Effects of theacrine on the astringency of EGCG by affecting salivary protein − EGCG interactions through different molecular mechanisms

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ABSTRACT

(−)-Epigallocatechin gallate (EGCG) and theacrine are involved in imparting tea with its astringent and bitter tastes. This study investigated the effect of theacrine on the astringency of EGCG and its molecular mechanism. Sensory evaluation was used to study the astringent intensities of EGCG solutions in the presence and absence of various concentrations of theacrine. The results indicated a considerable increase in the astringency values of EGCG–theacrine solutions compared with those of EGCG solutions alone. Furthermore, dynamic light scattering (DLS) and molecular dynamics simulations (MD) were to explore the interaction mechanisms. The results revealed that theacrine increased the particle size of EGCG-proline-rich proteins (PRPs) aggregates with that of EGCG and PRPs alone. MD revealed that theacrine potentially acted as a bridge between EGCG and PRPs, promoting their interaction and intensifying the EGCG's astringency. However, theacrine could not bridge two or more mucins owing to the substantial spatial structure of mucin.

1. Introduction

Astringency and bitterness are crucial quality attributes in tea infusions and they significantly affect their taste and influence consumer acceptance and preference (Deng et al., 2022). While astringency and bitterness can enhance the flavor of tea, their excessive levels can negatively affect the desired taste and deter consumers (Ye et al., 2022). Human perception of bitterness and astringency typically starts with the interaction among taste compounds during oral exposure, however, the subsequent pathways for these two tastes are different. Bitterness is considered one of the most basic tastes, resulting from bitter substances binding to bitter taste receptors TAS2Rs and activating corresponding receptors (Zhang, Cao, Granato, Xu, & Ho, 2020). By contrast, astringency is commonly perceived as a tactile sensation rather than a taste. Previous studies have proposed three main pathways for perception of astringency: (1) the interaction of astringent compounds with salivary proteins form insoluble aggregates in the oral cavity, which further interact with the oral mucosa, leading to increased friction and reduced oral lubrication (Lei, Tang, Zheng, Ma, & Zhou, 2022). (2) Some small astringent substances partially bind to saliva proteins, forming a soluble assembly of protein complexes, which may bind to chemoreceptors or/ and mechanoreceptors in the epithelial cells of the oral mucosa, potentially altering membrane potential and triggering subsequent signaling (Huang & Xu, 2021).

(−)-Epigallocatechin Gallate (EGCG) and theacrine (1, 3, 7, 9-tetra-methyluric acid) are important for the astringent and bitter taste of tea. Specifically, EGCG plays a crucial role in the development of astringency by interacting with saliva proteins, with previous research examining its interaction with mucin (Ye et al., 2021). Mucins and proline-rich proteins (PRPs) are two salivary proteins that account for ~30% and ~ 70% of the total salivary proteins, respectively. In addition, these two proteins are vital in oral lubrication, and hence are involved in astringent perception (Rudge et al., 2021). Therefore, this study selected mucins and PRPs as model proteins to study the effect of EGCG on astringent taste of tea. Theacrine, a purine alkaloid recently discovered in tea at concentrations ranging from 0.1% to 4.2%, has been studied for its

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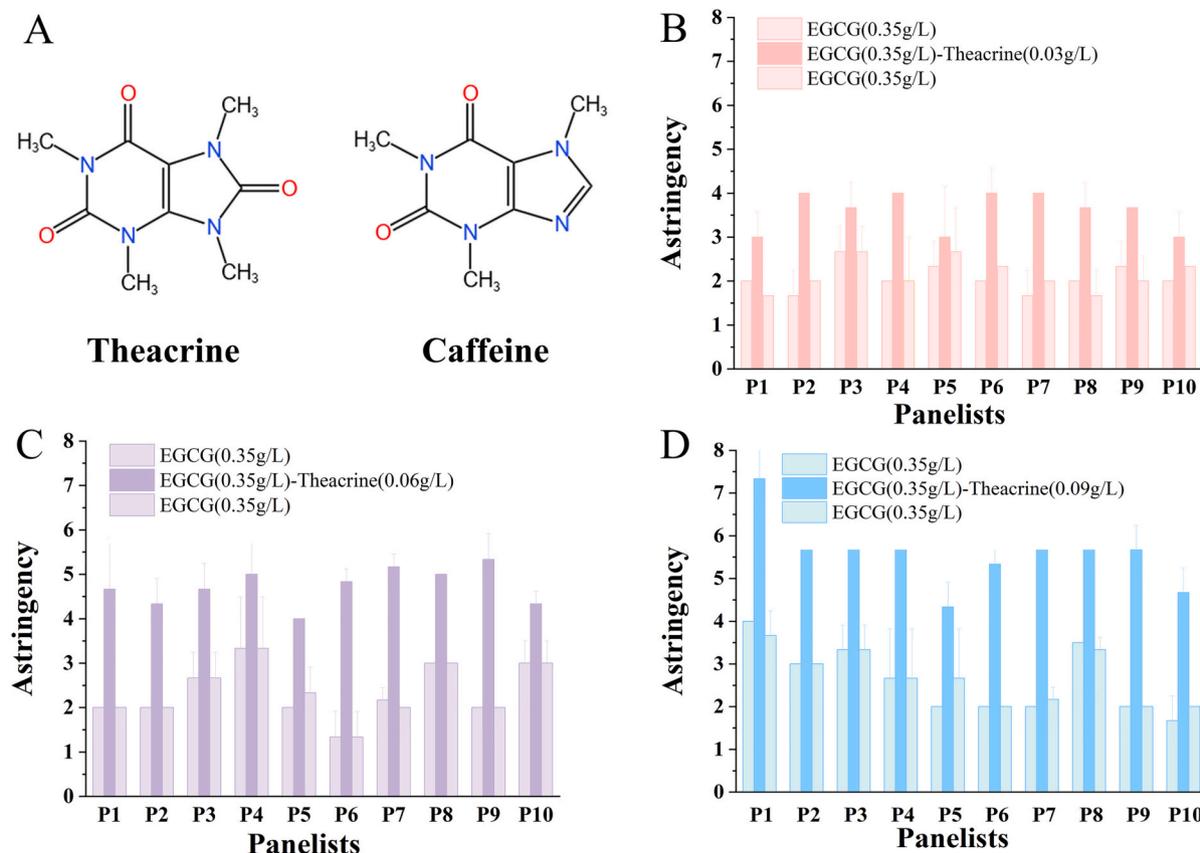[1] Jizhou Xie and Shi Yu should be considered co-first authors.

