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Full Length Research Paper

Analysis of the major chemical compositions in Fuzhuan brick-tea and its effect on activities of pancreatic enzymes *in vitro*

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Fuzhuan brick-tea, a fungal-fermented tea, is commonly consumed in northwest China; in places such as Sinkiang and Tibet and is thought to be helpful in digestion. To better understand Fuzhuan brick-tea and its function on digestion, the Fuzhuan brick-tea's chemical compounds were surveyed at pivotal process phases, and its effects on pancreatic enzymes *in vitro* were studied. Most of the changes in amino acids, proteins, polyphenols, catechins and organic acids were found during fungal fermentation phase. All the infusions of Fuzhuan brick-tea samples had promotional effects on pancreatic amylase and protease and no effect on pancreatic lipase. Correlation analysis and principle component analysis between the main compounds of Fuzhuan brick-tea and the activities of two pancreatic enzymes were performed. The results showed that among ten significantly related compounds, the catechins and organic acids were particularly correlated with these two pancreatic enzymes' activities. The present work confirmed the importance of microbial fermentation in the compositional changes of Fuzhuan brick-tea and its effects on two pancreatic enzymes *in vitro*, and suggested the possible application of microbial fermented tea such as Fuzhuan brick-tea in digestive aid.

Key words: Fuzhuan brick-tea, chemical composition, pancreatic enzyme, principal component analysis.

INTRODUCTION

Fuzhuan brick-tea is traditionally made with old and coarse green tea or dark tea materials. It is categorized as a compressed dark tea as Pu-erh tea. Fuzhuan brick-tea has a long history and is also known as "Hu Tea" or "Jinya Tea" (Chen, 1989). Its manufacturing process includes steaming, piling, pressing, fungal fermentation and drying; it is then compressed into a brick shape for sale or storage. This process has also been described carefully by Mo et al. (2008b). The fungal fermentation is an important phase, which uniquely controls the special qualities of Fuzhuan brick-tea, that is, it makes the Fuzhuan brick-tea looks like a golden 'fungal flora' on the surface and in the inside, is a symbol of high quality.

Fuzhuan brick-tea is also an indispensable beverage for people living in Sinkiang and Tibet areas where there are few vegetables and fruits, and it is regarded nutritional supplement. Usually, it is more important than meat and milk for the good help it renders in digestion. Its functions on digestive promotion, anti-disease and general health care were long believed by people living there. Since numerous researches indicated the promotional functions of Pu-erh tea on human health, the dark teas fermented with microorganisms have intrigued researchers. Many studies have reported the pharmacological efficacy of Pu-erh tea with respect to anti-obesity (Liu and Shi, 1994), lipid-lowering (Lu and Hwang, 2008), anti-

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Abbreviations: PCA, Principal component analysis; **HPLC,** high performance liquid chromatography; **CG,** catechin gallate; **GC,** gallocatechin; **EGC,** epigallocatechin; **EGCG,** epigallocatechin gallate; **GCG,** gallocatechin gallate; **ECG,** epicatechin gallate.

oxidation (Duh et al., 2004; Jie et al., 2006), anti-bacterial (Chosa et al., 1992) and anti-mutagen (Wu et al., 2007). The anti-bacterial properties of Fuzhuan brick-tea were also investigated by the research works on several food borne bacteria such as the spore forming bacteria; *Bacillus cereus*, *Bacillus subtilis*, *Clostridium perfringens* and *Clostridium sporogenes* (Mo et al., 2008a; Mo et al., 2008b). The carbonacenous, nitrogenous compounds and volatile compounds were identified to be important for Fuzhuan brick-tea's aroma and taste (Wang et al., 1991; Xu et al., 2007). However, there is a little experimental evidence on Fuzhuan brick-tea's function of aiding digestion.

To better understand Fuzhuan brick-tea and its possible impact on digestion in this work, the main chemical components were surveyed at the pivotal process phases of Fuzhuan brick-tea. Its effects on three pancreatic enzymes *in vitro* were tested and the relationship between chemical compounds of Fuzhuan brick-tea and the influences on pancreatic enzymes *in vitro* were analyzed. Principal component analysis (PCA), as a useful tool in exploratory data analysis and making predictive models was used here to predict the most correlative ingredients of pancreatic enzymes' activities *in vitro*, so as to provide an insight into the possible benefits of Fuzhuan brick-tea for digestion.

