Scientific Research Publishing

Food and Nutrition Sciences, 2023, 14, 1043-1056
https://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

Influence of Tea Leaf Particle Size on Catechin Extraction and Green Tea Sensory Acceptance

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How to cite this paper: Li, D.Y., Zhang, Y., Tamura, R., Nakajima, T. and Caballero, Y. (2023) Influence of Tea Leaf Particle Size on Catechin Extraction and Green Tea Sensory Acceptance. *Food and Nutrition Sciences*, **14**, 1043-1056.
https://doi.org/10.4236/fns.2023.1411066

Received: June 21, 2023
Accepted: November 24, 2023
Published: November 27, 2023

Abstract

The intensity of the bitterness of catechins increases with increased concentration, but the taste palatability decreased in green tea extract. The aim of this study was to investigate whether a blend of tea leaf particles of various sizes would result in a good balance between catechin content and appreciable taste. The control is common tea (CT) with 6 - 10 mm long leaves. Blend tea (BT) was prepared by mixing 5 mm and 120 - 130 mm long tea leaves in a ratio of 3:2. The catechin content of hot water extracts was analyzed by HPLC, and the sensory test was conducted with 99 volunteers. In BT, the total catechin content was significantly higher than that in CT. The sensory test results revealed that BT was less bitter and had more preferable color than CT. More catechins were extracted from BT, but it tasted less bitter. Thus, the recalibration of the tea leaf particle size can result in good balance between catechin content and palatability.

Keywords

Blend Tea, Catechin Content, Sensory Test, Bitterness, Palatability

1. Introduction

Green tea (*Camellia sinensis* L.) is one of the most popular beverages consumed worldwide [1]. The daily consumption of tea is associated with many important health benefits because of its high polyphenol content [2] [3] [4] [5]. To increase the functional activity of green tea, agitation, circulation, ultrasonic and microwave irradiation, and enzyme treatments have been conducted for enhancing the extraction efficiency of polyphenols from green tea leaves [6] [7]. It has also been demonstrated that reducing the particle size of tea leaves to increase the surface area improves polyphenol extraction efficiency [8]. Catechins are the most important functional polyphenols in green tea, and show biological benefits because of their strong antioxidant and anti-angiogenic activities as well as their potential to inhibit cell proliferation and to modulate carcinogen metabolism [5] [9] [10] [11]. Dry tea leaves contain 14% - 25% catechins [12]. The main constituents of green tea catechins are (−)-epigallocatechin gallate (EGCg), gallocatechin (GC), epicatechin (EC), epigallocatechin (EGC), and epicatechin gallate (ECG) [13] [14] [15].

In addition, the caffeine content of tea leaves ranges from 3.9 to 4.6 mg/g, depending on the age of the leaf—higher caffeine concentrations are found in younger leaves [16] [17]. Caffeine positively stimulates the cognitive function and has the potential to improve task performance in our daily work [18]. Both catechins and caffeine confer bitterness and astringency to green tea, which are the main constituents that contribute to the green tea taste. Therefore, the taste of green tea can change depending on the catechin and caffeine contents [19] [20]. Polyphenol-rich green tea with less taste of bitter and astringent is ideal for daily consumption and is readily meets the commercial needs of green tea market.

The international market for Japanese green tea shows an annual increase. The world trade of green tea increased from 1000 to 2000 tons in 10 years from 2005 to 2015 (Japan Tea Central Public Interest Incorporated Association). A previous study showed that the preference for green tea among non-Asians was different from that among Asians, because sensory perception varies among cultures [21]. Even among Asians, who consume green tea every day as a beverage, the preference for green tea varies depending on the countries [22]. Furthermore, studies comparing impressions and appreciation of green tea between countries are limited, especially between Japanese and non-Japanese Asians.

