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Fate and Antioxidant Activity of Matcha Catechins during Lactic Acid Bacteria Fermentation

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Abstract

Matcha is widely applied in fermented foods because of its abundant catechins, dietary fiber and health effects, but the fate of catechins during lactic acid bacteria (LAB) fermentation and its underlying mechanisms remain poorly understood. Hence, the effects of LAB (Lactobacillus bulgaricus and Streptococcus thermophilus) fermentation on the catechins release from matcha, and its relationship with antioxidant capacity were investigated. The results showed that aqueous extraction yielded 43% free catechins, whereas 70% methanol recovered nearly 90%. LAB fermentation increased the concentrations of TPC, catechin, CG, GC, GCG, EGC, EGCG, gallic acid, and caffeine. Lactose co-fermentation dramatically elevated catechin release (230.8%) and gallic acid (127.4%) while reducing the proportion of total epicatechin-type catechins. Mechanistically, LAB fermentation promotes EGCG and ECG conversion into EGC, catechin, and gallic acid via multiple pathways. Antioxidant assays revealed that DPPH, •OH, and $\bullet O_2^-$ scavenging capacities and total reducing power correlated positively with the epicatechin-type catechins concentrations, especially EGCG and EGC. Overall, matcha catechins release during LAB fermentation is co-regulated by acidochemical desorption, dietary fiber degradation, and enzymatic bioconversion. Matcha dosage, lactose availability, and strain metabolic activity collectively determine the final catechins profile, release degree, and antioxidant performance. These findings provide a theoretical basis and practical guidance for fermented matcha products.

1. Introduction

Matcha is an ultrafine green tea powder produced from shade-cultivated tea leaves through steaming, drying, destemming, and grinding, and it differs distinctly from common green tea powder in raw material, processing, and consumption manner [1, 2]. The components of major interest in matcha mainly include polyphenols, dietary fiber, caffeine, and L-theanine. Among these, the polyphenol content ranges from 56 to 270 mg GAE/g, predominantly catechins, mostly in free form; EGCG is a representative catechin with relatively high content and strong activity, at approximately 19 – 75 mg/g [3–6]; dietary fiber content is about 30–58 g/100g, but water-soluble dietary fiber accounts for less than 10% of total dietary fiber [5, 7]. Studies have shown that matcha possesses good antioxidant activity[8], anti-obesity effects [9, 10], and cardiovascular health benefits [11]. Research has demonstrated that matcha polyphenols exert antioxidant and anti-inflammatory effects by scavenging free radicals and chelating metal ions [5, 12], while matcha dietary fiber promotes intestinal peristalsis and modulates the composition and diversity of gut microbiota[13, 14]. Because matcha can be consumed as whole leaves, it has considerable potential in functional food development [4].

However, some catechins in matcha, such as EGCG, GCG and CG, have low water solubility, and some catechins bind to macromolecules such as dietary fiber through covalent or non-covalent bonds [5, 15]. Therefore, unlike consuming tea infusions, the fiber–polyphenol complexation and low water solubility when ingesting whole-leaf matcha may limit the bioaccessibility of catechins in the upper gastrointestinal tract, thereby weakening their antioxidant and cardioprotective effects. These fiber-bound and low-water-soluble catechins compounds are considered promising components in matcha,

and enhancing their release is expected to improve the bioavailability and health benefits of matcha polyphenols [4].

