Inhibitory Effect of the Theabrownin and Tea Polysaccharide Extracts of Dark Tea on Lipase

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Abstract. In order to investigate the effect of theabrownin and tea polysaccharide of dark tea on reducing fat and weight, theabrownin and tea polysaccharide extracted from dark tea of the same level in different regions were studied in this experiment and p-nitro phenol method and enzyme kinetics method was used to analyze the inhibition effects of theabrownin and tea polysaccharide on lipase, and the inhibition mechanism of tea theabrownin and tea polysaccharide on lipase was explored. The results showed that theabrownin and tea polysaccharide of dark tea extracts had a good inhibitory effect on lipase. Among them, theabrownin extracts of Pu-erh tea had the best inhibitory effect, and the IC$_{50}$ of the semi-inhibitory concentration was the smallest at 24.21mg · mL$^{-1}$, which was significantly less than semi-inhibitory concentrations of theabrownin extracts from dark tea in the other three regions( P<0.01). The inhibitory effect of polysaccharide extract of Liubao tea was the best, and the IC50 of the semi-inhibitory concentration was the smallest, which was 58.57mg · mL$^{-1}$. It is not significantly different from the semi-inhibitory concentration of the tea polysaccharide of Pu-erh and Kang brick, but it was significantly greater than the semi-inhibitory concentration of Fu-brick tea polysaccharide (P <0.01). The results of kinetic studies showed that the catalyst type of tea theabrownin extract of all teas was a mixed type, which contained competitive and non-competitive type in a reversible inhibition type. The catalyst type of tea polysaccharide extract of Pu-erh tea, Liubao and Tibetan tea tea were mixed types, which contained competitive and noncompetitive type in a reversible inhibition type. The catalyst type of polysaccharide extract of Fu-brick tea was a reversible inhibition type and its inhibition type was competitive inhibition. In conclusion, the theabrownin extract of Pu-erh tea and tea polysaccharide extract of Liubao tea had the best inhibitory effect on the lipase.

1. Introduction

The World Health Organization (WHO) defines obesity as abnormal fat distribution or excessive fat accumulation. A large number of studies had shown that obesity not only increases the incidence of chronic diseases such as hypertension, type 2 diabetes, breast cancer, and colon cancer, but also causes an increase in mortality and seriously affects people's health [1]. At present, about one-third of the...
global population is obese, and China had 16.3% of male obese patients and 12.4% of female obese patients. Therefore, it is urgent to find a healthy, safe and effective way to lose weight.

Lipase, also known as glyceride hydrolase, is a general term for a class of enzymes that hydrolyze fats and belongs to carboxyl ester hydrolase [2]. Studies show that lipase is closely related to obesity. The use of lipase inhibitors can effectively inhibit the activity of lipase in the intestine, reduce the accumulation and synthesis of fat in the body, and achieve the goal of weight loss [3], [4]. Therefore, screening for materials that inhibit lipase activity had become a hot topic.

Dark tea belongs to post-fermented tea. It is the only one of the six major teas with microorganisms involved in its processing and quality formation. It is mainly produced in Hunan, Yunnan, Sichuan, Guangxi and other provinces (regions). Dark tea uses four leaves and five leaves, and even pruned branches and leaves. The content of tea polysaccharides is the first of the six major teas. Studies had found that dark tea had a variety of pharmacological effects [5-8], especially its good effect of reducing fat and losing weight had been proven from many aspects such as cells, animals and humans, but its ingredients and effects The mechanism is unclear. Theabrownin is the main pigment in dark tea and an important functional component. It is made from theaflavin and theaflavin and oxidized and polymerized. It had a good effect of reducing fat and losing weight [9-12]. Tea polysaccharide is a general term for active polysaccharides extracted from tea leaves. Studies had found that tea polysaccharides had the effect of reducing free fatty acids [13]. At present, the inhibitory effects of dark tea theabrownin and tea polysaccharides on lipase activity and enzymatic kinetic reactions had not been reported. Therefore, this paper intends to study the inhibitory effects of theabrownin and tea polysaccharides on lipase activity and make the theabrownin the health effects of tea polysaccharides and their further development and utilization provide scientific basis and provide new ideas for finding natural lipid-lowering materials.

