文章编号:1001-6880(2014)Suppl-0056-05

普洱熟茶发酵过程多酚、咖啡碱以及茶色素的变化研究

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摘　要:后发酵是云南地理标志产品普洱熟茶品质形成的关键,但该过程的化学物质变化仍需研究。本文应用高效液相色谱分析发现随着发酵进行包括表儿茶素[(-)-epicatechin,EC)]、表没食子儿茶素[(-)-epigallocatechin,EGC)]、表儿茶素没食子酸酯[(-)-epicatechin-3-*O*-gallate,ECG]、表没食子儿茶素没食子酸酯[(-)-epigallocatechin-3-*O*-gallate,EGCG]在内的儿茶素含量以及酚酸类物质茶没食子素(theogallin,TG)和水解单宁类物质strictinin(STR)、1,4,6-tri-*O*-galloyl-*β*-D-glucose(1,4,6-tri-G-G)的含量降低;与之相反,没食子酸(GA)和咖啡碱(CAF)的含量增加。另外在发酵样品中未检测到与红茶样品相同的茶色素,表明红茶与普洱熟茶的色素不同。

关键词:普洱熟茶;多酚;咖啡因;色素;发酵

中图分类号:T272　　　　**文献标识码**:A

Content Variation of Polyphenols, Caffeine and Pigment during Fermentation of Pu-erh Shucha

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Abstract: Pu-erh shucha, a traditional beverage in Yunnan Province, China, is popular in East Asia. The key process for the development of characteristic flavors of Pu-erh shucha is "post-fermentation"; however, little is known about the content variation of chemical components during this process. In this work, the content variation of tea polyphenols, caffeine and pigments during the fermentation process of Pu-erh shucha was monitored by high performance liquid chromatography (HPLC). The HPLC analysis revealed that the concentrations of (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-*O*-gallate (ECG), (-)-epigallocatechin-3-*O*-gallate (EGCG), theogallin (TG), strictinin (STR) and 1,4,6-tri-*O*-galloyl-*β*-D-glucose (1,4,6-tri-G-G) decreased, while the concentrations of gallic acid (GA) and caffeine (CAF) increased during the fermentation process. It was inferred that gallate catechins hydrolyze to GA during the fermentation process. In addition, the fact that no black tea pigments were detected in the fermenting samples of pu-erh shucha suggested that different pigments existed in the different teas.

Key words: Pu-erh shucha; polyphenols; caffeine; pigment; fermentation

Pu-erh tea is a traditional beverage, mainly produced in Yunnan Province (China) from sun-dried green tea leaves [*C. sinensis* var. *assamica* (JW Masters) Kitamura][1]. It can be categorized as non-fermented (Pu-erh shengcha) and post-fermented (Pu-erh shucha) tea. For its special taste and various health benefits, Pu-erh tea is popular in Hong Kong and Taiwan, as well as many other areas of East Asia. The health benefits of Pu-erh shucha included anti-oxidant [2], anti-infective[3], anti-microbe[4] and anti-cancer properties[5], lowering the risk of atherosclerosis[6] and as an effective hypoglycemic[7] agent.

Tea polyphenols are important functional ingredients and are responsible for the bitterness and astringency of tea liquor[3]. Several reports have highlighted the differences of polyphenols in Pu-erh tea and other teas[8,9], and the changes induced in tea polyphenols during fermentation[8-11]. These studies have shown that

Received: September 27, 2013　Accepted: March 18, 2014
Funding Item: National Natural Science Foundation (No. 31160173)
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tea polyphenols significantly decrease during Pu-erh tea fermentation[8-11]. Polyphenols are thought to oxidize to high-molecular-weight pigments, e. g. thearubigins (TRs), theaflavins (TFs) and theobromines (TBs) and it is suggested that the polyphenol oxidation accounts for the characteristic red-brown liquor and mellow taste of Pu-erh shucha. Clarify the changes of tea polyphenols and pigments help to explain how the sensory qualities of Pu-erh shucha develop[10]. In this work, the variation in the contents of tea polyphenols, caffeine and pigments were investigated.

Materials and Methods

Chemicals

Chemical standards of (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin 3-*O*-gallate (ECG), (-)-epigallocatechin 3-*O*-gallate (EGCG), theogallin (TG), strictinin (STR), 1,4,6-tri-*O*-galloyl-β-D-glucose (1,4,6-tri-G-G), gallic acid (GA) and caffeine (CAF) were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile (ACN) and phosphoric acid used for the mobile phases were HPLC-grade reagents from Tedia Co., Inc., (Fairfield, OH). Methanol and acetone used for extraction were of analytical reagent grade.

Instruments

Deionized water EPED-T purification system (Yi Pu Yi Da Co., Nanjing, Jiangsu, China), grinder (Midea Co., Fuoshan, Guangdong, China), MCI gel CHP-20 chromatography column (Mitsubishi Chemical Corporation), Rotary evaporator with water bath (type SR-650; Tokyo Rikakikai Tokyo, Japan). HPLC system consisting of a TSK-gel ODS-80TM column (4.6 mm × 250 mm, 5 μm, Tosoh), a CCPM pump, a UV-970-detector set at 280 nm (Tosoh Co., Tokyo, Japan).

