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Research Article

Stability of Dark Tea Infusion and their Biochemical Components

[1, 2]Xiaoqiang Chen, [3]Junfeng Du, [2]Yongwen Jiang, [2]Haibo Yuan, [1]Zhouhe Wu, [1]Shan Li, [1]Yuntian Zhang, [1]Yu Du and [1]Zhengqi Wu

[1]Hubei Collaborative Innovation Center for Industrial Fermentation, Hubei University of Technology, Wuhan 430068,

[2]Tea Research Institute, China Academy of Agricultural Sciences, Hanghzou 310008,

[3]Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430077, China

Abstract: This research investigated the stabilityof Pu'er tea infusion and Fangbao tea infusion and their contents of biochemical components, such as TP (tea polyphenols), caffeine, water-soluble saccharides, free protein, free amino acids. Compared with green tea, there are a small number of TP and free amino acids in the above two kinds of dark tea and no free amino acids were detected in Fangbao tea. Pu'er tea infusion and Fangbao tea infusion were respectively stored at 2, 30 and 60°C, respectively for 1, 2, 6, 12, 16, 24 and 48 h, respectively to evaluate their stability. The turbidity of Pu'er tea infusion and Fangbao tea infusion began to increase after stored for 6 h and its stability was the worst at 30°C.

Keywords: Biochemical components, dark tea, stability, tea infusions

INTRODUCTION

Both Pu'er tea and Fangbao tea belong to dark tea that is one the six types of tea in China. Pu'er tea was made of dying green tea in Yunnan province of China, refined by fermentation treatment and gradually favored by consumers at home and abroad due to its special characteristics and recognized physical effects (Gong *et al*., 2007; Yang and Hwang, 2006; Xu *et al*., 2005; Kuo *et al*., 2005). Fangbao tea was sold in the border in western Sichuan province of China. It had many processing procedures, including steaming, griping, weighing, stirring, packaging, sealing, heating, airing and so on. The tea was made of aged fresh leaves from ripe branches of one or two years old, immediately drying them in the sun. And there are a lot of peduncles (Chen, 1986). This research investigated the chemical components of both Pu'er tea and Fangbao tea, as well as their stability of tea infusions at different temperatures, whose research results will provide a theoretical basis for the development of dark tea beverages.

MATERIALS AND METHODS

Samples of Pu'er tea, Fangbao tea and experimental reagent: Pu'er tea, four-year storage period; and Fangbao tea, ten-year storage period,. Anthranone and ninhydrin, Sinopharm Chemical Reagent Co., Ltd. Gallic acid standard substances, caffeine standard substances, sigma company. Protein determination kit, Beyotime institute of biotechnology.

Sample treatment and content analysis of biochemical components: Sample treatment analysis of TP and caffeine: 2.000 g of tea sample was put into 100 mL 50% ethanol solution, placing it at 25°C water bath for 12 h and shaking it for many times during this period, then, to reduce pressure for suction filtration and the filtrate volume to 250 mL. To fetch each sample for three times and measure their average value. Sample treatment analysis of free amino acids, free protein as well as water-soluble sugars: 1.500 g of tea samples was put into 200 mL ultra pure water, placing it at 85°C water bath for 45 min and shaking it for many times during this period, then, to reduce pressure for suction filtration and the filtrate volume to 250 mL.

The measurement of free amino acids content in the tea was applied with ninhydrin development process according to national standard (GB/T 8314-2002).

The measurement of TP content was applied with foline-phenol colorimetric method according to national standard (GB/T 8313-2008).

The measurement of caffeine content was applied with ultraviolet visible spectrophotometry according to national standard (GB/T 8312-2013).

Water-soluble sugars content took glucose as a standard substance was applied with anthranone-sulfuric acid method to measure total water-soluble

Corresponding Author: Yongwen Jiang, Tea Research Institute, China Academy of Agricultural Sciences, Hangzhou, China, Zhouhe Wu, Zhengqi Wu, Hubei University of Technology, Wuhan 430068, China

sugars content in the tea according to the documents (Chen *et al*., 2010, 2009).

The measurement of free protein content was applied with Bradford method and Beyotime protein determination kit.