2. Materials and Methods

2.1. Theoretical

The taste characteristics of catechins consist primarily of the astringency and bitterness of tea, and the intensity increases with catechin concentration. However, catechins have tastes that consisted primarily of astringency and bitterness, thus the palatability decreases with the increasing of the catechin content [23]. Herein, we hypothesized that a blend of tea particles of various sizes would result in a good balance between catechin content and the appreciable taste of the tea extract. Moreover, a sensory test was conducted to assess the preference of tea among people living in the United States, including Japanese and non-Japanese Asians.

2.2. Evaluation of the Sensory Taste

The green tea samples were kindly provided by the Tohoen Tea Company (Tokyo, Japan). Tea leaves were cut 5 mm and 120 - 130 mm long tea leaf particles by the cutting machine in the company used commercially. Blend tea (BT) samples were prepared with 60% of 5 mm and 40% of 120 - 130 mm long tea leaf particles. One common Japanese green tea product, made of 6 - 10 mm long tea leaf particles, has been used as a common tea (CT) sample. The BT and CT samples were measured 3 g of each in a glass pot and pour 200 mL of hot water (80˚C) and put without agitation for 3 minutes. After that, the pot was calmly swung for three turns and pour cups to make them the same concentration. Subsequently, to simulate the repetitive tea-making habits of many people, the tea leaves from the first brewing underwent a second brewing under the same conditions as those utilized in the first brewing. There were four samples in total: the first brewed samples of CT and BT and the second brewed samples of CT and BT.

Ninety-nine volunteers, including Japanese and non-Japanese subjects, participated in the sensory test conducted in a university, a shopping mall, and a seminar room in California, United States. The four tea samples were used simultaneously in the test. And the volunteers rinsed their mouths with water between samples.

The volunteers were instructed to evaluate five items: aroma strength, color preference, bitterness, sweetness, and overall impression. Before drinking, we asked the following two questions about the aspect and aroma of tea extraction: Do you like or dislike this color? How strong or weak is the aroma? After drinking, the volunteers were asked to evaluate the bitterness, sweetness, and overall impression of the four samples. The Likert scales from 0 to 6 were used for evaluation: from none or dislike to strong or like.

It was explained to the panelists that the sweetness was not from sugar but from the leaves themselves. Astringency was excluded because it could not be identified with bitterness among non-Asian people in our pilot study. Volunteers were asked to sign an informed consent if they agreed to participate in the survey. The protocol was approved by the Ethical Committee of Ochanomizu University (permission number: 2017-45).

2.3. Reagents and Chemicals

Standards of caffeine (>98.5%), EC (>99.0%), ECG (>99.0%), EGC (>99.0%), and EGCg (>99.0%) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). HPLC-grade methanol and acetonitrile used in HPLC were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

The tea extract samples were prepared according to the sensory test by brewing 3 g of leaves in 200 mL of 95˚C hot water for 3 min (n = 3). After the first brewing, the tea leaves were filtered and subjected to the second brewing under the same conditions as those utilized for the first brewing.

2.4. HPLC Analysis

The standard catechins were diluted in 10% ethanol (v/v) and diluted with Milli Q water. The tea extracts were filtered with 0.45 mL acrylic styrol filter, and diluted 10 times with Milli Q water. For green tea component analysis, 10 mL of the samples was loaded into the HPLC system. Each sample was analyzed in triplicate. The HPLC conditions were set according to the method published in the Shimadzu application news No. L304B. A Shim-park FC-ODS column (75 mm × 4.6 mm ID) was used for the separation (Shimazu, Kyoto, Japan). Mobile phase A was 10.0 mM (sodium phosphate buffer (pH 2.6)), and acetonitrile was used as mobile phase B. The flow rate was set as 1.0 mL/min. The column temperature was maintained at 40˚C. The UV detection wavelength was 270 nm. The gradient separation procedure was set as 7% phase B from 0.00 to 6.00 min, 20% phase B from 6.00 to 20.00 min, 50% phase B from 20.01 to 25.00 min, and 7% phase B at 25.01 min.

