Comparison of the main compounds in Fuding white tea infusions from various tea types

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Abstract The purpose of this study was to compare the main components, and particularly catechins, caffeine, theanine, free amino acids, and water extracts, in Bai Hao Yin Zhen, Bai Mu Dan, and Shou Mei Fuding white tea infusions brewed at different temperatures, and their contributions to taste were estimated by dose-over-threshold value. Infusion temperature had a distinct effect on the main components extracted, and 100 °C was found to be optimal for extracting catechins and caffeine. However, the effect of temperature on theanine, free amino acids, and water extracts varied with tea type. Bai Mu Dan and Shou Mei yielded a higher content of the major compounds than did Bai Hao Yin Zhen. Thus, infusion temperature had a large effect on extracting the main compounds, and the differences in content between the three white teas presumably reflected differences in the harvest time, processing method or leaf shape.

Keywords White tea · Catechin · Caffeine · Theanine · Free amino acid · Water extract

Introduction

Tea, a beverage prepared by infusing the leaves of *Camilla sinensis* into hot or cold water, is the second most popular drink in the world after water (Damiani et al., 2014; Yu et al., 2014). Green, black, oolong, dark, and white tea are the most commonly consumed tea varieties (Damiani et al., 2014). Tea contains large quantities of polyphenols, caffeine, amino acids, vitamins, volatile oils, and other compounds that have unique biological activities and health benefits (Kocadağlı et al., 2013).

White tea, which is receiving increasing attention (Damiani et al., 2014), is the least processed form. It is made exclusively from very young tea leaves or buds covered with tiny, silvery hairs, which are harvested before fully opening (Azman et al., 2014; Hajiaghaalipour et al., 2015; Nunes et al., 2015; Rusak et al., 2008). Compared with other teas, the preparation and manufacture of white tea is significantly different (Ning et al., 2016), since it involves only two simple processes termed withering and drying (Ning et al., 2016; Tan et al., 2017). This minimal process ensures white tea retains a substantial amount of catechins, amino acids, and other constituents (Ning et al., 2016; Nunes et al., 2015). The strong antioxidant activity of white tea could therefore be related to the high concentrations of several major constituents, explaining its many health benefits (Azman et al., 2014; Nunes et al., 2015; Zielinski et al., 2016). Indeed, many health beneficial activities of white tea, such as antioxidative, antimicrobial, anticarcinogenic, and antimutagenic properties, have been reported (Dias et al., 2013; Song et al., 2015; Tan et al., 2017), but research on white tea remains limited.

White tea is generally divided into four grades from high to low; Bai Hao Yin Zhen (Silver Needle), Bai Mu Dan (White Peony), Gong Mei, and Shou Mei (Ning et al.,

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2016; Tan et al., 2017). Grading is based on the tenderness of fresh tea shoots, and different grades vary in performance, flavour, and taste (Ning et al., 2016). Bai Hao Yin Zhen, the most famous and expensive white tea, is made only from the unopened buds and includes no leaves. It has a silver-white colour, long, thin needles, and infusions are light yellow with a delicate flavour. Bai Mu Dan is produced from buds with one or two leaves, and infusions have a light golden-brown colour and a pleasing roasted aroma (Damiani et al., 2014; Ning et al., 2016). Finally, Gong Mei and Shou Mei are made from a single bud with two or three leaves, and mature leaves, respectively (Ning et al., 2016; Tan et al., 2017).

Ning et al. (2016) analysed the chemical components of differing quality of white tea and reported that total catechins and esterified catechins declined with the growth of new shoots. Tan et al. (2017) concluded that catechins, hydrolysable tannins, phenolic acids, theanine, and caffeine were more abundant in Bai Hao Yin Zhen produced in early spring than in Bai Mu Dan in late spring and Shou Mei in autumn. Despite this knowledge, studies on the chemical compounds analysis of Fuding white tea infusions from various tea types are limited.

The quality and quantity of polyphenols, caffeine, and amino acids are closely correlated with the quality of tea infusions. However, many factors may affect the amount of these constituents in tea infusions, such as the maturity of the tea leaves, the processing methods, and the brewing conditions (Kocadağlı et al., 2013; Saklar et al., 2015). White tea is receiving increasing attention, but these compounds extracted in Fuding white tea infusions from various tea types and how they are correlated with infusion conditions remain poorly understood. Thus, in the present work, we compared the levels of catechins, caffeine, theanine, free amino acids, and water extracts in Fuding white tea infusions from different tea types brewed under different temperatures, and their contributions to taste were estimated by dose-over-threshold (Dot) value. We aimed to identify differences in the main components among Fuding white teas under different brewing temperatures and provide a theoretical basis for brewing and drinking white tea.