Stability of tea infusion:

Preparation of tea infusion: Pu'er tea infusion and Fangbao tea infusion were respectively prepared in accordance with sample treatment analysis of free amino acids, free protein and water-soluble sugars.

To fetch respectively an equal amount of tea infusion to beakers and seale with the fresh-keeping film, then, separately store them in 2°C refrigerator springhouse, 30°C calorstat and 60°C calorstat, then, to fetch the samples for 1, 2, 6, 12, 16, 24 and 48 h, respectively to measure their absorbances at the wavelengths of 380, 520 and 660 nm, respectively. Finally, to draw a coordinate curve chart of absorbance and time and make a comparative analysis.

RESULTS AND ANALYSIS

Biochemical components in the tea infusion: According to Table 1, compared with chemical component contents of green tea and oolong tea, there are a small number of TP and free amino acid in the above two kinds of dark tea (Chen *et al*., 2007) and no free amino acid were detected in Fangbao tea. Tea polyphenols, free amino acids, water-soluble sugars and free proteins content in Pu'er tea were higher than Fangbao tea, because it was related to longer storage period and less tea stems of Fangbao tea. Content of water-soluble sugars in Pu'er tea was higher than green tea and red tea, which was related to post-fermentation process of dark tea. The action of microorganisms caused polysaccharide in the tea to decomposition into water-soluble sugar. During this process, both sweet flavour and thickness of dark tea have been enhanced.

There are rich oxidation matrix in dark tea, such as TP, saccharides etc., which laid a good material foundation for its characteristics of strong flavor, pure mellow, sweetness as well as brewing. As for the content of caffeine, these two teas were close to green tea, oolong tea and red tea (Chen *et al*., 2007).

Stability of Pu'er tea infusion at different temperatures: According to Table 2, Fig. 1 to 3, as for Pu'er tea infusion at different temperatures, its absorbance increased more obvious after storied for 6 h and the longer time, the bigger absorbance and it suggests the less stable tea infusion. Among which, Pu'er tea infusion storied at 30°C had maximum variation tendency of absorbance, tea infusion storied at 60°C ranks the second and minimum at 30°C, It could be observed that Pu'er tea infusion storied at low temperatures was the most stable, while the least stable at 30°C.

Table 1: Main component contents of Pu'er tea and Fangbao tea (mg/g)

	Pu'er Tea	Fangbao Tea
Free amino acids content	4.45±0.41	-
Caffeine content	31.87±1.82	34.69±2.75
Polyphenol content	50.50±5.33	42.10±7.91
Water-soluble sacchairdes	57.46±2.88	18.77±2.08
Free protein	38.73±4.29	20.17±2.33

-, not detected

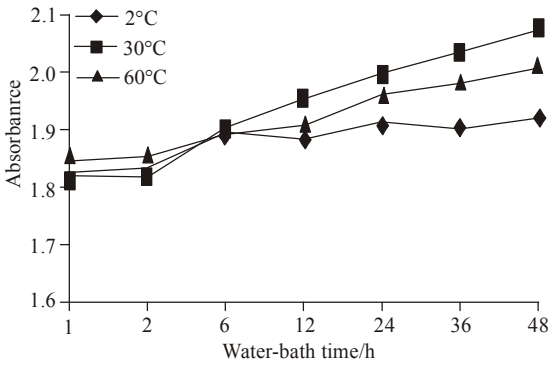

Fig. 1: Turbidity changes of Pu'er tea infusion stored at 2, 30 and 60°C (recorded at 380 nm)

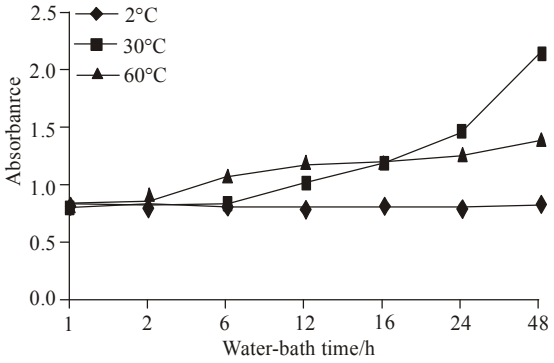

Fig. 2: Turbidity changes of Pu'er tea infusion stored at 2, 30 and 60°C (recorded at 520 nm)