2. Materials and methods

2.1. Materials and reagents

This test was taken from dark tea samples produced in different dark tea main producing areas of the country as experimental materials. These samples all had the quality characteristics of regular dark tea and were seen as following in the table 1 for details.

| Tea         | Years | Origin           | Factory                                                                 |
|-------------|-------|------------------|-------------------------------------------------------------------------|
| Pu-erh tea  | 2014  | Shimonoseki, Yunnan | Yunnan Shimonoseki Tea (Group) Co., Ltd.                               |
| Tibetan tea | 2014  | Ya'an, Sichuan    | Sichuan Lucky Tea Co., Ltd. (Ya'an Tea Factory)                        |
| Fu-brick tea| 2014  | Baishaxi, Hunan   | Hunan Baishaxi Tea Factory Co., Ltd.                                   |
| Liubao tea  | 2014  | Wuzhou, Guangxi   | Guangxi Zhuang Autonomous Region Wuzhou Tea Factory                    |

Note: The blackness and tenderness of the dark tea samples were basically the same; the Pu-erh tea materials in this article were all Pu-erh tea.

Lipase (extracted from pig pancreas, enzyme activity ≥30000U / g, PPL, typeII), Sigma Company, USA; 4-nitrophenylbutyrate (p-NPB), p-nitro phenol (p-NP), dimethyl sulfoxide (DMSO), Tris, absolute ethanol, 95% ethanol, anhydrous acetone, anhydrous Diethyl ether, ethyl acetate, chloroform, n-butanol, etc. were all domestic analytical grade. Instruments and equipment SHY-2A water bath constant temperature oscillator Changzhou Guoyu Instrument Manufacturing Co., Ltd; SPX-250 Biochemical Incubator Shanghai Shixin Constant Temperature Equipment Factory. High-speed refrigerated centrifuge American Sigma; UV-2300 UV-visible spectrophotometer Hitachi Japan, Precision micropipette Shanghai Jia’an Analytical Instrument Factory; RE52-86A rotary evaporator Shanghai Yarong Biochemical Instrument Factory.
2.2. Experimental method

2.2.1. Solution preparation. Preparation of lipase solution [14-15]: 0.5000g of lipase was accurately weighed in a mortar, and was added to a little Tris-HCl buffer (pH = 8.0), and grinded and added to a 100 mL volumetric. It was flacked, stood, frozen and centrifuged at 12000 r / min for 1min at 4℃, and saved. The supernatant was stored in a refrigerator at -20°C until it was used as an enzyme stock solution with a concentration of 5 mg mL⁻¹.

Substrate preparation: a certain amount of ρ-NPB was accurately weighed, and it was prepared with DMSO to configure a stock solution with a concentration of 0.01mol·L⁻¹, and was put it in a -2℃refrigerator for future using.

Preparation of p-nitro phenol solution: a certain amount of ρ-NP was accurately weighed and prepared to a 10 μmol mL⁻¹ solution with DMSO for later using.

Preparation of 0.05 mol·L⁻¹ Tris-HCl buffer (pH = 8.0): 6.0575g Tris-HCl was accurately weighed and put in water to make up to 250 mL. 25mL of Tris solution was token and added to a 100mL volumetric flask containing 25mL of 0.1mol·L⁻¹ HCl.

2.2.2. Preparation of theabrownin [16]. 40g each of dark tea and tea samples was weighed, and added to boiling distilled water at 1:20 tea-to-water ratio, and extracted for 30min, it was repeated for 3 times and the extracts were combined, and filtered through 4 layers of gauze, and the resulting filtrated under vacuum was filtered, and concentrated under reduced pressure on a rotary evaporator. To obtain an extract, the extract was extracted three times with equal volumes of n-butanol, chloroform, and ethyl acetate. The aqueous phases were combined, concentrated under reduced pressure, and dried. The theabrownin content was determined by systematic colorimetry [17].