Lactic acid bacteria (LAB) fermentation may be an effective approach to enhance the bioaccessibility and nutritional functions of matcha catechins[16, 17]. For example, Yunhong Ren [18] found that after black tartary buckwheat was fermented by *Bacillus* sp. DU-106, the contents of free kaempferol and quercetin, as well as total polyphenols increased significantly, while the contents of rutin, epicatechin, and chlorogenic acid decreased significantly; moreover, the fermented product was more effective in improving blood lipid metabolism. Rocchetti, Miragoli [19] reported that after sweet buckwheat was fermented by *Lactobacillus paracasei* A1 2.6, the total polyphenol content decreased by approximately 23%, but the in vitro antioxidant activity was significantly enhanced. Similarly, fermentation with LAB strains (*Lactobacillus casei* NCU215) increased the content of free polyphenols such as gallic acid in mango juice, and this effect was closely associated with pectin degradation [20]. Frutos [21] found that after pomegranate juice was fermented by *Lactobacillus*, new phenolic derivatives such as catechin and α-punicalagin were produced, which originated from the metabolism of ellagitannins, epicatechin, and catechin by LAB. Furthermore, compared with unfermented green tea, tea fermented with *Lactobacillus paracasei* subsp. paracasei NTU 101 had higher contents of EGCG, EGC, and chlorogenic acid, and could inhibit lipid accumulation in mature 3T3-L1 adipocytes by stimulating lipolysis [22].

In summary, LAB can alter the composition and content of plant phenolic compounds through multiple pathways and enhance their health benefits. However, matcha differs from tartary buckwheat, fruit juices, and green tea in raw material characteristics, chemical composition, and catechins content. Moreover, matcha is often mixed with milk to prepare fermented yogurt, a process mainly involving *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, whose fermentation characteristics differ from those of the LAB commonly used in the above plant-based fermentations. In particular, LAB fermentation causes a low-pH environment may affect the stability, existing forms, and release of catechins from the fiber matrix. Currently, systematic research is lacking on the effects of fermentation by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* on the composition, content, and antioxidant activity of matcha catechins.

Therefore, we hypothesized that the growth and metabolism of LAB can alter the composition, content, and antioxidant activity of catechins in matcha. Considering that catechins exhibit different stability and existing forms under different solvents and pH conditions, we investigated the effects of solvent type and pH on the catechin content of matcha to exclude interference from non-fermentation factors. Subsequently, we analyzed the effects of fermentation with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* on the composition, content, and antioxidant activity of matcha catechins, and preliminarily explored the potential mechanisms, aiming to provide a basis for the development and nutritional evaluation of matcha-based functional fermented foods.

2. Materials and methods

2.1. Materials, isolation of polyphenol

The matcha (3#, European standard grade) was purchased from Guizhou Tongren Guicha Tea Co., Ltd. (Guizhou, China). Its basic chemical composition includes 4.12% moisture, 29.6 g/100 g protein, 5.5 g/100 g fat, 55.6 g/100 g carbohydrate and 5.3 g/100 g ash. Caffeine and polyphenol standards including gallic acid, catechin, gallocatechin (GC), catechin gallate (CG), gallocatechin gallate (GCG), epicatechin (EC), epigallocatechin (EGC), epicatechin-3-O-gallate (ECG), and epigallocatechin 3-O-gallate (EGCG) were purchased from Macklin (Shanghai, China). HPLC-grade absolute acetic acid and acetonitrile were purchased from Sigma-Aldrich Co., Ltd. (St. Louis, Missouri, USA). The lactic acid bacteria strains strains contained *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* (1:1, w: w) were purchased from (Angel Yeast Co., Ltd., Yichang, China). All other chemicals were of analytical graded.

The extract of matcha catechins was conducted based on a previous method established by Kan, Capuano [23] with some modifications. 2 g of matcha powder samples were mixed with 100 mL 70% methanol solution, followed by ultrasonic treatment for 10 min (100 W) at room temperature (18–20°C). The mixture was centrifuged (4000 ×g, 10 min) and the residue was re-extracted twice. The supernatants were collected and concentrated to 25 mL using a rotary evaporator (Yarong Biochemical Instrument Factory, Shanghai, China) at 35°C. The matcha extract was then stored at -20°C until analysis. To investigate the effects of solvent and pH, catechins were extracted separately using distilled water at various pH (4.0, 5.0, 6.0, and 7.0) adjusted by using 1 M HCl solution according to the procedures described above.

