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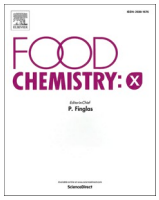

# Probing the binding mechanism of tea polyphenols from different processing methods to anti-obesity and TMAO production-related enzymes through *in silico* molecular docking

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ABSTRACT

Tea polyphenols possess anti-obesity properties and reduce TMAO levels. However, the variability of tea polyphenols under different processing methods and their preventive efficacy requires further exploration. This study systematically evaluated the antioxidant, hypoglycemic, and hypolipotropic enzyme capacities of GT, YT and DT through UPLC-ESI-MS/MS analysis of catechin profiles. OPLS, correlation analysis, and molecular docking were employed to investigate the compounds and inhibitory mechanisms targeting hypoglycemic, hypolipidemic, and TMAO-producing enzymes. GT exhibited significantly lower $IC_{50}$ values for biological activity and higher catechins contents compared to YT and DT ($p < 0.05$). Strong positive correlations were observed between EGCG, CG, and ECG and biological activities ($r \geq 7.4$, $p < 0.001$). Molecular docking results highlighted the establishment of stable hydrogen bonds and hydrophobic interactions between EGCG, CG, ECG, and the receptor. These findings contribute novel insights into the mechanisms by which tea polyphenols prevent obesity and inhibit TMAO production.

## 1. Introduction

Westernized countries, such as the United States, Canada, and Europe, where a diet high in fats predominates. On the one hand, previous studies have shown that this type of diet can lead to obesity (Yoshitomi et al., 2021). On the other hand, Western diet promotes the production of choline, which can be metabolized by several different microbial enzyme complexes in the gut, includ ing CutC/D and CntA/B, to produce TMA, which is metabolized by the hepatic enzyme flavin monooxygease-3 (FMO3) and converted to trimethylamine *N*-oxide (TMAO) in the liver (Li et al., 2021). Furthermore, the abundance of saturated fatty acids, in Western diet affects mitochondrial bioenergetics by inducing hydrogen peroxide production in mitochondria, in addition, negatively affects the physiological state of the gut (Yoo et al., 2021). In contrast, in the colon, the high consumption of oxygen by the mitochondria is crucial to keeping the epithelium in a hypoxic state. The maintenance of hypoxia promotes the growth of specialized anaerobic bacteria in the intestine, this also leads to the suppression of the overgrowth of parthenogenic bacteria, such as Enterobacteriaceae (Litvak, Byndloss, & Bäumler, 2018). This further causes intestinal flora to be disrupted, this affects the conversion of choline to TMA and increases the content of TMAO, both of which lead to cardiovascular disease (CVD).

Tea is a traditional drink in China. Drinking tea can mitigate the

*Abbreviations:* GT, Green tea; YT, Yellow tea; DT, Dark tea; GA, Gallic acid; C, Catechin; EC, Epicatechin; GC, Gallocatechin; EGC, Epigallocatechin; ECG, Epicatechin-3-O-gallate; EGCG, Epigallocatechin gallate; ECG, Epicatechin gallate; GCG, Gallocatechin gallate; EA, Ellagic acid; TPC, Total polyphenols content; TFC, Total flavonoid content; TMA, Trimethylamine; TMAO, Trimethylamine *N*-oxide; FMO3, Flavin monooxygease-3; CVD, Cardiovascular disease; Vc, Vitamin C; PL, pancreatic lipase; CE, cholesterol esterase; OPLS, orthogonal partial least squares; UPLC-ESI-MS/MS, Ultra performance liquid chromatography electrospray ion source mass spectrometry.

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effects of CVD because of its bioactive components (Brimson et al., 2023). First, the primary bioactive components in tea that have a strong antioxidant capacity, such as EGCG, which are 10 times more potent than vitamin C (Vc). Additionally, tea's polyphenols can prevent the digestion of fat and carbohydrates by blocking the activity of digestive enzymes, such as pancreatic lipase (PL) and amylases (Riyaphan et al., 2021). The prevention of obesity can be achieved by targeting the activity of digestive enzyme and the metabolizing of lipid.

According to different processing methods, tea can be divided into unfermented tea (green tea), slight fermented tea (white and yellow tea), semi fermented tea (Oolong tea), fermented tea (black tea) and post-fermented tea (dark tea) (Liu et al., 2022). Different methods of processing may alter the composition or bioactivity of tea; for example, the content of tea polyphenols decreases as the degree of fermentation increases (Ge et al., 2019). As a result, the content alterations of tea polyphenols, the capacity of tea polyphenols to inhibit the development of CVD should be determined at different levels of fermentation, additionally, the effect of tea polyphenols on reducing blood sugar and lowering blood lipids should be assessed in order to identify the most effective fractions for preventing CVD.

Currently, molecular docking is typically employed to calculate the affinity of a protein for a ligand via noncovalent interactions, such as hydrogen bonds, van der Waals, and Coulomb's forces, these are then used to predict the conformation of the ligand in the binding site of the protein, the predicted conformation is then employed as the docking score (Cakmak et al., 2021). Although studies have suggested the inhibitory effect of the main component of tea polyphenols on hypoglycaemic and hypolipidaemic enzymes and the mechanism of action through molecular docking (Shahidi & Dissanayaka, 2023), a comprehensive evaluation of the changes in tea polyphenols and their ability and mechanism on TMAO-producing enzymes has not been reported yet.

This study chose three different types of tea derived from three different methods of processing in Yaan, Sichuan Province that were intended to extract tea's polyphenols. The antioxidant capacity and inhibition ability of tea polyphenols against α-amylase, α-glucosidase, PL and CE were evaluated *in vitro* through comparison of chemical composition. Molecular docking was employed to explore the binding energy and mechanism of action between the main chemical components of tea and α-amylase, α-glucosidase, PL, CE, CutC and FMO3 inhibition ability to determine the most potential components of tea polyphenols obtained from different processing methods.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Gallic acid (GA), catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), epicatechin-3-O-gallate (ECG,), epigallocatechin gallate (EGCG), epicatechin gallate (ECG), gallocatechin gallate (GCG and ellagic acid (EA) were purchased from Sigma-Aldrich (Saint Louis, MO, USA) with a purity of > 98 %. Folin-Ciocalteu phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tri-(2-pyridyl)-1,3,5-triazine (TPTZ), dinitrosalicylic acid (DNS), 4-nitrophenyl-a-D-glucopyranoside (pNPG), porcine pancreatic α-amylase (10 U/mg), *Saccharomyces cerevisiae* α-glucosidase (100 U/mg), porcine pancreatic lipase (30 U/mg) and porcine pancreatic cholesterol esterase (100 U/mg) were obtained from Beijing Solarbio Science & Technology Co. Ltd. (Beijing, China). All other reagents were of analytical grade and were commercially available.

### 2.2. Tea materials

Three different processing teas including green tea (GT), yellow tea (YT) and dark tea (DT) were collected from a local market (Yaan, Sichuan, China). GT, YT and DT represent Ganlu, Huangya and Tibetan teas, respectively (Supplementary Material Fig. S1). Fresh tea leaves were obtained from Monding mountain, Mingshan District, Yaan, Sichuan, China (N 29°58E 103°) in May 2022. The GT and YT maturities of fresh tea shoot were buds, and the fresh tea maturity of DT was one bud with three leaves or old leaves. The three tea samples were stored in the dark at −20 ℃.

