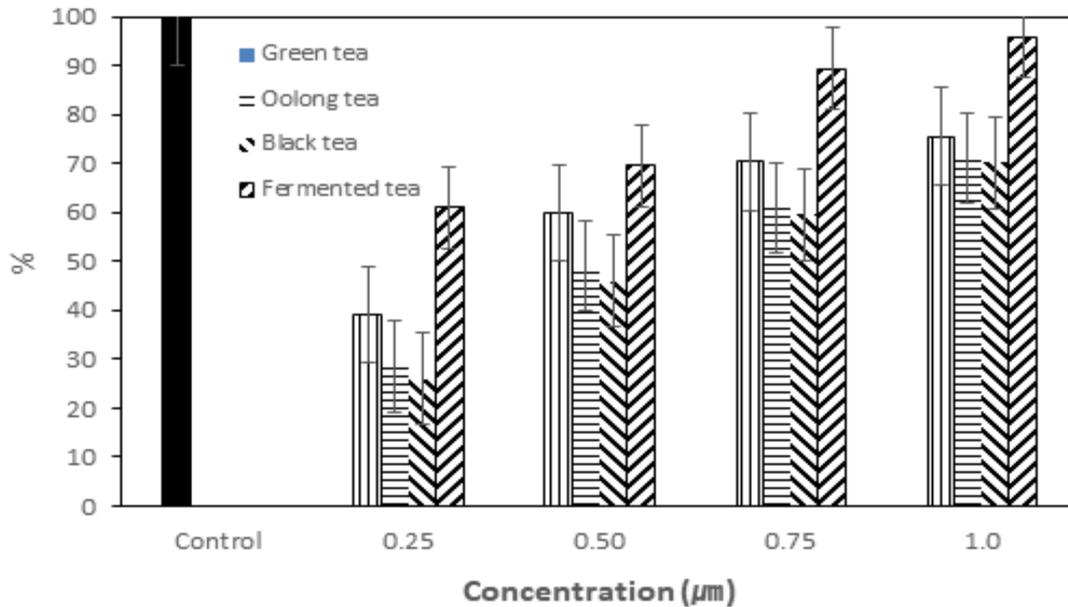


Figure 2. The rate of ABTS inhibitory of Trolox (positive control) and relative inhibitory rate for green, oolong, black, and fermented teas at different concentrations.

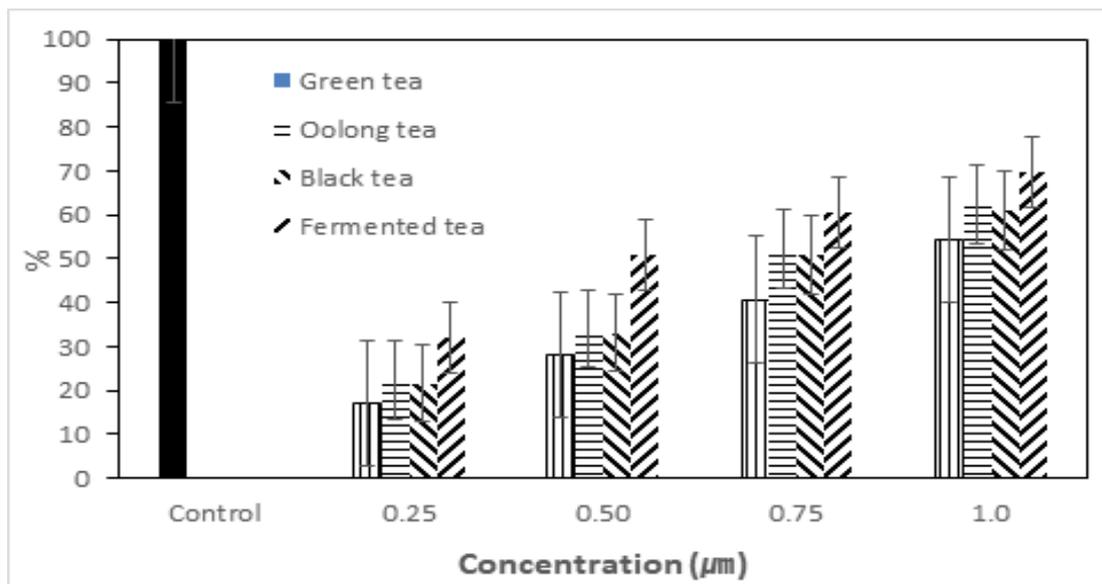


Figure 3. The rate of APX inhibitory of H₂O₂ (positive control) and relative inhibitory rate for green, oolong, black, and fermented teas at different concentrations.

DISCUSSION

Fresh tea leaf is unusually rich in the flavanol group of polyphenols known as catechins which may constitute up to 30% of the dry leaf weight.^[15] Other chemicals that contribute to the flavor and effects of tea (but to a lesser degree) include caffeine and amino acids, mainly theanine. Caffeine is present at an average level of 3% along with very small amounts of the other common methylxanthines, theobromine and theophylline. Gramza et al.^[16] suggest that green tea activity is the result of the presence of considerable quantities of catechins. In black tea, however, catechins occurred in considerably lower quantities. The catechin and caffeine contents of Jeju fermented tea were 13.41 and 4.42 mg/g, respectively.

Thus, catechin and caffeine contents of Jeju fermented tea were higher than those of unfermented (green) tea and black tea. The chemical composition is different in each type of tea. The chemical quality parameters and sensory evaluation of oolong or black teas changed with method of plucking. This is because of chemical changes during processing of the fresh leaves, mainly caused by fermentation as well as oxidation. Jeju 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. There have been many fermented teas in Korea since the past. For example, Ddeok-cha is the traditional one that Korean nation have produced and had from the era of the Three Kingdoms to the years of 1940 and naturally fermented one convenient to carry and keep.^[17]

Some research investigated that black, oolong, and green teas possess the antioxidant activity.^[18] The antioxidant activity of green tea extracts was higher than oolong tea and black tea extracts.^[16] The radical scavenging DPPH of the oolong and black tea extract is more weakness than green and white tea extract.^[19] The antioxidative effect of five tea extracts (green tea, black tea, oolong tea 861, oolongtea732, and jasmine green tea) that protect against oxidation and melanoma production, with green tea and jasmine green tea showing the lowest cell viability when tested against malignant melanoma cells.^[20]

DPPH, ABTS, and APX scavenging activities were shown a significant difference between Jeju fermented tea and other three teas ($p < 0.05$). Tea extracts, generally in powder or paste form, may be prepared from fresh green tea or from fermented tea (black tea). The number of extraction stages depends on the efficiency of the equipment used.^[21] It is the method that is being employed industrially for large scale production today. After basic processing, teas may be altered through additional processing steps before being sold and is often consumed with additions to the basic tea leaf and water added during preparation or drinking. Examples of additional processing steps that occur before tea is sold are blending, flavoring, scenting, and decaffeination of teas. The Jeju fermented tea is the bottle tea on an industrial scale. In the process of setting the standard for absorbance, the Jeju fermented tea was found that it was manufactured from a concentrate much thicker than other tea products. It was judged that these strong concentrate had a high antioxidant function. The effect of additives to tea may also depend on the type of tea, but the few studies on this topic have mostly focused on black tea.

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