Fig. 1. Chemical structure of theacrine and caffeine; the astringency of EGCG solutions before and after adding the theacrine. (A) Chemical structure of theacrine and caffeine, respectively; The astringency of EGCG solutions before and after adding the theacrine; (B) the concentration of the theacrine solution was 0.03 g/L; (C) the concentration of the theacrine solution was 0.06 g/L; (D) the concentration of the theacrine solution was 0.09 g/L. The concentration of each EGCG solution was 0.35 g/L. The P1 to P10 represented panel 1 to panel 10, respectively. There was a significant difference before and after adding the theacrine in the astringency of the EGCG solution ($P < 0.05$). There was a significant difference in the increase of astringency value among the three theacrine addition groups ($P < 0.05$). The data was shown in Table S2.

sensory properties and exhibits a distinct bitter taste. The bitter taste threshold of theacrine is 18.45 mg/L at 25 °C and 23.24 mg/L at 45 °C, which is considerably lower than some bitter compounds found in tea (Shi et al., 2022).

Recent studies on taste perception mechanisms have revealed that taste components can interact with each other to enhance or inhibit their taste properties (Keast & Breslin, 2003). Synergistic relationship analysis has indicated that caffeine can substantially enhance the astringency of EGCG and the response value increases notably with caffeine concentration (Yin et al., 2014). Interestingly, the chemical structure of theacrine closely resembles that of caffeine, as shown in Fig. 1A (Ouyang et al., 2021). This similarity suggests that theacrine may also play a crucial role in the astringency of EGCG. However, whether theacrine contributes to the astringency of tea infusions remains unclear.

This study aimed to validate the potential effects of the astringency of EGCG using varying concentrations of theacrine through quantitative descriptive analysis (QDA). In addition, the impact of theacrine on the binding to PRPs or mucin proteins was studied by measuring aggregate size using dynamic light scattering (DLS). Moreover, molecular dynamics (MD) was employed to elucidate the interaction mechanism between theacrine and EGCG. This study provides new insight into the molecular mechanism of theacrine affecting the astringency of EGCG. In the future, in-depth studies on other purine alkaloids can be conducted to enhance the astringency of EGCG as well as that of other substances to facilitate a deeper understanding of the taste interactions among various components in tea.

2. Materials and methods

All solvents used in this study were of high-performance liquid chromatography grade, and all chemicals were analytical reagent grade. Sodium hydroxide and hydrochloric acid were purchased from Yuexiang Chemical Industry Company Limited (Chongqing, China), trifluoro-acetic acid was purchased from Honeywell Company (Charlotte, USA), porcine gastric mucin Type II was purchased from Darui Chemical Company (Shanghai, China), theacrine and tannin were obtained from Bide Company (Shanghai, China), and EGCG was obtained from Chembee Company (Shanghai, China).

2.1. Sensory evaluation

The effect of theacrine addition on the astringency of EGCG solutions was studied using QDA. The samples were evaluated at a room temperature of 25 °C by a panel of 10 well-trained participants, comprising 3 men and 7 women aged 20–29 years, from the School of Food Science at Southwest University. All procedures involving the human participants followed the ethical standards of the institutional and national research committees. All human volunteers signed a declaration of informed consent (the consent was presented in Supplementary Material). The participants could withdraw from the survey at any time without giving a reason. The products tested were safe for consumption. The materials used in the sensory evaluation were of food grade quality and the panelists were instructed not to swallow the sample solutions during the tests. After scoring each sample, the panelists were requested to wash their mouths with pure water.

The standard reference for astringency was tannin, and the series concentrations of standard tannin solutions were 0.288 g/L (2-point scale), 0.576 g/L (4-point scale), 0.864 g/L (6-point scale), 1.152 g/L (8-point scale), and 1.440 g/L (10-point scale), respectively (Liu et al., 2018; Xu et al., 2017). The panelist's sensory responses for astringency were assessed on a 10-point scale (where 8–10 was "extremely strong", 6–8 was "strong", 4–6 was "neutral", 2–4 was "weak" and 0–2 was "extremely weak") following a previously reported method (Ye et al., 2021).

During the test, three cups of the sample solution were given to the panelists at the same time, each cup measured ~30 mL and was labeled with a random three-digit number. Three cups of the sample solution comprised one set of tests: two of the three cups contained EGCG solutions, and the third cup contained theacrine–EGCG mixed solution. EGCG and theacrine solutions were prepared using ultrapure water. The concentration of each EGCG solution was 0.35 g/L, and the concentration of theacrine was 0.03, 0.06 and 0.09 g/L. In addition, the concentration of EGCG in the tea ranged from 0.1% to 15%, and the concentration of theacrine ranged from 0.1 to 4.2% (Huang, 2008; Shi et al., 2022). Herein, the concentrations of EGCG and theacrine were selected within this range. During sensory evaluation, the panelists were asked to select the cup containing the solution with the highest astringent intensity and then score the astringency taste of the solutions contained in the three cups. There was a 6-min rest period between tasting the solution in each cup and between participating in each test group to minimize the sensory fatigue and carry-over effects. The sessions were conducted two to three times per week, each lasting 2 h (seven sessions). Three samples were tested in each session and each sample was repeated three times.

2.2. Preparation of PRP solutions

2.2.1. Collection of saliva

The method used to isolate proline-rich proteins (PRPs) from human saliva was adapted from a previously developed method (Rudge et al., 2021). The study participants involved five men and five women from the School of Food Science, Southwest University, all of whom were healthy, nonsmoking, and 20–29 years old. All human subjects had signed a declaration of informed consent.