### 2.3. Sample preparation

All kinds of teas were crushed in an ultrafine grinder (FW100, Beijing Ever Bright Medical Treatment Instrument Co., LTD) to obtain tea powder. About 10 g of tea powder was added to 200 mL of 70 % methanol and extracted by hot reflux at 80 ℃ while sonicating for 1 h. The sample was cooled and precipitated in a refrigerator at 4 ℃. The supernatant was subsequently centrifuged at 8000 rpm for 15 min (Thermo Sorvall ST 16 ST16R, Thermo Fisher Scientific). Total polyphenol content (TPC) in the supernatant was determined using Folin–-phenol method with gallic acid as the standard. Total flavonoid content (TFC) was quantified using aluminium trichloride-sodium nitrite colorimetric assay with catechin as the standard (Wang et al., 2020). Rotary evaporation (RE-52AA, Shanghai Yarong Biochemistry Instrument Factory) was used to remove methanol in the supernatant, and the concentrate was extracted with twice the volume of trichloromethane. The upper layer of liquid was extracted again with twice the volume of ethyl acetate. Ethyl acetate was removed by rotary evaporation, and the sample was lyophilised to obtain tea polyphenols (Zheng et al., 2021). The three types of tea polyphenols were stored at −80 ℃ until biological activity analysis.

### 2.4. In vitro antioxidant activity assays of tea polyphenols

#### 2.4.1. DPPH radical scavenging assay

DPPH radical scavenging was determined using our previous methods with some modification (Wu et al., 2022). In brief, 100 μL of tea polyphenol solution with various concentrations (0–1 mg/mL) was mixed thoroughly with 500 μL of 0.1 mM DPPH in micro EP tubes. After incubation in the dark for 30 min, absorbance was recorded at 517 nm by using a microplate reader. Vc was used as a positive control, methanol was used instead of the sample for the blank group and anhydrous ethanol was used instead of DPPH-ethanol solution for the background group. DPPH radical scavenging ability was calculated and expressed using Equation (1):

$$\text{Scavenging rate}\% = \frac{A0 - (AS - A1)}{A0} * 100\% \quad (1)$$

where $A_0$ is the absorbance of the control reaction (containing all reagents except the test compound), $A_1$ is the absorbance of the background reaction (containing all reagents except DPPH ethanol solution), and $A_S$ is the absorbance with the test compound.

#### 2.4.2. ABTS radical scavenging assay

ABTS radical scavenging was determined using our previous methods with some modification (Wu et al., 2022). Potassium persulfate (2.45 mM) was mixed with ABTS (7 mM) at a ratio of 1:1, and the $ABTS^+$ reaction solution was obtained after 12 h of reaction away from light. The $ABTS^+$ reaction solution was diluted with anhydrous ethanol until a value of 0.7 ± 0.002 was reached at 732 nm absorbance. About 200 μL of Vc solution or tea polyphenol solution (0–1 mg/mL) and 800 μL of $ABTS^+$ reaction solution were added to the micro EP tubes. After incubation in the dark for 30 min, absorbance was recorded at 734 nm. Methanol was used instead of the sample for the blank group, and anhydrous ethanol was used instead of the ABTS solution for the background group. ABTS radical scavenging ability was calculated and expressed as Equation (1).

#### 2.4.3. OH radical scavenging assay

OH radical scavenging was determined using our previous methods with some modification (Wu et al., 2022). Firstly, 200 μL of the sample (0–1 mg/mL), $FeSO_4$ (9 mM) and $H_2O_2$ (8.8 mM) were mixed. The reaction was initiated by adding 200 μL of salicylic acid (9 mM) and water. The mixture was reacted at 37 ℃ for 30 min under protection from light, and absorbance was recorded at 510 nm. The sample was replaced by water in the blank group, and the $H_2O_2$ solution was replaced by ultrapure water in the background group. OH radical scavenging ability was calculated and expressed as Equation (1).

#### 2.4.4. FRAP assay

FRAP was determined using our previous methods with some modification (Wu et al., 2022). The FRAP working reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM, dissolved in 40 mM HCl) and $FeCl_3$-$6H_2O$ solution (20 mM) at a ratio of 10:1:1 (v/v) at 37 ℃. About 50 μL of the sample solution (0–0.1 mg/mL) and 100 μL/1.5 mL of FRAP working reagent were added into the micro EP tube. Absorbance was recorded at 593 nm after incubation for 30 min at 37 ℃ away from light. The blank group used methanol instead of the sample, and the background group used acetate buffer solution instead of the FRAP working reagent. FRAP rate was expressed by the standard curve.

### 2.5. In vitro enzyme activity assays of tea polyphenols

#### 2.5.1. α-Amylase inhibitory activity assay

α-Amylase inhibitory assay was based on a previous method (Xiong et al., 2022) with slight modifications. In short, 1 % starch solution, α-amylase (1 U/mL) and samples were incubated at 37 ℃ for 30 min. Dinitrosalicylic acid (DNS) was added and boiled for 5 min to terminate the reaction. Distilled water was added to each tube, and absorbance was recorded at 540 nm.

$$Inhibition(\%) = \left(1 - \frac{A_1 - A_2}{A_3 - A_4}\right) \times 100\% \quad (2)$$

where $A_1$ is the sample solution, enzyme solution and substrate; $A_2$ is the sample solution, buffer solution and substrate; $A_3$ is water, enzyme solution and substrate; and $A_4$ is water, buffer solution and substrate.

#### 2.5.2. α-Glucosidase inhibitory activity assay

The assay of α-glucosidase inhibitory activity was referred to a previous method. α-Glucosidase (1.5 U/mL) and the samples were incubated at 37 ℃ for 10 min. The solution was added with pNPG and incubated at 37℃ for 20 min. The $Na_2CO_3$ solution was added to terminate the reaction. Absorbance of the reaction solution was recorded at 405 nm. α-Glucosidase inhibitory activity was calculated and expressed as Equation (2).

#### 2.5.3. Pancreatic lipase inhibitory activity assay

Pancreatic lipase (1 mg/m L) and the samples were incubated at 37 ℃ for 30 min. The sample was added with pNPB (2 mg/mL) and incubated for 20 min. Absorbance of the reaction solution was recorded at 405 nm. Pancreatic lipase inhibitory activity was calculated and expressed as Equation (2) (Zhang et al., 2020).

#### 2.5.4. Cholesterol esterase inhibitory activity assay

Potassium phosphate buffer was added into the centrifuge tube and incubated at 37 ℃ for 5 min. Cholesterol esterase (300 U/mL) was added and incubated at 37 ℃ for 30 min. Finally, the sample (cholesterol esterase solution: tea polyphenol solution = 1:1) was added. Absorbance was recorded at 500 nm. Cholesterol esterase inhibitory activity was calculated and expressed as Equation (2).