The separation of bound catechins in matcha powder was performed based on our previous method [24] with some modifications. Briefly, the residue after free catechins extraction was dissolved in 15 mL of 70%methanol-1 M sodium hydroxide solution (90:10, v/v) and incubated at 60°C for 20 min. Then 80 mL of ethyl acetate was added and mixed for 10 min to remove the organic fraction, followed by discarding the organic phase using a pipette. Subsequently, the aqueous fraction was pooled and concentrated to 25 mL as described above, followed by storing at -20°C until analysis.

2.2. Batch-culture fermentation

Matcha powder (1.5, 2.0, or 2.5 g) was added to separate 150 mL sterilized test tubes, and the corresponding groups were designated the 1.5, 2.0, 2.5 groups. Then, 99.0 mL of autoclaved basal nutrient medium (pH 7.0), consisting of peptone 10.0 $g \cdot L^{-1}$, beef extract 10.0 $g \cdot L^{-1}$, yeast extract 4.0 $g \cdot L^{-1}$, triammonium citrate 2.0 $g \cdot L^{-1}$, sodium acetate 5.0 $g \cdot L^{-1}$, $MgSO_4 \cdot 7H_2O$ 0.2 $g \cdot L^{-1}$, $MnSO_4 \cdot 4H_2O$ 0.05 $g \cdot L^{-1}$, KH_2PO_4 2.0 $g \cdot L^{-1}$ and Tween-80 1.0 $g \cdot L^{-1}$ was added to each tube. The test tubes were then inoculated with 1 mL of lactic acid bacteria suspension. Batch cultures were incubated under anaerobic conditions for 8 h, after which samples were collected. The non-fermented group (2.0 + pre-ferm. group) and the fermentation group with 2.0 g matcha and 2.0 g lactose (2.0 + Lac group) were used as the negative and positive controls, respectively. Each fermentation was performed in triplicate.

2.3. Determination of bacterial counts, pH, and carbohydrates content

Colony-count of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* in all fermented samples was carried out according to the procedures validated by ISO 7889:2003. Briefly, 25 mL of the fermented soup was transferred into a sterile Erlenmeyer flask (containing several sterile glass beads) containing 225 mL of sterile saline by using a sterile pipette. The flask was shaken vigorously to obtain a 1:10 diluted fermented soup. Then, 1 mL of the diluted sample was carefully transferred into a sterile test tube containing 9 mL of sterile saline to prepare a 1:100 diluted fermented soup, ensuring that the pipette tip does not contact the diluent. For each subsequent 10-fold serial dilution, the procedure described above was followed to prepare the next dilution level of the fermented soup, changing to a fresh sterile tip with each dilution step.

Further, 1 mL of the sample from three consecutive appropriate dilutions was transferred into a sterile plate, based on the estimated viable count of *Streptococcus thermophilus* in the test sample. Immediately, 15 mL of MC agar medium (cooled to 48°C) was added to the plate, and the contents were mixed by gentle wrist rotation to ensure even distribution of the sample. The plate was then incubated aerobically at 36°C for 72 h, after which the colonies were enumerated. For the enumeration of *Lactobacillus delbrueckii subsp. Bulgaricus*, three consecutive appropriate dilutions were selected, and 1 mL of each diluted sample was aseptically transferred into a sterile plate. Subsequently, 15 mL of MRS agar medium (cooled to 48°C) was added and mixed within 15 min of the dilution step. The plates, placed in an anaerobic chamber containing an oxygen indicator and a sufficient number of anaeropack sachets, were incubated at 36°C for 72 h. Colonies were then enumerated. The pH value of the fermented soups was measured by using a pH meter (ST5000, Ohaus Instruments Co., Ltd., China). The contents of reducing sugar and total soluble sugar in supernatant of fermentation broth were detected by using GOPOD Kit and total sugar content assay Kit (Nanjing Jiancheng Bioengineering Institute, China), respectively.