The saliva samples of each participant were collected at ~2:00 pm. The participants were instructed to refrain from eating or drinking for 2 h before sampling. Before saliva collection, the participants rinsed their mouths with water. The collected saliva samples were centrifuged at 10,000 ×g for 30 min at 4 °C to remove debris and the supernatant was collected for PRP solution preparation.

2.2.2. Preparation of PRP solutions

The PRPs were isolated from human saliva, following a previously developed method (Rudge et al., 2021). A 10% (1.3 mol/L) trifluoroacetic acid (TFA) stock solution was added to the saliva samples, resulting in a final concentration of TFA at 0.1%. Then the tubes were immediately centrifuged for 10 min at 12,000 ×g at 4 °C to remove the precipitated larger proteins and any residual particles. Next, the supernatant obtained after centrifugation was dialyzed against running deionized water for 48 h using a Spectra/Por 3 Cellulose Membrane (SpectrumLabs, Sigma-Aldrich) with an exclusion size of 3.5 kDa, and the water was changed every 8 h. The dialyzed solution was frozen for at least 24 h and freeze dried overnight to obtain a dry PRP-rich protein concentrate. The PRP concentrate was redispersed in water at 0.2% to obtain a similar concentration present in saliva.

2.3. Dynamic light scattering

To investigate the effect of theacrine addition on enhancing the reaction between EGCG and salivary proteins, the particle size of the aggregates was measured using a Zetasizer Nanoseries (Malvern Instruments) for DLS.

All liquids used to dilute the sample (dispersant and solvent) were filtered (0.02 umol/L) before use. The EGCG, theacrine, and NaOH solutions were prepared at concentrations of 0.35, 0.03, and 0.1 M, respectively. Mucin Type II is often used as a representative of human saliva. Subsequently, mucin and PRP concentrations were prepared at 0.1 wt% (weight units) and 0.2%, respectively, and the pH was adjusted to 6.8 using NaOH solution, similar to the pH of human saliva. Mucin, PRP proteins, and EGCG particle sizes were first measured after a hundredfold dilution. Following that, the particle size was assessed in the presence of a ratio of astringent substances and proteins (mucin and PRP) of 1:1, and the change when theacrine was added to the mixture of proteins and EGCG was determined.

2.4. Molecular dynamics simulation

Two systems were constructed to represent two peptide models, including a fragment of PRP and mucin. The amino acid sequence of IB937 was SPPGKPQGPPPPQGGNQPQGPPPPPGKPQGPPPPPQQGGNR and the amino acid sequence of mucin was MKTLPLFVCICALSACFSFSE-GRERDHELRHRRHHHQSPKSHFEL-PHYPGLLAHQKPFIRK-SYKCLHKRCRPKLPPSPNNPPKFPNPHQPPKHPDKNSSVVNPTLVATT-QIPSVTFPSASTKITTLPNVTFLPQNA (Manjon, Bras, Garcia-Estevez, & Escribano-Bailón, 2020).

The original structure of EGCG and theacrine was determined using GaussView software (Gaussian Inc), following which four different molecular systems were built: (1) 6 chains of the PRP fragments (IB937) and 10 EGCG molecules (named IB9_EGCG), (2) 6 chains of the mucin protein fragments and 10 EGCG molecules (named MUC_EGCG), (3) model one and 4 theacrine molecules (named IB9_EGCG-THE), and (4) model two and 4 theacrine molecules (named MUC_EGCG_THE).

MDs were performed using GROMACS 2018.4 (Van der Spoel et al., 2005) with constant temperature and pressure ensembles and periodic boundary conditions. The GAFF force field was used for heptane molecules, while the Amber14SB all-atom force field was used for peptides, in conjunction with the TIP3P water model (Jorgensen, Chandrasekhar, Madura, Impey, and Klein (1983); Maier et al., 2015). The linear constraint solver algorithm was used to constrain all bonds containing hydrogen in the entire course of dynamic simulations, and the integration time step was 2 fs (Hess, Bekker, Berendsen, & Fraaije, 1997). The particle mesh Ewald method was used to calculate electrostatic interactions (Darden, York, & Pedersen, 1993). The nonbonding interactions were truncated at a cutoff of 10 Å and updated every 10 steps. The simulation temperature was 298.15 K and controlled using the V-rescale temperature coupling method (Berendsen, Postma, Vangunsteren, Dinola, & Haak, 1984). The pressure was maintained at 1 bar using the Parrinello–Rahman pressure coupling method (Martonak, Laio, & Parrinello, 2003).

First, the energy of the two binary systems was minimized using the steepest descent method, eliminating contacts between atoms. Then, a 1 ns equilibration was performed in NVT and NPT at 298.15 K. Finally, 100 ns MD was conducted, and the conformations were stored every 10 ps. All MD visualization analyses were performed using the GROMACS software and the visual molecular dynamics program.