### 2.6. Quantification of phenolic composition by UPLC-ESI-MS/MS

The sample was digested with 2 M HCl at 80 °C for 3 h. An aliquot (200 μL) of the supernatant was evaporated under a gentle stream of $N_2$. For UHPLC-ESI-MS/MS analysis, the samples were dissolved in 1 mL of 30 % methanol (vol/vol) and transferred to insert-equipped vials. The sample extracts were analysed using an UPLC-Orbitrap-MS system (UPLC, Vanquish; MS, QE). The analytical conditions were as follows, UPLC: column, Waters ACQUITY UPLC HSS T3 (1.8 μm, 2.1 mm × 50 mm); column temperature, 40℃; flow rate, 0.3 mL/min; injection volume, 2 μL; solvent system, water (0.1 % formic acid): acetonitrile (0.1 % formic acid); gradient program, 90:10 V/V at 0 min, 90:10 V/V at 1.0 min, 65:35 V/V at 6 min, 5:95 V/V at 8 min, 5:95 V/V at 10 min, 90:10 V/V at 10 min, 90:10 V/V at 12 min. HRMS data were recorded on a Q Exactive hybrid Q-Orbitrap mass spectrometer equipped with a heated ESI source (Thermo Fisher Scientific) using SIM MS acquisition methods. The ESI source parameters were set as follows: spray voltage, −2.8 kV; sheath gas pressure, 40 arb; aux gas pressure, 10 arb; sweep gas pressure, 0 arb; capillary temperature, 320 ℃; and aux gas heater temperature, 350 ℃. Data were acquired on Q-Exactive by using Xcalibur 4.1 (Thermo Scientific), processed using TraceFinder™4.1 Clinical (Thermo Scientific). Quantified data were output into Excel format.

### 2.7. Molecular docking assay

Firstly, the 2D structures of small molecular compounds were downloaded from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and converted into 3D structures by ChemBio3D Ultra 14 software for minimum free energy conversion. The 3D structures of α-amylase (1OSE) (Chang et al., 2011), α-glucosidase (3A4A) (Cakmak et al., 2021b), pancreatic lipase (1ETH) (Bajes et al., 2020), cholesterol esterase (1F6W) (Varghese et al., 2019), CutC (5FAU) and FMO3 (2VQ7) (Ma et al., 2022) were downloaded from the PDB database (https://www.rcsb.org/). AutoDockTools 1.5.7 were used to treat the protein structure by successively adding hydrogen, gasteiger charge and merging non-polar hydrogen. The active pocket of the protein was used as the centre of the search box. The central coordinates of α-amylase were set as 37.5, 38.0, and −1.9, the lattice points in each direction of XYZ were fixed as 25.7 × 22 × 26.2. The central coordinates of α-glucosidase were set as 21.5, −7.5 and −24.4, and the lattice points in each direction of XYZ were fixed as 40 × 40 × 40. The central coordinates of pancreatic lipase were set at 81.7, 40.0 and 162.6, and the lattice points in each direction of XYZ were fixed at 50 × 20 × 4. The central coordinates of cholesterol esterase were set at 6.6, 3.3 and 18.4, and the lattice points in each direction of XYZ were fixed at 25 × 25 × 25. The central coordinates of CutC were set at –33.9, −20.6 and 106.5, and the lattice points in each direction of XYZ were fixed at 30 × 30 × 30. The central coordinates of FMO3 were set at −14.3, 180.8 and 14.0, and the lattice points in each direction of XYZ were fixed at 30 × 30 × 30. Auto Dock vina was used for automated semi-flexible docking and minimised energy calculation based on MMFF94s force field. During docking, all twistable bonds of the ligand were released and the exhaustiveness was set to 50. After the docking, the best conformation of the complex was selected according to the binding pose and binding energy for further analysis. The docking results were visualised by PyMOL software and Discovery Studio software 2019 to obtain 3D and 2D images, respectively.

### 2.8. Statistical analysis

One-way analysis of variance (ANOVA) was performed using SPSS (version 24.0), to determine differences among groups. Data were presented as mean ± standard deviation (SD). Values at $p < 0.05$ were considered statistically significant. Principal component analysis (PCA) and orthogonal projections to latent structures (OPLS) were performed using SIMCA-P software (14.1, Umetrics Umea, Sweden). Correlation

analysis heatmaps were generated by Origin 9.1.

## 3. Results and discussion

### 3.1. Changes in TPC and TFC of tea extracts

Table 1 shows significant changes in the TPC and TFC of the three methanol-extracted tea leaves ($p < 0.05$). The TPC of GT extract was the greatest, while the TPC of the DT extract was the least. The TFC of the three types of tea extracts exhibited a similar pattern. In general, the amount of catechins in young leaves is greater than in mature leaves (Xu et al., 2021). GT and YT in our sample were derived from fresh leaves. In addition, the technology involved in processing has a significant impact on the changes in TPC and TFC. During the fermentation process of tea, microbial degradation, polymerisation, oxidation and other chemical reactions will occur due to the high temperature and humid environment, resulting in the oxidation and dimerisation of catechins in tea (Liu et al., 2022).

### 3.2. Changes in antioxidant activity and enzymatic activity in tea polyphenols

The changes in antioxidant activity are shown in Fig. 1(A–D). The trends of the four indices of antioxidant capacity, namely, DPPH, ABTS, OH and FRAP, were related to the degree of fermentation. Unfermented GT polyphenols had the strongest antioxidant activity, and the antioxidant capacity was stronger than that of the Vc control, followed by YT polyphenols; the antioxidant capacity of DT polyphenols was the weakest among the three kinds of tea. The strong antioxidant capacity of green tea polyphenols has been reported (Oliveira et al., 2023). In addition, YT named Eagle tea, exhibits strong antioxidant properties (Liu et al., 2020).

α-Amylase and α-glucosidase are therapeutic targets for the regulation of postprandial blood glucose (Ghauri et al., 2021). As shown in Fig. 1(E, F), GT polyphenols showed the strongest inhibitory capacity for α-amylase and α-glucosidase, but the inhibitory capacity was lower than that for acarbose. The $IC_{50}$ values of GT, YT and DT polyphenols on α-amylase inhibition were 0.425, 0.785 and 0.927 mg/mL, respectively. The $IC_{50}$ values of GT, YT and DT polyphenols on α-glucosidase inhibition were 0.196, 0.416 and 0.927 mg/mL, respectively. Previous research reported that green tea inhibited α-amylase and α-glucosidase ability (Liu et al., 2017). Pu 'er tea, a type of DT, has been reported to have inhibitory α-amylase and α-glucosidase activity (Armstrong et al., 2020).

PL and CE are important factors associated with the development of hyperlipidaemia and related complications (Soares et al., 2015). In the present study, orlistat and simvastatin were used as inhibitors of PL and CE. As shown in Fig. 1(G, H), GT and YT polyphenols showed the strongest inhibitory ability ($p > 0.05$), which were stronger than the controls. The inhibitory ability of DT polyphenols to inhibit enzyme activity was the weakest and lower than that of the controls. In another study, green tea, white tea and red tea extracts caused a direct *in vitro* inhibition of PL and CE (Gondoin et al., 2010; Zhang et al., 2020).

**Table 1**
Total polyphenols content (TPC) and total flavonoids content (TFC) in different teas of dry weight (DW).

| Component (mg/g DW) | GT | YT | DT |
|---|---|---|---|
| TPC | $190.52 \pm 3.04^{a}$ | $165.93 \pm 2.23^{b}$ | $80.65 \pm 0.67^{c}$ |
| TFC | $261.13 \pm 3.35^{a}$ | $65.79 \pm 1.83^{b}$ | $37.39 \pm 0.86^{c}$ |

Data presented are means ± SD (n = 3). Means with different letters within a line are significantly different ($p < 0.05$). DW: dry weight; GT: Green tea; YT: Yellow tea; DT: Dark tea.