Plant materials and Pu-erh shucha fermentation

Sun-dried green tea, used as a raw material for the production of Pu-erh shucha, was purchased from JingGu in Yunnan province, China[12]. About 10 tons of the sun-dried green tea leaves were mixed with tap water to give a solid content of ~70% (w/v) and were kept in a fermented room. During the fermentation, the leaves were mixed every seven days to ensure homogeneity of the mixture and tap water was added to keep the solid content at 65%-75% (w/v). The fermentation process was stopped when the fermented tea mass was reddish-brown and free from any astringent taste (~42 days). Samples were collected on day 0, 7, 14, 21, 28, 35 and 42 of the fermentation process.

Extraction of tea polyphenols, caffeine and pigments

Extractions of tea polyphenols, caffeine and pigments were carried out according to the reports of Hashimoto[13]. Tea samples of 5.0 g were ground into powder with a grinder. Tea powder (1.5 g) was extracted with 60 mL of 80% acetone at room temperature. After 24 h extraction, the extracts were filtered to remove rough particles and concentrated by rotary evaporator at 40 ℃. The concentrated solution was injected into a MCI gel CHP-20 chromatography column, followed by successive elution with dd H_2O, 50% methanol and 70% methanol. All eluents were concentrated by rotary evaporator and were freeze-dried to obtain the powder. 3.0 mg of the powder was dissolved in 10 mL of 70% methanol. The solution was filtered through a 0.45 μm nylon membrane and subjected to HPLC to determine the contents EC, EGC, ECG, EGCG, TG, STR, 1,4,6-tri-G-G, GA and CAF.

HPLC conditions

Tea extracts were determined using a Tosoh HPLC system. The mobile phases were (95% 0.05 M H_3PO_4-H_2O, 5% CH_3CN) (A) and (20% 0.05 M H_3PO_4-H_2O, 80% CH_3CN) (B). The gradient elution program was as follows: 0-55 min, 9%-53% (B); 55-56 min, 53%-100% (B); 56-70 min, 100% (B); 70-71 min, 100%-9% (B), 71-81 min, 9% (B). The flow rate was 0.7 mL/min and the temperature of the column oven temperature was maintained at 40 ℃. The injection volume was 10 μL.

Calculation of component contents

The average contents were calculated using the standard curve method, and the data were transformed into the contents of tea polyphenols and caffeine.

Statistical Analysis

One-way ANOVA was used to identify statistical differences, followed by the least-significant difference

(LSD) method for paired data. Results were expressed as mean ± SD. All data were analyzed using SPSS 19.0 software packages (SPSS Inc., Chicago, IL), and $P < 0.05$ was considered to be significant.

Results and Discussion

Variation of tea polyphenol and caffeine contents during the fermentation of Pu-erh shucha

The contents of EGCG, ECG, GA, TG, STR, 1,4,6-tri-G-G and CAF were detected using HPLC during the fermentation of Pu-erh shucha (Table 1). The content variation of EGCG, ECG, EGC, EC, and GA during the fermentation was shown in Fig. 1a, and the content variation of TG, STR, 1,4,6-tir-G-G, and CAF was shown in Fig. 1b. The results showed that EGCG and ECG in all tea samples decreased significantly during fermentation ($P < 0.01$); EC and EGC initially increased ($P < 0.01$) from 0 d to 7 d, then decreased; GA increased from 28 d after fermentation; caffeine content increased with the increasing of fermentation process; TG, STR, and 1,4,6-tri-G- G significantly decreased ($P < 0.05$) (Fig. 1).

Table 1 Tea polyphenols and caffeine contents during the fermentation process ($n = 3$)

Fermentation period (day)	EGCG	ECG	EGC	EC	GA	TG	STR	1,4,6-tri-G-G
0	36.4 ± 5.5	41.4 ± 5.1	13.2 ± 1.3	21.1 ± 2.6	2.1 ± 0.7	8.7 ± 0.9	9.7 ± 1.8	1.2 ± 0.5
7	15.9 ± 3.7	21.9 ± 3.0	15.3 ± 1.4	22.0 ± 0.8	22.5 ± 1.7	5.5 ± 1.3	5.2 ± 1.3	0.6 ± 0.2
14	8.4 ± 2.7	11.2 ± 1.9	10.4 ± 2.9	16.5 ± 1.2	22.5 ± 4.0	3.4 ± 0.8	2.5 ± 0.8	0.2 ± 0.1
21	5.3 ± 1.2	7.8 ± 2.6	9.1 ± 0.5	15.2 ± 1.5	25.3 ± 3.3	2.6 ± 0.5	1.6 ± 0.4	0.2 ± 0.0
28	4.0 ± 1.3	4.7 ± 1.5	6.3 ± 1.0	10.0 ± 0.7	22.1 ± 3.8	2.2 ± 0.4	0.7 ± 0.7	0.1 ± 0.0
35	2.3 ± 0.7	4.3 ± 1.8	4.0 ± 0.5	9.2 ± 1.5	15.9 ± 2.9	1.9 ± 0.2	0.3 ± 0.5	0.1 ± 0.1
42	1.1 ± 0.2	1.6 ± 0.5	3.0 ± 0.4	5.4 ± 0.9	11.9 ± 3.5	1.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0

