ISSN: 2347-467X, Vol. 12, No. (2) 2024, Pg. 926-939

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Comparison of Waste from Different Types of Tea to Dried Butterfly Pea Flower

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Abstract

At present, the bioactive compounds of teas are well established and there are more attempts to apply tea as a functional drink or food supplement. In addition, during tea processing, tea waste, including broken tea leaves, buds and stems, is generated and disposed to the environment. The present study aimed to compare the quality of tea waste generated in full fermentation (raw Pu-erh tea, ripen Pu-erh tea and Anhua dark tea) and non-fermentation (green tea) processings in comparison with dried butterfly pea flower (DBPF) (non-*Camellia sinensis*). The results showed that total flavonoid (TFC) was found to be the highest in raw Pu-erh tea at 474.470 ± 47.173 mg RE/g, followed by ripened Pu-erh tea, green tea, Anhua dark tea, and DBPF, respectively. Similarly, raw Pu-erh tea also had the highest total phenolic (TPC) at 608.090 ± 2.795 mg GAE/g, followed by green tea, Anhua dark tea, ripen Pu-erh tea, and DBPF, respectively. On the other hand, total polysaccharides (TPS) content in DBPF was the highest among samples, whereas that of raw Pu-erh tea was the lowest. According to the HPLC analysis, caffeine (CF) was the major catechin found in all types of tea waste and the total catechin content was in the order of raw Pu-erh > ripen Pu-erh tea > green tea > Anhua dark tea which was in agreement with the TPC results. However, the types of catechin were different in each sample. It is noteworthy that there was no CF in DBPF, and major catechin found in DBPF was catechin gallate. Likewise, antioxidant ability determined by DPPH was the most superior in raw Pu-erh tea, followed by green tea, ripen Pu-erh tea, Anhua dark tea and DBPF, respectively. ORAC antioxidant capacity

Article History
Received: 12 January 2024
Accepted: 17 May 2024

Keywords
Antioxidant;
Catechins;
Dried butterfly pea flower;
Fermented tea.

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Doi: https://dx.doi.org/10.12944/CRNFSJ.12.2.34

was highest in the green tea, while that in raw Pu-erh tea, ripen Pu-erh tea and Anhua dark tea was lower but not significantly different, as expected, DBPF possessed the lowest ORAC antioxidant capacity. Finally, it can be said that the potential for tea waste to be a valuable source of antioxidants varies depending on the tea processing, and the plant variety also had an impact on the characteristics of the tea samples.

Introduction

Tea is produced from *Camellia sinensis* plant. It has a long history of medicinal use in Asian countries such as China, Japan, India, and Thailand as ancient as 500,000 years ago. Tea is the second most consumed beverage globally, behind water, with a daily intake of 500 mL per person, owing to its pleasant sensory aspects, extensive health advantages, and distinctive sociocultural traits.[1, 2] Tea leaves are popularly consumed in unfermented (green tea, Hunan), fully chemical and microbial fermented (Pu-erh tea, Yunnan) and fully microbiological fermented (Anhua dark tea, Hunan) forms.[3] Among the fully fermented tea products, Pu-erh tea needs a long fermentation time (60-70 days), whereas Anhua dark tea only needs 8-10 h. The different processes determined their unique chemical and sensory properties. The chemical compositions of tea mainly include total polyphenols (TPC), total polysaccharide (TPS), chlorophyll, and alkaloids.[3] Raw Pu-erh tea, ripen Pu-erh tea, green tea and Anhua dark tea have been documented for their advantageous constituents and antioxidant capacities.

Previous findings indicate that the tea processing produces a significant quantity of tea waste while converting fresh tea leaves into marketable products, these tea waste were primarily disposed of landfills or burnt, potentially it could lead to environmental harm.[4, 5] In recent decades, tea research mainly focused on the bioactive components of different tea varieties, while there was still a large gap in research on the utilization of waste tea biomass.[6]