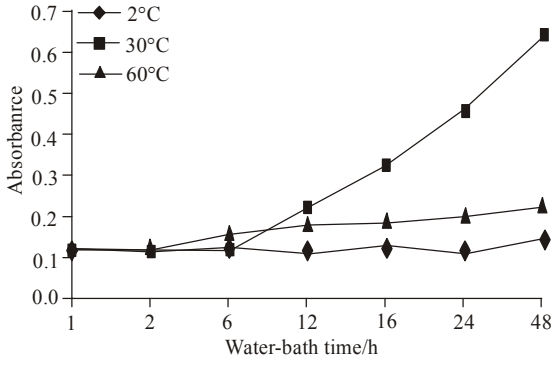

Fig. 3: Turbidity changes of Pu'er tea infusion stored at 2, 30 and 60°C (recorded at 660 nm)

Stability of Fangbao tea at different temperatures: According to Table 3, Fig. 4 to 6, at the wavelengths of 380, 520 and 660 nm, respectively absorbance of

Table 2: Turbidity changes of Pu'er tea infusion storied at different temperatures

		1 h	2 h	6 h	12 h	16 h	24 h	48 h
2°C	380 nm	1.831	1.836	1.898	1.89	1.913	1.905	1.928
	520 nm	0.781	0.78	0.771	0.775	0.791	0.772	0.809
	660 nm	0.113	0.11	0.114	0.109	0.12	0.109	0.137
30°C	380 nm	1.82	1.822	1.903	1.957	2.005	2.038	2.081
	520 nm	0.784	0.787	0.805	0.986	1.161	1.426	2.121
	660 nm	0.114	0.113	0.116	0.217	0.318	0.455	0.641
60°C	380 nm	1.851	1.855	1.89	1.916	1.964	1.986	2.016
	520 nm	0.834	0.881	1.05	1.162	1.184	1.227	1.371
	660 nm	0.111	0.122	0.151	0.174	0.187	0.19	0.22

Table 3: Turbidity changes of Fangbao tea infusion storied at different temperatures

		1 h	2 h	5 h	6 h	12 h	16 h	24 h	48 h
2°C	380 nm	1.831	1.836	1.853	1.898	1.89	1.913	1.905	1.928
	520 nm	0.781	0.78	0.777	0.771	0.775	0.791	0.772	0.809
	660 nm	0.113	0.11	0.111	0.114	0.109	0.12	0.109	0.137
30°C	380 nm	1.82	1.822	1.856	1.903	1.957	2.005	2.038	2.081
	520 nm	0.784	0.787	0.801	0.805	0.986	1.161	1.426	2.121
	660 nm	0.114	0.113	0.115	0.116	0.217	0.318	0.455	0.641
60°C	380 nm	1.851	1.855	1.905	1.89	1.916	1.964	1.986	2.016
	520 nm	0.834	0.881	0.961	1.05	1.162	1.184	1.227	1.371
	660 nm	0.111	0.122	0.132	0.151	0.174	0.187	0.19	0.22

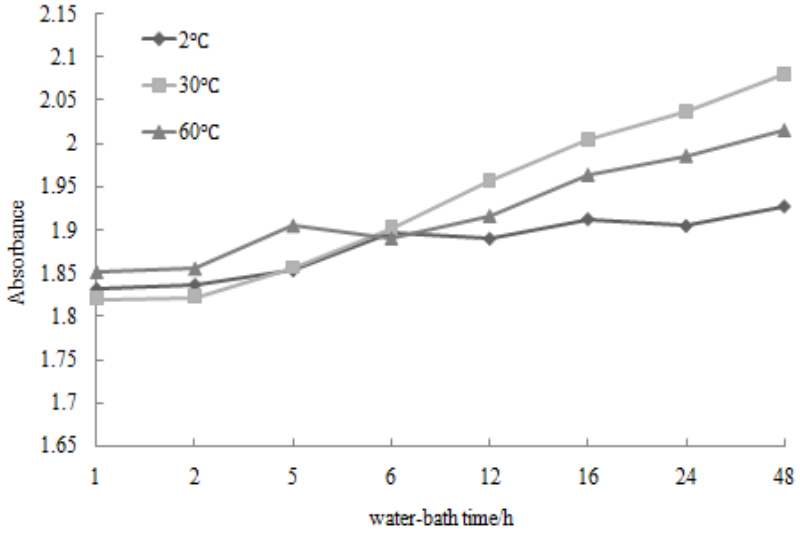

Fig. 4: Turbidity changes of Fangbao tea infusion stored at 2°C, 30 and 60°C (recorded at 380 nm)