2.4. Catechins composition analysis by HPLC

The composition of catechins in matcha and fermented samples were determined by HPLC (U1tiMate 3000, Thermo Fisher) equipped with a diode array detector and a Phenomenex C18 column (5 μm, 250 mm × 4.6 mm) (Phenomenex, Cheshire, UK) according to the reported methods [7, 23, 25] with modifications. The UV detecting channel and column temperature was set at 278 nm and 25°C, respectively. The injection volume was 10 μL. A binary gradient of water with 9% acetonitrile, 2% acetic acid, and 0.2% EDTA-2Na (10 $mg{\cdot}mL^{-1}$) (eluent A) and water with 80% acetonitrile, 2% acetic acid, and 0.2% EDTA-2Na (10 $mg{\cdot}mL^{-1}$) (eluent B) was used to run on the system at a flow rate of 1 mL min^{-1}. The gradient started at 100%A, remained at 100%A for 10 min, and decreased to 68% over 5 min, then remained at 68% for 10 min, then climbed to 100% over 5 min, and remained at 100% for 2 min to wash the column. Standard curves (Table 1S) for quantification were prepared with standard compounds dissolved in 70% methanol at concentrations between 5–300 $\mu g{\cdot}mL^{-1}$. The polyphenol content in

matcha and fermented samples were expressed as mg per gram of dried matcha powder and mg per milliliter sample, respectively.

2.5. Determination of TPC

The TPC were detected with Folin-Ciocalteu according to our previous method [24]. Briefly, 100 μL Folin-Ciocalteu were added to the diluted matcha extract or fermented soup (125 μL) with distilled water. 1000 μL of 7% Na_2CO_3 solution was added after 6 min. the mixture was then incubated at 40°C for 10 min, followed by measuring the absorbance of the sample at 765 nm using a microplate reader (BioTek ELx800, Biotek, USA). The TPC were calculated by using the calibration curve ($y = 3.027x + 0.057,\ R^2 = 0.9893;\ x : 0.05 \sim 0.30\ mg \bullet\ mL^{-1}$) established with gallic acid and expressed as gallic acid equivalent (GAE) per mg or mL.

2.6. Bioaccessibility evaluation and in vitro antioxidant activity

The release degree of catechins and TPC from matcha was calculated based on the follow formula:

$$Release\ degree\ \% = C_1 \times\ V_1 / \left(C_{max} \times\ M_0\right) \times\ 100\%$$

Where C_1represents the concentration of TPC and catechins dissolved in the fermentation broth after fermentation, mg/mL. V_1 represents the volume of fermentation broth, mL. C_{max} and M_0 represent the detected max-concentration of polyphenols extracted from match, mg/g, and the weight of matcha flour used in vitro fermentation, g, respectively.

The determination for DPPH radical scavenging capacity of fermented and unfermented sample was conducted based on a previous method [26]. The assays for •OH, T-AOC, and $•O_2^-$ radicals scavenging rate were performed accordance to the respective kit instructions (Nanjing Jiancheng Bioengineering Institute, China).

2.7. Statistical analysis

Assays for each experiment were conducted independently in triplicate. Data were expressed as means ± standard deviation of duplicates. Data were processed using OriginPro 8.0 (OriginLab Co., Northampton, MA, USA) and GraphPad Prism software version 8.0.1 (Inc., USA). Pearson correlation analysis coupled with Mantel test was conducted by using Python 3.10 software. Significant differences were statistically analyzed using IBM SPSS V.24.0 software (Chicago, IL, USA) and considered at $P < 0.05$.

3. Results

3.1. Content and composition of TPC and catechins in matcha

Based on retention time matching with HPLC standards (Fig. 1A) and their regression equations listed in Table S1, ten target compounds, including catechin, GC, CG, EC, and EGCG, were successfully identified and calculated in the matcha samples (Fig. 1B). As shown in Fig. 1C, the solvent type did not alter the retention times. However, the 70% methanol extract exhibited significantly higher response intensities at most characteristic peaks than the aqueous extract, indicating its superior extraction capacity for catechins As shown in Fig. 2A, 70% methanol extracts showed significantly more free phenolics (177.8 mg GAE/g) than aqueous solution (155.1 mg GAE/g), which is consistent with the well-known higher solubility of medium-to-low polarity polyphenols in hydro-organic solvents (8). In both types of extracts, the content of free TPC was higher than that of bound TPC, yet the 70% methanol extract yielded a higher total TPC content (Fig. 2B–C), which was in basically agreement with the results reported previously (6).