According to the beneficial compounds in tea, there has been extensive research to identify the most efficient extraction method for the economical utilization of tea waste[4, 7-9] By examining the characteristics of these tea waste materials, an opportunity to utilize these tea wastes can be optimized, furthermore, it is possible to increase the value of these waste products.[4, 10] Among many putative bioactive compounds, polysaccharides found in tea and dried butterfly pea flower have several beneficial properties, such as preventing cardiovascular diseases, reducing inflammation, fighting against viruses, managing diabetes, controlling weight, protecting the nervous system, lowering blood pressure, and modulating the immune system.[9, 11] It also possesses antibacterial, anticarcinogenic, and antioxidant properties.[12, 13] Furthermore, proteins, polysaccharides, lipids, and terpenes are examples of natural polymers that have potential applications in food packaging.[14] Among many tea types, Pu-erh tea has become one of the popular and widely consumed teas because of its health benefits due to various bioactive compounds that are microbially produced during Pu-erh tea production. Pu-erh tea can be classified into two types: raw Pu-erh tea and ripen Pu-erh tea. Raw Pu-erh tea is made by drying the tea leaves, similar to green tea, and then allowing microbial fermentation to occur during storage. On the other hand, ripen Pu-erh tea is made by rehydrating the dried tea leaves to stimulate microbial activities, followed by a second drying process.[13] So far, biological activities have been observed in studies of Pu-erh tea including lipid adjustment, decreased blood sugar and cholesterol levels, anti-obesity and anti-inflammatory activity, immunity regulation, cytotoxic effects, and the prevention of metabolic syndrome.[15] Anhua dark tea undergoes a full microbiological fermentation process without the initial drying stage, which sets it apart from raw and ripened Pu-erh tea processing. After several days of microbial fermentation, the fermented tea leaves are dried and pressed to form a brick shape. Produced and consumed mostly in Hunan province in China, it facilitates the breakdown of fat, prevents blood clotting, dissolves fibrinogen, relaxes blood vessels, and inhibits the formation of atherosclerotic plaques. As a result, it reduces blood pressure, improves the flexibility of blood vessels, and helps

prevent cardiovascular diseases.[16] Anhua dark tea is gaining increasing attention recently,[3] because it contains various bioactive compounds with diverse biological functions and microbial fermentation plays a critical role in forming the numerous health benefits and flavor characteristics of this dark tea.[17-20] Besides, green tea is a non-fermented tea, it has a cold nature with good anti-inflammatory effects. According to previous research, green tea had the ability to hinder the rise of cholesterol levels in the human body by aiding in the breakdown of fat and green tea catechins may boost fat oxidation, energy expenditure, and insulin sensitivity;[21, 22] green tea polyphenols can lower blood lipids and blood sugar levels, as well as prevent the development of cardiovascular diseases, diabetes, and other illnesses,[23, 24] thus, consuming green tea can help prevent the onset of certain illnesses and enhance overall health.[3] Whereas, butterfly pea flower is a tropical vine plant that blooms throughout the year. It is mainly produced in Chiang Rai, Thailand, and also being found in Xishuangbanna, Yunnan, China. Dried butterfly pea flowers are abundant in vitamins A, C, and E and have a high concentration of anthocyanins, which can enhance the immune system, stimulate skin elasticity and collagen synthesis, and exert antioxidant properties.[25] Thai people and Xishuangbanna use it to make tea for drinking or dye food. With the continuous expansion of the use of dried butterfly pea flowers, the market demand for dried butterfly pea flowers is gradually increasing.

Based on prior research, it was found that tea contains significant amounts of biologically active components.[19, 26] With the increased consumption of tea and its extracts, the amount of tea waste is fast growing as a by-product generated during its processing which includes old tea leaves, broken tea leaves, stems, dust, and discolored tea leaves. The disposal of tea wastes not only creates massive biomass loss but also increases environmental stress. In recent years, there has been a lot of interest in using tea waste biomass.[27, 28]

The current study aimed to investigate total flavonoid (TFC), total phenolic (TPC), total polysaccharides (TPS), catechin, DPPH ability and ORAC antioxidant capacity of different types of tea waste from diverse tea manufacturings namely raw Pu-erh tea, ripen Pu-erh tea, green tea and Anhua dark tea and dried butterfly pea flower. It also explores the differences of these properties with dried butterfly pea flowers. Finally, the results of this study might promote the utilization of tea waste, therefore, bolstering sustainable practices within the tea sector.

Materials and Methods

Materials

Tea Samples

Five types of samples including the waste from fully chemical and microbial fermented tea (raw and ripen Pu-erh tea, Yunnan), fully microbial fermented tea (Anhua dark tea, Hunan), non-fermented tea (green tea) and dried butterfly pea flower were employed in this study.

