Journal of Oral Biology and Craniofacial Research 16 (2026) 93–100

Contents lists available at ScienceDirect

Journal of Oral Biology and Craniofacial Research

journal homepage: www.elsevier.com/locate/jobcr

Evaluation of chlorogenic acids and melanoidin interactions with salivary proteins and their effect on tooth discoloration

Trianna Wahyu Utami [a,*], Muhammad Raka Aqila Zaky [b], Margareta Rinastiti [c], Diatri Nari Ratih [c], Sri Budi Barunawati [d], Nunuk Purwanti [a], Yosi Bayu Murti [e]

[a] Department of Dental Biomedical Science, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia
[b] Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia
[c] Department of Conservative Dentistry, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia
[d] Department of Prosthodontics, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia
[e] Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia

ARTICLE INFO

Keywords:
Tooth discoloration
Coffee melanoidins
Chlorogenic acid
Salivary proteins
Molecular dynamics simulation
Colorimetry

ABSTRACT

Background: Tooth discoloration is a multifactorial process that is significantly influenced by the interaction between dietary polyphenols and salivary proteins. This study investigates coffee-induced discoloration using in vitro staining models and in silico simulations.

Materials and methods: For 14 days, human premolar teeth were soaked in coffee solution (27 g/450 mL) with daily replacement. Lab and ΔE measurements were used to quantify color changes before and after immersion using ultraviolet–visible spectroscopy. Melanoidin compounds docked with salivary proteins (statherin, histatin, and proline-rich protein [PRP]). Hex 8.0 was used for molecular docking, whereas Discovery Studio Visualizer and UCSF Chimera were used for molecular dynamics analysis.

Results: Molecular docking revealed the strong binding affinity of chlorogenic acid with PRP (−251.66 kJ/mol), histatin (−245.4 kJ/mol), and statherin (−240.5 kJ/mol), stabilized by hydrogen bonds with glycine/arginine/tyrosine residues. The molecular dynamics simulations confirmed complex stability with statherin (RMSD 4.2–4.6 Å) and histatin (RMSD 4.4–4.6 Å). Chromameter analysis showed significant color changes after coffee exposure ($\Delta Eab = 12.286 \pm 3.645$), with a decrease in lightness (L: 83.569 → 71.873) and an increase in redness (a*: 1.375 → 2.992) and yellowness (b*: 15.848 → 18.585). Paired samples correlation analysis revealed statistically significant changes in the a* and b* values ($p < 0.001$), while the L* value showed no significant change ($p = 0.257$).

Conclusion: Chlorogenic acid drives discoloration through stable protein interactions and measurable color shifts, suggesting that targeted inhibition of these molecular pathways could prevent coffee-induced discoloration.

1. Introduction

Tooth staining is a complex process that is influenced by the interactions between polyphenols and salivary proteins. Polyphenols derived from food exhibit a high affinity for salivary proteins, particularly those rich in basic proline. These proteins facilitate the binding of polyphenols, such as anthocyanins and theaflavins, to the hydroxyapatite surface of teeth. This interaction results in cross-linking between polyphenols and proteins in the pellicle region, which becomes the primary site of staining following the consumption of pigmented foods and beverages.[1] The pellicle, which forms naturally on the tooth surface, can serve as an adhesion site for extrinsic stains. Substances found in food, beverages, tobacco, mouthwash, and various medications are major sources of such staining.[2]

The protein pellicle initially forms on enamel through the activity of salivary proteins that coat the oral cavity. The acquired salivary pellicle is generated through a dynamic and selective process influenced by adsorption and desorption. Key precursor proteins, such as proline-rich proteins (PRPs), statherin, and histatin, play essential roles in this process.[3] The pellicle is critically involved in tooth discoloration. PRPs, are major components and can account for up to 70 % of total salivary proteins. Statherin, histatin, and PRPs exhibit high affinity for

* Corresponding author. Department of Dental Biomedical Science, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia.
E-mail address: triannautami@ugm.ac.id (T.W. Utami).

https://doi.org/10.1016/j.jobcr.2025.11.006
Received 7 August 2025; Received in revised form 23 October 2025; Accepted 4 November 2025
Available online 14 November 2025

hydroxyapatite surfaces and contribute to the formation of the pellicle on enamel. Basic PRPs and histatin, in particular, display a strong binding affinity to food-derived polyphenols, thereby playing a major role in the development of tooth staining.[4] The salivary pellicle is resistant to mechanical procedures such as toothbrushing. Although the globular layer of the pellicle may undergo changes, the basal layer remains firmly attached to hydroxyapatite.[5]

Free amino acids, peptides, and proteins in green coffee beans serve as the primary precursors of volatile nitrogen-containing compounds. Coffee proteins act as activators in sugar degradation and serve as donors of ammonia or hydrogen sulfide after the breakdown of amino acid residue. This role highlights the significant contribution of proteins to the formation of volatile compounds during roasting. The Maillard reaction, which occurs during roasting, involves interactions between free amino acids or oligopeptides and reducing sugars, ultimately generating compounds such as aldehydes via Strecker degradation.[6]

Melanoidins and chlorogenic acid (CGA) in coffee contribute significantly to tooth discoloration by binding to the enamel and potentially eroding its surface, leading to extrinsic stains ranging in color from yellow to dark brown. Melanoidins are formed through the Maillard reaction during coffee roasting. These complex compounds are composed of polysaccharides such as galactomannan and arabinogalactan, 11S-type storage proteins, and key phenolic compounds such as CGA. Coffee bean proteins undergo denaturation and degradation during roasting, altering the profile of identifiable amino acids and producing melanoidin fractions containing amino acids such as alanine, aspartic acid, glutamic acid, glycine (GLY), and hydroxyproline. Hydroxyproline is also found in arabinogalactan-proteins extracted from green coffee beans, suggesting its involvement in the formation of the melanoidin structure.[7]

In this study, we integrate in vitro tooth-staining and whitening models with in silico simulations to explore the molecular mechanisms underlying stain formation and removal, with a particular focus on CGA–protein interactions. While previous research has explored the individual roles of polyphenols like chlorogenic acid and salivary proteins in tooth discoloration, our study provides a novel, comprehensive approach by combining experimental models with computational simulations. This integrated methodology allows for a more detailed understanding of the interactions between chlorogenic acids, melanoidins, and salivary proteins—an aspect that has not been fully addressed in existing literature. By providing insights into both the formation and potential removal of stains at the molecular level, our study offers a fresh perspective on the management of coffee-induced tooth discoloration.