2.5. Statistical Analysis

All values are presented as mean ± standard error (SE) (n = 3). The data for the four samples were compared using an independent t-test with the SPSS software (IBM version 23). Scores of the sensory test were compared using one-way ANOVA with a Bonferroni post-hoc test. Principal component analysis (PCA) was performed to integrate all items and identify the characteristics of each sample.

3. Results

3.1. Sensory Test Result Evaluation

Table 1 shows the demographic characteristic of participants in this study. **Figure 1** shows the scores of the preference and strength of taste for all participants. As is shown in **Figure 1**, the score of the color preference of the first brewing BT sample was the highest, and there was a significant difference between first brewing BT and second brewing CT samples ($P < 0.05$).

The first and second brewing CT samples were bitterer than the first and second brewing BT samples; there was a significant difference in their scores and those of the second brewing BT samples ($P < 0.05$). There were no significant differences in the scores of sweet intensities among the four samples. However, the second brewing BT samples was the sweetest. The average score of aroma for the first brewing CT sample was the highest. However, there were no significant differences in the scores for the two groups. The scores of the overall impression were almost the same among the four samples, with no significant difference.

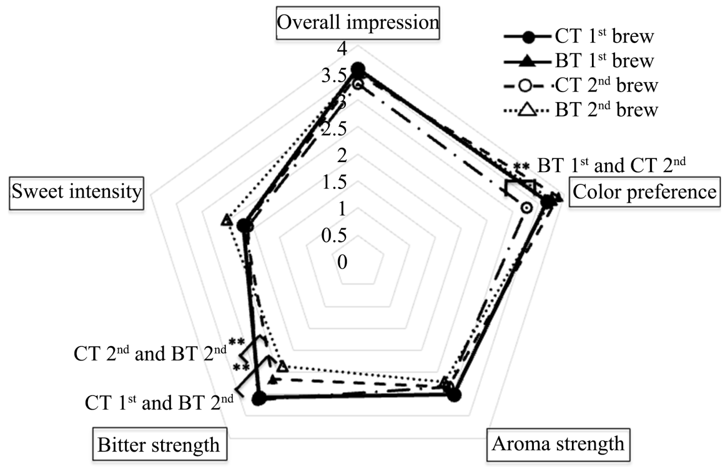


Figure 1. Preference and strength of taste (n = 99). Scale of overall impression and color preference: 6 = like extremely, 0 = dislike extremely; scale of sweet intensity and aroma strength: 6 = very strong, 0 = none. Statistical differences were determined using one-way ANOVA with the Bonferroni post-hoc test. ** $P < 0.01$.

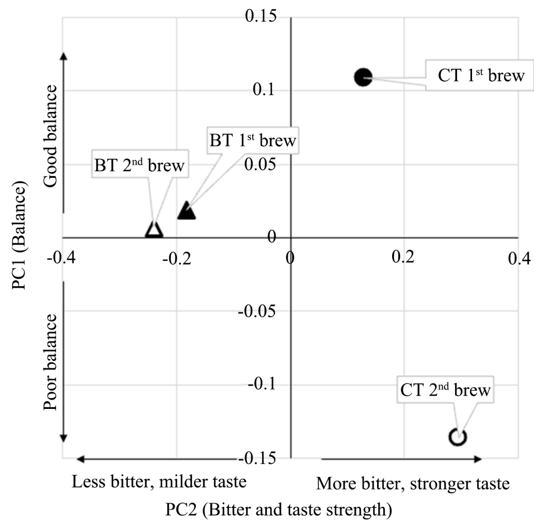
Table 1. Demographic characteristic of participants (n = 99).