Raw Pu-erh tea waste, ripen Pu-erh tea waste, green tea waste and Anhua dark tea waste were collected from the local tea producers. Pu-erh tea waste was obtained from Puer, Yunnan, China. Green tea waste and Anhua dark tea waste were obtained from Hunan, China. Dried butterfly pea flower was collected from local company in Chiang Rai, Thailand. The tea plants and butterfly pea flower were cultivated by local farmers and processed in May 2021 and the wastes were collected in the same year. The selection of the tea producers was carried out after an information survey taking into account the location of the tea factory, the availability of quality certificates, and the different types of tea grades produced by the tea factory. The tea wastes were then collected and homogeneously mixed from each stage of processing. The samples were maintained at a temperature of 5 °C under a vacuum condition in a bag made of nylon/ linear low-density polyethylene before being analyzed within a period of 3 months.

Chemical Reagents

The study employed a range of reagents, such as sodium fluorescein, gallic acid, rutin, folinol, sodium nitrite, aluminum nitrate, calcium chloride, and potassium dihydrogen phosphate, which were procured from McLean Biotechnology Co., Ltd. located in Shanghai, China. The following chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (China): sodium bicarbonate, magnesium chloride, potassium dihydrogen phosphate, ammonium carbonate, sodium hydroxide, sulfuric acid, DPPH reagent, anhydrous sodium acetate, glacial acetic acid,

ferric chloride solution, hydrochloric acid solution, and 240 mM AAPH ((2,2'-azobis (amidinopropane) dihydrochloride)). Tea polyphenol standards, including catechin (C), catechin gallate (CG), gallocatechin (GC), gallocatechin gallate (GCG), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG), were acquired from Shanghai Yuanye Biotechnology Co., Ltd. located in Shanghai, China. All the other substances used in this investigation were of analytical grade.

Methods

Extraction of Tea Samples

According to the experimental method of Yue Liu with slight modifications,[29] 50 g of samples were added with 1000 mL of water and heated at 70 °C for 30 min. The aqueous extract was then filtered using Whatman filter paper No.1, removed water by rotary evaporator (70 °C) and freeze-dried to obtain the extract powder. The extracts were stored at -20 °C for further analysis. Then according to different reaction systems, use powder to configure extraction solutions with different concentrations.

Total Flavonoid Content

With slight adjustments, the previously established sodium nitrite-aluminum nitrate colorimetry was employed to determine the total flavonoid concentration.[30] Rutin solutions at the concentration ranged 0, 50, 100, 200, 400, 600, 800 and 1000 µg/mL in 80% ethanol were used as the standard of the total flavonoid content. Briefly, the extract powder was dissolved in deionized water, then the solution (1.0 mL) was mixed with 0.3 mL of 5% sodium nitrite, followed by 0.3 mL of 10% aluminum nitrate and 2.0 mL of 4% sodium hydroxide.

After incubation at room temperature for 15 min, absorbance was recorded using a 96-well plate reader at 510 nm. Based on the concentration of Rutin, the total flavonid content was determined and expressed as mg of Rutin equivalent (RE) per g of tea powder.

Total Phenolic Content and Catechin Profile

Total phenolic content was determined by using Folin-Ciocalteu reagent. Gallic acid was used as standard Gallic acid was used as standard and treated in the same manner.[28] Briefly, each tea waste extract solution (0.1 mL) was mixed with 2 mL of distilled water, followed by 0.2 mL of Folin phenol and 0.9 mL of 20% sodium carbonate. After incubation at room temperature with darkness for 2 hours, absorbance was recorded using a 96-well-plate reader at 756 nm. Based on the concentration of gallic acid, the total phenolic content was determined and expressed as mg of gallic acid equivalent (mg GAE) per g of tea powder.

Then catechin contents were measured by high-performance liquid chromatography (HPLC). Agilent 1260 HPLC system was used in this analysis (Agilent Technologies, Santa Clara, USA). The column used was a Zorbax SB-C8 with a UV detector at 280 nm. The mobile phases were methanol (25%) and trifluoroacetic acid (TFA) (57%) with the injection volume at 10 µL. The catechins were reported in µg/mL of tea extract.[31]

Total Polysaccharides

The TPS content was determined according to the method described previously.[32] Briefly, each tea extract solution (1 mL) was mixed with 1 mL of 5% phenol solution, followed by 5 mL of sulfuric acid solution at room temperature for 10 min. Then it was incubated in water bath at 30 °C for 20 min. Absorbance was recorded using a microplate reader with a 96-well-plate reader at 490 nm. Based on the mass concentration of glucose, the total polysaccharides were calculated.

