Changes in Polyphenols and Antioxidant Activities of Yingshan Yunwu Tea during Digestion *in Vitro*

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Abstract [**Objectives**] To explore the change rule of polyphenol content and antioxidant activity of coarse old leaves of Yingshan Yunwu Tea in the process of human digestion. [**Methods**] The coarse and old leaves of Yunwu tea in Yingshan, Huanggang, Hubei Province were selected as the research object, and their digestion *in vitro* was simulated. The total polyphenol content was determined by Folin-phenol reagent colorimetric method, and the DPPH radical scavenging activity and total antioxidant activity were determined. [**Results**] After simulated gastrointestinal digestion *in vitro*, the polyphenol content and antioxidant activity of coarse old leaf tea soup showed a downward trend. After gastrointestinal digestion, the polyphenol content in tea infusion separately decreased by 31.8% and 8.5%; the scavenging rate of DPPH free radical was 97% before digestion, decreased to 92% after gastric digestion and 65% after intestinal digestion, which decreased by 5% and 27%, respectively; after gastrointestinal digestion, the total antioxidant capacity of tea soup decreased by 4.7% and 3.1%, respectively. [**Conclusions**] This study provided a reference for the development and application of coarse old leaves of Yingshan Yunwu tea, and provided a reference for the nutritional value evaluation and comprehensive utilization of coarse old leaves, so as to make the best use of coarse tea leaves and reduce the waste of resources.

Key words Coarse old leaves of Yunwu tea, Polyphenol, Digestion *in vitro*, Antioxidant activity

1 Introduction

Yingshan Yunwu Tea is produced in Tiantangzhai at the southern foot of the main peak of Dabie Mountains in the northeast of Hubei Province. As a geographical indication agricultural product, it is rated as "Ten Famous Teas in Hubei Province"[1]. China is rich in tea resources, but the utilization rate is low, except for a small amount of tea processed into high-grade commercial tea, the rest are leftovers and coarse old leaves[2]. The taste of coarse old leaves is bitter and astringent, and there are a variety of bioactive components such as tea polyphenols, tea polysaccharides and caffeine. Studies have shown that tea polyphenols are the general name of polyphenolic compounds and their derivatives in tea, which are the most important components in tea. They have antioxidant, antiviral and anti-radiation effects, and are widely used in food and medicine fields. Antioxidant capacity is the core of tea polyphenols to play a variety of effects[3]. According to previous studies, the content of tea polyphenols in coarse old tea leaves is 6% - 15%[4].

The antioxidant effect of tea polyphenols is due to its strong ability to supply hydrogen, which can combine with free radicals and convert them into inert compounds, so as to interrupt the chain reaction of free radicals and eliminate free radicals in human body[5]. Feng Liqin *et al.*[6] studied the antioxidant activity of tea polyphenols from old leaves of Ziyang selenium-enriched tea, and the results showed that it had strong scavenging capacity for DPPH free radicals and hydroxyl free radicals. Gao Renjin *et al.*[7] studied the antioxidant activity of tea polyphenols from tea waste, and the results showed that the inhibition rate of tea polyphenols on ·OH could reach 78.13%, and the inhibition rate of tea polyphenols on O^{2-} could reach 60%, which had good inhibition effect on olive oil rancidity and reducing power, and the antioxidant effect was slightly better than that of Vc. Extensive studies have shown that polyphenols in tea have good antioxidant capacity[8], and polyphenols will undergo structural modification or degradation in the gastrointestinal tract before being absorbed by specific organs[9], and their biological activities may also change during digestion[10].

The digestion *in vitro* simulates the digestive environment of human gastrointestinal tract, which can be used to predict the digestibility of food, the release of food components, the change of structure, *etc.*[11]. Zhou Yao *et al.*[12] studied the brewing and drinking effect of ultramicro green tea powder by simulating digestion *in vitro*, and the results showed that it was more conducive to improving the comprehensive utilization rate of tea by human body than the traditional way of brewing tea. Cong Yanli *et al.*[13] studied pears by simulating digestion *in vitro*, and the results showed that pepsin, pancreatin and gastric acid could promote the release of antioxidant active substances in the process of simulated gastrointestinal digestion of pears. Bioactive substances must be released from the food matrix in the form of absorbable in the process of human digestion, absorbed by the gastrointestinal tract and enter the blood circulation system to participate in metabolism, in order to play their nutritional value. As a common tool in food and drug analysis[14], the *in vitro* digestion model has been widely used in health and nutrition research, with the advantages of low cost,