2.5. Statistical analysis

All results were recorded as the mean of three replicates. The analysis of significant differences ($p < 0.05$) between the means was performed via one-way analysis of variance. The panel performance was evaluated using PanelCheck (Version 1.4.2, Nofima Mat and DTU-Informatics and Mathematical Modelling, Norway). Statistical analyses were performed using SPSS Statistics software (Version 20, SPSS Inc., Chicago, IL, USA) and OrignPro 2021 (Version 2021, OriginLab Inc., Northampton,
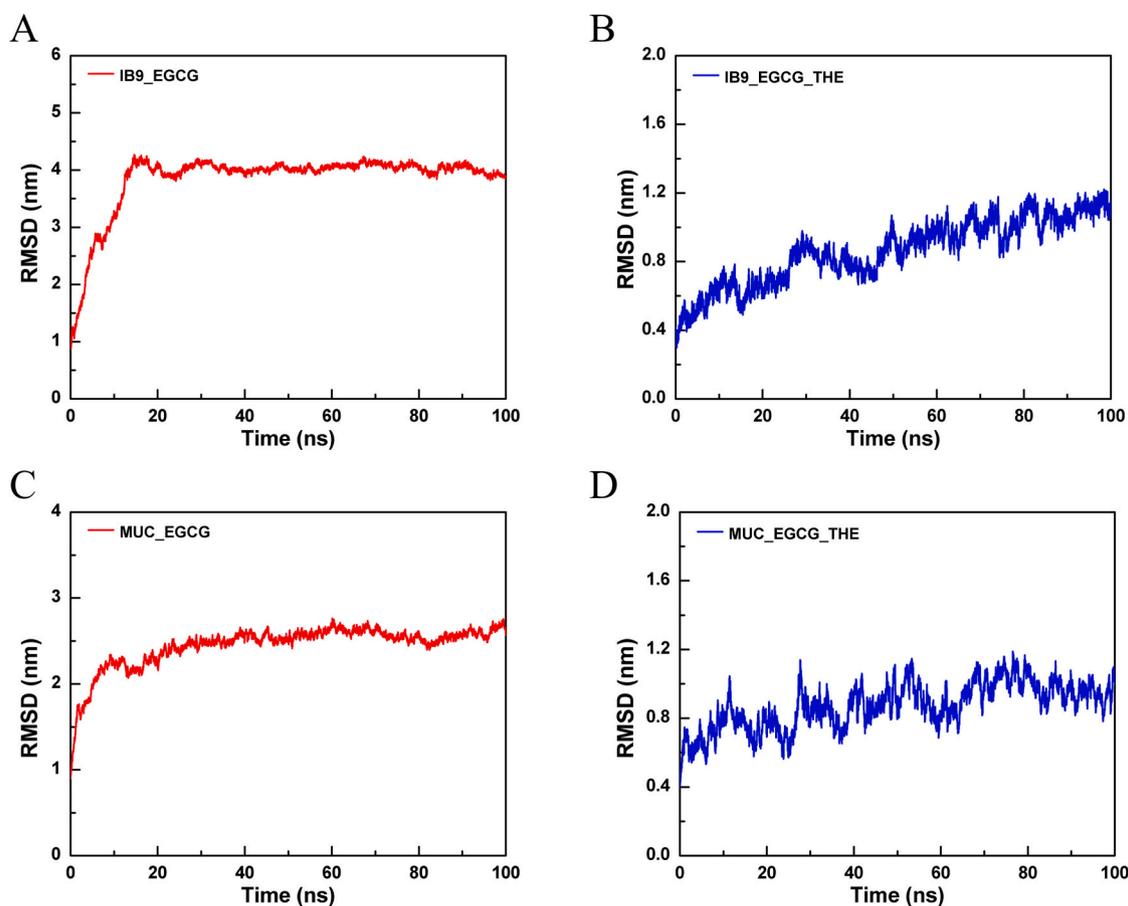
Fig. 2. The RMSD values of four systems over time.

(A) the RMSD values of system IB9_EGCG over time; (B) the RMSD values of system IB9_EGCG_THE over time; (C) the RMSD values of system MUC_EGCG over time; (D) the RMSD values of system MUC_EGCG_THE over time.

After the system reached its stable state, the average RMSD values of the four systems were 4.03 ± 0.07 (A), 0.93 ± 0.14 (B), 2.54 ± 0.08 (C), and 0.91 ± 0.11 nm (D), respectively.

Massachusetts, USA).

3. Results and discussion

3.1. Theacrine enhanced the astringency of EGCG

Sensory evaluations were performed to validate the effect of varying theacrine concentrations in EGCG solutions on astringent intensity. The astringent intensity of the EGCG control solution was compared with that of the EGCG solutions to which varying concentrations of theacrine were added. Previous studies have reported that theacrine solutions exhibited distinct bitterness, suggesting it potential as a reliable bitterness standard (Shi et al., 2022). The QDA results revealed that the astringent intensity of EGCG solutions after adding various concentrations of theacrine was stronger than the EGCG control solution (Fig. 1B, C, D), indicating that theacrine had an important impact in enhancing the astringency of EGCG. Significant differences in astringency values were observed between the control EGCG and EGCG–theacrine solutions. Specifically, after the addition of different theacrine concentrations, the astringent intensities of the EGCG-theacrine solutions were higher than those of the control EGCG solution across all the three groups. Meanwhile, the astringency values significantly increased when the concentration of the added theacrine increased from 0.03 g to 0.09 g/L, suggesting that the pathway by which theacrine promotes the astringency of EGCG is concentration-dependent.

There are three main types of interactions between taste substances, namely, simple chemical reactions between taste compounds, effect of one compound on the receptors of another compound, and combined perception of the mixed taste compounds in the cerebral cortex (Keast & Breslin, 2003). For example, mannoproteins can suppress the astringency of flavanol by inhibiting the flavanol–PRP interaction (Manjon et al., 2020). We hypothesized that theacrine affects the astringency of the EGCG solutions by promoting the interactions between EGCG and salivary proteins. To investigate this hypothesis further, the differences in EGCG and salivary protein interactions before and after the addition of theacrine were assessed.

3.2. Theacrine promotes the binding reaction of EGCG to salivary proteins

One prevailing model explaining oral astringency suggests that astringents can interact with human saliva proteins, inducing salivary protein aggregation. These aggregates further interact with oral mucus surfaces, consequently increasing friction. Studies have reported that the astringency of EGCG is produced by binding to mucins and PRPs, resulting in the perception of astringency (Rudge et al., 2021).