Antioxidant Activity Determination

Oxygen Radical Absorbance Capacity (ORAC) Assay

According to the previous description,[33] 1 mmol/L of Trolox was used as a stock solution, and the serial concentrations of 2.5, 12.5, 25, 50 and 100 µmol/ were prepared. Each well of the plate was filled with 75 µL of 1.9 M fluorescein in a 75 mM sodium acetate buffer (pH 5.44) before 50 µL of the sample, a Trolox control (50 µM), or buffer was added. The peroxyl radical generator, 240 mM AAPH (2,2'-azobis (amidinopropane) dihydrochloride), was then added to the plate after the plate was incubated at 37 °C for 10 min. This reaction started the process in which fluorescein was slowly oxidized, resulting in a decrease in fluorescence emission. As soon as possible, the plate was put into the microplate reader to begin the kinetic assay, which involved taking fluorescence values every 60 sec for 90 min. Excitation and emission wavelengths were

adjusted to 485 nm and 518 nm, respectively, to observe fluorescence quenching. The ORAC value was determined by comparing the net area under the fluorescence curve for each sample to a Trolox reference. A depiction of the antioxidant strength in terms of mmol Trolox per g sample was produced after adjusting for the blank and comparing the net AUC supplied by each sample to that of Trolox.

DPPH Scavenging Assay

50 µL of the sample solution was mixed with 150 µL of DPPH (0.4 mmol/L, made with methanol), which was then left to stand at room temperature in the dark for 30 min before the absorbance at 517 nm was measured with a spectrophotometer.34 Trolox concentrations ranging at 0, 50, 100, 200, 300, 400, 500 µM/mL were used to assess the DPPH scavenging.

Statistical Analysis

The experiment was performed in triplicate. Data was presented as means ± standard errors of the mean (SEM). Differences between groups were examined through one-way analysis of variance, SPSS software was used to conduct analyses of variance base on the General Linear Model and LSD test was used to evaluate the differences between means ($p < 0.05$). Graphpad prism10 was used for visual analysis of all data ($p<0.05$).

Results and Discussion

Quantitative Analysis of Total Flavonoids (TF), Total Phenolic Content (TPC) and Total Polysaccharides (TPS) Content in Different Tea Waste and DBPF

According to the tea processing technology and fermentation degree, tea can be divided into different teas: green tea is unfermented tea, raw and ripen Pu-erh tea are fully chemical and microbial fermented tea, and Anhua dark tea is post-fermented or microbially fermented tea. Under moist and oxygen conditions, polyphenol oxidase in tea leaves and fermentation can oxidize tea polyphenols into corresponding oxidation products, producing various tea leaves with different flavors and qualities.[35] Fermentation has a dynamic effect on the accumulation of tea compounds, and different tea samples can be distinguished based on changes in compounds.[3]

Figures 1-3 show the TF, TPC and TPS, respectively, in different tea wastes and DBPF. The highest content of TF values was observed in Raw being 474.47±47.17 mg RE/g followed by Ripen, Green, Anhua and DBPF, respectively. The lowest TF content was seen in DBPF being 24.47±0.74 mg RE/g. Similarly, the highest TPC was also found in Raw at 608.09±2.80 mg GAE/g, followed by Green, Anhua, Ripen and DBPF, respectively, whereas that in DBPF was noted at the lowest value being 48.09±1.67 mg GAE/100g. TPS content, on the other hand, was found the highest in DBPF (0.053±0.007 g/100g) whereas Raw had the lowest content (0.014±0.001g/100g) compared with the rest of tea waste samples. These results indicate that tea variety (compared between tea plant and flower tea) and production process (different tea types) directly affect the content of TF, TPC and TPS. Previous study also discovered that different tea types had different TPC and TF values. Ripen and raw Pu-erh tea contained TPC at 23.68 g/100 g and 53.26 g/100 g, respectively, whereas TF values were 11.61 and 16.52 g/100 g, respectively (extraction at 100 °C for 15 min).36 Likewise, it was reported that TPC values were 182 mg/g and 110 mg/g, and the TF values were 98 mg/g and 49 mg/g, respectively in raw and ripen Pu-erh tea macerated for two hours using 60% aqueous acetone extracts.[37] Similar to the current findings, raw Pu-erh tea had the superior amount of TPC than ripen Pu-erh tea. The previous research reported that the amount of TF and TPC was in the order of raw Pu-erh tea, green tea, white tea, yellow tea, oolong tea, black tea and dark tea, and the differences between tea types were significant,[38] which was consistent with the trend of the results of this experiment. Processing techniques form different categories of tea, and the degree of fermentation is the key factor for differentiation. Green tea is an unfermented tea, while Pu-erh tea needs a long fermentation time (60-70 days), whereas fully microbiological fermentation (Anhua) only needs a short time (8-10 h).[39-41] As the degree of tea fermentation continues to deepen, the average content of total polyphenols in chemical and microbial fermented tea (Raw and Ripen) is higher than that in microbiological fermented tea (Anhua), indicating that the fermentation process has a great impact on tea polyphenols. During the fermentation process, polyphenol oxidase