Materials and methods

Materials and chemicals

Fuding in Fujian province is the most important production area of white tea and is regarded as the “hometown of Chinese white tea”. Three different kinds of Fuding white tea (Bai Hao Yin Zhen, Bai Mu Dan, Shou Mei) were provided by the Fujian Bamin Tea Shop (Fig. 1). All three types were cultivated in the same tea plantation, and

processed in the same factory using the traditional method, as well as naturally preserving in the same condition: dry, sealed and avoid from light and smell. The moisture content was 7.02, 7.10, and 7.17 g/100 g, respectively. Catechin, caffeine, and theanine standards, and acetonitrile and methanol used in high performance liquid chromatography (HPLC) were purchased from Sigma–Aldrich Chemical Co. (Shanghai, China). Infusions were prepared with pure water provided by Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China). All other chemicals used were of analytical grade.

Preparation of tea infusions

White tea (3.0 g) was brewed using 150 mL water at 80, 90, or 100 °C for 5 min using porcelain tea pots and lids. White tea infusions were filtered through a tea strainer prior to analysis.

Analysis of the main compounds

The main compounds in Fuding white tea infusions, including catechins, caffeine, theanine, free amino acids, and water extracts, were analysed. Analysis of catechins and caffeine was carried out by HPLC according to ISO 14502-2:2005. Epigallocatechin gallate (EGCG), epicatechin (EC), epicatechin gallate (ECG), and epigallocatechin (EGC) were quantified using a caffeine standard and the Relative Response Factors listed in ISO 14502-2:2005. Levels of catechin and caffeine were calculated in units of mg/L. Theanine in Fuding white tea infusions was analyzed by heating water extraction, purification and decolorization, and derivatization, then was determined by HPLC according to national standards (GB/T 23193-2008, China). The free amino acid content was determined according to national standards (GB/T 8314-2013, China). The water extracts content was analysed according to national standards (GB/T 8305-2013, China).

Statistical analysis

All samples were prepared and analysed in triplicate, and the results were presented as mean $\pm$ standard deviation. Statistical analysis was performed using analysis of variance (ANOVA) in SPSS (version 20.0). A p value < 0.05 was considered statistically significant.

Fig. 1 Picture of Fuding white tea (from left to right: Bai Hao Yin Zhen, Shou Mei, Bai Mu Dan)

Results and discussion

Catechin content

The catechins EGCG, EC, ECG, and EGC are major polyphenolic compounds in tea (Narukawa et al., 2010), and their levels were determined and correlated with brewing temperature (Fig. 2). EGCG was the major catechin extracted in all tea infusions, and brewing temperature played an important role in determining the amount of EGCG extracted (Saklar et al., 2015), in agreement with the present study. The EGCG content increased with increasing temperature in all three Fuding white tea infusions [Fig. 2(A)]. A sharp increase in EGCG was observed in the Bai Mu Dan tea infusion, from 115.11 ± 5.76 mg/L at 80 °C to 263.56 ± 13.18 mg/L at 100 °C, while the EGCG content increased more slowly in the Bai Hao Yin Zhen tea infusion, from 89.92 ± 4.50 mg/L at 80 °C to 115.61 ± 5.78 mg/L at 100 °C [using data on Bai Hao Yin Zhen from our previous study (Zhang et al., 2017)]. The trend for the Shou Mei infusion was intermediate between Bai Mu Dan and Bai Hao Yin Zhen. EGCG levels peaked at 100 °C in all three tea infusions, confirming that a high temperature is beneficial for extracting EGCG. Similarly, the amount of ECG and EC increased with increasing brewing temperature, and reached maximum values at 100 °C [Fig. 2(B), (C)]. The EGC content also reached maximal values at 100 °C [Fig. 2(D)].

However, at the highest brewing temperature of 100 °C, EGCG, ECG, and EC levels in tea infusions decreased in the order of Bai Mu Dan > Shou Mei > Bai Hao Yin Zhen, while EGC content decreased in the order of Shou Mei > Bai Mu Dan > Bai Hao Yin Zhen, and levels of all four catechins were generally higher in Bai Mu Dan and Shou Mei infusions than in Bai Hao Yin Zhen infusions. However, silver-needle white tea and white-peony white tea were reported to produce infusions with low amounts of EGCG (AlHafez et al., 2014). Our results differ somewhat from this previous finding, presumably due to differences in tea leaves grown at different locations or to differences in handling and processing.