To better understand how theacrine promotes the astringency of EGCG, changes in particle size of the aggregates formed by the binding of EGCG to PRPs or mucins before and after the addition of theacrine were measured **(Table S1)**. The results indicated that the addition of theacrine significantly increased the particle size of aggregates formed by the binding of EGCG to PRPs, whereas the particle size of the aggregates formed by the binding of EGCG to mucins showed no significant change. This indicates that theacrine promotes the binding of EGCG to PRPs but not to mucin, thereby enhancing the astringency of EGCG.
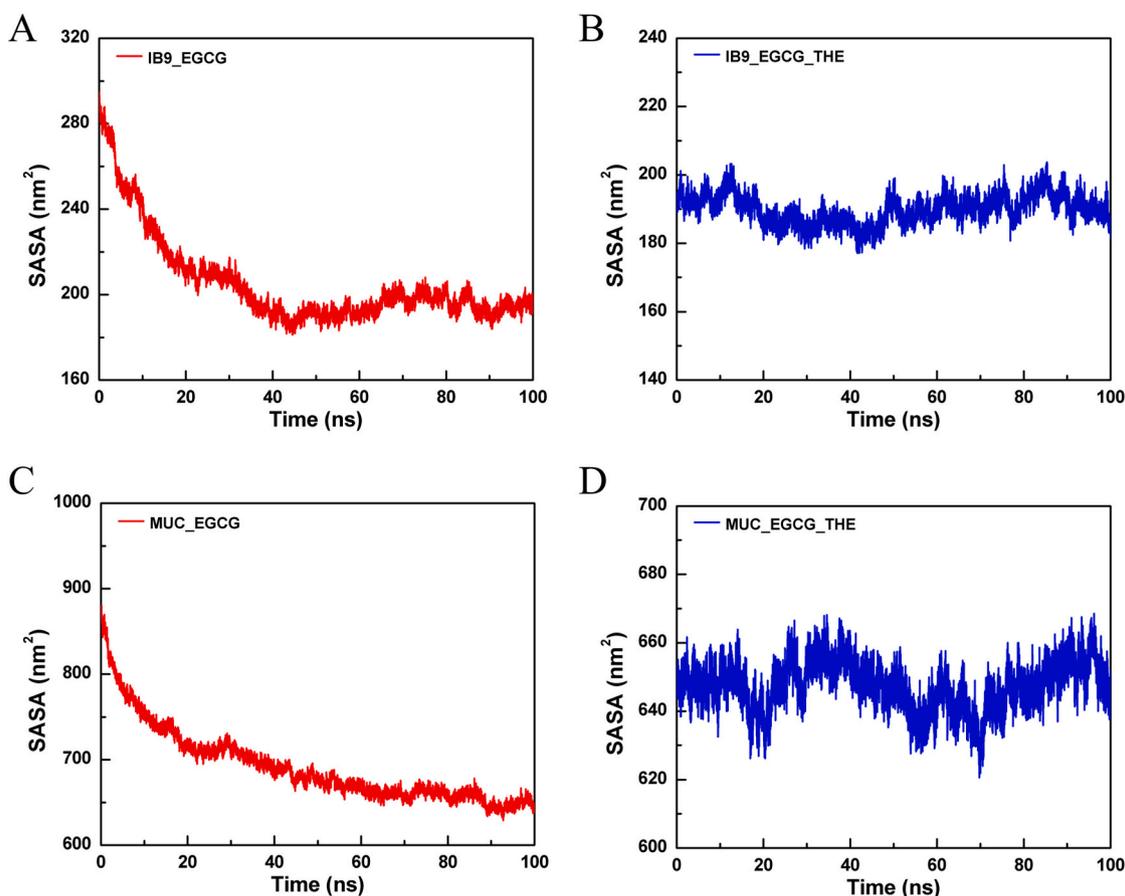
Fig. 3. The SASA values of four systems over time.

(A) the SASA values of system IB9_EGCG over time; (B) the SASA values of system IB9_EGCG_THE over time; (C) the SASA values of system MUC_EGCG over time; (D) the SASA values of system MUC_EGCG_THE over time.

After the system reached its stable state, the average SASA values of the four systems were 194.60 ± 6.93 (A), 183.14 (B), 651.31 ± 22.31 (C), and 645.33 nm2 (D), respectively.

Next, MD was used to compare the effect of theacrine addition on the interactions between EGCG and PRPs or mucins at the molecular level.

3.3. Theacrine promoted the interactions between EGCG with salivary proteins

MD has previously been used to satisfactorily elucidate the pathway of salivary protein-flavanol interactions, which are responsible for astringency (Ferrer-Gallego et al., 2015; Ferrer-Gallego et al., 2016). Thus, MD was used to determine how theacrine affected the interactions between EGCG and PRPs or mucin proteins.

3.3.1. Root mean square deviation analysis

Root mean square deviation (RMSD) of protein structure over time is an important indicator for monitoring the system equilibrium process and protein structure stability (Sun, Wang, Wang, Xia, & Kong, 2021). Fig. 2 shows the changes in RMSD over time for all the atoms of the four systems. The RMSD of two binary systems to the initial conformation tended to be stable after 20 ns, indicating that the protein chains and EGCG had formed a stable complex (Fig. 2A, C).

Theacrine molecules were added after the formation of the stable complex (Fig. 2B, D). Within 100 ns of simulation, the RMSD value of the system IB9_EGCG_THE increased, thereafter remaining stable from 80 ns. The observed changes in the RMSD value after the addition of theacrine may be attributed to some conformational change in the complex resulting from the interaction of theacrine with EGCG and IB937 peptides. In comparison, the RMSD fluctuating value of the MUC_EGCG_THE system was significantly lower than that of the IB9_EGCG_THE system. This discrepancy may be explained as the overall structural size of mucin is relatively large, and thus, the addition of theacrine cannot change its conformation. Consequently, the RMSD values of the four measured systems tended to be stable throughout the entire simulation process, indicating that the dynamic simulation based on this field parameter is stable and reliable, and can be used for further analysis.

3.3.2. Solvent accessible surface area analysis

The solvent accessible surface area (SASA) of a molecule is its surface area in contact with the solvent in the system. Higher values of SASA indicate a larger and more loosely structured protein, suggesting a more flexible conformation of the protein (Zhu, Wang, Vanga, & Raghavan, 2021).

To further study the overall picture of the extent of change in the complex system throughout the simulation, the SASA value of the complex system was analyzed. The curve depicting the change of SASA versus time is shown in Fig. 3, and the average values of the two binary systems (IB9_EGCG and MUC_EGCG) were 194.60 ± 6.93 and 651.31 ± 22.31 nm^2 between 80 ns and 100 ns simulations, respectively. The SASA values of the IB9_EGCG and MUC_EGCG systems plateaued after 40 and 80 ns during simulation, respectively. Mucin protein had an overall larger structure and may need more time to form stable aggregates.