MATERIALS AND METHODS

Preparation of Fuzhuan brick-tea infusions

All Fuzhuan brick-tea samples were provided by Jiangnan Chengmao brick-tea Ltd of Xinchang, Zhejiang province. Tea samples were obtained from the major process phases, including raw materials, piling tea, fungus-fermented tea for 5 and 18 days and made tea, and another eight Fuzhuan brick-tea samples were from different process phases randomly. The tea samples were extracted with fresh boiled water in a weight ratio of 3:500 for 45 min. After being filtered, the infusions were ready for analysis.

Chemical compounds analysis of Fuzhuan brick-tea samples

The measurement of total tea polyphenols, tea polysaccharides and amino acids were followed by the Chinese National standard methods (Zhong, 1989), while the Coomassie brilliant blue (G-250) method was performed to determine the crude protein contents (Chen, 2002). The contents of caffeine and catechins in Fuzhuan brick-tea infusions were determined by High Performance Liquid Chromatography (HPLC) method as described by Tu et al. (2005). The concentrations of organic acids (including oxalic, pyruvic, D-malic, α-keto-glutaric, ascorbic, lactic, acetic, citric, L-malic and succinic acid) were detected with the modified HPLC method, which used 0.10 mol L^{-1} of KH_2PO_4 (pH = 2.5) as the flow solution for a better separation of ten kinds of organic acids (Ding et al., 2005).

Pancreatic enzymes activities assays

Amylase activity assay

The method used to test the α-amylase activity was described by Cao et al. (2001). The reaction solution tube which contained 1 ml of starch solution (0.4 mg ml^{-1}) and 0.1 ml of the amylase solution (4.0 Uml^{-1}) were incubated at 40°C for 5 min. Each Fuzhuan brick-tea extraction of different contents (0, 0.5, 1.0, 1.5, 2.0 mg dry weight) were added separately in each tube and incubated at 40°C for 7.5 min. After that, iodine diluents (1 ml) were added to end the reaction and deionized water was added to dilute the solution to an appropriate concentration for measuring the absorbance at 660 nm. Subsequently, each sample was analyzed in triplicate.

Protease activity assay

The pancreatic protease activity assay was performed essentially as described by Stellmach (1992). 1 ml protease solution (0.5 Uml^{-1}) and 2 ml boric acid buffer were mixed and incubated at 40°C for 10 min. Then 5 ml preheated casein solution (15 mg ml^{-1}) and 0.5 ml Fuzhuan brick-tea extract of different concentrations (0, 0.5, 1.0, 1.5 and 2.0 mg dry weight) were added to the above reagent system. After incubating at 40°C for 30 min, the reaction was stopped by TCA reagent (5%). After being shaken and filtered, the absorbance of the reaction solution was measured using a spectrophotometer at 275 nm and the control used deionized water instead of Fuzhuan brick-tea infusion.

Lipase assay

The lipase activity assay was performed according to the method described by Xu (1994). Olive oil (4 ml) was mixed with 7.5 g arabica acacia, and after being mashed and filtered, the solution was used as the substrate emulsion. Following the Fuzhuan brick-tea, extraction with a linear content (0, 0.5, 1.0, 1.5 and 2.0 mg dry weight) was added to the substrate solution, then 5 ml of Tris-HCl buffer (pH = 9.0) was mixed with the above reagents, and finally 1 ml (12 U ml^{-1}) of the lipase solution was added and incubated at 37°C for 15 min. The changes of fatty acid content were tested by NaOH solution.

Statistical analysis

All experiments were performed with independent triplicate analysis and data were analyzed with MS Excel 2003 and Statistical Analysis System (SAS) system. Statistical significances among contents of chemical compositions in Fuzhuan brick-tea infusion were examined by Duncan SSR test, and the correlations between the chemical compositions' contents and pancreatic enzymes' activities were analyzed by linear regression assay. As a result, the SPSS software was applied for principal component analysis.