2.2.3. Preparation of dark tea polysaccharide [18]. 20.0g of tea samples was weigh, crushed and extracted for 30min under the condition of tea-to-water ratio of 1:20 and temperature of 70℃, suction filtration while hot, the extraction was repeated 3 times and the filtrates was combined, and concentrated to 20% of the original volume. The concentrated tea soup was mixed with 3 times the volume of 95% ethanol for 1 hour, and then centrifuged at 7000 r / min for 5 minutes. The precipitate was washed twice with anhydrous ethanol, acetone, and ether, and vacuumed at 70℃, dried. The tea polysaccharide content was determined through anthrone-sulfuric acid colorimetry [19].

2.2.4. Determination of lipase activity [20-21]

1) Drawing of p-nitro phenol standard curve. 10 μmol.mL⁻¹ p-nitro phenol 1.0, 2.0, 3.0, 4.0, 5.0mL were added to 6 test tubes, and then made up to 5mL with DMSO. A blank solution without p-nitro phenol was use as a control at 405 nm. In the colorimetric comparison, the standard curve of p-nitro phenol was obtained with the absorbance value as the ordinate and the concentration of p-nitro phenol as the abscissa.

2) Determination of enzyme activity. 2100 μL of 0.05mol·L⁻¹ Tris-HCl buffer (pH = 8.0) and 300 μL of enzyme solution was put in a 37°C incubator for 15 minutes, and then injected into 100 μL of substrate solution, and shaked for 15 minutes, and 5 mL of water ethanol with the lipase-free solution as a blank control was immediately added, and measured at a wavelength of 405 nm. Using the absorbance value as the ordinate and the concentration of p-nitro phenol as the abscissa, a standard curve for p-nitro phenol was obtained.

3) Calculation of enzyme activity. The lipase activity unit was defined as: Under certain conditions, the amount of enzyme that releases 1 μmoL of p-nitro phenol per minute was defined as an enzyme activity unit (U). And calculated as follows:

\[ X = \frac{cV}{tV'} \]

\[ X = \frac{cV}{tV'} \] (1)

X was lipase activity, U·mL⁻¹; c was the concentration of p-nitro phenol, μmoL·mL⁻¹; V was the
4) Determination of inhibitory activity of lipase inhibitor. Lipase inhibitory enzyme activity was determined according to the method of 1.3.4. First, quantitative Tris-HCl buffer solution (pH = 8.0), theabrownin (tea polysaccharide) extract and lipase solution were added, and preheated, and then added to the substrate solution, and then the reaction was stopped, the remaining enzyme activity was measured, and the inhibition rate was calculated as follows:

\[
\text{Inhibition rate} = \frac{(\text{Lipase activity} - \text{Post-inhibition vitality})}{\text{Lipase activity}} \times 100\% \quad (2)
\]

2.2.5. Determination of semi-inhibitory concentration. The inhibition rate of lipase activity was measured at the concentrations of the theabrownin extracts of dark tea (tea polysaccharides) at 0, 1.25, 2.5, 5.0, 10.0, 20.0, 40.0, 80.0mg mL\(^{-1}\), Tea polysaccharide) concentration extract was the abscissa, the lipase inhibition rate was the ordinate, and a regression curve was obtained to obtain the semi-inhibitory concentration IC\(_{50}\). The smaller the semi-inhibitory concentration, the stronger the inhibitory ability.

2.2.6. Determination of lipase inhibition kinetics \([22-23]\)

1) Determination of the type of suppression. A certain amount of enzyme solution with a mass concentration of 0, 2, 3, 4, 5 mg mL\(^{-1}\) without no inhibitor was added to appropriate concentration of the theabrownin extracts of each dark tea (tea polysaccharide) extract according to the above 1.3.4 method, and the absorbance was measured and the reaction rate was calculated. Dixon mapping method was used to plot the reaction rate against lipase concentration to determine the type of inhibition.