Item	Japanese (n = 55)	Non-Japanese Asian (n = 23)	Non-Asian (n = 19)	Blank (n = 2)
Male	18	10	15	0
Female	35	13	2	0
Blank	2	0	2	2
Under 20	5	5	0	0
20’	4	15	11	0
30’	16	1	1	0
40’	5	2	4	0
50’	5	0	2	0
More than 60	10	0	0	0
Blank	5	0	1	2

Figure 2 shows the PCA results, which is an integration of the sensory test. PC1 accounted for 39.7% of all factors, and the values for each item were almost the same. Therefore, we named PC1 as a balance. PC2 accounted for 21.0% of the total. The bitterness values showed the highest contribution of all, and sweet intensity was relatively high. In contrast, the color preference values showed the lowest contribution. Thus, we named PC2 as the taste strength (bitterness and sweetness). The first brewing CT sample showed the highest level of PC1. In contrast, the second brewing CT sample showed the lowest level in PC1. The values for the first and second brewing BT samples were not as high as those of CT in both PC1 and PC2. However, they did not change drastically from the first to second brewing.

Component	PC1	PC2
Contribution (%)	39.7	21.0
Color preference	0.34	-0.49
Aroma strength	0.36	0.18
Bitter strength	0.20	0.74
Sweet intensity	0.26	0.24
Overall impression	0.39	-0.28

(a)



(b)

Figure 2. PCA results of the sensory test. Mean scores of the first and second brewing CT and BT samples in all participants (n = 99). PC1 indicated good balance because it consisted of almost the same values of items; PC2 indicated taste strength because values of bitter strength and sweet strength were high.

To clarify the ethnicity difference in tea preferences, the average scores of the five items were compared for each tea type. **Table 2** shows the mean scores of sensory attributes among ethnicities. Color preference and overall impression showed significant differences only in the first brewing CT sample. Non-Japanese Asians liked the color and overall impression of the first brewing CT sample more than participants of other ethnicities.

Furthermore, the score of overall impression for the first brewing CT sample among non-Japanese Asians was higher than that for any other tea sample. In contrast, the scores of color preference and overall impression for the first brewed CT samples by the non-Asians were the lowest because the first brewed CT samples were more bitter and more yellow than any other samples.

3.2. Concentrations of Catechins and Caffeine by HPLC

Use a zero before decimal points: "0.25", not 0.25". Use "cm^3", not "cc".

Figure 3(a) shows the HPLC analysis results of standards. Caffeine, EC, ECG, EGC, and EGCg were separated under the HPLC conditions described in the method section of HPLC analysis. The catechin component results are shown in **Figure 3(b)** and **Figure 3(c)**.

Table 2. Mean scores of sensory attributes for different ethnicities.

Tea type	Brewing time	Item	Japanese (n = 57)	Non-Japanese Asian (n = 21)	Non-Asian (n = 20)	Total (n = 98)
CT	First	Color preference	3.67 ± 1.27^{ab}	4.19 ± 1.21^{a}	3.00 ± 1.84^{bc}	3.64 ± 1.43
		Aroma strength	3.10 ± 1.16	3.00 ± 1.34	2.75 ± 1.59	3.01 ± 1.29
		Bitter strength	3.00 ± 1.41	3.00 ± 1.55	3.35 ± 1.87	3.07 ± 1.53
		Sweet intensity	2.42 ± 1.29	2.24 ± 1.58	1.75 ± 1.25	2.24 ± 1.36
		Overall impression	3.65 ± 1.36^{ab}	4.10 ± 1.26^{a}	2.68 ± 1.86^{c}	3.56 ± 1.51
BT	First	Color preference	3.99 ± 1.11	3.60 ± 1.31	3.75 ± 1.55	3.86 ± 1.25
		Aroma strength	2.99 ± 1.24	2.75 ± 1.07	2.45 ± 1.36	2.83 ± 1.24
		Bitter strength	2.84 ± 1.28	2.15 ± 1.27	2.65 ± 1.84	2.66 ± 1.42
		Sweet intensity	2.25 ± 1.20	2.15 ± 1.35	2.05 ± 1.19	2.19 ± 1.22
		Overall impression	3.56 ± 1.27	3.60 ± 1.10	3.05 ± 1.57	3.46 ± 1.31
CT	Second	Color preference	3.12 ± 1.26	3.13 ± 1.39	3.65 ± 1.23	3.23 ± 1.29
		Aroma strength	2.92 ± 1.32	2.78 ± 1.44	2.55 ± 1.39	2.81 ± 1.36
		Bitter strength	3.26 ± 1.44	2.70 ± 1.58	3.15 ± 1.27	3.10 ± 1.44
		Sweet intensity	2.23 ± 1.28	1.83 ± 1.40	2.20 ± 1.01	2.13 ± 1.26
		Overall impression	3.11 ± 1.53	3.30 ± 1.33	3.70 ± 1.08	3.28 ± 1.41
BT	Second	Color preference	3.62 ± 1.48	3.65 ± 1.27	4.20 ± 1.11	3.75 ± 1.37
		Aroma strength	2.83 ± 1.34	2.52 ± 1.40	2.45 ± 1.32	2.69 ± 1.35
		Bitter strength	2.42 ± 1.23	1.76 ± 1.00	2.64 ± 1.03	2.32 ± 1.18
		Sweet intensity	2.42 ± 1.26	2.76 ± 1.34	2.63 ± 1.30	2.54 ± 1.28
		Overall impression	3.35 ± 1.40	3.76 ± 1.34	3.68 ± 1.20	3.50 ± 1.35