RESULTS AND DISCUSSION

The changes of chemical compounds during Fuzhuan brick-tea's process phases

Fuzhuan brick-tea samples at different phases of processing (raw material, piling tea, 5 days of fungal fermented tea, 18 days of fungal fermented tea and dried tea) were infused for measuring the chemical compounds. The results showed that there were great changes of the compounds including total tea polyphenols, catechins, amino acids, polysaccharides and organic acids during fungal fermentation (Figure 1). There were about 20%

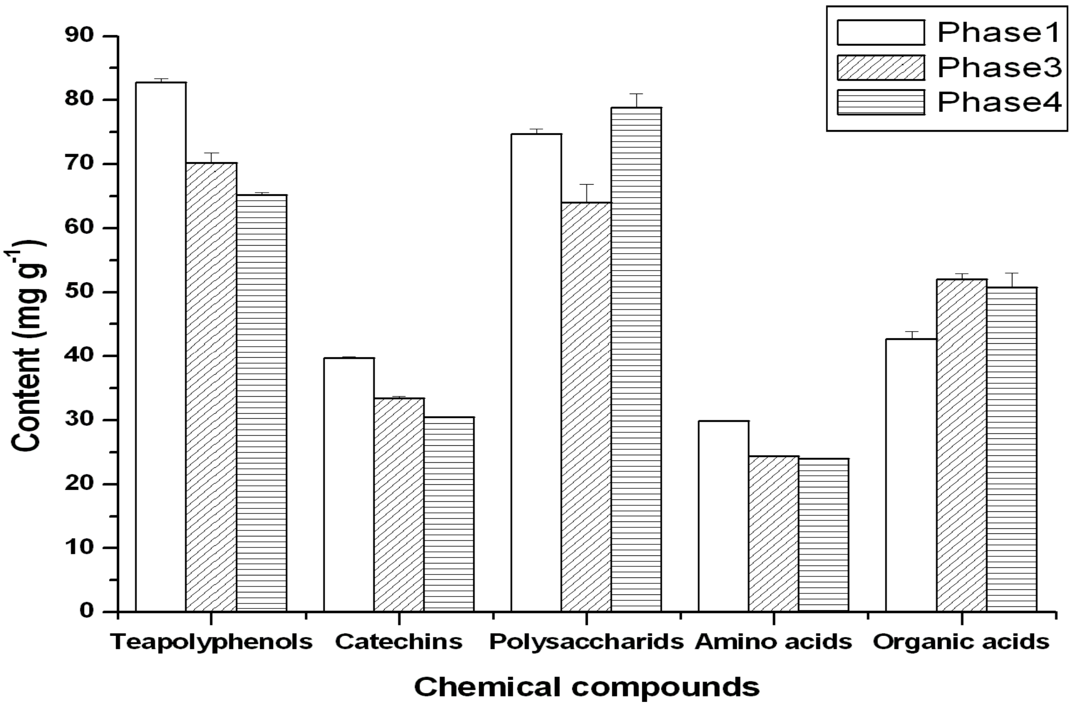

Figure 1. Chemical components changes during Fuzhuan brick-tea fermentation. Five Fuzhuan brick-tea samples were collected from different process phases; the data of fermentation phase compared with unfermented tea sample were shown. The contents are expressed as mg of material per g of dry weight. The values shown are the means from three independent experiments. Error bars indicate the standard deviations. Phase 1: Raw tea without fermentation; Phase 3: fungus-fermented tea for 5 days; Phase 4: fungus-fermented tea for 18 days.

decreases of tea polyphenols, catechins and amino acids. For catechin monomers, their decreases were parallel with total catechins, except for (-)-catechin gallate (CG) that was too low to be detected (Figure 2).

The compounds that increased during the fungal fermentation were total organic acids and polysaccharides. As shown in Figure 3, five organic acids were tested to contribute to the overall increase in organic acids. These include pyruvic, D-malic, lactic, acetic and citric acid. Citric acid increased mostly, followed by acetic acid, which became the most prominent organic acids after fungal fermentation. D-malic and lactic acid showed significant increases of 13 and 28%, respectively. The other organic acids showed decreases in the range of 10 - 40%: oxalic (18%), alpha-keto-glutaric (39%), ascorbic (24%), L-malic (11%) and succinic acid (29%).

These changes probably resulted from secreting enzymes such as cellulases, hemicellulases, proteases and α-amylases during the fungal fermentation, which were proven in Pu-erh tea's fermentation (Abe et al., 2008; Ward et al., 2005). The aldehyde compounds of stale aroma and terpene alcohols of flower aroma which specially increased after the fermentation process were from decarboxylation and oxidative deamination of amino acids (Xu et al., 2007). The degradations of proteins and polyphenols during fungal fermentation are good for the formation of Fuzhuan brick-tea's taste and its special aroma. The increase in organic acids is also the result of fungal metabolism, which might be helpful on some bio-functions and is worthy for further research work.