Following the addition of theacrine molecules, the SASA of the two ternary (IB9_EGCG_THE and MUC_EGCG_THE) systems was first
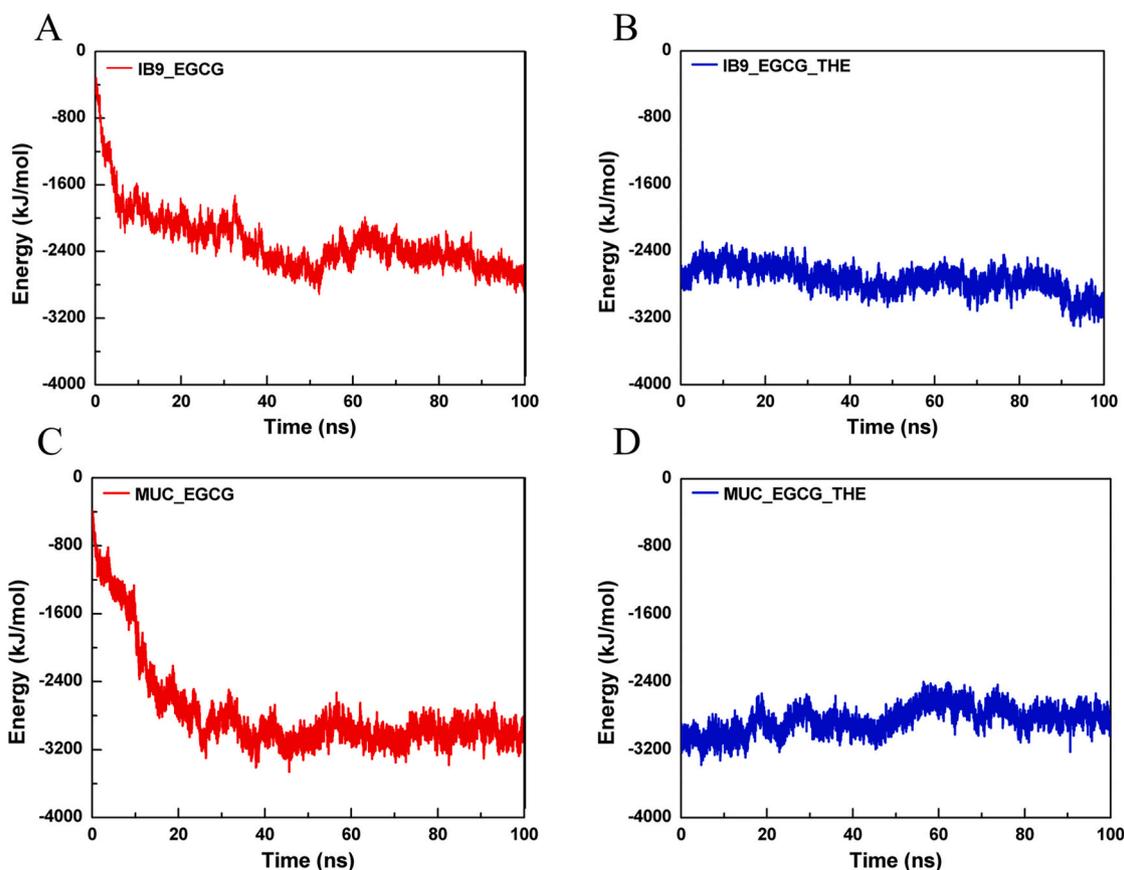
Fig. 4. The interaction energies of four systems over time.
(A) the interaction energies of system IB9_EGCG over time; (B) the interaction energies of system IB9_EGCG_THE over time; (C) the interaction energies of system MUC_EGCG over time; (D) the interaction energies of system MUC_EGCG_THE over time. After the system reached its stable state, the average interaction energies of the four systems were − 2463.86 ± 196.95 (A), −2993.64 ± 140.57 (B), 2813.05 ± 142.45 (C), and − 2747.87 ± 133.32 kJ/mol (D), respectively.

stabilized in the 10 ns simulation. This process could be supported by the fact that free theacrine molecules had not yet interacted with the proteins. In the subsequent simulations, the SASA values of systems IB9_EGCG_THE and MUC_EGCG_THE decreased from 193.46 to 183.14 nm^2 and 648.34 to 645.33nm^2, respectively, whereas the SASA value of MUC_EGCG_THE only decreased by 0.47%. The SASA of IB9_EGCG_THE was significantly reduced, indicating that the overall protein structure tended to stabilize. A relatively stable complex may be formed between theacrine and IB9_EGCG, similar to that reported by a previous study (Zhan et al., 2020).

3.3.3. Interaction energy analysis

After a preliminary conformational analysis as described earlier, the effect of theacrine addition was different in various protein systems. On this basis, the study further analyzed the interaction energies between EGCG and different proteins before and after the addition of theacrine molecules (Fig. 4). For the systems IB9_EGCG and IB9_EGCG_THE, the interaction energies of EGCG and IB9 were constantly increasing during the initial 50 ns of simulation, plateauing after 50 ns (Fig. 4A). Within the 50–100 ns simulation, the average energy values of the two systems were − 2463.86 ± 196.95 kJ/mol and − 2813.05 ± 142.45 kJ/mol before and after the addition of the theacrine molecules. The negative binding free energy (ΔG) indicated that the binding process occurred spontaneously (Sargolzaei, 2021). The free energy value was more negative after the addition of theacrine, indicating that the IB9-EGCG interaction was improved.

The interaction energies of the EGCG with mucin proteins before and after the addition of theacrine are shown in Fig. 4C. For the systems MUC_EGCG and MUC_EGCG_THE, the interaction energies of EGCG and

mucin proteins were constantly increasing during the initial 50 ns simulation, plateauing after 50 ns (Fig. 4C, D). During the 50–100 ns simulation, the average values of the MUC_EGCG interaction energies were − 2993.64 ± 140.57 kJ/mol. After the addition of theacrine, the average energy values initially plateaued and started to decrease after 20 ns, stabilizing at −2747.87 ± 133.32 kJ/mol. These data suggest that the addition of theacrine appears to weaken the MUC_EGCG interactions.