2) Reversible inhibition kinetics. The theabrownin (tea polysaccharide) extract of each dark tea was diluted to a certain concentration, and 300 μL was added to 300 μL enzyme solution, and then it was added to the substrate concentration of 0.001, 0.002, 0.003, 0.004mmol·L\(^{-1}\) reaction system, and the enzyme reaction rate was measured. Line weaver-Burk double reciprocal method was used to plot \(1 / V\) vs. \(1/S\) to determine the type of reversible inhibition of lipase by theabrownin (tea polysaccharide) extract.

2.3. Data processing

SPSS 22.0 was used for significance analysis. Comparison between different groups was performed by LSD method for analysis of variance. All data were expressed as ± s. The results were significantly different at P <0.05 and extremely significant at P <0.01.

3. Results and analysis

3.1. Dark tea theabrownin and tea polysaccharide content and analysis of crude product purity

Table 2 showed the contents of the theabrownin and tea polysaccharides of four kinds of dark tea and the purity analysis of crude products. It can be seen that under the same theabrownin conditions, the theabrownin content of Pu-erh tea was the highest, reaching at 8.17%, which was significantly higher than that of Liubao, Tibetan tea, and Fu-brick tea (P <0.01). The theabrownin content of Fu-brick tea was the lowest at 3.17%. The theabrownin content of Fu-brick and Tibetan tea were not significantly different; Under the same conditions for the determination of tea polysaccharides, the content of polysaccharides in Pu-erh tea was the highest, being 2.83%, which was significantly higher than that of theabrownin in Liubao, Tibetan tea, and Fu-brick (P <0.01), There was no significant difference in polysaccharide content between Liubao tea and Tibetan tea. Fu-brick tea had the lowest polysaccharide content at 1.46%. Among the crude theabrownin products, the purity of Pu-erh tea theabrownin was the highest, being 54.97%, which was significantly higher than that of other dark theabrownin products; Among the crude tea polysaccharide products, there was no significant difference between the purity of Pu'er, Liubao and Tibetan tea polysaccharides, which was
significantly higher than that of Fu-brick tea polysaccharides (P < 0.01).

### Table 2. The content and purity analysis of theabrownin and tea polysaccharide of dark tea (±s, %)

| Sample       | Pu-erh tea | Liubao tea | Tibetan tea | Fu-brick tea |
|--------------|------------|------------|-------------|--------------|
| Theabrownin content | 8.17±0.09aA | 5.91±0.07bB | 3.18±0.11cC | 3.17±0.00cC |
| Theophylline purity | 54.97±0.03aA | 47.91±0.08bB | 27.04±0.03cC | 26.94±0.05cC |
| Tea polysaccharide content | 2.83±0.00aA | 2.32±0.01bB | 2.19±0.01bB | 1.46±0.01cC |
| Tea polysaccharide purity | 19.51±1.33aA | 18.00±0.04aA | 18.91±0.07aA | 15.56±0.07bB |

Note: For horizontal comparison at the same level, lowercase letters indicate significant differences, P < 0.05; uppercase letters indicate significant differences, P < 0.01

### 3.2. Effects of tea theabrownin and tea polysaccharide extracts on lipase activity

Under different concentrations of p-nitro phenol solution, using a blank solution without p-nitro phenol as a control, the standard curve of p-nitro phenol was $y = 0.0637x - 0.0185$, and $R^2 = 0.9995$, as shown in Figure 1.