2. Materials and Methods

2.1. Research design

This study is an experimental and in silico investigation with the aim of evaluating changes in tooth color resulting from immersion in coffee solution and examining the interaction mechanisms between melanoidin compounds and salivary proteins (statherin, histatin, and PRPs) involved in stain formation. The in silico approach used molecular docking to analyze ligand–receptor interaction affinity.

2.2. Tooth specimen preparation

The study sample consisted of permanent premolar teeth that met the inclusion criteria, namely teeth with an intact crown structure, free from caries and restorations, with fully developed roots, and extracted for orthodontic reasons. Teeth showing any structural defects, severe discoloration, or previous restorations were excluded from the study. Premolar teeth were cleaned using a prophylaxis paste and a prophy brush. The apical area was sealed with sticky wax and nail polish to prevent penetration of the solution other than through the crown. Each specimen was labeled and fixed in a plastic holder with the buccal surface facing upward.

2.3. Immersion in coffee solution

Specimens were immersed in a robusta coffee solution (27 g ground coffee/450 mL water) for 14 days, with the solution replaced every 24 h. After immersion, the teeth were rinsed with distilled water and dried. Robusta coffee contains higher levels of chlorogenic acids and caffeine compared to Arabica coffee, which contribute to higher staining potential.[8] The inclusion of robusta coffee allows for a more accurate representation of the staining potential of coffee-derived compounds in the context of real-world consumption. Moreover, robusta coffee is commonly consumed in many regions, making it a relevant choice for this study on tooth discoloration induced by polyphenol-rich beverages.

2.4. Tooth color measurement

We measured the initial and final tooth color using a UV-2401PC spectrophotometer (Shimadzu, Kyoto, Japan) based on the CIE Lab* scale and ΔE values. Specimens were placed in a black Bakelite holder with the buccal surface directed toward the light source.

2.5. Protein and ligand structure preparation

The three-dimensional (3D) structures of the salivary proteins—statherin, histatin, and PRP—were obtained from AlphaFold (https://alphafold.ebi.ac.uk/). Ligand structures (melanoidin-related compounds: alanine, arabinogalactan, asparagine, CGA, galactomannan, glutamine [GLN], GLY, and hydroxyproline) were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov) in *.sdf format.

2.6. Docking simulation and visualization

We performed the docking simulations using Hex 8.0 with the following parameters:

- Correlation type: shape only
- Fast Fourier transform mode: 3D
- Sampling method: range angles
- Grid dimension: 0.6
- Number of solutions: 2000

We visualized the results using Discovery Studio Visualizer to identify the hydrogen bond types and binding locations. Docking outputs were exported and further analyzed using UCSF Chimera to conduct molecular dynamics (MD) simulations. Chimera enabled the real-time visualization of molecular movement, monitoring of protein conformational changes, and analysis of intermolecular forces during simulation, to evaluate ligand–protein interaction stability and dynamics after docking.

2.7. Interaction analysis

The analysis included evaluating the E-total values as indicators of binding affinity, as well as visualizing ligand–protein interactions. The green color denoted hydrogen bond acceptors, whereas magenta indicated hydrogen bond donors. We used these findings to assess the potential of melanoidin compounds to form tooth stains through interactions with salivary proteins.

3. Results

Table 1 shows the total interaction energies (in kJ/mol) between melanoidin compounds in coffee and different salivary proteins, statherin, histatin, and PRP. Among all compounds tested, CGA exhibited the lowest (most negative) interaction energy across all proteins: −240.5

Table 1
Total interaction energy (kJ/mol) of coffee melanoidin compounds with salivary proteins.

	Alaine	Arabinogalactan	Aspargin	Chlorogenic acid	Galactomannan	Glutamin	Glycin	Hydroxyprolin
Statherin	−103.1	−224.5	−123.0	−240.5	−240.2	−143.8	−85.3	−107.3
Histatin	−101.9	−238.8	−132.1	−245.4	−234.4	−144.7	−91.5	−109.95
PRP	−106.88	−261.6	−130.35	−251.66	−248.6	−140.0	−100.3	−111.77

(statherin), −245.4 (histatin), and −251.66 (PRP). Lower interaction energy values indicate stronger binding affinity and higher complex stability; thus, we selected CGA for further analysis through interaction visualization and MD simulations.

The docking visualization revealed that CGA forms several hydrogen bonds and noncovalent interactions with key residues on the surface of the PRP proteins, including GLY, arginine (ARG), tyrosine (TYR), and GLN, which are involved in stabilizing the complex. The 2D interaction diagram (Fig. 1) indicated hydrogen bonds between the hydroxyl groups of CGA and residues GLY A:38 and TYR A:40 as well as electrostatic interactions with positively charged residues such as ARG A:28 and ARG A:32.