The chemical composition of tea reportedly varies with many factors, such as tea variety, growing conditions, harvest time, and processing method (Hajiaghaalipour

et al., 2016; Song et al., 2015). However, in the present study, all three teas were cultivated in the same tea plantation and processed using the traditional method in the same factory. Tea processing method might play an important role in the compounds content of tea leaves, which further affecting the amount of these compounds in tea infusions.

However, the harvest time of Bai Hao Yin Zhen, Bai Mu Dan, and Shou Mei Fuding white tea in this study were different, from early to late was ordered Bai Hao Yin Zhen > Bai Mu Dan > Shou Mei, which might affect the chemical composition. Tan et al. (2017) reported that harvest season affected the quality of tea and found that total catechins content decreased with relevance to the harvest season of tea leaves. Thus, the observed differences in bioactive compounds might be due to differences in the harvest time, which is reported to affect the quality of tea (Tan et al., 2017).

The shape of the leaves (whole, broken, ground, etc.) might also account for differences in the amount of compounds released in tea infusions (AlHafez et al., 2014). For example, the shape of rolled leaves retards the extraction kinetics and slows diffusion of components, and a longer steeping time causes the leaves to unroll, resulting in the exposure of a larger surface area available for mass diffusion (Lantano et al., 2015). It was found that silver-needle white tea yielded extracts with the highest total polyphenol content, but these compounds were present in low abundance in tea infusions, presumably due to the hydrophobic hairs (AlHafez et al., 2014). Bai Hao Yin Zhen mostly consists of tea buds covered with fine white hydrophobic hairs, whereas Bai Mu Dan is a mixture of buds and small or broken leaves, and Shou Mei contains ground tea leaves, and their large reactive surface improves the release of compounds into the infusion (AlHafez et al., 2014). This could explain why EGCG, ECG, EC, and EGC levels were higher in Bai Mu Dan and Shou Mei tea infusions than in Bai Hao Yin Zhen in the present study.

Caffeine content

Caffeine is a major component in tea (Ramalho et al., 2013), and it was the most abundant compound among those analysed in the present study. As shown in Fig. 3,

Fig. 2 Content of catechins in tea infusions of various Fuding white tea under different brewing temperatures. **(A)** EGCG; **(B)** ECG; **(C)** EC; **(D)** EGC; **(E)** non-gallated catechins; **(F)** gallated catechins

caffeine levels increased with increasing infusion temperature, and reached a maximum of 386.58 ± 19.33 , 610.96 ± 30.55 , and 555.76 ± 27.79 mg/L in Bai Hao Yin Zhen, Bai Mu Dan, and Shou Mei at 100 °C, respectively. These results showed that temperature affected the extraction of caffeine, and a higher temperature was more effective, presumably because heat treatment made the cell walls more permeable to solvent molecules and other compounds, and increased the solubility and diffusion coefficients of tea compounds (Saklar et al., 2015). As

observed with catechins, the caffeine content was ordered Bai Mu Dan > Shou Mei > Bai Hao Yin Zhen (from high to low), possibly due to differences in the harvest time, processing method or leaf shape of these three white teas. Bai Hao Yin Zhen contained the lowest concentration of caffeine at all three temperatures. This was again likely to be due to the hydrophobic hairs that floated on the water surface. A higher temperature was favourable for hydrating these hairs and consequently increasing the caffeine

Fig. 3 Caffeine content in tea infusions of various Fuding white tea under different brewing temperatures

content of Bai Hao Yin Zhen infusions, as reported previously (AlHafez et al., 2014).

Theanine content

Theanine accounts for more than 50% of free amino acids (Kocadağlı et al., 2013; Yu et al., 2014). Bai Hao Yin Zhen, Bai Mu Dan, and Shou Mei Fuding white tea infusions were brewed at different infusion temperatures, and differences in theanine content were apparent (Fig. 4). The amount of theanine extracted from Bai Hao Yin Zhen increased with increasing brewing temperature, and peaked at 100 °C. Theanine levels in the Shou Mei infusion increased more slowly with increasing temperature. This behaviour might be explained by the infusing rate and side reactions. When the temperature was between 80 and 90 °C, the theanine content was low, and the compound was potentially destroyed by side reactions. With increasing temperature, more theanine was extracted, resulting in a higher content. However, unlike Bai Hao Yin Zhen and Shou Mei, a significant change was observed in the Bai Mu

Fig. 4 Theanine content in tea infusions of various Fuding white tea under different brewing temperatures

Dan tea infusion, which reached a maximal value of 148.5 ± 7.43 µg/mL at 80 °C. Apart from the three white teas at 90 °C, theanine levels were highest in Bai Mu Dan infusions, followed by Shou Mei, and lowest in Bai Hao Yin Zhen. These differences in theanine were probably due to differences in harvest time, processing method or the shape of leaves, as described above.