Different letters indicate significant differences by one-way ANOVA with the Bonferroni post-hoc test at $P < 0.05$.

The overall concentration of catechins was significantly higher in the first brewing BT samples than in the first brewing CT samples ($P = 0.042$). The catechin content of the second brewing samples decreased compared to that of the first brewing samples, but there was no difference between the BT and CT samples (**Figure 3(b)**).

In the first brewing tea samples, the EGC content of the BT sample increased significantly compared with that of the CT sample ($P = 0.019$). Additionally, EC and EGCg contents were 12.1% and 19.8% higher in the BT extract than those in the CT, respectively. The GC, ECG, and caffeine contents were similar in the first and second brewed BT and CT extracts. The catechin and caffeine contents of the second brewing samples decreased significantly compared with those of the first brewing samples. No difference was observed between the second brewing BT and CT samples (**Figure 3(c)**).

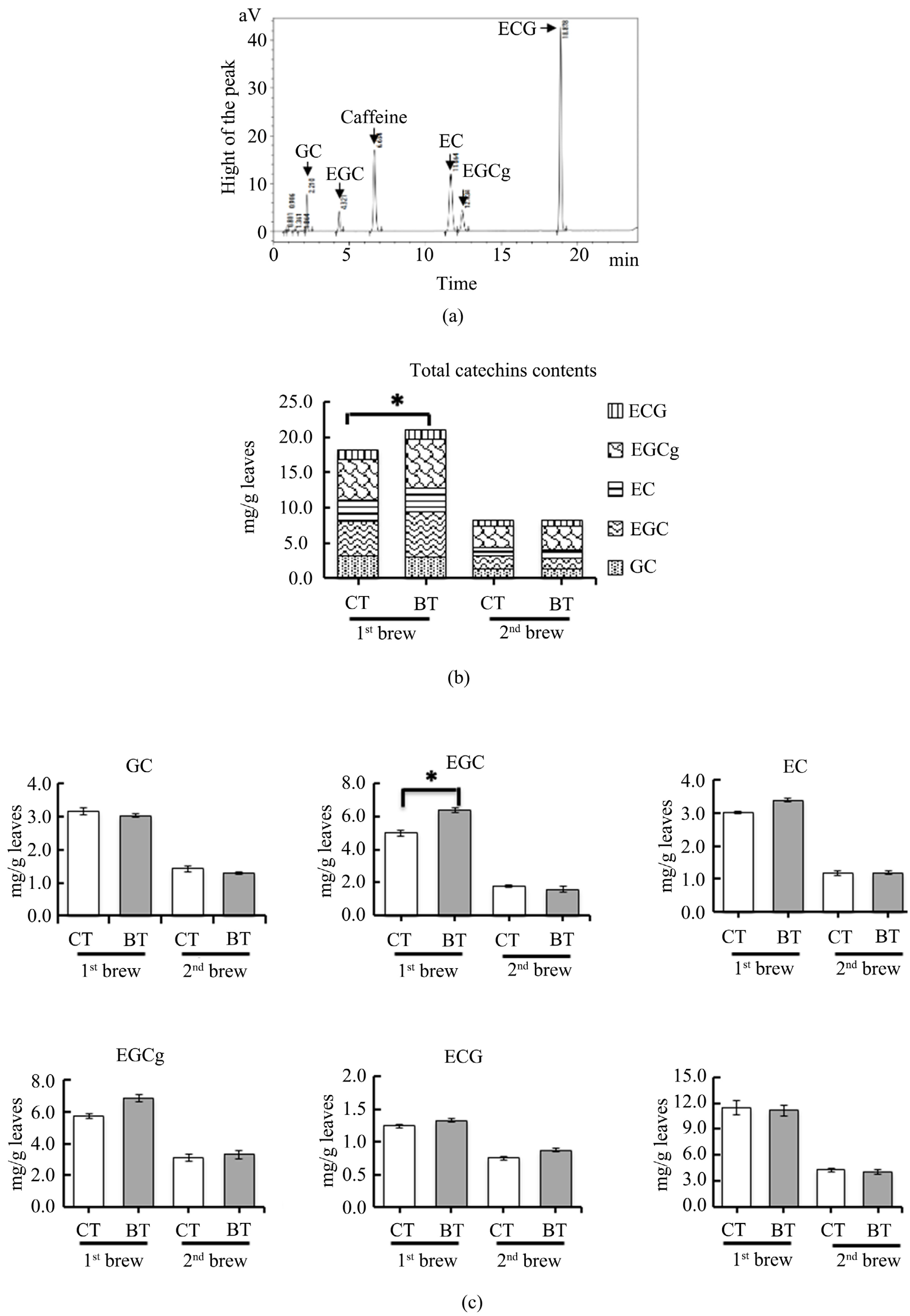


Figure 3. Results of catechins and caffeine components analysis. A. Standards of the components separated by HPLC; B. total catechin contents of the BT and CT samples; C. GC, EGC, EC, EGCg, ECG, and caffeine contents of the BT and CT samples. All values are expressed as means ± SE (n = 3). *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

4. Discussion

In this study, we compared blend teas with different leaf particle sizes with normal Japanese Sencha by using component analysis and sensory test. The BT sample possessed higher catechin content than the CT sample, and no difference in the overall acceptance was observed.

In our study, the particle size and mixing ratio were assumed to be optimal based on previous studies [24] [25] [26]. The milled tea leaves showed the highest antioxidant activity after extraction, but incurred a bitter and astringent taste unlike that of normal tea leaves [24]. Moreover, tea particles ranging from 100 to 180 μm had the highest catechin content (33.5 mg/g leaves) and the highest antioxidant activity. It seems that smaller tea particles (<100 μm) cause less catechin to be extracted, which may be due to the grinding conditions. During grinding, the high temperature increases biochemical alterations in food materials and may result in the modification of their physicochemical properties [25]. Thus, we chose tea particles of approximately 120 μm in size. The most popular type of green tea is Sencha, which is most commonly manufactured in Japan [27] [28]. Before the tea is brewed, the quality of Sencha can be evaluated based on the shape, color, and aroma of the tea leaves. However, during the manufacturing process, the tea leaves can be reduced into pieces and even powder during processes such as tapping and roasting [29]. Sencha with a mixture of large and small sizes is considered to be of low quality [30]. In this study, we found that mixing tea particles of different sizes release more antioxidants upon simple hot water brewing. Thus, unrefined Sencha can be promoted widely because of its easy manufacturing processes, low price, and high antioxidant yield.