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short test cycle and strong repeatability of results[15], which can effectively reflect the changes in the process of digestion and absorption of food in the human body[16]. In this study, the coarse old leaves of Yingshan Yunwu tea were taken as the research object, and the *in vitro* gastrointestinal digestion model was used to explore the change law of polyphenol content and antioxidant activity of coarse tea during human digestion[17], so as to provide a theoretical basis for the development and utilization of coarse and old leaves of Yingshan Yunwu tea[18].

2 Materials and methods

2.1 Materials

2.1.1 Materials and reagents. Coarse old leaves of Yingshan Yunwu Tea: produced in Yingshan, Hubei; gallic acid (analytically pure): Tianjin Damao Chemical Reagent Factory; Folin phenol reagent, pepsin, pig bile salt, 1,1-diphenyl-2-picrylhydrazyl (DPPH, analytical reagent), ferric chloride (analytical reagent), anhydrous sodium carbonate (analytical reagent): Sinopharm Chemical Reagent Co., Ltd.; Trypsin: Shanghai Yuanye Bio-Technology Co., Ltd.; hydrochloric acid (analytically pure): Kaifeng Dongda Chemical Co., Ltd. of China Pingmei Shenma Energy & Chemical Group Co., Ltd.; anhydrous ethanol (analytically pure): Tianjin Beilian Fine Chemicals Development Co., Ltd.; potassium ferricyanide (analytically pure): CANSPEC CHINA; trichloroacetic acid (analytically pure): Shanghai Macklin Biochemical Technology Co., Ltd.

2.1.2 Instrument. 722S Visible Spectrophotometer: Shanghai Jinghua Technology Instrument Co., Ltd.; THZ-C Constant Temperature Oscillator: Taicang Experimental Equipment Factory; CP413 Electronic Balance: Ohaus Instrument (Changzhou) Co., Ltd.; AL204 Electronic Balance: Mettler-Toledo Instruments, Inc; GZX-9240MBE Electric Heating Constant Temperature Blast Drying Oven: Shanghai Boxun Medical Biological Instrument Co., Ltd.

2.2 Tea soup extraction The coarse old leaves were dried and pulverized, and 3 g of tea powder was weighed and put into a tea bag, which was put into 150 mL of boiling water and stirred continuously for 5 min. The tea bag was taken out and the tea soup was cooled for later use.

2.3 *In vitro* digestion simulation

2.3.1 *In vitro* simulation of gastric digestion. With reference to the methods of Xing Huiying *et al.*[19] and Li Gou *et al.*[20], we made slight changes to prepare simulated gastric juice. First, we dissolved 0.04 g of pepsin in 10 mL of 0.1 mol/L hydrochloric acid solution. The *in vitro* simulation of gastric digestion referred to the method of Liu Jingmin *et al.*[21], with slight changes. We took 150 mL of tea soup, adjusted the solution system to pH 2 with 1 mol/L hydrochloric acid, added 5 mL of simulated gastric juice, shook evenly, and placed it in a constant temperature oscillator, shook at 37 ℃ for 2 h to simulate the gastric digestion process. Samples were taken every 30 min during gastric digestion, and the digestive juice was heated in a water bath at 90 ℃ for 5 min to inactivate digestive enzymes. After standing and cooling for 5 min, the supernatant was taken and stored at 4 ℃ for later use.

2.3.2 *In vitro* simulation of intestinal digestion. With reference to the methods of Xing Huiying *et al.*[19] and Li Gou *et al.*[20], the simulated intestinal juice was prepared with slight changes. We dissolved 0.02 g of trypsin and 0.125 g of pig bile salt in 10 mL of 0.1 mol/L $NaHCO_3$ solution. The *in vitro* simulation of intestinal digestion referred to the method of Liu Jingmin *et al.*[21], with slight changes. Repeated the process of gastric digestion, took 100 mL of digestive juice after simulated gastric digestion without enzyme inactivation, adjusted the solution system to pH 7 with 1 mol/L $NaHCO_3$ solution, added 5 mL of simulated intestinal juice, shook evenly, and placed it in a constant temperature oscillator, shook at 37 ℃ for 2 h to simulate the process of intestinal digestion. Samples were taken every 30 min during intestinal digestion. Took out each digestive juice and heated it in a water bath at 90 ℃ for 5 min to inactivate the digestive enzyme. After standing and cooling for 5 min, took out the supernatant and stored it at 4 ℃ for later use.