![Figure 1. Standard curve for p-nitro phenol](image)

According to the method of measuring the semi-inhibitory concentration of lipase activity as shown in 1.3.5, the theabrownin and tea polysaccharide extracts of Pu-erh tea, Liubao tea, Fu-brick and Tibetan tea were measured at concentration gradients of 0, 1.25, 2.5, 5, 10, 20, 40, 60, 80 mg mL\(^{-1}\) conditions for lipase inhibition, The results were shown in Figures 2 and 3. After multivariate linear fitting and SPSS analysis, the IC\(_{50}\) of the theabrownin and tea polysaccharide extracts of each of dark tea of inhibiting lipase activity was obtained, as shown in Table 2.

It can be seen from Figures 2 and 3 that the theabrownin and tea polysaccharide extracts of different dark teas all had a certain inhibitory effect on lipase activity. With the increase of the concentration of theabrownin pigment and tea polysaccharide extract, the inhibitory effect on lipase in a certain concentration range showed an upward trend. It can be seen from Figure 2 that with the increase of the concentration of the theabrownin extract, the inhibition rate of lipase by the theabrownin extract of Pu-erh was significantly higher than that of the other three types of dark theabrownin extract (P < 0.01); When the theabrownin extract concentration was 10, 20, 40 mg mL\(^{-1}\), the inhibition rate of lipase activity of Liubao theabrownin extract was significantly higher than that of Tibetan tea and Fu-brick theabrownin (P < 0.01). Tibetan tea theabrownin extract had a significantly higher lipase inhibitory rate than Fu-brick theabrownin extract. Fu-brick theabrownin extract had the lowest inhibitor. When the concentration of the theabrownin extract was 80 mg mL\(^{-1}\), the inhibition rate of lipase activity of Liubao theabrownin extract was significantly higher than that of Tibetan tea theabrownin extract, and was significantly higher than that of Fu-brick theabrownin extract. There was
no significant difference in the inhibition rate of lipase activity between Tibetan tea theabrownin and Fu-brick theabrownin.

![Figure 2. Effect of different concentrations of dark tea theabrownin extracts on lipase](image_url)

It can be seen from Figure 3 that when the concentration of tea polysaccharide extract was 10 mg·mL⁻¹, the lipase inhibition rate of the polysaccharide extract of Liubao tea was significantly higher than that of Pu-erh tea polysaccharide and Tibetan tea polysaccharide extract, and Pu-erh tea polysaccharide and Tibetan tea polysaccharide. There was no significant difference in the inhibitory rate of lipase activity between the extracts, all of which were significantly higher than the inhibitory rate of lipase activity by Fu-brick tea polysaccharide extract (P < 0.01); When the concentration of tea polysaccharide extract was 20 mg·mL⁻¹, there was no significant difference in the inhibition rate of lipase activity between Liubao tea polysaccharide and Pu-erh tea polysaccharide extract, which was significantly higher than Tibetan tea polysaccharide extract, Pu-erh tea polysaccharide and Tibetan tea polysaccharide. There was no significant difference in the inhibitory rate of lipase activity between the extracts, and the inhibitory rates of the three were significantly higher than those of Fu-brick tea polysaccharides (P < 0.01); When the concentration of tea polysaccharide extract was 40 mg·mL⁻¹, the inhibition rate of lipase activity of Pu-erh tea polysaccharide extract was the highest, which was significantly higher than that of Liubao tea polysaccharide extract, and extremely significantly higher than Tibetan tea and Fu-brick tea polysaccharide extract (P < 0.01), Fu-brick tea polysaccharide extract had the lowest inhibition rate, which was significantly lower than that of the other three types of dark tea polysaccharide extract on lipase activity (P < 0.01); When the concentration of tea polysaccharide extract was 80 mg·mL⁻¹, the inhibition rate of lipase activity between Pu-erh tea polysaccharide and Liubao tea polysaccharide extract was not significant, which was significantly higher than the other two types of dark tea polysaccharide extract (P < 0.01), Pu-erh tea polysaccharide and Tibetan tea polysaccharide extract were significantly different, Tibetan tea polysaccharide extract had a significantly higher inhibition rate of lipase than Fu-brick tea polysaccharide extract.
Figure 3. Effects of different dark tea polysaccharide extracts on lipase activity