Effects of Fuzhuan brick-tea extraction on pancreatic enzymes activity

The pancreas is the main digestive gland in the human body that secretes the enzymes such as amylase, protease and lipase, which can hydrolyse starch, protein and triglycerides, separately. Here, eight randomly selected samples of Fuzhuan brick-teas were extracted with water and were then added in three pancreatic enzymes systems at different contents (0, 0.5, 1.0, 1.5 and 2.0 mg dry weight). As a result, their effects on three pancreatic enzymes *in vitro* were assayed as described above. The results showed that all Fuzhuan brick-tea extractions had promotional effects on the activities of pancreatic amylase and protease, but no effect was found on that of pancreatic lipase. The activities of these two pancreatic enzymes increased with the additional amounts of Fuzhuan brick-tea extractions. There were good corre-

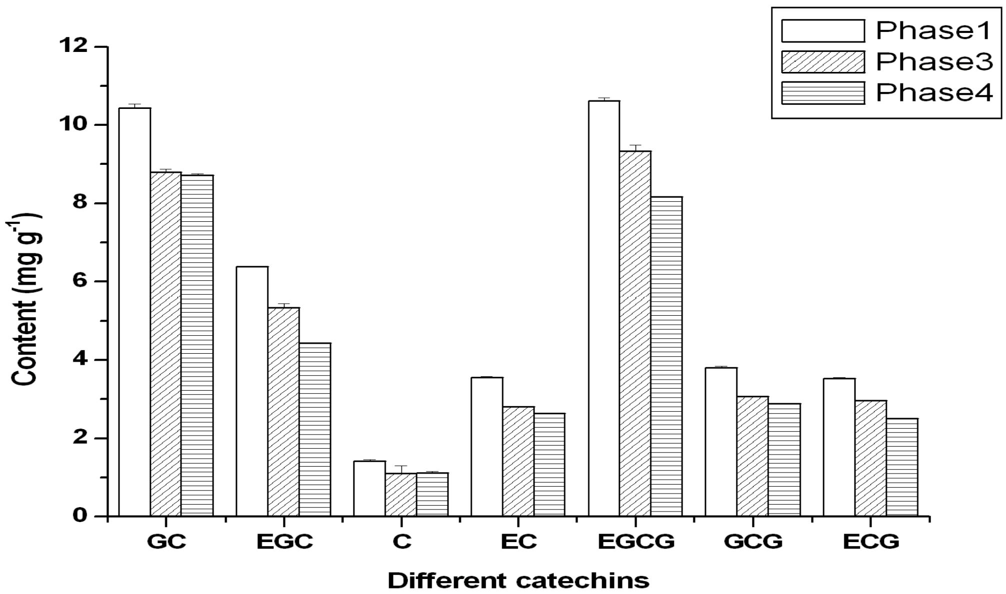

Figure 2. Catechin monomers changes during Fuzhuan brick-tea fermentation. Five Fuzhuan brick-tea samples were collected from different process phases; the data of fermentation phase are shown. Values are the means from three independent experiments. Phase 1: Raw tea without fermentation; Phase 3: fungus-fermented tea for 5 days; Phase 4: fungus-fermented tea for 18 days.