2.4 Determination of total polyphenol content The determination method referred to the method in standard GB/T 8313-2018[22], and the total polyphenol content in coarse tea was determined by Folin-phenol reagent colorimetric method. With gallic acid as the standard, a standard curve was prepared based on the absorbance (A) of the gallic acid working solution and the gallic acid concentration of each working solution. Transferred 1.0 mL of digestive juice of coarse tea leaves at different time stages into 10 mL volumetric flasks, added 5.0 mL of Folin phenol reagent into each volumetric flask, and shook up. Within 3 – 8 min of reaction, 4.0 mL of 7.5% sodium carbonate solution was added, and the mixture was shaken to a constant volume. Placed at room temperature for 60 min. The absorbance was measured with a spectrophotometer at 765 nm using a 10 mm cuvette.

2.5 Determination of antioxidant capacity

2.5.1 Determination of DPPH free radical scavenging activity. With reference to the methods of Chen Jin'e *et al.*[23] and Chen Wei *et al.*[24], we took 2.0 mL of the digestive solution to be tested into a 10 mL volumetric flask, added 2.0 mL of 2×10^{-4} mol/L DPPH solution, shook up, placed in the dark for 30 min, measured the absorbance Ai at 517 nm, and replaced the blank with 2.0 mL of absolute ethanol; took 2.0 mL of the digestion solution to be tested, put it into a 10 mL volumetric flask, added 2.0 mL of absolute ethanol, shook up, placed it in the dark for 30 min, and measured the absorbance A_j at 517 nm; separately took 2.0 mL of DPPH solution and absolute ethyl alcohol, shook up, placed them in the dark for 30 min, measured the absorbance A_0 at 517 nm, measured each digestive solution to be measured for three times, took the average value, and calculated the scavenging rate using the following formula.

$$\text{DPPH free radical scavenging rate (\%)} = 1 - \frac{A - A_j}{A_0}$$

2.5.2 Determination of total antioxidant capacity. With refer-

ence to the methods of Li Meiling *et al.* [25] and Wang Min *et al.* [26], took 1.0 mL of the digestion solution to be tested into a 10 mL centrifuge tube, and then separately added 2.5 mL of 0.2 mol/L (pH 6.6) sodium phosphate buffer solution and 2.5 mL of 1% potassium ferricyanide solution. After the centrifuge tube was bathed in 50 ℃ water for 20 min, added 2.5 mL of 10% trichloroacetic acid, and shook up. Took 5 mL of the mixed solution in the centrifuge tube into a 10 mL volumetric flask, added 4.0 mL of deionized water and 1.0 mL of 0.1% ferric chloride solution, and mixed well, then measured the absorbance A at 700 nm. The higher the absorbance, the stronger the total antioxidant capacity.

2.6 Data processing With the aid of Excel 2010 software, we plotted curves for analysis, and used SPSS 24.0 software for ANOVA one-way analysis of variance, and the significance level was $P<0.05$.

3 Results and analysis

3.1 Standard curve of gallic acid We plotted the standard curve with the different concentrations of gallic acid standard stock solution as the abscissa (X) and the absorbance A measured at the wavelength of 765 nm as the ordinate (Y), as shown in Fig. 1.