In the aqueous extracts, the calculated apparent contents of bound CG, GCG, and EGCG were significantly higher than those of their free counterparts. In contrast, for the 70% methanol extracts, the contents of all tested bound catechins, except for GCG, approached zero (Fig. 2D–O). For instance, the free EGCG content reached 54,388 μg/g, representing an increase of approximately 2.5 fold, while CG (15,667.1 μg/g) exhibited the highest increase of about 5.4-fold. These findings are generally consistent with previous reports (1, 6). Quantitative analysis (Fig. 2L) revealed that the total catechin content in the aqueous extract was 87,603.2 μg/g, of which only 43.2% was present in the free form, whereas that in the 70% methanol extract significantly increased to 95,542.0 μg/g, with free catechins accounting for nearly 90% (EGC was not detected in the bound form in either solvent). Nevertheless, the levels of EGCG, ECG, and CG were lower than those reported in some previous studies (1, 6), which may be attributable to differences in raw material characteristics. Regarding the epicatechins composition (Fig. 2M), the proportion of total epicatechin-type catechins in the aqueous extract (approximately 88.3%) was significantly higher than that in the 70% methanol extract (81.2%). However, the proportion of EGCG in the aqueous extract (40.0%) was markedly lower than that in the 70% methanol extract (58.7%). In all, 70% methanol solution is the preferred solvent for enabling the efficient release of EGCG, CG, GCG, and EC.

3.2. Effects of pH on the content of TPC and catechins

Based on the HPLC chromatograms (Fig. 1D), the number of chromatographic peaks remained essentially unchanged across different pH conditions, indicating that no new compounds were generated or that the original components did not degrade below the detection limit. However, a systematic shift to earlier retention times was observed for certain target compounds (e.g., GC shifted from 5.4 min to 5.2 min). Notably, no significant differences in peak profiles were observed among the pH 6.0, 5.0, and 4.0 groups, suggesting that, under the present chromatographic conditions, acidity variation did not deteriorate the chromatographic separation performance.

TPC measurements (Fig. 3A–C) revealed that the content of free TPC (167.2 mg GAE/g) increased significantly only at pH 5.0 compared to the value of 157.8 mg GAE/g at pH 7.0, accompanied by a corresponding decrease in bound TPC, whereas total TPC did not differ significantly among all pH

treatments ($p > 0.05$). This result indicates that acidic conditions did not substantially improve the overall extraction yield of matcha polyphenols, but rather promoted the desorption of polyphenols from the matrix or the conversion of weakly bound forms to free forms, i.e., altering the distribution ratio between free and bound fractions rather than increasing the absolute extractable amount. For individual catechin monomers (Fig. 3D–O), the effects of acidic conditions (pH 4.0–6.0) on free contents exhibited monomer specificity. For instance, the free forms of CG, GC, GCG, EC, EGCG, and total free catechins increased significantly ($p < 0.05$). Among them, GCG showed the largest increase (2.9-, 2.7-, and 3.1-fold higher than at pH 7.0, respectively), followed by CG (1.1-, 0.8-, and 1.2-fold), and EGCG (0.59-, 0.48-, and 0.63-fold). In contrast, free catechin, ECG, gallic acid, and caffeine decreased significantly ($p < 0.05$). The changes in bound catechins were generally opposite to those of their free counterparts (except for bound GCG, which was not detected). Furthermore, at pH 4.0, the free contents of CG, GC, EC, ECG, and total catechins reached their highest levels among all treatment groups. At pH 6.0, however, the free EGCG content (24,732.9 μg/g) was significantly higher than that at other pH groups, representing an increase of approximately 63.3% relative to pH 7.0. No significant differences ($p > 0.05$) were observed for free GCG and ECG across the pH 4.0–6.0 range. Total free catechins increased significantly at both pH 4.0 and pH 6.0 (by approximately 40%), but no statistical difference was found between these two pH values (Fig. 3L), indicating that pH 4.0 and pH 6.0 exerted comparable enhancing effects on total free catechins, albeit with different dominant monomers.