### 3.3. Differences in the main chemical composition of tea polyphenols

Catechins are the main functional component of tea and include EGCG (epigallocatechin gallate), EGC (epigallocatechin), EC (epigallocatechin) and their respective non-differential isomers GC (epigallocatechin), CG (catechin gallate), C (catechin) and GCG (epigallocatechin gallate). We assumed that the difference in antioxidant capacity and inhibitory enzyme activity was caused by the difference in the above components; as such, eight major chemical components were identified by UPLC-ESI-MS/MS (Table 2). As shown in Fig. 2, the composition of polyphenols significantly differed among GT, YT and DT. In the PCA score chart (Fig. 2A), GT and YT are close to each other, indicating that they have similar principal components. t (1) line separates BT from GT and YT, indicating that the principal component of BT is different from that of GT and YT. The PCA loading diagram (Fig. 2B) shows that EA, CG, ECG and EGCG are the main components that distinguish DT from GT and YT. The quantitative results showed that the content of three ester catechins in GT polyphenols was higher, the content of EGCG reached 67.34 mg/g dw and the content of ECG was second highest (10.08 mg/g dw), followed by CG (9.72 mg/g dw). The content of YT polyphenols in tea subjected to slight fermentation was also higher in EGCG, ECG and CG. However, in DT polyphenols, the contents of C, EGC and GC were higher, indicating that the content of ester catechin was significantly less than that of non-ester catechin. Previous reports found that the chemical composition of tea differs significantly depending on the degree of fermentation (Salman et al., 2022). Under deeper fermentation, the ester catechin content was lower and the reduction of EGCG was usually accompanied by an increase in the contents of gallic acid and EGC. The C-3 ester bond of EGCG is hydrolysed by microbial extracellular enzymes during fermentation (Peng & Shahidi, 2023). Therefore, the chemical composition of the three different tea polyphenols leads to different biological activities.

### 3.4. OPLS analysis

OPLS is a regression modelling method of multiple dependent variables to multiple independent variables (Chen et al., 2023). An OPLS regression model was established with the antioxidant capacity and inhibitory enzyme activity of the three kinds of tea polyphenols as dependent variable (Y) and absolute quantification of main chemical components as independent variable (X). In the OPLS scoring chart (Fig. 3), red to blue indicates activity values from high to low. The regression line between DPPH ABTS, OH, FRAP, α-amylase, α-glucosidase, pancreatic lipase and cholesterol esterase inhibition and the three kinds of tea polyphenols is shown in Supplementary Material Fig. S2 ($R^2$ = 0.9998, 0.984, 0.9973, 1, 1, 0.9742, 0.9992 and 0.9814, respectively). The prediction results of the OPLS model were found to be reliable. Based on the VIP value (VIP > 1) and P value ($p < 0.05$), OPLS could predict the correlation between components and antioxidant capacity and inhibitory enzyme activity of several important bioactive metabolites (Yang et al., 2020).

#### 3.4.1. OPLS analysis between main chemical composition and antioxidant activity

As shown in Table 3, EGCG, ECG and CG as the major chemical components in tea are associated with antioxidant capacity. Structure–activity relationship studies showed that antioxidant capacity has a strong relationship to the molecular structure; that is, an increasing number of phenolic hydroxyl groups indicates stronger antioxidant capacity (Kongpichitchoke et al., 2015). EGCG, EGC and CG have numerous hydroxyl groups. ECG and CG have the same chemical structure, except that they are isomers of each other. The structure of ECG and CG contains 3 '4′ -dihydroxyl group (catechol group) or 3 '4′ 5 '-trihydroxyl group (gallic group) in the other B ring, 3′ gallic group in the C ring and the 5′ and 7 'hydroxyl groups in the A ring. The structure of EGCG has 3 '4′ 5 '-trihydroxyl groups (pyrogallol group) in the B ring,

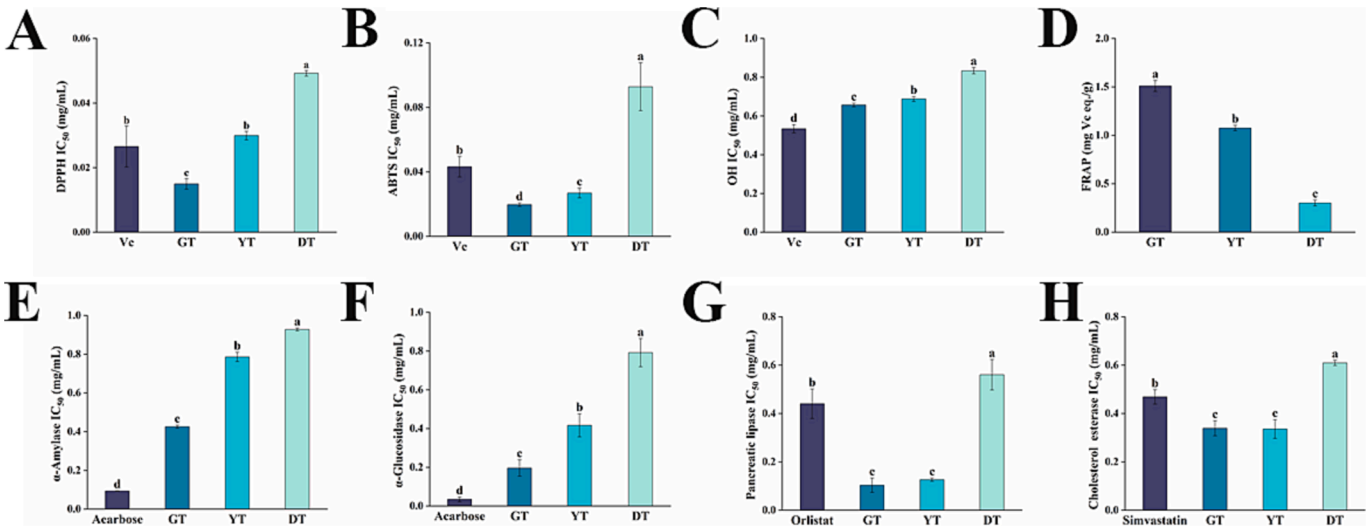


**Fig. 1.** *In vitro* bioactivity in tea polyphenols of the three types of tea extracts. (**A**) Results based on DPPH (**B**) ABTS (**C**) OH radical scavenging ability, (**D**) FRAP antioxidant activity. (**E**) α-amylase (**F**) α-glucosidase (**G**) pancreatic lipase (**H**) cholesterol esterase inhibition ability. Note: columns and error bars represent the mean ± standard deviation; different letters a-d indicated significant difference ($p < 0.05$); GT, green tea; YT: yellow tea; DT: DT; Vc: vitamin C.