Molecular visualization revealed that CGA interacts with several key residues in the histatin protein, primarily through hydrogen bonding and hydrophobic interactions (Fig. 2). Conventional hydrogen bonds were formed between the carboxyl and hydroxyl groups of CGA and residues ARG A:25, ASP A:27, and GLY A:28, indicating strong and specific binding within the active site of histatin. In addition, we observed π-alkyl interactions between the aromatic ring of CGA and residues LYS A:32 and ARG A:31, further enhancing the stability of the complex via hydrophobic forces. Van der Waals interactions were also identified at residues LYS A:30, TYR A:29, PHE A:33, and HIS A:34. However, we detected an unfavorable steric clash (unfavorable bump) at residue HIS A:26, suggesting spatial incompatibility at that site, which may have a negative effect on the overall binding affinity and stability of the CGA–histatin complex.

Molecular visualization showed that CGA interacts with PRP via GLY residues (GLY A:132, A:136, A:137) and proline residues (PRO A:133, A:134, A:135, A:138) (Fig. 3). The binding mechanism was predominantly mediated by conventional hydrogen bonding between the hydroxyl/carboxyl groups of CGA and the oxygen/nitrogen atoms of proline residues, along with van der Waals interactions. We also observed C–H hydrogen bonds between the hydrogen atoms of proline residues and the carbonyl group of CGA, contributing to complex stabilization. However, we noted a steric clash (unfavorable bump) at residue PRO A:196, indicating a possible spatial mismatch at that location. This could affect the affinity and stability of the CGA binding to PRP as a whole.

Root mean square deviation (RMSD) analysis indicated that the system maintained structural stability throughout the MD simulation. The RMSD values ranged from 4.2 to 4.6 Å (Fig. 4), with no sharp fluctuations indicative of major conformational changes. Although the RMSD values were relatively high, the consistently stable trend we observed after the initial simulation phase shows that the system reached equilibrium. This indicates that the complex remained structurally stable relative to its reference conformation during the simulation period.

The RMSD analysis of the histatin–CGA complex revealed an initial value of approximately 4.1 Å, which gradually increased and stabilized between 4.4 and 4.6 Å throughout the simulation (Fig. 5). This stable conformation was achieved relatively early, indicating that the complex maintained structural integrity over time. Minor fluctuations remained below the 5 Å threshold, suggesting that the histatin–CGA interaction was stable and did not undergo significant conformational changes.

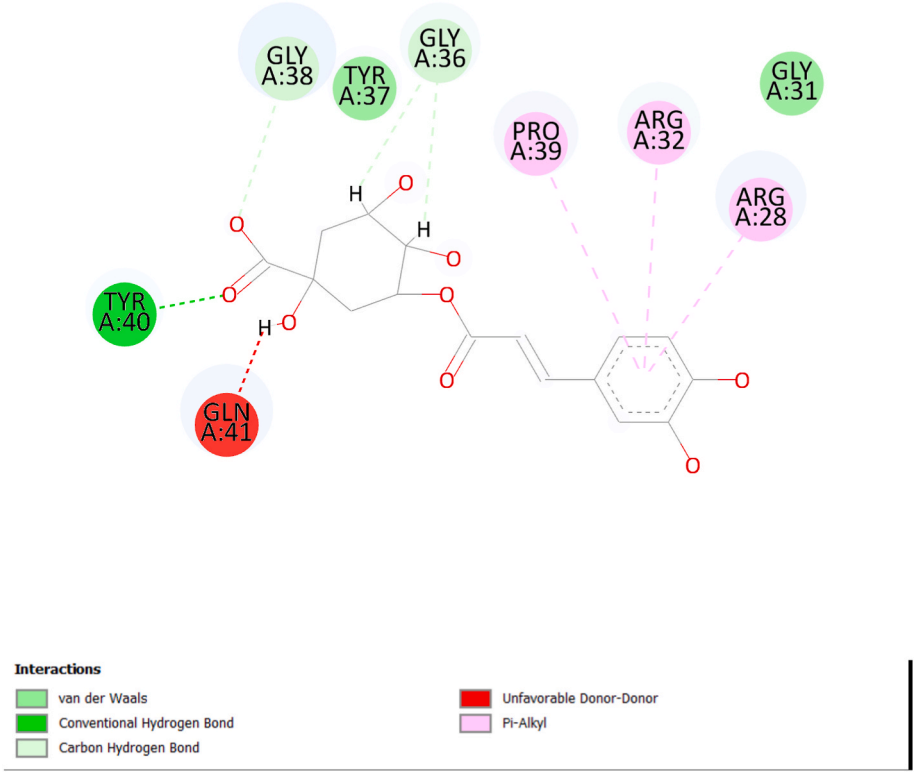

Fig. 1. Interaction of chlorogenic acid with statherin.

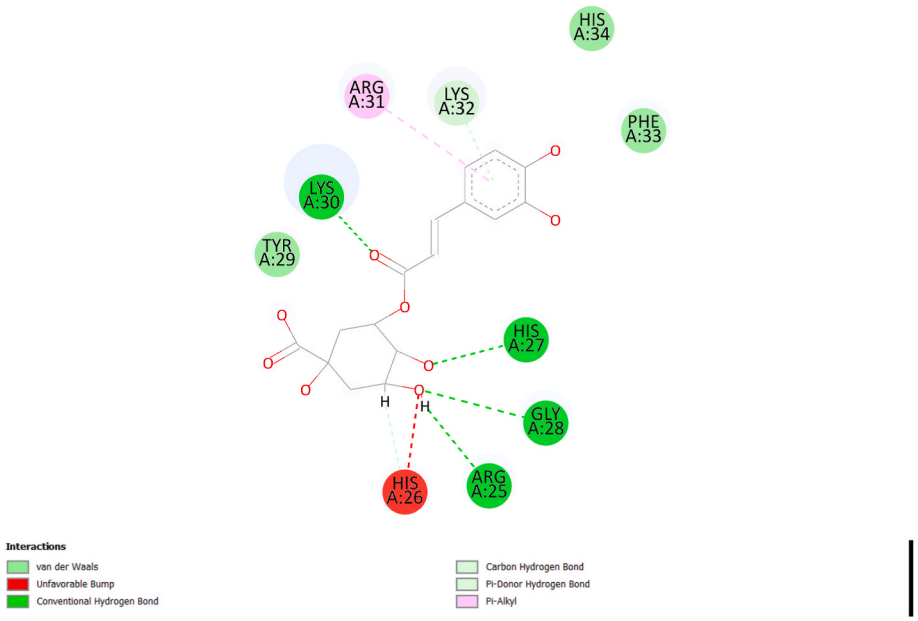

Fig. 2. Interaction of chlorogenic acid with histatin.