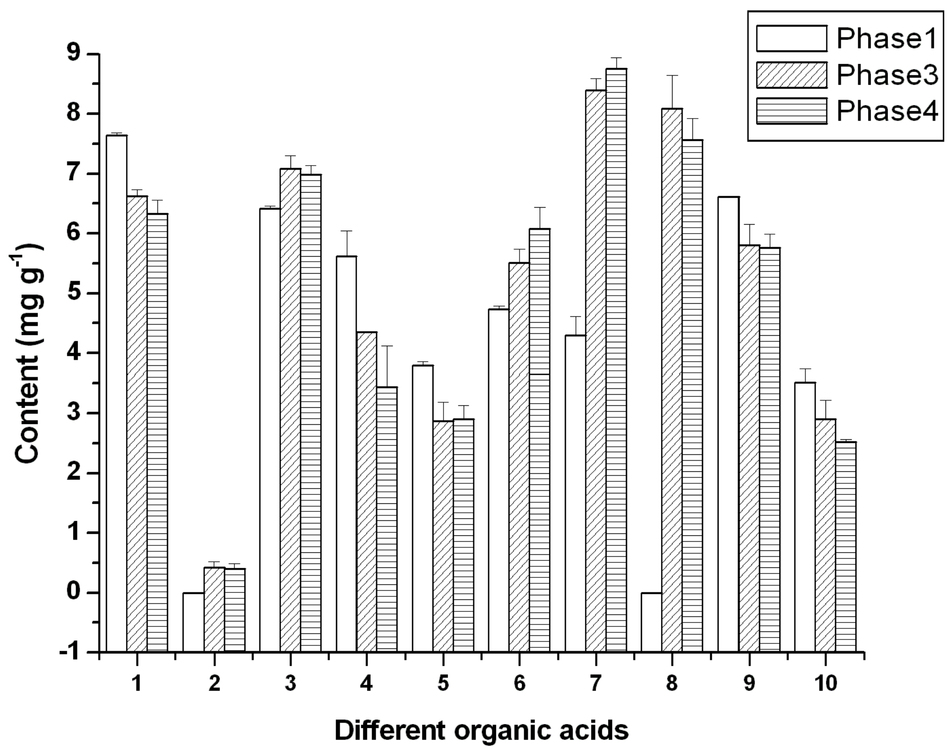

Figure 3. Organic acids changes during Fuzhuan brick-tea fermentation. Five Fuzhuan brick-tea samples were collected from different process phases; the data of fermentation phase are shown. Values are the means of three independent experiments. Phase 1: Raw tea without fermentation; Phase 3: fungus-fermented tea for 5 days; Phase 4: fungus-fermented tea for 18 days; 1: oxalic acid; 2: pyruvate acid; 3: D-malic acid; 4: α-keto-glutaric acid; 5: ascorbic acid; 6: lactic acid; 7: acetic acid; 8: citric acid; 9: L-malic acid; 10: succinic acid.

Table 1. The correlation between added amounts of Fuzhuan brick-tea infusion and activities of two pancreatic enzymes.

Sample	Pancreatic amylase		Pancreatic protease	
	Regression equation	Correlation coefficient	Regression equation	Correlation coefficient
1	Y=50.78X	0.8637**	Y=55.17X	0.8999**
2	Y=42.45X	0.8819**	Y=48.34X	0.8922**
3	Y=52.42X	0.8178**	Y=61.23X	0.8916**
4	Y=47.64X	0.8153**	Y=55.50X	0.9087**
5	Y=42.80X	0.9025**	Y=44.80X	0.8781**
6	Y=40.44X	0.9144**	Y=46.11X	0.8958**
7	Y=46.54X	0.8285**	Y=49.83X	0.8815**
8	Y=45.81X	0.8754**	Y=51.68X	0.8633**

** Significant linear correlation ($p < 0.01$).

Table 2. The linear correlation coefficient between the chemical compounds of Fuzhuan brick-tea and the activities of the pancreatic amylase and protease.

Chemical compound	Pancreatic protease	Pancreatic amylase
Amino acids	0.63	0.62
Protein	0.47	0.59
GC	0.76*	0.85**
EGC	0.89**	0.88**
C	0.39	0.41
Caffeine	0.69	0.61
EC	0.69	0.76
EGCG	0.89**	0.94**
GCG	0.78*	0.87**
ECG	0.89**	0.95**
Total catechins	0.86**	0.92**
Tea polysaccharid	-0.07	0.16
Tea polyphenol	0.84**	0.94**
Oxalic acid	0.87**	0.93**
Pyruvate	-0.33	-0.48
D-malic acid	-0.05	-0.16
a-keto-glutaric acid	0.64	0.63
Ascorbic acid	0.71**	0.85**
Lactic acid	-0.62	-0.65
Acetic acid	-0.52	-0.57
Citric acid	-0.29	-0.44
L-malic acid	0.69	0.83*
Succinic acid	0.83*	0.68
Total organic acid	0.33	0.23

*,** Significant difference from other chemical components at $p < 0.05$ and $p < 0.01$, respectively.

lations between the additive amounts and the activities of two pancreatic enzymes (Table 1). These results suggested that the Fuzhuan brick-tea might be the digestion aids in the food which is full of starch and protein.

Chemical compounds and their relationship with two pancreatic enzymes' activities

It is interesting to find out the compounds, which might be related with the activities of two pancreatic enzymes in Fuzhuan brick-tea. The linear correlation analysis between the contents of chemical compounds and the activities of two enzymes were adopted. As shown in Table 2, the contents of gallocatechin (GC), epigallocatechin (EGC), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), epicatechin gallate (ECG), total catechins, total tea polyphenols, oxalic acid, L-malic acid and ascorbic acid were correlated with the activity of pancreatic amylase, and the contents of GC, EGC, EGCG, GCG, ECG, total catechins, total tea polyphenols, ascorbic acid, succinic acid and oxalic acid were correlated with the activity of pancreatic protease.