**Table 2**
Main chemical composition in different processing methods teas.

| Component (mg/g DW) | GT | YT | DT |
|---|---|---|---|
| EGCG | $67.34 \pm 1.17^{a}$ | $46.43 \pm 0.82^{b}$ | $3.02 \pm 0.06^{c}$ |
| ECG | $10.08 \pm 0.07^{a}$ | $6.74 \pm 0.06^{b}$ | $0.48 \pm 0.02^{c}$ |
| CG | $9.73 \pm 0.30^{a}$ | $6.07 \pm 0.23^{b}$ | $0.44 \pm 0.03^{c}$ |
| C | $6.15 \pm 0.15^{b}$ | $5.12 \pm 0.09^{c}$ | $7.92 \pm 0.07^{a}$ |
| EGC | $5.02 \pm 0.12^{c}$ | $4.72 \pm 0.13^{c}$ | $7.90 \pm 0.06^{a}$ |
| EC | $2.47 \pm 0.04^{b}$ | $2.29 \pm 0.03^{c}$ | $3.86 \pm 0.06^{a}$ |
| EA | $1.66 \pm 0.13^{a}$ | $0.95 \pm 0.04^{b}$ | ND |
| GC | $1.43 \pm 0.04^{b}$ | $1.45 \pm 0.06^{b}$ | $5.57 \pm 0.05^{a}$ |
| GCG | ND | ND | ND |

Data presented are means ± SD (n = 3). Means with different letters within a line are significantly different ($p < 0.05$). DW: dry weight; GT: Green tea; YT: Yellow tea; DT: Dark tea; C: catechin; EC: epicatechin; GC: gallocatechin; EGC: epigallocatechin; ECG: epicatechin gallate; GCG: gallocatechin gallate; EGCG: epigallocatechin gallate; CG: catechin gallate; EA: ellagic acid; ND: not detected.

5′ and 7 ’hydroxyl groups on the A ring and the gallate moiety at the 3′ position in the C ring. The structure also gives them the ability to chelate metal ions, such as iron and copper, thereby contributing to their antioxidant function (Weinreb et al., 2009). The two points of attachment of transition metal ions to flavonoid molecules are the o-diphenolic groups in the 3′,4′-dihydroxy positions in the B ring and the keto structure 4-keto, 3-hydroxy or 4-keto and 5-hydroxy in the C ring of the flavonols (van Acker et al., 1996). EGCG and EC bond dissociation energies (BDEs) have been studied. Free radical scavenging activity is due to the ability of a compound to provide hydrogen atoms or electron donors to form intramolecular hydrogen bonds. Boulmokh et al. (2021) calculated bond dissociation energies of EGCG and EC; the bond dissociation energies of EGCG4 ’–OH are lower than those of the 4′ –OH ECB ring, suggesting that EGCG has a higher antioxidant capacity than EC. Therefore, EGCG, ECG and CG play an important role in antioxidant capacity.

### 3.4.2. *OPLS analysis between main chemical composition and enzymatic activity*

As shown in Table 4, EGCG, ECG and CG significantly inhibited α-amylase, α-glucosidase, PL and CE. EGCG showed greater inhibitory α-amylase effect than ECG. Catechins that contain CG in green, oolong and black tea extracts were reported to play an important role in the inhibition of α-glucosidase (Sun et al., 2016; Yilmazer-Musa et al., 2012). The inhibitory effect of EGCG and ECG on PL was already reported, that is, the IC50 values were 0.016 and 0.027 mmol/L, respectively; the structure of the enzyme also changed (Liu et al., 2020). The structure–activity relationship illustrated that galloylated catechins had higher inhibition effect than nongalloylated catechins; catechol-type catechins were stronger than pyrogallol-type catechins; the inhibition activities of catechins with 2, 3-trans structure were higher than those of

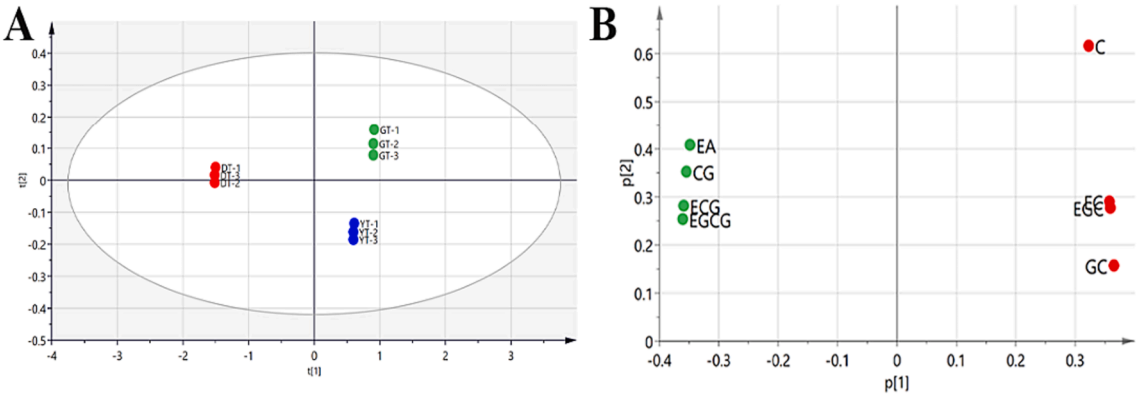


**Fig. 2.** (**A**) Principal component analysis (PCA) score plot, (**B**) partial least squares discrimination analysis (PLS-DA) loading plot for tea polyphenols of three different teas.