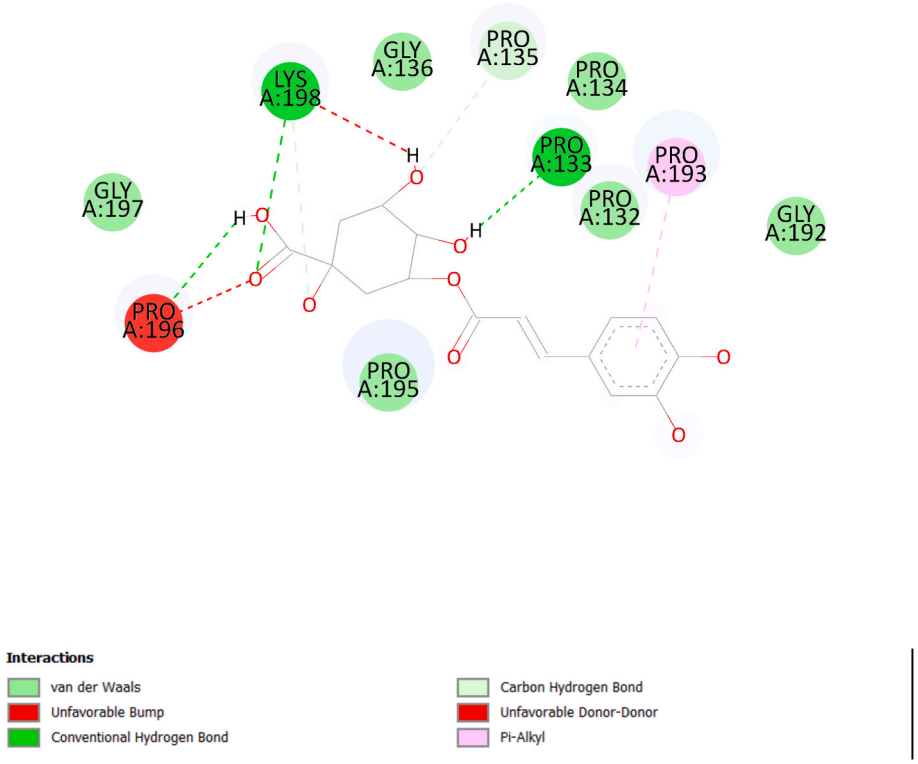

Fig. 3. Interaction of chlorogenic acid with Proline-Rich Protein (PRP).

We could not perform the MD simulation of the CGA–PRP complex due to the highly flexible and extended structure of the PRP, which resulted in minimal stable interactions with the ligand. Consequently, the system failed during the initial preparation phase, and the RMSD data as a measure of complex stability could not be obtained.

After immersion of the specimens in coffee, we observed significant changes in the color parameters (Fig. 6). The lightness value (L*) decreased from 83.569 ± 1.98 to 71.873 ± 3.236, indicating that the surfaces of the specimens became darker due to coffee exposure. The a* value, representing the red–green axis, increased from 1.375 ± 0.867 to 2.992 ± 0.784, indicating a shift toward the red spectrum. The b* value, which reflects the yellow–blue axis, increased from 15.848 ± 1.928 to 18.585 ± 1.682, indicating enhanced yellow coloration on the surface. The total color change (ΔE*ab) was recorded at 12.286 ± 3.645, which exceeds the clinically perceptible threshold, signifying a visually noticeable discoloration (Table 2).

Overall, our results demonstrate that immersion in coffee caused the specimens to become significantly darker, redder, and yellowerm, which is consistent with the typical staining pattern associated with pigmented beverages such as coffee.

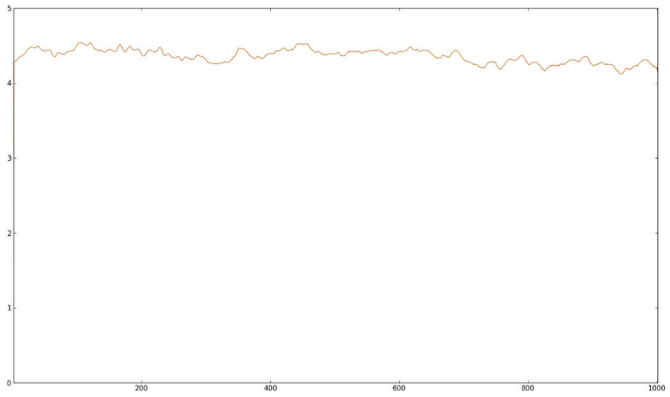

Fig. 4. Molecular dynamics simulation of chlorogenic acid with statherin.