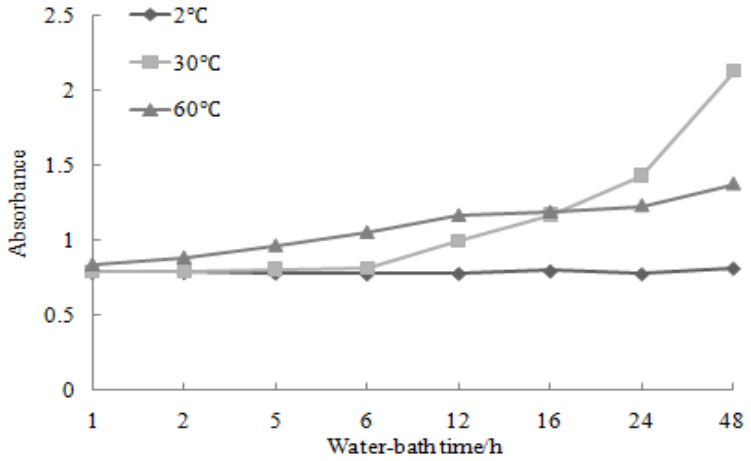

Fig. 5: Turbidity changes of Fangbao tea infusion stored at 2°C, 30 and 60°C (recorded at 520 nm)

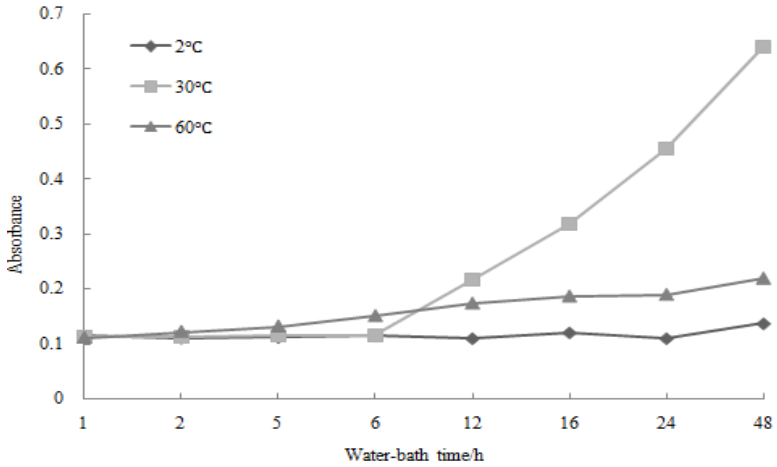

Fig. 6: Turbidity changes of Fangbao tea infusion stored at 2, 30 and 60°C (recorded at 660 nm)

Fangbao tea increased over time, the bigger absorbance, the muddier the tea infusion, which suggested the less stable the tea infusion. However, storied at 30°C, absorbance changes of Fangbao tea infusion was obviously bigger than that of 2 and 60°C and it was the smallest trend at 2°C. Therefore, it was the least stable for Fangbao tea infusion at 30°C and it was the most stable at 2°C.

CONCLUSION

From the experimental results, compared with green tea and oolong tea, tea polyphenols and free amino acids content in this two kinds of dark tea were low. Tea polyphenols, free amino acids, water-soluble sugars and free proteins content except affeine content in Pu'er tea were higher than Fangbao tea, because it was related to longer storage period and less tea stems of Fangbao tea. In the tea infusion stability, the turbidity of Pu'er tea infusion and Fangbao tea infusion began to increase after stored for 6 h and tea infusion was the most stable stored at 2°C, easy to storage; second at 60°C; it was the least stable at 30°C.

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