From Table 3, it can be seen that the IC$_{50}$ of Pu-erh theabrownin extract inhibiting lipase activity was the smallest, which was 24.21 mg mL$^{-1}$, which was significantly lower than the dark theabrownin extract half inhibitory concentration in the other three regions (P <0.01 ), The best inhibitory effect; Fu-brick theabrownin extract had the largest IC$_{50}$ of semi-inhibitory concentration of lipase activity, which was 64.31 mg mL$^{-1}$, which was significantly higher than other dark theabrownin extracts, which was significantly higher than Liubao tea Lignin and Pu-erh theabrownin extract (P <0.01) had the worst inhibitory effect. The comparison of the inhibitory effect of each dark tea polysaccharide extract on lipase activity. The IC$_{50}$ of Liubao tea polysaccharide extract had the smallest IC$_{50}$ of 58.57 mg mL$^{-1}$, which had the best inhibitory effect. There was no significant difference in the semi-inhibitory concentrations of the substances, which was extremely significantly greater than the semi-inhibitory concentrations of Fu-brick tea polysaccharide extract (P <0.01); the brick tea polysaccharide extract had the largest IC$_{50}$, which was 897.06 mg mL$^{-1}$, with the worst inhibitory effect.

Table 3. IC$_{50}$ of theabrownin and tea polysaccharide extracts on inhibiting lipase activity (mg·mL$^{-1}$)

| sample          | Fu-brick           | Tibetan tea        | Liubao tea         | Pu-erh tea         |
|-----------------|--------------------|--------------------|--------------------|--------------------|
| Theophylline IC$_{50}$ | 64.31±4.50aA       | 56.82±1.67bA       | 40.79±1.43cB       | 24.21±0.12dC       |
| Tea polysaccharide IC$_{50}$ | 897.06±85.70aA    | 98.36±19.57bB     | 58.57±4.72bB       | 71.87±5.48bbB      |

Note: For horizontal comparison at the same level, lowercase letters indicate significant differences, P <0.05; uppercase letters indicate significant differences, P <0.01

3.3. Types of dark tea theabrownin and tea polysaccharide extracts inhibiting lipase

According to the mode of action and binding characteristics of inhibitors and enzymes, the types of inhibition of lipases by inhibitors can be divided into reversible inhibition and irreversible inhibition. Liu et al. [24] found that there was no inhibitor in the system by adding quantitative inhibitors to the enzyme activity measurement system, measuring enzyme activity at different enzyme concentrations, and plotting the enzyme concentration as the abscissa and the reaction rate as the ordinate. Straight through the origin. When a certain amount of reversible inhibitor was present in the system, a straight line with a relatively low slope across the origin can also be obtained. Therefore, it can be seen from Figure. 4 that the types of inhibition of lipase by the dark tea theabrownin extracts were all reversible. The types of inhibition of lipases by each dark tea polysaccharide extract were shown in Figure. 5. The types of lipase inhibition by Pu-erh tea, Liubao tea, Fu-brick and Kangzhuan tea polysaccharides were all reversible.
Figure 4. The Dixon diagram of inhibitory effect of dark tea theabrownin extracts on lipase

Figure 5. The Dixon diagram of inhibitory effect of dark tea polysaccharide on lipase
3.4. Types of reversible inhibition of lipase by tea theabrownin and tea polysaccharide extracts

Figure 6 was the result of a double reciprocal Line weaver-Burk plot of reversible inhibition of lipase by each tea black brown extract. In the test system, the concentration of theabrownin extracts of Pu'er, Liubao tea, Fu-brick and Tibetan tea were 2.5 and 5 mg ml\(^{-1}\), respectively, and the straight lines intersected in the second quadrant, indicating that the reversible inhibition type was competitive and non-Competitive hybrid [25].