can convert polyphenols into compounds such as theaflavins and epigallocatechin dimeric quinones, and the pyrogallol structure is more easily oxidized than the catechol and monophenol structures in the B ring.[42] The tea total polyphenols of raw Pu-erh tea were higher than those of ordinary green tea, because Pu-erh is a large-leaf tea, and the content of polyphenols is relatively high. In addition, most of the tea total polyphenols of ripen Pu-erh tea has been converted into other components that may be beneficial to the human body, such as tea pigments and tea polysaccharides. However, some tea polyphenols have not been completely transformed, but they are far smaller than ordinary green tea. Thus, the TPC content was found in Raw > Green> Anhua > Ripen. The polyphenols in tea are mainly the catechin EGCG (with a pyrogallol structure), resulting in a significant decrease in the total polyphenols content in the tea samples after chemical fermentation.[43] The TPC of DBPF extract was significantly lower than the other tea samples (48.090±1.669 mg GAE/g) which was close to the range reported earlier (41- 65 mg GAE/g).[44] The plant species clearly influenced the bioactive chemicals found in the samples, likely because of the varying levels of polyphenol accumulation in various parts of the plant. This accumulation is closely tied to the activities of these compounds throughout the plant's life cycle and development stages.[45, 46] Not only TPC and TFC are recognized as crucial bioactive chemicals in tea and DBPF, TPS has also been shown to be present in tea and DBPF, contributing to their health benefits.[47] Several studies have demonstrated that TPS exhibited anti-inflammatory and antioxidant properties.[48, 49]

It has been reported that different fermentation degrees and preparation methods led to the alteration of the physical structure and TPS. Based on dry tea weight, the polysaccharide content of oolong tea (4.6% ± 0.2%) was higher than that of green tea polysaccharide (4.0% ± 0.3%) and black tea polysaccharide (4.2% ± 0.3%),47 while the polysaccharide content of Pu-erh tea was 1.21%,[48] which was consistent with the trend of the results of this experiment, the ranking of TPS content was in the order of green tea waste > Anhua dark tea waste > ripen Pu-erh tea waste > raw Pu-erh tea waste. When comparing the aforementioned TPS contents in the tea products with that found in the waste in this current work, TPS contents in green tea waste and black tea waste were lower than their tea products being 0.036 ± 0.002% and 0.023 ± 0.001%, respectively, and that in the waste of raw Pu-erh tea and ripen Pu-erh tea were lower than the value reported in Pu-erh tea product being 0.014 ± 0.001 % and 0.017±0.001 %, respectively.[47, 48] Currently, there are no reports comparing the TPS content in tea products and their wastes. However, one of the variables that influences the TPS concentration in tea is the plant organs. Typically, the TPS content in young leaves and leaf buds is lower compared to mature tea leaves.[49] The reduced TPS contents in the tea wastes in this experiment are attributed to this cause, as the waste comprised fragmented segments of young tea leaves. DBPF had the lowest TPC and TF as it is a non-*Camellia sinensis* plant, but it had the highest content of TPS. Some studies have indicated that plant polysaccharides primarily enhance macrophage immune responses, resulting in immunomodulation, anti-tumor effects, wound healing, and other therapeutic benefits.[50]

Among these benefits, the hypoglycemic effect has been extensively researched.[51] TPS may alleviate diabetic symptoms and prevent the condition by inhibiting digestive enzyme activity, improving insulin resistance, regulating gene and protein expression, and influencing gut flora.[52] In addition, it was demonstrated that TPS diet could lower blood sugar levels, reduce inflammation, and lower cholesterol levels, thereby reducing the risk of cardiovascular disease.[35, 53] Therefore, polysaccharide can be one of the bioactive compounds in the tea waste and DBPF that possesses potential health benefits. It is important to mention that the bioactivity of DBPF might be greatly due to its TPS content, which is different from tea waste where the major bioactive substances encompass TPC and TFC.[54]