Free amino acids content

The effect of brewing temperature on free amino acids was investigated, and brewing temperature had a significant effect (Fig. 5), although increasing the infusion temperature did not necessarily increase the concentration. Specifically, the free amino acids content first increased then decreased with an increasing temperature, and 90 °C was more beneficial for extracting free amino acids than the hotter or cooler temperatures. A brewing temperature of 90 °C yielded 82.34 ± 4.12 , 617.85 ± 30.89 , 512.54 ± 25.63 µg/mL free amino acids in Bai Hao Yin Zhen, Bai Mu Dan, and Shou Mei infusions, respectively. We hypothesize that the hairs on tea leaves become hydrated and compounds gradually leach into the infusion more efficiently at higher temperatures, but extracts were destroyed by side reactions such as oxidation by catechin o-quinone and subsequently Strecker degradation, leading to a decrease in free amino acids (Ning et al., 2016). These results suggested high temperature led to the degradation of compounds, as reported previously (Lantano et al., 2015).

Although the content of free amino acids in tea infusions was correlated with the brewing temperature, many other factors affect free amino acid levels, such as the type of tea. At temperatures of 80 and 90 °C, the free amino acids content was ordered Bai Mu Dan > Shou Mei > Bai Hao Yin Zhen, while at 100 °C the order was Shou Mei > Bai Mu Dan > Bai Hao Yin Zhen (from high to low).

Fig. 5 Free amino acids content in tea infusions of various Fuding white tea under different brewing temperatures

Differences between Bai Mu Dan/Shou Mei infusions and Bai Hao Yin Zhen were significant ($p < 0.05$). This could be because Bai Mu Dan and Shou Mei were of moderate maturity, resulting in rapid metabolism followed by hydrolysis of water-soluble proteins into free amino acids, since previous research demonstrated that the highest amino acid content could only be reached when the maturity was in a moderate condition (Ning et al., 2016).

Water extracts content

The three white tea infusions were brewed at different temperatures, and the water extracts content was measured (Fig. 6). When the brewing temperature increased from 80 to 90 °C, a sharp increase was observed in the Bai Mu Dan infusion, but the increase in Shou Mei and Bai Hao Yin Zhen was subtler. The water extracts content continued to increase in the Shou Mei infusion upon a further increase in temperature, but this was not the case in Bai Mu Dan and Bai Hao Yin Zhen infusions. Thus, the water extracts content peaked at 90 °C for Bai Mu Dan (3.95 ± 0.20 mg/mL) and Bai Hao Yin Zhen (1.88 ± 0.09 mg/mL), and at 100 °C for Shou Mei (3.44 ± 0.17 mg/mL). As observed with the other compounds analysed in this study, the water extracts content was higher in Bai Mu Dan and Shou Mei infusions than in Bai Hao Yin Zhen. Again, this might be due to differences in harvest time, processing method or the shape of tea leaves.

Taste contribution of the main compounds

To evaluate and compare the taste contribution of these analysed compounds in white tea infusions, the Dot values were calculated as the ratio of the compound concentration to the taste threshold reported by previous research (Yu et al., 2014) (Table 1). The following equation was used:

$$\text{Dot value} = \frac{\text{The compound concentration in tea infusion}}{\text{Taste threshold}}$$

Catechins are mainly responsible for bitterness and astringency (Zhang et al., 2017). Among catechins, the Dot values of EC, ECG, and EGC were less than 1.0, while exclusively those of EGCG in all the samples were equal to or greater than 1.0 ranging from 1.03 to 3.03, showing that EGCG might make a significant contribution to the taste of tea infusions. Caffeine represents compounds imparting bitter taste (Zhang et al., 2017). The concentrations of caffeine in white tea infusions exceeded its threshold value with the Dot values greater than 2, indicating a possible contribution towards bitter taste by caffeine. Theanine is reported to contribute sweet and umami taste (Zhang et al., 2017), however, the Dot values of theanine were found to be less than 1.0.

Similarly, Yu et al. (2014) reported that the Dot values of catechins and L-theanine were less than 1.0, while those of caffeine were equal to or greater than 1.0. Meanwhile, Scharbert and Hofmann (2005) found that the Dot values of EGCG and caffeine were above 1.0, while the concentrations of all the other catechins were below their threshold values.