The chemical composition is associated with the taste of teas in terms of bitterness, astringency, sweetness, and umami [31]. Catechins are known to confer astringency and bitterness to green tea, and the taste of catechin-rich tea tends to be bitter, unlike that of normally processed tea [24]. To reduce the astringency of tea extracts caused by these reduced tea particles, approximately 5 mm of leaves were blended into the tea samples. Moreover, 5 mm and 120 - 130 μm long tea leaf particles mixed in a ratio of 3:2 resulted in the desirable taste. According to the results of the component analysis, more catechins were extracted from BT samples. It was necessary to assess the acceptability of the product's taste among consumers. The taste analysis of catechins by human sensory evaluation and sensory test performed using the TS-500Z taste recognition system fitted with an AE1 and reference probes showed that ECG had the strongest taste intensity, followed by EGCg, EC, and EGC [23]. In our study, the EGC content of the BT samples increased significantly in relation to that of the CT samples, but induced less bitterness in the BT samples than that of the CT samples in the sensory test (**Figure 1**). It may also be induced by water-soluble carbohydrates and amino acids such as theanine, which contribute to the lower bitterness [32]. In fact, a previous study showed that extraction from smaller tea leaf particles facilitated an increase in the number of amino acids and water-soluble carbohydrates than that from larger ones [33].

EGC and EGCg are the main catechins in the green tea extract. In boiled water, the EGCg and EGC contents were approximately 30% and 40%, respectively [34]. EGCg is a predominant catechin and has several biological and pharmacological properties [35]-[41]. In our study, the EGCg content of the BT samples was higher than that of the CT samples (**Figure 3(b)**), which indicates that the beneficial effects of green tea were strong in the BT samples.

In the sensory test, there was no significant difference about overall impression though there was a significant difference in the bitter strength. It might because other factors like aroma also contributed to overall impression, and they offset the bitter strength. Ethnicity differences were expected between Asians and non-Asians. However, it was observed only in CT first brewing samples, while other types of tea did not show any significant differences between ethnicities. This result was different from some previous studies showing large differences between ethnicities in the palatability of green teas [21] [22]. This may be because the place where we conducted the study was California, the southern part of Los Angeles, where many Japanese restaurants are franchised, and green tea might have been commonly consumed in this region. A previous study reported that people who drink green tea regularly prefer green tea with higher flavor intensities, bitterness, and astringency [42]. Another study showed that the range of palatability was related to familiarity with green tea rather than cultural differences [22].

In our study, some non-Asians wrote that they did not like the color of green tea because it was similar to that of urine. The first and second brewing BT samples were greener than the first and second brewing CT; this might reflect the color preference. The participants were not asked to evaluate astringency intensity because many panelists in our trial survey could not understand the concept of astringency. Therefore, the relationship between each item from the sensory test and chemical components combined with catechins, caffeine, and amino acids should be further analyzed, especially in terms of bitterness and astringency.

In the PCA, the scores for the first and second brewing BT samples did not change in either PC1 or PC2 (**Figure 2**). This suggests that the second brewing BT sample maintains the same quality of taste and flavor as the first brewing BT sample, which may enhance its commercial value.

In this study, the first brewing BT sample had more catechins than the first brewing CT sample. If the catechin content of the tea samples is the same, the catechin content may decrease in the second brewing samples. However, in this study, no differences in the sensory test and component analysis results were observed between the second brewing CT and BT samples (**Figure 1** and **Figure 3**).

5. Conclusion

In conclusion, this study demonstrated that the tea extracts prepared from a blend of tea leaves with various particle sizes (*i.e.*, both 120 - 130 mm and 5 mm long) contain more catechins than those usually found in commercial tea products. Moreover, the higher antioxidant content did not alter the overall acceptance of the tea taste. Therefore, it is suggested to recalibrate the size of the tea leaves to small values to favor the extraction of catechins with antioxidant properties in commercial tea products.

Acknowledgements

We greatly appreciate Dr. Nami Fukutome's suggestions and guidance for this study. This study was supported by the Program for Leading Graduate Schools in Ochanomizu University, supported by the Ministry of Education, Culture, Sports, Science and Technology and the Japan Society for the Promotion of Science (JSPS).

Conflicts of Interest

All authors declare that they have no conflict of interest or financial conflicts to disclose.

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