3.3.4. Overall changes in conformations of the four systems and analysis of interaction ways

To intuitively compare the effect of the addition of theacrine to the IB9_EGCG and MUC_EGCG complexes, this study analyzed the overall changes in the conformations of complexes throughout the simulations process (Fig. 5). For the IB9_EGCG complexes, hydrogen bonding interactions and π–π stacking were observed. In addition, hydrogen bonds were formed between the EGCG–hydroxyl and the IB9 chain, promoting the interaction of the IB9_EGCG complex (Fig. 5A).

When theacrine molecules were added to the complexes during a 100 ns simulation process, six IB9 protein chains tended to cluster together into stable aggregates, and EGCG molecules are adsorbed on the surface of chains. The protein chains tended to aggregate more readily, resulting in smaller complexes compared with that formed without the addition of theacrine (Fig. 5A), consistent with the SASA results in section 3.3.2. This result demonstrates that the addition of theacrine molecules promoted the aggregation of the complexes and made them more stable. Further analyses suggested that theacrine possibly served as a bridge between EGCG and salivary proteins and further improved the interaction between the complex (Fig. 5C).
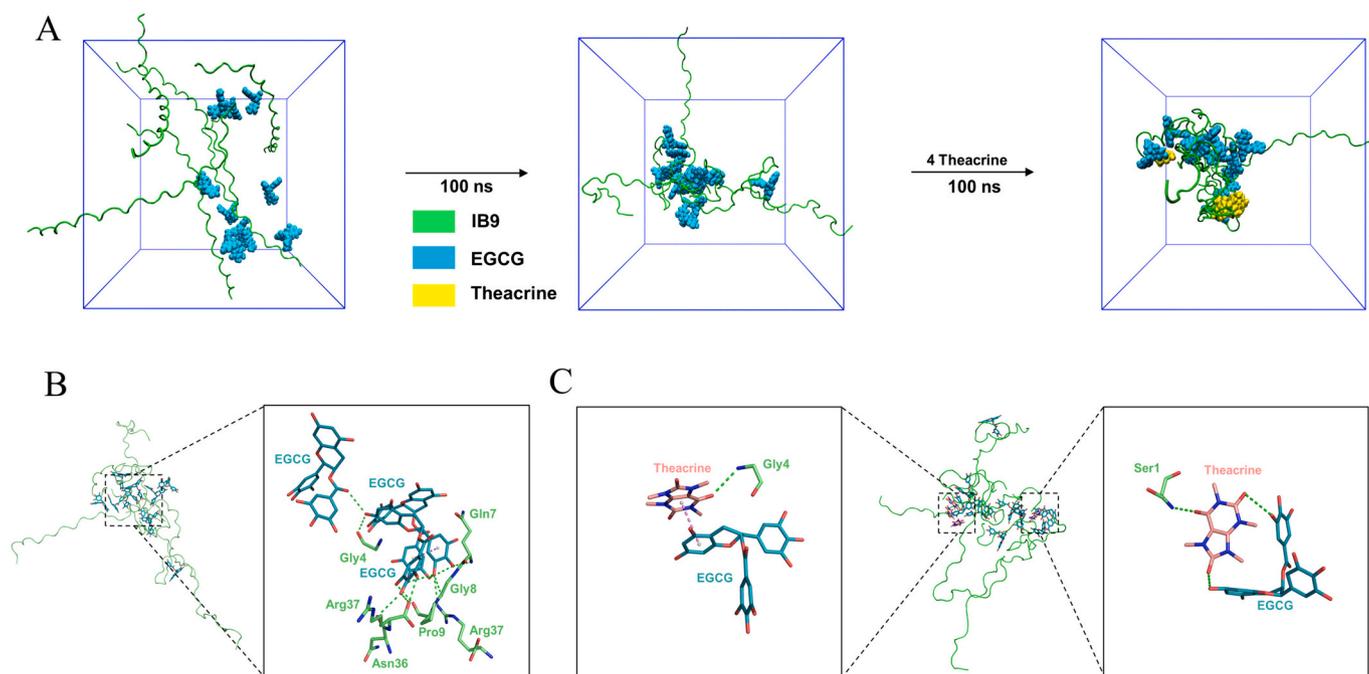
Fig. 5. The overall changes in conformations of IB9_EGCG systems and the analysis of interaction ways.
(A) the overall changes in conformations of IB9_EGCG systems before and after adding the theacrine; (B) the analysis of interaction ways between EGCG and IB9 peptide; (C) the analysis of interaction ways between theacrine with EGCG and IB9 peptide. The green peptide chain indicated IB9, the colored spherical represented EGCG molecular, and the purple spherical represented theacrine molecular. Hydrogen bonds were indicated as green dashed lines and π-π interactions were indicated as purple dashed lines. The study performed 100 ns long molecular dynamics (MD) simulations MD simulations for the IB9_EGCG system, and then MD simulations were run for 100 ns again when the theacrine molecules were added. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Furthermore, theacrine appeared to bridge two or more IB9_EGCG complexes together, forming even larger aggregates, resulting in smaller surface areas of the overall structure, as validated from the reduced SASA values of the IB9_EGCG_THE system.

For the MUC_EGCG complex, after 100 ns simulation, six mucin protein chains tended to cluster together into stable aggregates such that EGCG molecules are adsorbed on the surface of chains (Fig. 6). However, owing to the large structure of mucin, EGCG was relatively scattered on the surface of buddled mucin chains through the formation of hydrogen bonding between the EGCG–hydroxyl group and the mucin peptide chain.

On the addition of theacrine molecules to the MUC_EGCG system, there were no obvious changes in the aggregation of the complexes, and theacrine had also relatively scattered on the surface of the buddled mucin chains (Fig. 6A). In addition, one EGCG molecule transitioned from a bound state to an unbound state, indicating that the addition of theacrine weakens the interactions between EGCG and mucin proteins. Furthermore, theacrine did not act as a bridge between EGCG and mucins. Instead, it interacted with the amino acid residues of mucins or EGCG molecules, respectively (Fig. 6C). Owing to the relatively large spatial structure of the mucin protein, theacrine could not bridge two or more MUC_EGCG complexes together. Consequently, the addition of theacrine did not significantly affect EGCG–mucin protein interactions.