Principal component analysis is a useful tool for reducing the dimensionality and retaining enough variations and visualization of the datasets. Here, ten dimensions from the aforementioned datasets were reduced to two dimensions due to the score of the first two components (PC1 and PC2). Their cumulative variances are 96.424 and 97.357% corresponding to pancreatic amylase and protease, respectively (Tables 3 and 4) and this appeared to provide enough information for the datasets. Further analysis showed that total catechins (component score = 0.994) and L-malic acid (component score = 0.400) were selected as the representative components for PC1 and PC2 to pancreatic amylase (Table 3). To pancreatic protease, they were total catechins (component score=0.997) and oxalic acid (component score = 0.336), respectively (Table 4). Two regression models of the two pancreatic enzymes' activities on the above two principal components were proposed as follows, and they both showed highly significant relation-

Table 3. Total variance distribution by principal component analysis and elements score (amylase).

Component	Total	% of Variance	Cumulative (%)
1	9.268	92.678	92.678
2	0.375	3.746	96.424
3	0.180	1.798	98.222
4	0.132	1.324	99.546
5	0.033	0.326	99.872
6	0.011	0.106	99.978
7	0.002	0.022	100.000
8	0.000	0.000	100.000
9	0.000	0.000	100.000
10	0.000	0.000	100.000

Element	PC1	PC2
GC	0.980	0.023
EGC	0.938	-0.249
EGCG	0.990	0.003
GCG	0.983	0.091
ECG	0.976	-0.158
Total catechins	0.994	-0.081
Tea polyphenols	0.993	0.077
Oxalic acid	0.911	-0.274
Ascorbic acid	0.968	0.176
L-malic acid	0.888	0.400

Extraction method used is the principal component analysis.

Table 4. Total variance distribution by principal component analysis and elements score (protease).

Component	Total	% of Variance	Cumulative (%)
1	9.453	94.532	94.532
2	0.283	2.825	97.357
3	0.144	1.440	98.797
4	0.068	0.684	99.481
5	0.037	0.368	99.849
6	0.013	0.130	99.979
7	0.002	0.021	100.000
8	0.000	0.000	100.000
9	0.000	0.000	100.000
10	0.000	0.000	100.000

Element	PC1	PC2
GC	0.982	-0.120
EGC	0.945	0.209
EGCG	0.989	0.016
GCG	0.984	-0.163
ECG	0.979	0.115
Total catechins	0.997	0.024
Tea polyphenols	0.988	-0.030
Oxalic acid	0.911	0.336
Ascorbic acid	0.964	-0.232
Succinic acid	0.979	-0.127

Extraction method used is the principal component analysis.

ship:

$$Y_1 = 23.025 + 0.572X_1 + 0.742X_2 \quad (1)$$

Where Y_1 is the pancreatic amylase activity, X_1 is total catechins, X_2 is L-malic acid, $R^2 = 0.921$ and $p < 0.01$.

$$Y_2 = 10.192 + 0.386\, X_1 + 4.189 X_3 \quad (2)$$

Where, Y_2 is the pancreatic protease activity, X_1 is the total catechins contents in Fuzhuan brick-tea, X_3 is the oxalic acid content in Fuzhuan brick-tea, $R^2=0.888$ with $p < 0.05$.

Tea polyphenols, especially tea catechins, were supposed to help digestion for a long time. Here, in addition to tea catechins, the organic acids of Fuzhuan brick tea, which were specially increased after fungal fermentation, were proposed to promote the activities of two digestive enzymes.

Conclusion

Tea, as a popular beverage in the world, is famous for its health benefits. Fuzhuan brick-tea is a special kind of Chinese tea which has a fungal fermentation process as Pu-erh tea. The present work confirmed the importance of microbial fermentation in the compositions changes of Fuzhuan brick-tea, which resulted in the formation of its special aroma and taste. Once more, the good promotion of Fuzhuan brick-tea on pancreatic amylase and protease *in vitro* were obtained from the present work. In addition to catechins, the organic acids which were the products of fungal fermentation were highly correlated with the activities of two pancreatic enzymes. These results supported the possible application of microbial fermented tea such as Fuzhuan brick-tea in digestive aid. Further studies on the regulating mechanism of catechins and organic acids in Fuzhuan brick-tea on digestive enzymes *in vitro* and *in vivo* would be interesting.

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