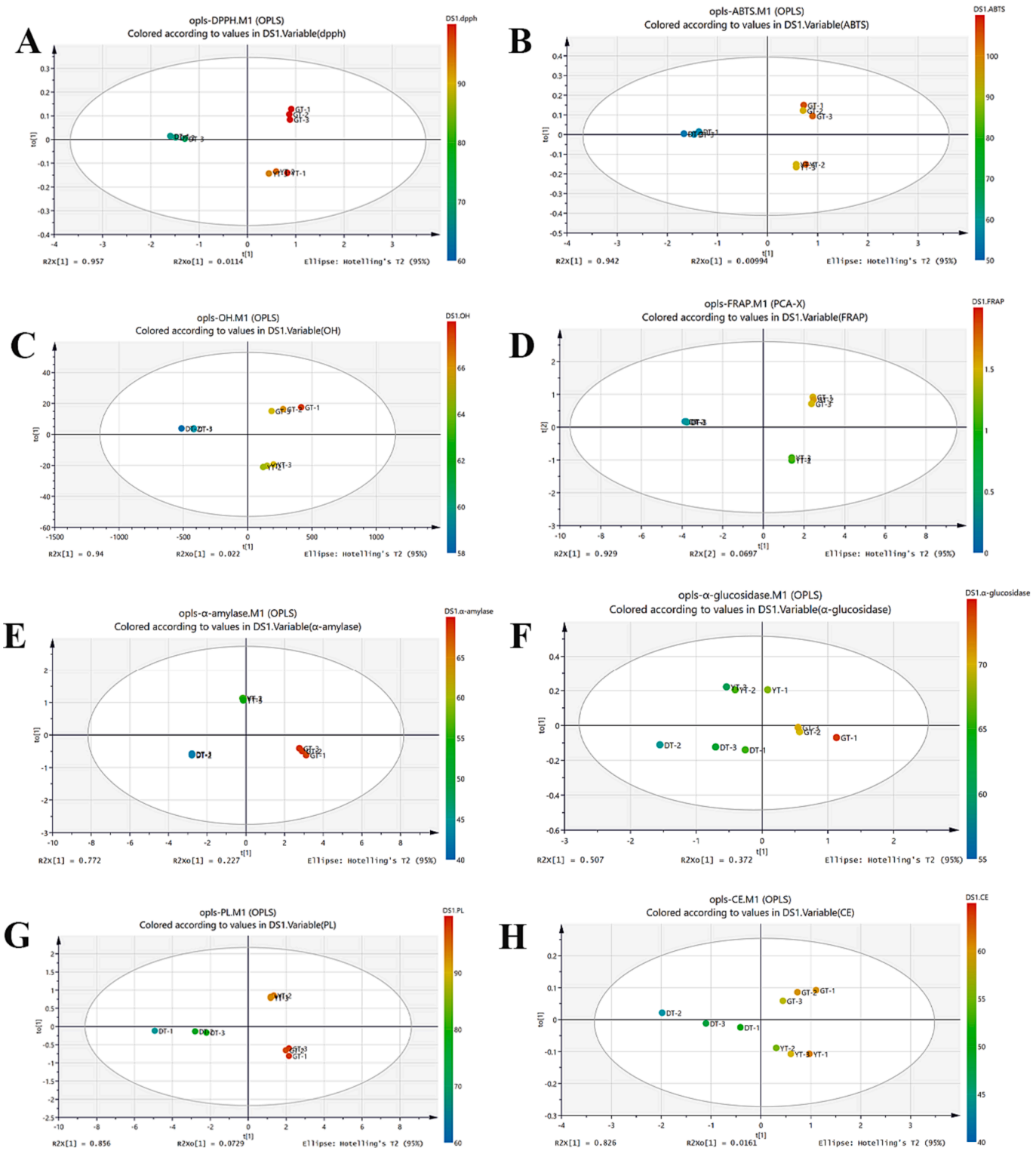


**Fig. 3.** OPLS model results based on main chemical composition in three kinds of tea. (**A**) Score plot colored according to DPPH, (**B**) ABTS, (**C**) OH, (**D**) FRAP antioxidant activity. (**D**) Score plot colored according to α-amylase, (**E**) α-glucosidase, (**F**) pancreatic lipase, (**H**) cholesterol esterase inhibitory activity.

catechins with the 2, 3-cis structure (Xiao et al., 2013). EGCG, ECG and CG are galloylated catechins. CG and ECG have a dihydroxyl group (catechol-type), which is unique to them, this enables the selection of EGCE, ECG and CG as potential inhibitors of the activity of α-amylase, α-glucosidase, PL and CE.

### 3.5. *Correlation analysis*

Heat maps of the correlations between EGCG, ECG and CG with antioxidant activity and inhibitory enzyme activity are shown in Fig. 4. Pancreatic EGCG, ECG and CG were significantly positively correlated with α-amylase, α-glucosidase, pancreatic lipase and cholesterol esterase ($p < 0.001$). The $r^2$ values of EGCG and pancreatic lipase and cholesterol

**Table 3**
The main chemical composition with significant correlation with antioxidant activity.

| main chemical composition | DPPH | | ABTS | | OH | | FRAP | |
|---|---|---|---|---|---|---|---|---|
| | VIP | P | VIP | P | VIP | P | VIP | P |
| EGCG | 1.57399 | 0.00998532 | 1.57611 | 0.00994382 | 1.57086 | 0.0115097 | 1.57144 | 0.0105146 |
| CG | 1.55047 | 0.0194364 | 1.5498 | 0.0214053 | 1.55205 | 0.0227578 | 1.55238 | 0.0181456 |
| ECG | 1.53791 | 0.00396411 | 1.53982 | 0.00355521 | 1.53587 | 0.00287328 | 1.53652 | 0.00339483 |

**Table 4**
The main chemical composition with significant correlation with enzymatic activity.

| main chemical composition | α-amylase inhibitory activity | | α-glucosidase inhibitory activity | | pancreatic lipase inhibitory activity | | cholesterol esterase inhibitory activity | |
|---|---|---|---|---|---|---|---|---|
| | VIP | P | VIP | P | VIP | P | VIP | P |
| EGCG | 1.57278 | 0.00828315 | 1.56473 | 0.0149275 | 1.57332 | 0.00735391 | 1.57203 | 0.0132168 |
| CG | 1.55894 | 0.0125587 | 1.57252 | 0.0180104 | 1.5489 | 0.0156527 | 1.5541 | 0.0221901 |
| ECG | 1.53978 | 0.00325459 | 1.53683 | 0.00421719 | 1.53809 | 0.00282021 | 1.53669 | 0.00394636 |

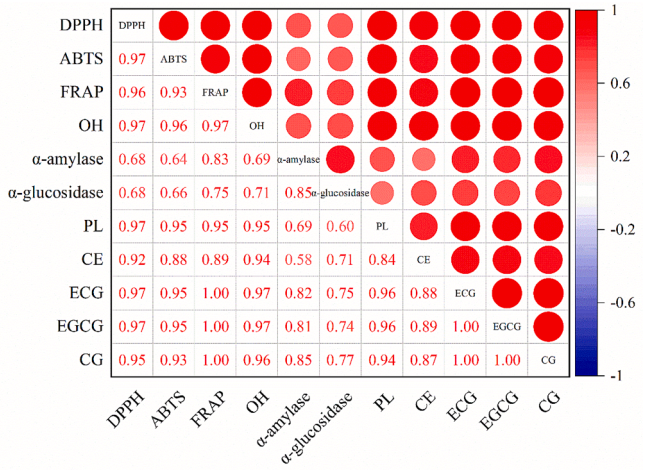


**Fig. 4.** Results of correlation analysis between biological activity and main chemical composition in three kinds of tea extracts. Red and blue colors represent positive and negative correlation, respectively.

esterase were the highest (0.958 and 0.889). The results showed that EGCG, ECG and CG had a strong relationship to the activity of inhibitory enzyme; that is, the higher the content is, the stronger the inhibitory ability will be.

### 3.6. Molecular docking analysis

Molecular docking was employed to further identify the potential active substances in the three types of tea polyphenols with α-amylase, α-glycosidase, PL, CE, CutC and FMO3 as receptor. The results of binding affinities of main chemical composition in the three kinds of tea with six receptors are shown in Table 5. The binding affinity values of EGCG with α-amylase and α-glycosidase were −9.1 and −10.5 kcal/mol (obviously superior to acarbose). The binding affinity of ECG and CG was followed by EGCG with α-amylase and α-glucosidase, indicating that EGCG, ECG and CG have good potential inhibitory activity against α-amylase and α-glycosidase. The binding affinity of CG with CE was −8.1 kcal/mol, followed by ECG and EGCG. The binding affinity of the main chemical composition with PL were higher than that of orlistat (-4.8 kcal/mol). Although the EGCG, ECG and CG absolute binding affinity were lower than main chemical compounds with PL, their contents in the three tea extracts were high. Choline as a dietary precursor for Cut/C cleavage binds with an affinity of −3.7 kcal/mol, while EGCG, ECG and CG have a higher affinity than choline as well as the inhibitor (DMB). We can see that the binding affinity of FMO3 as receptor and EGCG, ECG and CG as

**Table 5**
Molecular docking affinity (kacl/mol) results of main chemical composition in three different teas with α-amylase, α-glycosidase, pancreatic lipase (PL), cholesterol esterase (CE), CutC and FMO3.