4. Conclusions

Herein, QDA results verified that theacrine plays an important role in promoting the astringency of EGCG. Particle size experiments with and without the addition of theacrine revealed that theacrine promoted the binding of EGCG to saliva proteins rather than mucin proteins. Finally, MD suggested that theacrine primarily acted as a bridge between EGCG and PRPs, promoting their interactions. By contrast, the relatively large spatial structure of the mucin impeded the ability of theacrine to

enhance the binding between EGCG and mucins. Thus, theacrine promoted the astringency of EGCG by strengthening the interactions between EGCG and PRPs rather than with mucins.

In addition to theacrine, which was studied in this work, the predominant alkaloids in tea were primarily caffeine, theobromine and theophylline, all of which shared the basic chemical structure of the purine ring, while the main structural difference was in the number of methyl groups. Herein, we concluded that theacrine promoted the astringency of EGCG by strengthening the interactions between EGCG and PRPs and that hydrogen bonding interactions and π–π conjugation exist between theacrine and EGCG–saliva protein complexes. Based on structural similarities, we speculate that other purine alkaloids can also considerably improve the astringency of not only EGCG but also other astringent substances in tea. This phenomenon might be one of the reasons underlying the influence of the taste components of tea on one another. Furthermore, temperature could be an important factor influencing the bitterness and astringency taste of tea. Our research group will conduct further in-depth research in this area.

CRediT authorship contribution statement

Jizhou Xie: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yu Shi:** Writing – original draft, Methodology, Investigation, Conceptualization. **Wei Luo:** Writing – review & editing, Supervision. **Wei Fang:** Methodology, Conceptualization. **Liyong Luo:** Supervision, Funding acquisition, Conceptualization. **Liang Zeng:** Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence
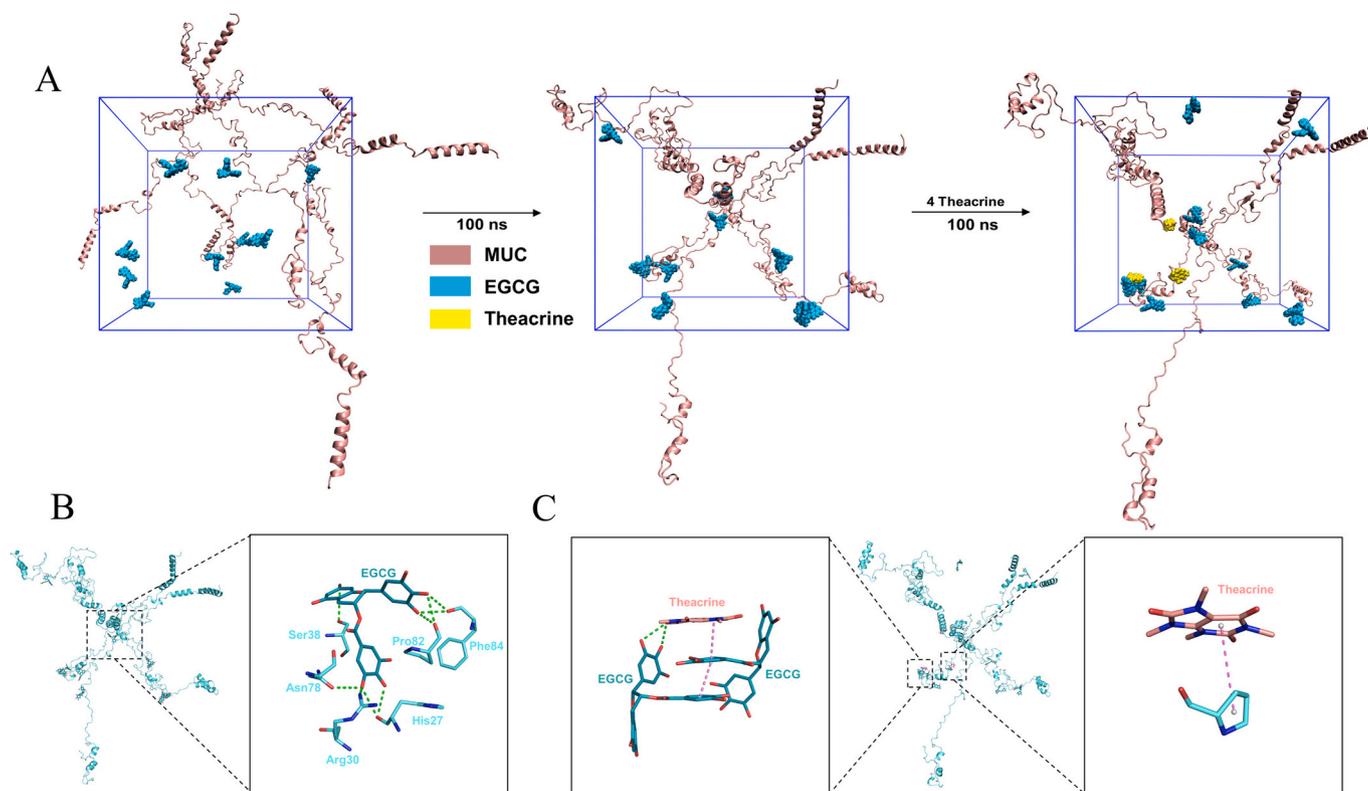
Fig. 6. The overall changes in conformations of MUC_EGCG systems and the analysis of interaction ways.
(A) the overall changes in conformations of MUC_EGCG systems before and after adding the theacrine; (B) the analysis of interaction ways between EGCG and mucin peptide; (C) the analysis of interaction ways between theacrine with EGCG and mucin peptide. The blue peptide chain indicated mucin, the colored spherical represented EGCG molecular, and the purple spherical represented theacrine molecular. Hydrogen bonds were indicated as green dashed lines and conjugated interactions were indicated as purple dashed lines. The study performed 100 ns long molecular dynamics (MD) simulations MD simulations for the MUC_EGCG system, and then MD simulations were run for 100 ns again when the theacrine molecules were added. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.fochx.2024.101474.

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