With the increasing of infusion temperature, the Dot values of catechins, caffeine, and theanine mostly increased. The Dot values of Bai Mu Dan and Shou Mei were higher than those of Bai Hao Yin Zhen, indicating that the taste of these three kinds of Fuding white tea might be different when brewed in the same condition. This might be because the differences in harvest time, processing method or the shape of tea leaves affected the content of taste compounds in tea infusions, and then led to the different taste of these three white teas. Hence, the different sensory evaluation of these three teas and the correlation between taste attributes and content of taste compounds could be explored next.

The non-gallated and gallated catechins results are presented in Fig. 2(E), (F). It was found out that the effect of brewing temperature on the non-gallated and gallated catechins content was similar with that of EGC and EGCG, respectively. Bai Hao Yin Zhen was relatively low in non-gallated and gallated catechins. Non-gallated catechins (EGC and EC) are reported to be less astringent than the gallated ones (EGCG and ECG), and specifically ECG can provide a bitter after taste while EGC is responsible for the slightly sweet of some teas, even when the concentrations are as low as 0.1% (w/v) (Kilel et al., 2013). According to Kilel et al. (2013), EGC affects the catechin quality index [(EGCG + ECG)/EGC]. The lower it is relative to the gallated catechins, the better is the quality index. The results of gallated/non-gallated catechin, catechin quality index, bitter and astringent index [(EGCG + ECG +

Fig. 6 Water extracts content in tea infusions of various Fuding white tea under different brewing temperatures

Table 1 Threshold and dot values of the taste compounds in Fuding white tea infusions

Fuding white tea	Infusion temperature	Taste compounds						Gallated/non-gallated catechin	Catechin quality index	Bitter and astringent index
		EGCG	EC	ECG	EGC	CAF	Theanine			
Bai Hao Yin Zhen	80	1.03	0.01	0.20	0.02	2.34	0.05	19.31	42.46	36.25
	90	1.28	0.02	0.28	0.03	3.02	0.06	14.88	29.37	31.19
	100	1.33	0.03	0.36	0.07	3.99	0.07	8.65	15.06	21.68
Bai Mu Dan	80	1.32	0.05	0.29	0.25	4.34	0.14	2.75	3.75	12.98
	90	1.76	0.05	0.47	0.12	6.18	0.11	6.22	11.07	15.49
	100	3.03	0.08	0.59	0.39	6.30	0.14	4.03	5.36	19.19
Shou Mei	80	1.60	0.06	0.34	0.31	3.49	0.11	2.76	3.63	14.70
	90	1.76	0.06	0.45	0.30	4.94	0.12	3.14	4.28	14.61
	100	2.50	0.07	0.48	0.45	5.73	0.11	2.99	3.77	18.32
Threshold (mg/L)		87	270	115	159	97	1045	–	–	–

ECG + GC)/(EC + C)] were shown in Table 1. Bai Hao Yin Zhen had higher catechin quality index, however, the gallated/non-gallated catechin and bitter and astringent index were also high. Bai Hao Yin Zhen had relatively higher indices than Bai Mu Dan and Shou Mei, might because of relatively lower non-gallated catechins. Hence, the relationship between these three indexes and taste attributes could be explored next to find an index to evaluate the bitter and astringent of tea infusions.

In conclusion, the present work compared the amount of catechins, caffeine, theanine, free amino acids, and water extracts in Fuding white tea infusions from different tea types brewed at different infusion temperatures, and their contributions to taste were estimated by dose-over-threshold value. Three different white tea types (Bai Hao Yin Zhen, Bai Mu Dan, and Shou Mei) were cultivated in the same tea plantation, and processed in the same factory. The results revealed that brewing temperature affected the levels of compounds extracted. In general, a higher infusion temperature increased the content of catechins and caffeine in all three infusions, while the trend in theanine, free amino acids, and water extracts varied depending on tea type. The EGCG, EC, ECG, and caffeine reached maximum values of 263.56 ± 13.18 , 20.50 ± 1.02 , 67.88 ± 3.39 , and 610.96 ± 30.55 mg/L in Bai Mu Dan at 100 °C, respectively. Moreover, Bai Mu Dan and Shou Mei infusions contained higher concentrations of all compounds tested than Bai Hao Yin Zhen, presumably due to differences in harvest time, processing method or the shape of tea leaves. The Dot values of EGCG and caffeine in all white tea infusions exceeded the threshold values, and Bai Mu Dan and Shou Mei showed the Dot values higher than those of Bai Hao Yin Zhen, indicating that the taste of these three Fuding white tea types might be different when brewed in the same condition. Thus, the content of the

main compounds in the white tea infusions varied, and water temperature played an important role.

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