| | α-Amylase | α-Glucosidase | PL | CE | CutC | FMO3 |
|---|---|---|---|---|---|---|
| Acarbose | −7.7 | −8.3 | – | – | – | – |
| Orlistat | – | – | −4.8 | – | – | – |
| Simvastatin | – | – | – | −7.4 | – | – |
| Choline | – | – | – | – | −3.7 | – |
| DMB | – | – | – | – | −4.8 | – |
| TMA | – | – | – | – | – | −6.5 |
| IMP | – | – | – | – | – | −8.9 |
| EGCG | −9.1 | −10.5 | −8.5 | −7.4 | −8.1 | −11.3 |
| ECG | −9 | −9.6 | −7.3 | −8 | −8.6 | −10.6 |
| CG | −9 | −9.6 | −7.3 | −8.1 | −8.6 | −10.6 |
| C | −8.9 | −8.6 | −8.8 | −6.9 | −7.5 | −9.4 |
| EC | −8.9 | −8.6 | −8.8 | −6.8 | −7.4 | −9.4 |
| GC | −9.1 | −8.5 | −7.9 | −7 | −7.3 | −9.9 |
| EGC | −9.1 | −8.5 | −7.9 | −6.5 | −7.3 | −9.9 |
| EA | −8.1 | −8.3 | −9.8 | −8 | −7.5 | −9.4 |

ligands is significantly better than the other ligands. The molecular docking results suggest that the receptors α-amylase, α-glycosidase, PL, CE, CutC and FMO3 may bind preferentially to EGCG, ECG and CG ligands in the presence of EGCG, ECG and CG ligands. The quantitative results showed that EGCG, ECG and CG were the main contents of tea polyphenols. Therefore, EGCG, ECG and CG are the main α-amylase, α-glycosidase, PL, CE, Cut/C and FMO3 inhibitors in tea polyphenols.

Molecular docking experiment visualization results are shown in Fig. 5 to further explain the structure–activity relationship of EGCG, ECG and CG with α-amylase, α-glycosidase, PL, CE, CutC and FMO3. Hydrogen bond interactions are thought to play a key role in stabilizing the enzyme-ligand complex for catalytic reactions (Yang et al., 2022). Previous studies have shown that the hydroxyl groups of flavonoids in different rings have different activities, with ring A having the weakest phenolic hydroxyl activity, ring B the strongest phenolic hydroxyl activity, and ring C also strong when double bonded (Liu et al., 2022). In terms of molecular structure, EGCG, ECG and CG compounds contain hydrogen bond acceptor or hydrogen donor groups. As shown in Fig. 5A (b), the B4-OH of EGCG formed a hydrogen bond with Arg195. As shown in Fig. 5F(b), B4-OH of ECG with Asp242 and Ser240, and D2-OH and D4-OH with Ser157 and Leu313 formed hydrogen bonds. As shown in Fig. 5H(b) and I(b), hydrogen bond with Arg257, Trp86, His152 and Asp80 on B-OH were found. As shown in Fig. 5P(b), seven hydrogen bonds were found on B-OH and C-OH with Ser213, Fad1451 and Trp34. This is consistent with our molecular docking results, and much of the reason why EGCG, ECG and CG have better affinity than other ligands depend on the number as well as the location of their OHs. At the same

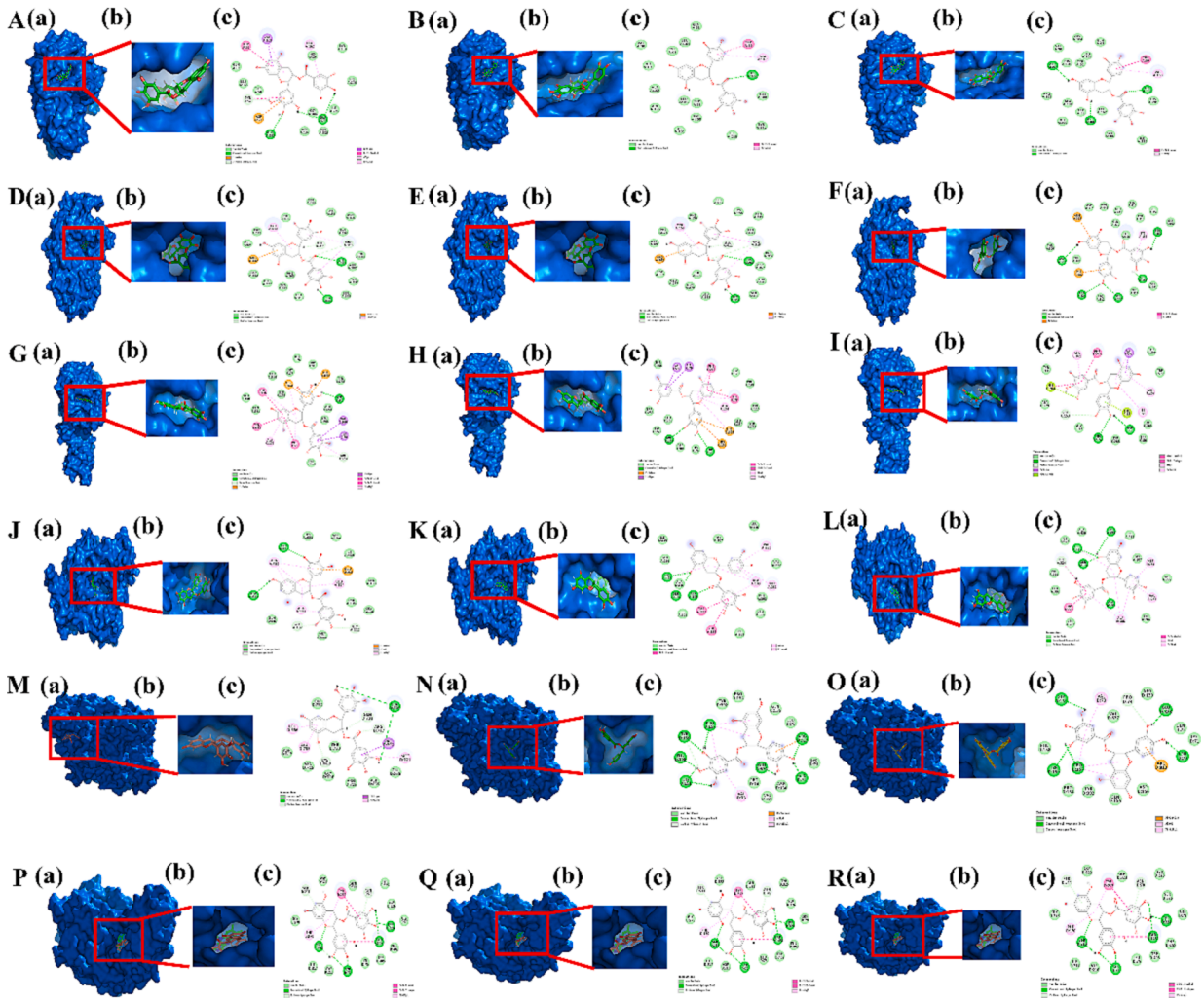


**Fig. 5.** Pattern of docking interactions between ligand and receptor. (**A**) EGCG, (**B**) CG, (**C**) ECG and α-amylase interaction; (**D**) EGCG, (**E**) CG, (**F**) ECG and α-glucosidase interaction; (**G**) EGCG, (**H**) CG, (**I**) ECG and pancreatic lipase interaction; (**J**) EGCG, (**K**) CG, (**L**) ECG and cholesterol esterase interaction; (**M**) EGCG, (**N**) CG, (**O**) ECG and CutC interaction; (**P**) EGCG, (**Q**) CG, (**R**) ECG and FMO3 interaction. (**a**) The 3D view of binding site with surface between ligand and receptor. (**b**) The 2D view of interactions between ligand and receptor.

time, the hydrophobic interactions between EGCG, ECG, CG and the receptor can be observed, including π-alkyl, π-π stacked and π-π *t*-shaped interactions. Our predicted enzyme active sites of receptor contained a higher proportion of hydrophobic amino acid residues (Trp, Val, Asp, Tyr, Arg, Cys, Gly, Phe, Ala, Pro, Ile, and His), which enables the hydrophobic compounds to bind more in the interior. In addition, as shown in Fig. 6A–R, EGCG, CG and ECG were located in the hydrophobic cavities of α-amylase, α-glucosidase, PL, CE, CutC and FOM3 but not in the hydrophilic cavities and interact with the C, O, C and N atoms of hydrophobic amino acids. In conclusion, we can find that the formation of stable hydrogen bonds between ligands and receptors are all related to the OH on the ligand. Meanwhile, EGCG, CG and ECG with C2 = C3 double bond were more effective to inhibit enzyme activity (Liu et al., 2022). Hydrophobic interactions can reduce the level of binding between enzymes and their substrates or hinder their access to the active sites of enzymes (Lee et al., 2023).