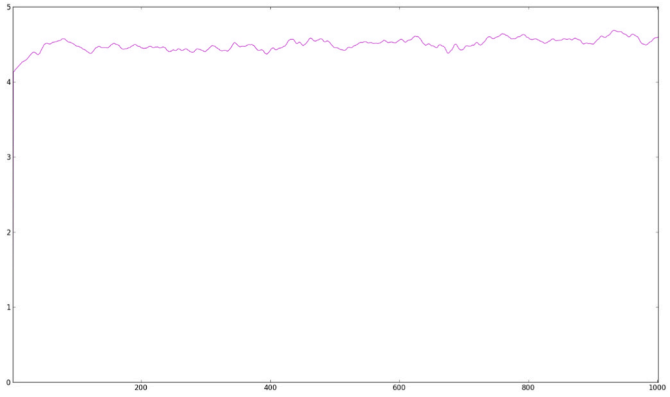

Fig. 5. Molecular dynamics simulation of chlorogenic acid with histatin.

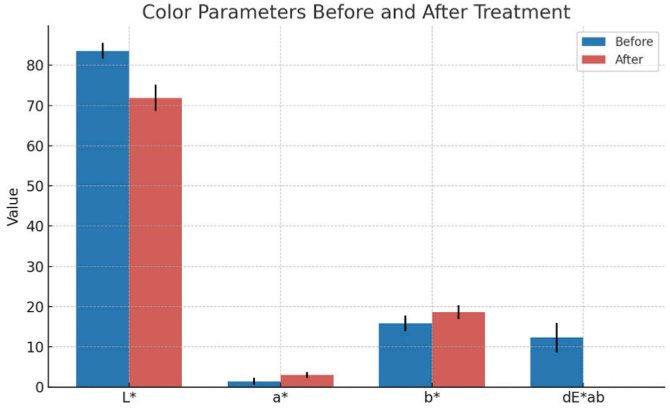

Fig. 6. Color measurement Results using a chromameter.

The paired samples correlation analysis (Table 3), revealed a weak and non-significant correlation between the pre- and post-treatment L* values ($r = 0.128$, $p = 0.257$), indicating inconsistent changes in lightness among samples. In contrast, moderate and statistically significant correlations were observed for both the a* ($r = 0.380$, $p < 0.001$) and b* ($r = 0.424$, $p < 0.001$) parameters, suggesting that chromatic variations in the red-green and yellow-blue axes occurred in a more consistent manner. These results imply that the treatment produced variable effects on brightness but more uniform tendencies in color tone changes.

4. Discussion

Based on the results of the molecular docking analysis, the three compounds that exhibited the highest binding affinity to salivary proteins were CGA, arabinogalactan, and galactomannan. These compounds showed the lowest total interaction energy (E-total), indicating the formation of the most stable complexes. The docking process functions by positioning a ligand at the target receptor site and calculating the binding energy, which reflects the energy required to form the interaction.[9] A lower energy value signifies a stronger interaction, and this interaction strength is directly correlated with the activity level of the ligand–receptor complex. The interaction between CGA and salivary proteins involves significant hydrogen bonding and hydrophobic interactions.

Further analysis of the MD trajectories revealed that CGA predominantly interacts with the N-terminal domain of statherin, which is rich in phosphorylation sites. This interaction is stabilized by the multiple hydrogen bonds between the hydroxyl groups of CGA and the phosphate groups of statherin. In addition, hydrophobic interactions between the aromatic ring of CGA and proline residues in statherin contribute to the stability of the complex. The spatial distribution of polar and nonpolar residues plays a key regulatory role in protein interactions. The hydrophobic effect, which causes nonpolar residues to cluster together, aids in maintaining the stability of the complex, while internalized polar residues are stabilized by intramolecular hydrogen bonds.[10] This interplay of forces may interfere with the normal function of statherin, which helps prevent the precipitation of calcium phosphate on the tooth surface, potentially contributing to tooth discoloration.

In contrast, MD simulations involving CGA and PRP proved challenging due to PRP's extensive and highly flexible structure, which demands significant computational resources. As the number of atoms in the system increases, both the computational time and memory requirements grow exponentially.[11] Furthermore, conventional MD simulations are typically limited to nanosecond-to-microsecond timescales, whereas important conformational changes in large proteins often occur over much longer durations.[12] The failure to achieve stable MD simulations for the CGA-PRP complex highlights the need for advanced computational techniques, such as enhanced sampling or replica exchange methods, to capture more accurate insights into the dynamic behavior of PRP during its interaction with CGA. Even with these limitations, the docking studies and simulations for statherin and histatin show that CGA can bind well to these proteins, providing useful insights into the mechanisms of tooth discoloration.

Table 2
Results of tooth color measurement before and after coffee immersion (chromameter analysis).

	Before	After
L*	83.569 ± 1.98	71.873 ± 3.236
a*	1.375 ± 0.867	2.992 ± 0.784
b*	15.848 ± 1.928	18.585 ± 1.682
dE*ab	12.286 ± 3.645	

Note: L* indicates lightness, ranging from 0 (black) to 100 (white). a* represents the position on the red–green axis; positive values indicate red, and negative values indicate green. b* represents the position on the yellow–blue axis; positive values indicate yellow, and negative values indicate blue. ΔE*ab is the total color difference between two samples, calculated using the CIE76 formula. Higher values indicate a greater color change.

Table 3
Paired Samples Correlations between Pre- and Post-Treatment CIE L*, a*, and b* Values.

Variables	N	Correlation (r)	Sig. (p)
L_0 & L_1	16	0.128	0.257
a_0 & a_1	16	0.380	<0.001
b_0 & b_1	16	0.424	<0.001

Note: Significant correlations were observed for a* and b* parameters ($p < 0.05$), whereas the L* parameter showed no significant relationship.