Phenolic Profile Analysis in Different Tea Waste and DBPF

From the results of total phenolic content (Figure 2), it is important to identify the type of catechin in the samples. Table 1 shows that raw Pu-erh tea extract had the highest total catechin content and it comprised four major compounds namely GC, EGC, EC, and CF while the ripen Pu-erh tea extract contained only CF. Green tea had the second rank of the catechin content including GC, EGC, EC, and CF which slightly higher than that in raw Pu-erh tea. Anhuah dark tea and DBPF had similar extent of

catechin content, however, GC and CF were found in Anhua dark tea extract whereas that of DBPF were GC and CG (Table 1). It can be seen that the dominant catechin in these samples was CF which is consistent with other studies reporting caffeine as the main compound observed in various tea samples.[55-57] the order of CF content in tea samples was raw Pu-erh > ripen Pu-erh tea > Green tea > Anhua dark tea. As expected, there was no CF detected in DBPF but the major compound found in DBPF was CG.

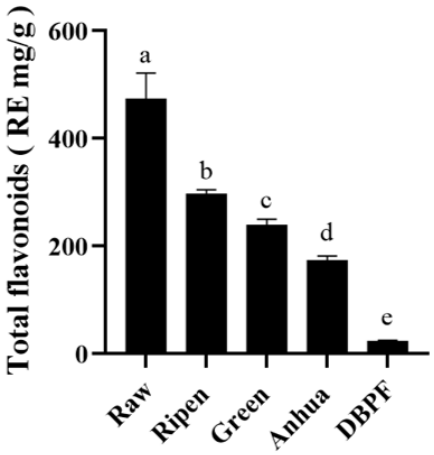

Fig. 1: Total flavonoid content (RE mg/g) in different tea waste and DBPF

Note: Raw = Raw Pu-erh tea, Ripen = Ripen Pu-erh tea, Green = Green tea, Anhua = Anhua dark tea, DBPF = Dried butterfly pea flower; Different letters indicate statistically significant difference ($p < 0.05$)

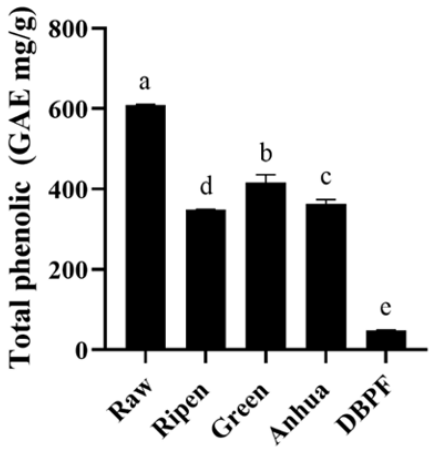

Fig. 2: Total phenolic content (GAE mg/g) in different tea waste and DBPF

Note: Raw = Raw Pu-erh tea, Ripen = Ripen Pu-erh tea, Green = Green tea, Anhua = Anhua dark tea, DBPF = Dried butterfly pea flower; Different letters indicate statistically significant difference ($p < 0.05$)

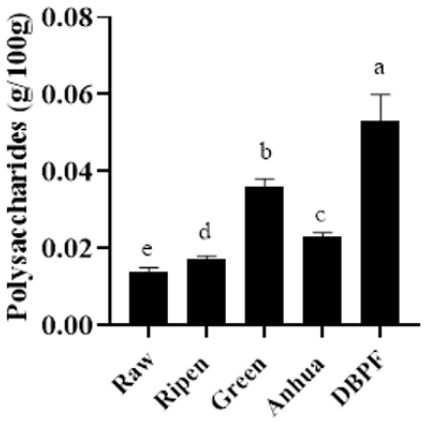

Fig. 3: Total polysaccharides content (g/100g) in different tea waste and DBPF

Note: Raw = Raw Pu-erh tea, Ripen = Ripen Pu-erh tea, Green = Green tea, Anhua = Anhua dark tea, DBPF = Dried butterfly pea flower; Different letters indicate statistically significant difference ($p < 0.05$)