## 4. Conclusion

Different methods of tea processing will change the content of catechin in tea. With increasing degree of fermentation, the content of catechin compounds in tea decreases, thereby affecting its antioxidant capacity and inhibitory activities against α-amylase, α-glucosidase, pancreatic lipase and cholesterol esterase. Based on OPLS and correlation analyses, EGCG, CG and ECG were the main compounds that affected the antioxidant capacity and inhibited the activities of α-amylase, α-glucosidase, pancreatic lipase and cholesterol esterase. In addition, molecular docking analysis found that the binding energy of EGCG, ECG and CG was higher than that of the other chemical compounds and formed hydrogen bond and hydrophobic interactions with crucial pocket amino acid residues of α-amylase, α-glucosidase, PL, CE, CutC and FMO3, attributed to the number and location of hydroxyl groups. Therefore, EGCG, ECG and CG were considered as active substances in tea polyphenols. EGCG, CG and ECG contents are higher in green tea, so green tea plays an important role in preventing obesity. These findings contribute novel insights into the preventive mechanisms of tea polyphenols against obesity and TMAO production, providing valuable implications for future research and applications in functional foods.

## CRediT authorship contribution statement

**Zhuo Wang:** Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Visualization, Writing – original draft. **Bin Chen:** Investigation, Methodology. **Xinyi Zhao:** Investigation, Methodology. **Shanshan Li:** Conceptualization, Investigation,

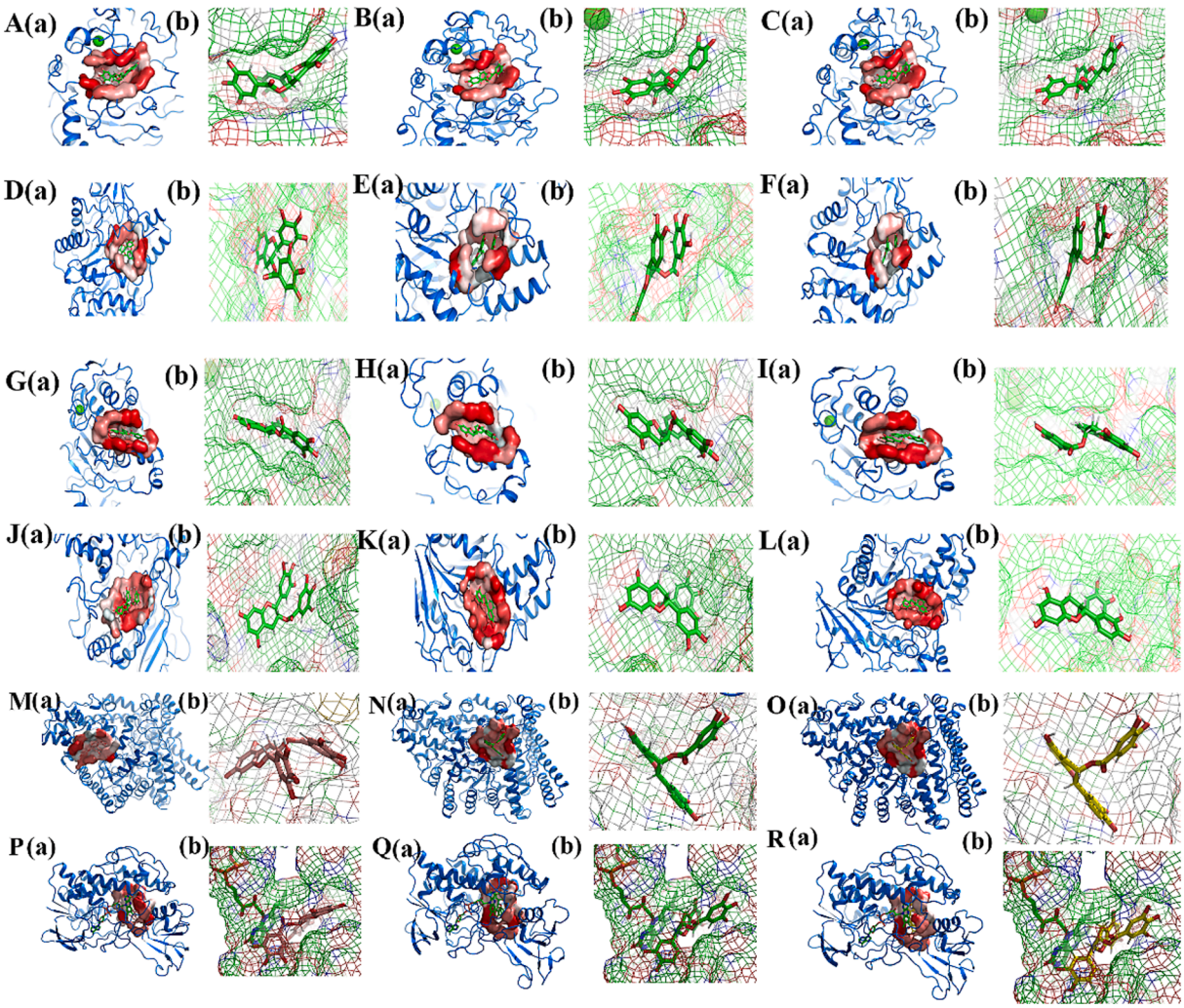


**Fig. 6.** Pattern of ligand binds to the hydrophobic cavity. (**A**) EGCG, (**B**) CG, (**C**) ECG and α-amylase hydrophobic cavity interaction; (**D**) EGCG, (**E**) CG, (**F**) ECG and α-glucosidase hydrophobic cavity interaction; (**G**) EGCG, (**H**) CG, (**I**) ECG and pancreatic lipase hydrophobic cavity interaction; (**J**) EGCG, (**K**) CG, (**L**) ECG and cholesterol esterase hydrophobic cavity interaction; (**M**) EGCG, (**N**) CG, (**O**) ECG and CutC hydrophobic cavity interaction; (**P**) EGCG, (**Q**) CG, (**R**) ECG and FMO3 hydrophobic cavity interaction. (**a**) Ligand binds to the hydrophobic cavity. Hydrophilic areas on the molecular surface are colored red, while hydrophobic areas are colored white. (**b**) Hydrophobic surface and the atom of surrounding amino acid residues in the binding area. The atom of C is colored green, the atom of O is colored red, the atom of H colored white, the atom of N is colored blue.

Methodology, Supervision, Validation, Writing – review & editing. **Zhengfeng Fang:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Yuntao Liu:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Writing – review & editing. **Zhen Zeng:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. **Cheng Li:** Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Hong Chen:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.101053.

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