CGA, as a phenolic compound, interacts with polar and aromatic residues on the protein surface through hydrogen bonds and π–π stacking interactions. Polar residues form hydrogen bonds with each other and with the surrounding solvent, whereas aromatic residues engage in van der Waals and π-stacking interactions, enhancing the binding affinity of CGA.[13] The π–π stacking mechanism enhances the binding affinity of small-molecule inhibitors to enzyme pockets containing aromatic residues. These aromatic interactions are critical for protein stability, constituting one of the major noncovalent intermolecular forces. This force arises from the intermolecular overlap of p-orbitals in conjugated π systems and becomes stronger as the number of π electrons increases.[14] Other studies have shown that CGA forms hydrogen bonds with residues such as GLN59, VAL43, and TRP19 in β-lactoglobulin as well as ASN50, ASN156, and SER160 in myofibrillar proteins, depending on the surrounding pH conditions. These interactions may alter the protein's structure and affect its functional properties.[15]

Colorimetric analysis using a chromameter has been shown to be effective and objective, particularly in the assessment of dental color. It operates based on a tristimulus color-sensing system (red, green, blue) and converts the measurements into L*, a*, and b* values within the CIELAB color space.[16] The immersion of the tooth samples in a coffee solution resulted in significant changes in tooth color parameters, marked by a decrease in lightness (L*), an increase in the a* value (toward red), and an increase in the b* value (toward yellow).[17] The average total color change (ΔE*ab) exceeded 20, indicating a visibly noticeable discoloration. This staining is attributed to the adsorption of melanoidin molecules from coffee, primarily polyphenolic compounds such as CGA, onto the tooth surface.[18] The discrepancies in the color measurements before and after coffee immersion could be minimized by soaking the specimens in distilled water for 48 h (2 × 24 h) to simulate the moist conditions of the oral cavity.[19,20]

The molecular interactions between CGA and salivary proteins, namely, statherin, histatin, and PRP, as evidenced by binding energy data, demonstrated strong affinities, with interaction energies reaching −240 kJ/mol or lower. In the molecular models, CGA forms multiple hydrogen bonds with polar residues such as GLN and TYR, as well as van der Waals interactions with residues such as GLY. Van der Waals forces occur when atoms are in very close proximity, producing weak, nonspecific attractive forces that operate effectively over only short distances.[21] Hydrogen bonds arise when a hydrogen atom in one molecule interacts with a more electronegative atom in another.[22] In contrast, hydrophobic interactions occur between nonpolar molecules that do not form hydrogen bonds with water.[23] Together, these interactions facilitate the adsorption of coffee-derived molecules onto the protective salivary protein layer on teeth, thereby enhancing pigment retention.

The phenolic –OH functional groups in CGA play a critical role in

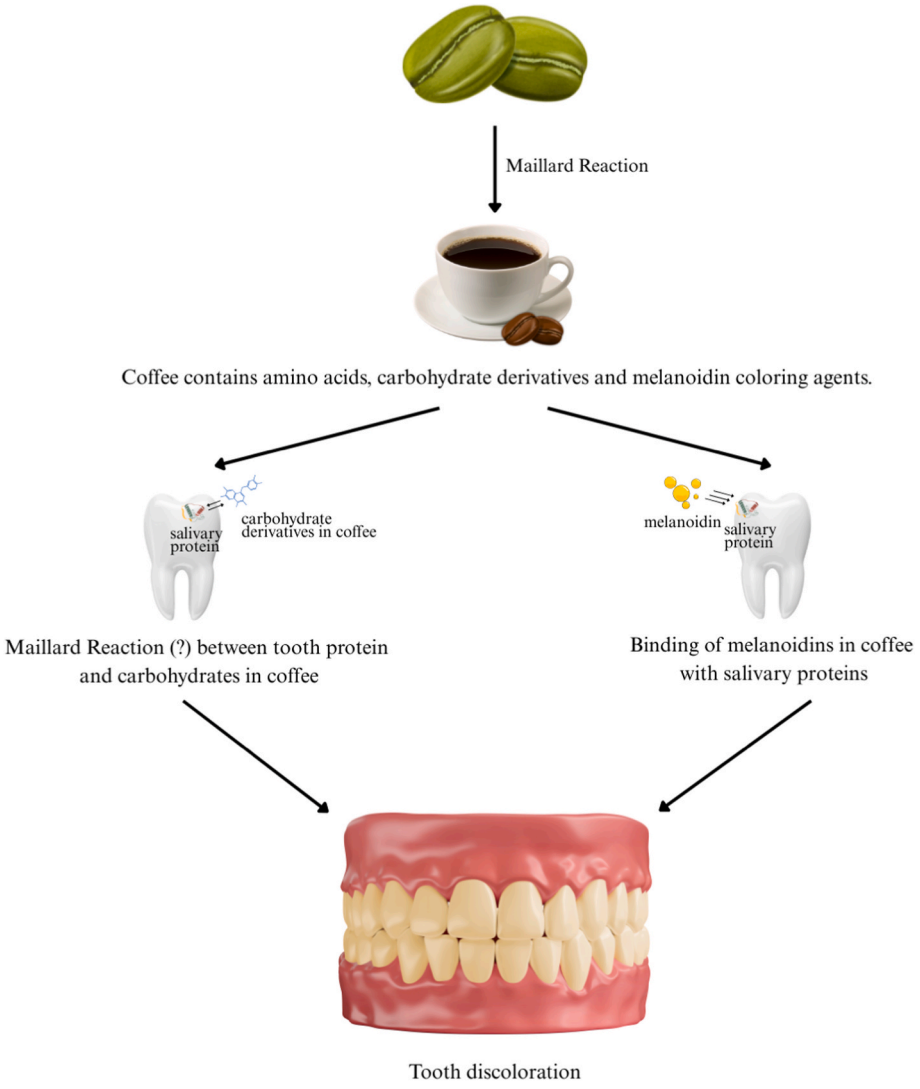

Fig. 7. Reaction mechanism of tooth discoloration induced by coffee.