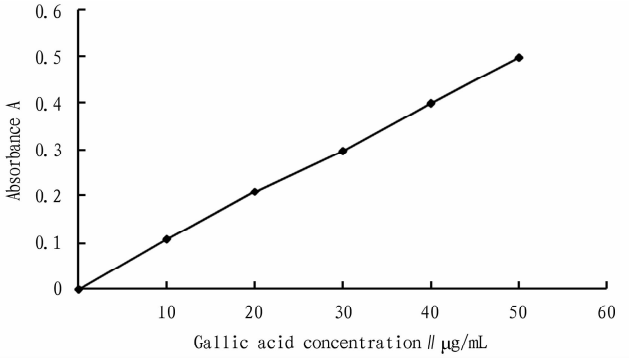

Fig. 1 Standard curve of gallic acid

The linear equation of gallic acid standard curve was $y=0.0098x+0.0062$, $R^2=0.9993$. The gallic acid solution showed a good linear relationship in the range of 0 – 50 μg/mL.

Total phenolic content results are denoted as gallic acid equivalents (gallic acid equivalents, GAE) and expressed in μg GAE/mL. As known from the calculation of the measured absorbance of the coarse tea sample, the content of total polyphenols in the decoction was 13.93 μg GAE/mL.

3.2 Changes in total polyphenol content in crude tea infusion after *in vitro* digestion Fig. 2A and Fig. 2B show the changes in polyphenols in the process of simulated gastric and intestinal digestion of coarse old leaf tea soup. The total polyphenol content decreased by 40.3% after simulated gastrointestinal digestion *in vitro*. As shown in Fig. 2A, the polyphenol content before digestion was 13.93 μg GAE/mL. In the gastric digestion stage, the polyphenol content suddenly decreased to 8.86 μg GAE/mL at 0.5 h after the start of gastric digestion, with a decrease of 36.4%, and there was a significant difference at each time point ($P<0.05$). However, with the extension of digestion time, the polyphenol content fluctuated slightly, rising to 9.10 μg GAE/mL after 1 h and 9.50 μg GAE/mL after 2 h, with an overall decrease of 31.8%. It can be seen from Fig. 2B that in the intestinal digestion stage, the polyphenol content showed a gradual downward trend, with significant differences between 0 – 1 h and 1.5 – 2.0 h ($P<0.05$), but the polyphenol content did not change significantly between 1.0 – 1.5 h ($P>0.05$). After digestion for 2 h, the polyphenol content decreased to 8.31 μg GAE/mL, with a decrease of 8.5%. Liu Jingmin *et al.* [21] found that the polyphenol content of four kinds of tea extracts showed a downward trend after gastrointestinal digestion *in vitro*, and the polyphenol content in the gastric digestion stage showed a more obvious downward trend, which was consistent with the results of this study. However, the downward trend of intestinal digestion stage was accompanied by fluctuations, and there was a rebound, which was slightly different from the trend of this study. Liu Chaoyue *et al.* [27] found that some acidic phenolic compounds may be degraded to other substances in neutral environment. The significant increase in the later stage may be due to the hydrolysis and release of polyphenol molecules combined with polysaccharides in the form of ester bonds to form glycosides under the action of enzymes[28], resulting in an increase in the overall phenolic hydroxyl content[29].

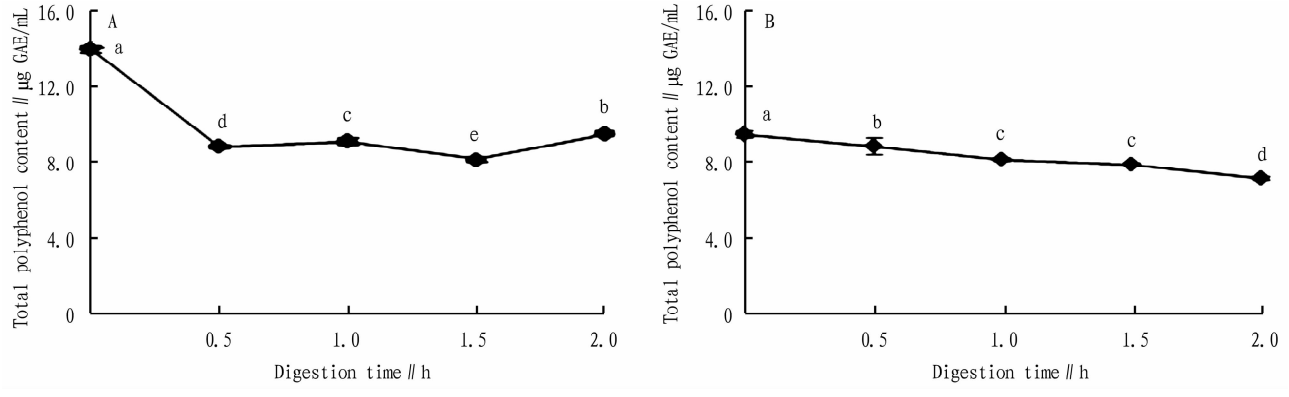

Fig. 2 Changes in total polyphenol content in crude old leaf tea soup during *in vitro* simulated gastric digestion (A) and intestinal digestion (B)