Between two types of Pu-erh tea, chemical and microbial fermentation products, it was observed that raw Pu-erh tea had greater levels of tea polyphenols and catechins (such as GC, EGC, EC, and CF). Previous study reported that polyphenols in raw Pu-erh tea samples were higher than ripen Pu-erh tea, with average concentrations of 269.23 ± 6.06 mg/g.[58] This outcome was in agreement with previous study where the previous research also discovered that the total catechin concentration of raw Pu-erh tea samples was much greater than that of ripen Pu-erh tea samples.[57, 59, 60] This might be due to the different processing of this two types of tea. The duration of the fermentation process and the impact of microorganisms determine how much of these substances are present. It should be noted that raw-Pu-erh tea is normally kept for very long fermentation time. Aspergillus fumigatus and Aspergillus niger that grow during fermentation may both raise the amount of gallic acid in tea when the fermentation process is prolonged, although

Aspergillus niger could decrease the caffeine level.[61] Raw Pu-erh tea (20.818 ± 0.320 µg/mL) had about three times higher total catechin concentration than that of Anhua black tea (7.752 ± 0.030 µg/mL) (Table 3). Based on this research, the unfermented green tea had a total catechin level of 15.676 ± 0.220 µg/mL, which was more than that of fully fermented tea samples. However, the decrease in total polyphenol levels after fermentation was not as significant as the decrease in catechin composition. This is thought to be due to conversion of catechins to polyphenols as a result of oxidation reaction occurred during fermentation. According to the previous research reported, one of the key criteria for categorizing the six main groups of tea is the degree of oxidation of the products.[62] Whereas, Anhua dark tea is a microbiological fermented tea, the addition of fungi during the microbiological fermentation process resulted in a notable increase in volatile organic compounds, as well as a stale taste and mushroom aroma, which enhanced the dark tea's sensory quality,[63] and total polyphenols, flavonoids, thearubigins, theaflavins, and gallocatechins were also significantly reduced.[64] Thus, total catechin order was Raw > Green > Ripen > Anhua >DBPF.

Table 1. Catechin content (µg/mL) in different tea wastes and DBPF determined by HPLC

Samples	GC	EGC	C	EC	EGCG	CF	GCG	ECG	CG	Total(µg/mL)
Raw	1.536±0.020	2.099±0.122	0	2.482±0.111	0	14.701±0.068	0	0	0	20.818± 0.320
Ripen	0	0	0	0	0	13.129±0.158	0	0	0	13.129±0.160
Green	1.330±0.144	3.286±0.019	0	0.772±0.025	0	10.288±0.036	0	0	0	15.676±0.220
Anhua	0.460±0.004	0	0	0	0	7.292±0.027	0	0	0	7.752±0.030
DBPF	0.973±0.013	0	0	0	0	0	0	0	6.524±0.003	7.497±0.020

Note: Raw = Raw Pu-erh tea, Ripen = Ripen Pu-erh tea, Green = Green tea, Anhua = Anhua dark tea, DBPF = Dried butterfly pea flower; Gallocatechin (GC), Epigallocatechin (EGC), Catechin (C), Epicatechin (EC), Epigallocatechin-3-gallate (EGCG), Caffeine (CF), Gallocatechin gallate (GCG), Epicatechin-3-gallate (ECG), Catechin gallate (CG)

DPPH and ORAC Assays in different Tea Wastes and DBPF

Antioxidant properties in all samples were analyzed. The antioxidant activity of tea is directly related to its phenolic type and content. The antioxidant effects of the tea extract could be regarded as early findings as there was a substantial body of research linking oxidative stress to a number of disorders.[65] According to previous research, it was reported that the DPPH and ORAC assays had been widely used to study the antioxidant activity of many food, such as blood orange juice and blueberries.[66, 67] Thus, in this work, extracts of Raw, Ripen, Green, Anhua tea wastes, and DBPF were tested for their in vitro antioxidant activities using DPPH and ORAC assays. It was observed that raw Pu-erh tea and green tea performed better than the other extracts in term of antioxidant potential (Figures 4 and 5). As shown in Figure 4, the antioxidant ability as determined by DPPH assay was found the highest in raw Pu-erh tea (271.752±19.782 µM TE/g) following with green tea (262.294±11.645 µM TE/g). Ripen Pu-erh tea, Anhua dark tea and DBPF showed less antioxidant property. Similarly, ORAC antioxidant capacity was found the highest in the green tea at 596.984±13.483 µmol/L, followed with raw Pu-erh tea, ripen Pu-erh tea, Anhua dark tea and DBPF. Obviously, DBFP had the lowest ORAC compared with all tea waste samples.