hydrogen bonding with salivary protein surfaces, while its aromatic ring promotes hydrophobic interactions, leading to stable aggregation on the tooth surface. As a result, coffee molecules adhere not only through physical interactions but also via stable chemical bonding, intensifying discoloration. The reduction in L* values reflects a loss of brightness due to the accumulation of dark pigments, while the increase in a* and b* values indicate a color shift toward red and yellow—hallmarks of coffee-induced staining. Dark brown pigmentation is a prominent physical characteristic of melanoidins.[24] Nonenzymatic browning is a key mechanism in extrinsic tooth discoloration (Fig. 7) and primarily involves the Maillard reaction—a chemical process between carbohydrates (e.g., pentoses and hexoses) and amino compounds (including amino acids, peptides, and proteins).[25] This reaction proceeds through condensation and polymerization steps, ultimately generating complex brown pigments known as melanoidins. The formation of melanoidins is accelerated under alkaline conditions (high pH) and in the presence of elevated concentrations of free amino groups.[26]

Additionally, we have explored the possibility of developing inhibitors that could prevent staining, suggesting specific strategies or types of compounds that could be further investigated. One possible direction is to develop small-molecule inhibitors or peptides that stop CGA from binding to salivary proteins. Another method is to modify CGA's ability to bind by implementing chemical modifications.[25] This could lead to the manufacturing of oral care products that reduce the staining effect of coffee and other drinks high in polyphenols. This approach offers a more comprehensive view of how our findings can be used in real-life applications to create oral health products that prevent tooth discoloration.

The in vitro experimental design provides valuable insights into the staining capacity of coffee and its interaction with salivary proteins; yet, it is essential to acknowledge the study's limitations. The key issue is that the settings in the lab are considerably different from those in the mouth, which is continually changing. In vitro models fail to fully replicate the many variables influencing staining in the mouth cavity, including saliva flow, which facilitates the dilution and removal of staining chemicals. Alterations in the pH of the oral cavity, influenced by dietary intake or the efficacy of saliva in neutralizing acids, might affect the adhesion of polyphenols such as chlorogenic acid (CGA) to tooth surfaces. The salivary pellicle, which naturally develops on the enamel surface, is crucial for preventing stains from entering the oral cavity. In our in vitro investigation, the absence of this natural pellicle may have led to more pronounced staining than would occur in the oral cavity. Future research should validate these molecular findings in dynamic oral situations using in situ or in vivo models.

5. Conclusion

This research offers compelling evidence that chlorogenic acid from coffee significantly contributes to tooth discoloration through its interaction with salivary proteins, notably proline-rich protein, histatin, and statherin. CGA and these proteins have strong and persistent interactions in molecular docking tests, with binding energies ranging from −240.5 to −251.66 kJ/mol, indicating high binding affinities; complex stability is also supported by MD simulations. Chromameter analysis of tooth color post-immersion in coffee showed significant discoloration, with a total color change (ΔEab) of 12.286, exceeding the clinically perceptible threshold.

These results indicate that focusing on the molecular interactions between CGA and salivary proteins may be a viable approach towards developing preventive strategies against coffee-related tooth discoloration. Designing inhibitors that disrupt these interactions could make oral care products work better, diminishing the detrimental impact of dietary polyphenols on tooth discoloration.

Patient's/Guardian's consent

Not available for this manuscript.

Author contributions

Trianna Wahyu Utami, Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, writing – original draft, writing – review and editing.

Muhammad Raka Aqila Zaky, Data curation, formal analysis, validation, writing – original draft.

Margareta Rinastiti, Conceptualization, data curation, formal analysis, writing – review and editing.

Diatri Nari Ratih, Conceptualization, data curation, formal analysis, supervision, writing – review and editing.

Sri Budi Barunawati, Conceptualization, data curation, formal analysis, writing – review and editing.

Nunuk Purwanti, Conceptualization, data curation, formal analysis, writing – review and editing.

Yosi Bayu Murti, Conceptualization, data curation, formal analysis, writing – review and editing.

All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Data availability statement

All data generated or analyzed during this study are included in this published article.

Sources of funding

This research is funded by an Academic Excellence Improvement Program, Universitas Gadjah Mada (Grant No. 7725/UN1.P.II/Dit-Lit/PT.March 01, 2023).

Ethical statement

This study was reviewed and approved by the Institutional Ethics Committee of the Faculty of Dentistry - Prof. Soedomo Dental Hospital, Universitas Gadjah Mada (Approval No. 134/UNI/KEP/FKG-UGM/EC/2023). Human teeth were obtained following routine dental extractions and were fully anonymized. No personal identifiers were recorded, and informed consent was not required as there was no direct involvement of human subjects.

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.

Acknowledgements

The authors acknowledge financial support for this work from an Academic Excellence Improvement Program, Universitas Gadjah Mada (Grant No. 7725/UN1.P.II/Dit-Lit/PT.March 01, 2023).

References

1. Mailart MC, Berli PC, Borges AB, Yilmaz B, Baumann T, Carvalho TS. Pellicle modification with natural bioproducts: influence on tooth color under erosive conditions. *Eur J Oral Sci*. 2022;130(5), e12886. https://doi.org/10.1111/eos.12886.
2. Hosdurga R. Extrinsic stains and management: a new insight. *J Acad Ind Res*. 2013;1(8):465–472.