![Figure 6](image1)

**Figure 6.** The line weaver-Burk diagram of reversible inhibition of theabrownin extracts of dark tea on lipase

Figure 7 was the result of a double reciprocal Line weaver-Burk plot of reversible inhibition of lipase by each dark tea polysaccharide extract. In Figures A and B, the concentration of polysaccharide extracts of Pu-erh tea and Liubao tea was 1.25 and 2.5mg ml\(^{-1}\), which intersect in the second quadrant. It was a mixed type of competitive and non-competitive inhibition. In Figure C, the concentration of Fu-brick tea polysaccharide extract was 2.5 and 5mg ml\(^{-1}\). In this Figure, Fu-brick tea polysaccharide extracts of different concentrations intersect at the same point on the 1 / V axis. Therefore, it was judged that Fu-brick tea polysaccharide extract and lipase were at Competitive inhibitors in this enzymatic reaction [26]; as shown in Figure D, the concentration of Tibetan tea polysaccharide extract was 2.5 and 5mg ml\(^{-1}\), and the group intersects in the second quadrant.
4. Discussion and conclusion

Under the same analysis conditions, Pu-erh tea theabrownin content was significantly higher than that of dark tea theabrownin in other regions. It was speculated that there may be the following reasons. First, Pu-erh tea mostly uses Yunnan large-leaf seed sun-green tea as raw material, and generally large-leaf species Small leaf species of tea contain more polyphenols; Secondly, compared with other dark teas, Pu-erh tea was more heavily fermented. During long-term fermentation or storage, the theabrownin pigment will accumulate in large amounts. As the content of tea polysaccharides increases significantly with the growth of tea tree leaves, under the same conditions of raw material grades [27], under the same extraction conditions of dark tea in different regions, the tea polysaccharide content does not differ significantly.

Dark tea theabrownin and tea polysaccharide extracts had obvious inhibitory effects on lipase activity, which was consistent with the results of studies by GAO Bin [28] and Wu [29]. From the semi-inhibitory concentration $IC_{50}$ of the theabrownin extract on lipase activity, it can be seen that there were significant differences in the inhibitory effects of different tea black brown extracts on lipase activity. Analysis of the reasons may be due to the different degrees of dark tea fermentation in different regions, and there were differences in the content of tea polyphenols and catechins during processing [27], which may lead to tea formed by the oxidative polymerization of catechins at Wodui. There were differences in the chemical structure and composition of lignin, which was consistent with the research results of He [30]. Fu-brick tea polysaccharide extract had the worst inhibitory effect on lipase activity. The possible reason was that under the same extraction conditions, tea polysaccharides extracted from dark tea in different regions had the same effective site for inhibiting lipase activity, which makes the difference not significant. There were differences in the composition and content of tea polysaccharides due to various factors [27], so Fu-brick tea polysaccharide extract and dark tea polysaccharide extract in the other three regions had significant differences in inhibition of lipase.
activity.

In summary, the extracts of theabrownin and tea polysaccharides had inhibitory effects on lipase activity. Pu-erh theabrownin extract had the lowest IC$_{50}$, which was 24.21 mg mL$^{-1}$. The semi-inhibitory concentration of theabrownin extracts from regional dark tea ($P < 0.01$), the inhibition effect was the best; The IC$_{50}$ of the semi-inhibitory concentration of Liubao tea polysaccharide extract was the smallest, which was 58.57 mg mL$^{-1}$, and the inhibition effect was the best, but the semi-inhibitory concentration of Pu-erh tea polysaccharide and Tibetan tea polysaccharide extract was not significantly different, and it was significantly greater than that of Pu-erh tea polysaccharide. Semi-inhibitory concentration ($P < 0.01$). Each dark tea theabrownin extract was a competitive and non-competitive mixed type of reversible inhibition type; Pu-erh tea, Liubao and Tibetan tea polysaccharide extracts were all competitive and non-competitive mixed types of reversible inhibition type, and Fu-brick tea polysaccharide extract inhibition type was reversible inhibition type competitive inhibition.

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