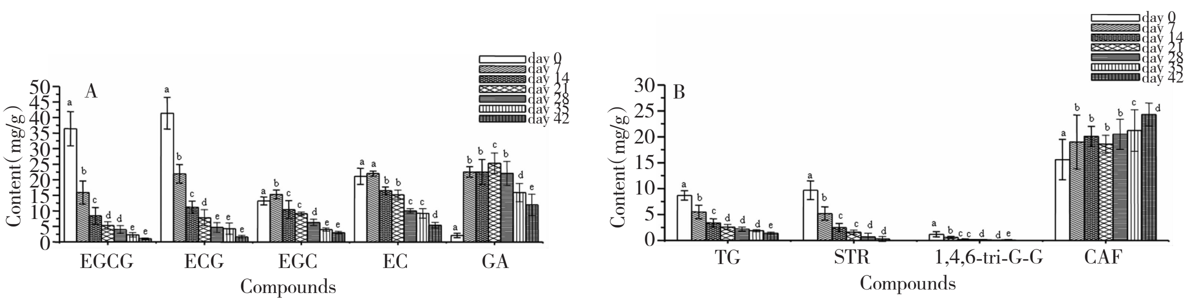

Fig. 1 Content variations of EGCG, ECG, EGC, EC and GA during fermentation process (a) and TG, STR, 1,4,6-tir-G-G, and CAF during fermentation process ($n = 3$) (b)

Note: Different letters on the same error bars indicated significant difference at 1% level by Fisher's test.

The HPLC analysis revealed that the contents of catechins including EGCG, ECG, EGC, and EC decreased during the fermentation process, while the content of GA increased during the fermentation process. This trend was consistent with the reports by Lv[14] and Gong[10]. In addition, Fumio Hashimoto[15,16] reported that the contents of tannins in Pu-erh tea were lower than that of other teas, especially flavone-3-ols. Our work found similar results and demonstrated that the content of GA significantly increased.

Pigments in Pu-erh shucha

Pu-erh shucha is a special variety of Chinese tea which undergoes a "post-fermentation" process. It was hypothesized that tea pigments such as TRs, TFs and TBs were synthesized during this "post-fermentation"; however, the characterization of these pigments is not clear. In this work, the HPLC results showed that no known black tea pigments were detected in fermented tea leaves (Fig. 2). This result showed that the contents of TRs, TFs and TBs in black tea and pu-erh tea were different. The possible reason was that tea pigments in black tea and pu-erh shucha were catalyzed by enzymes in fresh tea leaves and microbes, respectively.

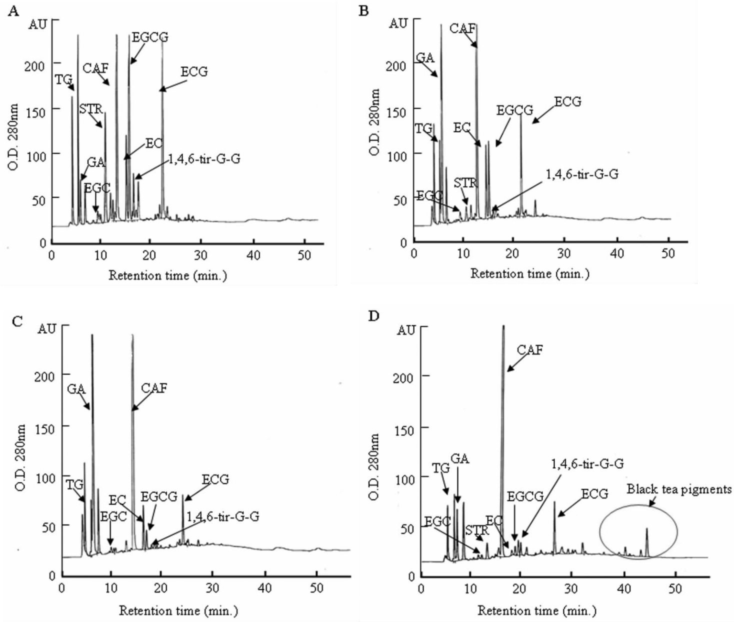

Fig. 2 HPLC Chromatograms of raw tea leaves (A), tea leaves collected on 21 d fermentation (B), tea leaves collected on 42 d fermentation (C) and black tea (D)

Conclusion

The HPLC detection revealed that the concentrations of EC, EGC, ECG, EGCG, TG, STR and 1,4,6-tri-G-G decreased, while the concentrations of GA and CAF increased during the fermentation of pu-erh shucha. In addition, our results also showed the pigments in black tea and pu-erh shucha were different.

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12 Gbt 22111-2008 Product geographical indication--Pu-erh tea.

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