3. Chawhuaveang DD, Yu OY, Yin IX, Lam WY, Mei ML, Chu CH. Acquired salivary pellicle and oral diseases: a literature review. *J Dent Sci*. 2021;16(1):523–529. https://doi.org/10.1016/j.jds.2020.10.007.
4. Proctor GB, Pramanik R, Carpenter GH, Rees GD. Salivary proteins interact with dietary constituents to modulate tooth staining. *J Dent Res*. 2005;84(1):73–78. https://doi.org/10.1177/154405910508400113.
5. Hannig M. The protective nature of the salivary pellicle. *Int Dent J*. 2002;52(Suppl 5):417–423. https://doi.org/10.1111/j.1875-595X.2002.tb00731.x.
6. Cardoso WS, Dias SR, Coelho VS, Pereira LL, Fioresi DB, Pinheiro FA. Maillard reaction precursors and arabica coffee (Coffea arabica L.) beverage quality. *Food Hum*. 2023;1:1–7. https://doi.org/10.1016/j.foohum.2023.01.002.
7. Moreira AS, Nunes FM, Domingues MR, Coimbra MA. Coffee melanoidins: structures, mechanisms of formation and potential health impacts. *Food Funct*. 2012;3(9):903–915. https://doi.org/10.1039/C2FO30048A.
8. Anh-Dao LT, Nguyen HTT, Tran TT, Pham TTH, Le TMH. Determination of caffeine, chlorogenic acid, total phenolic contents, and antioxidant capacities for Arabica and Robusta coffee from Vietnam. *Anal Lett*. 2024;57(17):2855–2869. https://doi.org/10.1080/00032719.2024.2304126.
9. Amrulloh LSWF, Harmastuti N, Prasetiyo A, Herowati R. Analysis of molecular docking and dynamics simulation of mahogany (Swietenia macrophylla King) compounds against the PLpro enzyme SARS-COV-2. *J Farm Ilmu Kefarm Ind*. 2023;10(3):347–359. https://doi.org/10.20473/jfiki.v10i32023.347-359.
10. Alberts B, Johnson A, Lewis J, et al. Molecular biology of the cell. In: *The Shape and Structure of Proteins*. fourth ed. New York: Garland Science; 2002. Available from https://www.ncbi.nlm.nih.gov/books/NBK26830/.
11. Hollingsworth SA, Dror RO. Molecular dynamics simulation for all. *Neuron*. 2018;99(6):1129–1143. https://doi.org/10.1016/j.neuron.2018.08.011. PMID: 30236283; PMCID: PMC6209097.
12. Durrant JD, McCammon JA. Molecular dynamics simulations and drug discovery. *BMC Biol*. 2011;9:71. https://doi.org/10.1186/1741-7007-9-71. PMID: 22035460; PMCID: PMC3203851.
13. Zhou HX, Pang X. Electrostatic interactions in protein structure, folding, binding, and condensation. *Chem Rev*. 2018;118(4):1691–1741. https://doi.org/10.1021/acs.chemrev.7b00305.
14. Zhao Y, Li J, Gu H, et al. Conformational preferences of π–π stacking between ligand and protein, analysis derived from crystal structure data geometric preference of π–π interaction. *Interdiscipl Sci Comput Life Sci*. 2015;7:211–220. https://doi.org/10.1007/s12539-015-0263-z.
15. Tarahi M, Gharagozlou M, Niakousari M, Hedayati S. Protein–chlorogenic acid interactions: mechanisms, characteristics, and potential food applications. *Antioxidants*. 2024;13(7):777. https://doi.org/10.3390/antiox13070777.
16. Li Y. Tooth color measurement using chroma meter: techniques, advantages, and disadvantages. *J Esthetic Restor Dent*. 2003;15(1):33–41. https://doi.org/10.1111/j.1708-8240.2003.tb00281.x.
17. Brook AH, Smith RN, Lath DJ. The clinical measurement of tooth colour and stain. *Int Dent J*. 2007;57(5):324–330. https://doi.org/10.1111/j.1875-595X.2007.tb00140.x.
18. Kim S, Son JE, Larnani S, et al. Effects of tea and coffee on tooth discoloration. *Ital J Food Sci*. 2024;36(4):64–71. https://doi.org/10.15586/ijfs.v36i4.2715.
19. Batista GR, Barcellos DC, Torres CR, Goto EH, Pucci CR, Borges AB. The influence of chemical activation on tooth bleaching using 10% carbamide peroxide. *Oper Dent*. 2011;36(2):162–168. https://doi.org/10.2341/09-280-L.
20. Dewiyani S, Sulistiyowati F. Effect of soaking cow's milk on tooth enamel surface roughness. *Int J Sci Res*. 2023;12(11):34–36. https://doi.org/10.36106/ijsr/3623361.
21. Luanphaisarnnont T. *Intermolecular Forces: Van der Waals Interaction*. Harvard University; 2009. Available from: http://evans.rc.fas.harvard.edu.
22. Arunan E, Desiraju GR, Klein RA, Sadlej JS. Definition of the hydrogen bond. *Pure Appl Chem*. 2011;83(8):1637–1641. https://doi.org/10.1351/PAC-REC-10-01-02.
23. Otto S, Engberts JBFN. Hydrophobic interactions and chemical reactivity. *Org Biomol Chem*. 2003;1(16):2803–2820. https://doi.org/10.1039/B304462D.
24. Li Y, Zhang Q, Xiao S, Yang Q, Wang L, Hao J. Review of melanoidins as by-product from thermal hydrolysis of sludge: properties, hazards, and removal. *Processes*. 2024;12(1):135. https://doi.org/10.3390/pr12010135.
25. Addy M, Moran J, Davies RM, Beak A, Levis A. The effect of single morning and evening rinses of chlorhexidine on the development of tooth staining and plaque accumulation: a blind cross-over trial. *J Clin Periodontol*. 1982;9(2):134–140. https://doi.org/10.1111/j.1600-051X.1982.tb01228.x.
26. Wang L, Pan X, Jiang L, et al. The biological activity mechanism of chlorogenic acid and its applications in food industry: a review. *Front Nutr*. 2022;9, 943911. https://doi.org/10.3389/fnut.2022.943911.