3.3 Changes in DPPH free radical scavenging rate of coarse tea soup after *in vitro* digestion Fig. 3A and Fig. 3B show the changes of DPPH free radical scavenging rate in the simulated gastric and intestinal digestion process of the coarse old leaf tea soup.

The ability of DPPH free radical scavenging decreased continuously after *in vitro* simulated gastrointestinal digestion, with an overall decrease of 32%. It can be seen from Fig. 3A that the DPPH radical scavenging rate before digestion was 97%, the change of DPPH radical scavenging rate within 0 – 1.5 h was not significant ($P > 0.05$), and the changes in DPPH radical scavenging rate within 0.5 – 2.0 h were significant ($P < 0.05$). In the gastric digestion stage, the DPPH free radical scavenging rate in the tea soup changed gently, and decreased to 92% at 2 h, decreased by 5%. It can be seen from Fig. 3B that in the intestinal digestion stage, the DPPH free radical scavenging rate in the tea soup showed a downward trend, which decreased to 65% at 2 h, a decrease of 27%, and there was a significant change in each period ($P < 0.05$). Liu Jingmin *et al.* [21] found that the DPPH free radical scavenging ability of four kinds of tea extracts showed a downward trend after gastrointestinal digestion, which was consistent with the change in this study.

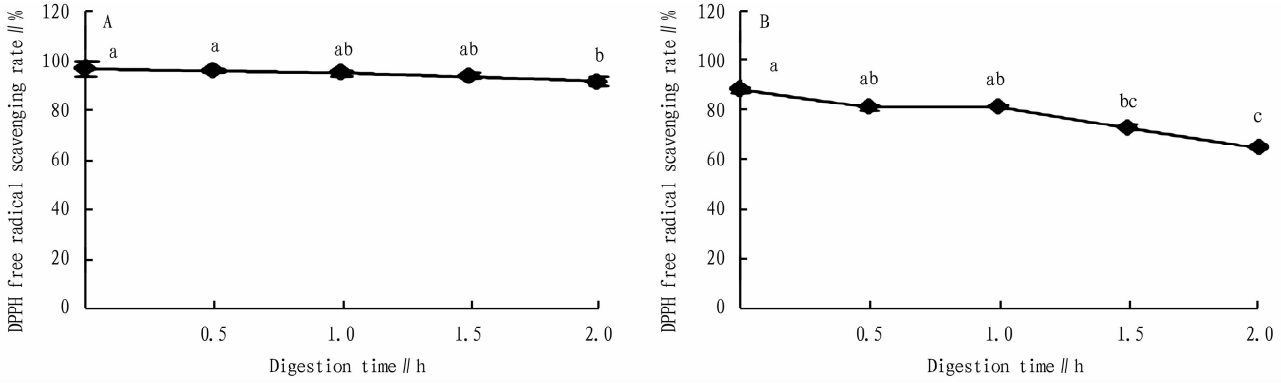

Fig. 3 Changes in DPPH free radical scavenging rate of coarse old leaf tea soup in *in vitro* simulated gastric digestion (A) and intestinal digestion (B)