The type and amount of catechin in the samples were different as mentioned earlier (Table1). Moreover, TPS also had been reported to possess antioxidant activity. It had a higher antioxidant activity in less fermented teas (i.e. green tea).68 Therefore, the antioxidant activity varies based on the TPS content: Green > Raw > Ripen > Anhua. According to certain studies green tea was superior to oolong tea and Pu-erh tea.[69] Previous report demonstrated that the antioxidant activity of processed tea from the same cultivar decreased with increasing oxidation degree.[70] This suggests that the process of manufacturing tea affects its properties. Furthermore, the utilization of butterfly

pea flower extract in the fermentation process of a kombucha-like beverage resulted in a progressive enhancement of antioxidant activity throughout the fermentation duration. This enhancement was primarily influenced by the concentration of phenolic compounds presented.[54] Thus, the antioxidant qualities of various tea varieties also differ when all of this is considered.

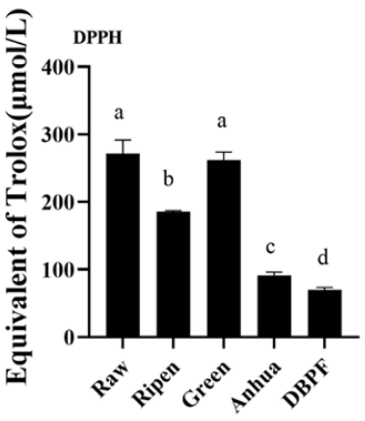

Fig. 4: DPPH scavenging property of different tea wastes and DBPF

Note: Raw = Raw Pu-erh tea, Ripen = Ripen Pu-erh tea, Green = Green tea, Anhua = Anhua dark tea, DBPF = Dried butterfly pea flower; Different letters indicate statistically significant difference ($p < 0.05$)

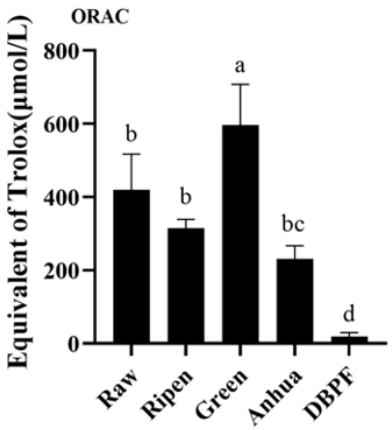

Fig. 5: The ORAC antioxidant capacity of different tea wastes and DBPF

Note: Raw = Raw Pu-erh tea, Ripen = Ripen Pu-erh tea, Green = Green tea, Anhua = Anhua dark tea, DBPF = Dried butterfly pea flower; Different letters indicate statistically significant difference ($p < 0.05$)

Conclusion

This study revealed that tea waste extract had a potential to be utilized as a good source of antioxidant, the properties were more superior when compared with DBPF. Even though raw Pu-erh tea had the highest content of TPC and TF as well as total catechin content but the FRAP and DPPH values were not significantly different from green tea indicating that not only the quantity but the type of bioactive compound also greatly affects functional properties of tea. In addition, DBPF had the highest TPS compared to tea wastes, which may partly contribute to their antioxidant effects. Further research should be carried out, in vitro and in vivo, to clarify more functions and their mechanisms of action including the inhibition of enzymes associated with blood sugar levels, anti-inflammatory effects, and immunomodulation. Conducting a thorough study on tea waste extracts allows us to discover the possible health benefits of these extracts and propose particular medical and functional applications for them in the future. It, therefore, has the potential to completely transform the nutraceutical sector, providing groundbreaking solutions for improving overall health and well-being. Furthermore, harnessing the bioactive components found in tea waste extracts can support sustainable practices in the tea business, encouraging environmentally responsible methods of waste management while optimizing the use of precious resources.

Acknowledgements

The authors would like to thank Mae Fah Luang University, Chiang Rai, Thailand, for the technical and financial support. Thanks also go to Laboratory of food function and nutrigenomics, College of food science and technology, Hunan Agricultural University, Changsha, China.

Funding Sources

This work was supported by Mae Fah Luang University.

Conflict of Interest

All authors have no conflict of interest.

Authors' Contribution

Each author mentioned has significantly and directly contributed intellectually to the project and has given its approval for its publication.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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