3.4 Changes in total antioxidant capacity of crude tea soup after *in vitro* digestion Fig. 4A and Fig. 4B show the changes in total antioxidant capacity of coarse old leaf tea soup during simulated gastric and intestinal digestion. The higher the absorbance, the stronger the total antioxidant capacity, so the total antioxidant capacity of coarse tea *in vitro* simulated gastrointestinal digestion was declining, with an overall decline of 7.8%. As shown in fig. 4A, the absorbance before digestion was 2.082. At the beginning of gastric digestion, the absorbance suddenly decreased to 2.000 at 1 h, with a decrease of 3.9%, and decreased to 1.984 at 2 h, with a total decrease of 4.7%, with significant changes at each time point ($P < 0.05$). It can be seen from Fig. 4B that in the intestinal digestion stage, the absorbance decreased first, then increased, and then decreased again. The total antioxidant capacity at each time point changed significantly ($P < 0.05$). The absorbance decreased to 1.950 after 0.5 h of intestinal digestion, increased to 1.999 after 1.0 – 1.5 h, and decreased to 1.920 after 2 h, with an overall decrease of 3.1%. The content of total polyphenols decreased continuously after *in vitro* simulated gastrointestinal digestion, with an overall decrease of 40.3%. As shown in Fig. 4, the total antioxidant capacity decreased with the decrease of polyphenol content during simulated gastrointestinal digestion. However, the trend of decline in the intestinal digestion stage was accompanied by fluctuations, and then declined after recovery, which was slightly different from the trend of polyphenol content, which may be related to the transformation of polyphenols in the intestine.

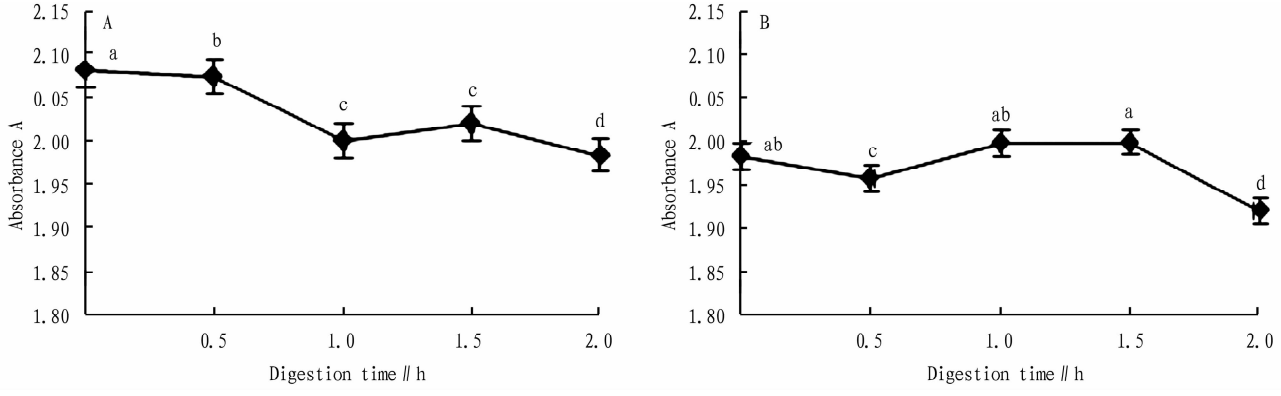

Fig. 4 Changes in total antioxidant capacity of coarse and old leaf tea soup in *in vitro* simulated gastric digestion (A) and intestinal digestion (B)

4 Conclusions

In this study, we analyzed the changes in polyphenols and their antioxidant activities in the coarse old leaves of Yingshan Yunwu Tea using the *in vitro* digestion method. The results indicated that the polyphenol content and antioxidant activity of coarse and old leaf tea soup decreased after simulated gastrointestinal digestion. The

polyphenol content of tea soup before digestion was 13.93 μg GAE/mL, decreased to 9.5 μg GAE/mL after gastric digestion, and decreased to 8.31 μg GAE/mL after intestinal digestion, which decreased by 31.8% and 8.5%, respectively, and the overall decrease was 40.3%, showing a significant change. The scavenging rate of DPPH free radical of tea soup before digestion was 97%, decreased to 92% after gastric digestion, and decreased to 65% after intestinal digestion, which decreased by 5% and 27%, respectively, and decreased by 32% as a whole, showing a significant change. After gastrointestinal digestion *in vitro*, the total antioxidant capacity of tea soup decreased by 4.7% and 3.1%, respectively, and decreased by 7.8% as a whole, showing a significant change. The above results are expected to provide a theoretical basis for the development and application of coarse old leaves, provide a reference for the nutritional value evaluation and comprehensive utilization of coarse old leaves, and lay a foundation for the further development and utilization of coarse old leaves of Yingshan Yunwu Tea[30].

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