From the perspective of epicatechins composition, EGCG accounted for 40.0% of total free catechins, EGCG plus EGC together represented 75.5%, and total epicatechin-type catechins accounted for 88.4% at pH 7.0. After acidic extraction, the proportions of epicatechin-type catechins and EGC decreased significantly, whereas the proportion of EGCG increased (up to 46.2% at pH 6.0). In summary, acidic conditions (pH 4.0–6.0) significantly increased the concentration of free catechins in matcha, with the increments primarily originating from GCG, CG, and EGCG. Although the proportion of total epicatechin-type catechins decreased, the absolute content and relative proportion of EGCG both reached their optima at pH 6.0, indicating that pH 6.0 represents a favourable acidity condition for balancing EGCG stability and extraction efficiency.

3.3. Carbohydrate metabolism and microbial growth

The color of the matcha infusion changed from green to yellowish-green after LAB fermentation, with no significant differences in color observed among the fermentation groups (Fig. 4). Regarding carbohydrate metabolism (Fig. 4B, C), trace amounts of reducing sugars were detected in the 2.0 group, while the reducing sugar content increased significantly in the 2.5 group. In the presence of lactose (2.0 + Lac group), the reducing sugar content was further elevated. Concurrently, the total soluble sugar content decreased modestly in the lactose-free groups (by 7.9% in the 2.0 group, and by 8.2% and 6.7% in the 1.5 and 2.5 groups, respectively), whereas a substantial reduction of 62.3% was observed in the 2.0 + Lac group (Fig. 4C). These results indicate that the LAB possessed a very limited capacity to degrade soluble dietary fibre in matcha, whereas lactose served as a preferred carbon source, substantially enhancing microbial metabolism and resulting in a high net consumption of total soluble sugars.

Colony count results (Fig. 4D) showed that Lactobacillus counts increased with increasing matcha concentration, but the growth rate slowed at 2.5%. Streptococcus counts were highest in the 2.0 group. When lactose was co-present with matcha, both bacterial counts increased significantly, which was consistent with the colony densities observed on agar plates (Fig. 4E). In summary, low matcha concentrations (1.5%–2.0%) moderately promoted bacterial proliferation, whereas the high concentration (≥ 2.5%), despite releasing more reducing sugars, inhibited the growth of LAB. This suggests that Streptococcus thermophilus and Lactobacillus bulgaricus lack key polyphenol-metabolising enzymes, such as flavonoid-cleaving enzymes and phenolic acid-degrading enzymes, and are therefore unable to utilise tea polyphenols for proliferation. Furthermore, the observation that Streptococcus counts peaked at 2.0% and declined at 2.5% implies that the two bacterial species may differ in their tolerance thresholds to match catechins.

3.4. Concentration and release degree of TPC and catechins

The pH, TPC, catechin profiles, gallic acid, and caffeine concentrations and their release efficiencies in matcha infusions fermented by LAB are presented in Fig. 5. Compared with the pre-fermentation values (2.0 + pre-ferm. group), the pH decreased significantly in all fermentation groups, with lower pH values corresponding to higher matcha dosages. However, no significant difference was observed between the 2.0 and 2.5 groups (Fig. 5A). The pH dropped to its lowest level (4.3) in the 2.0 + Lac group, which was consistent with the trends in total colony counts and plate densities shown in Fig. 4D and 4E. The TPC concentration increased with matcha dosage after fermentation, but its release degree decreased (Fig. 5B). No significant differences in TPC concentration or release efficiency were found between the 2.0 and 2.0 + Lac groups, suggesting that the addition of lactose did not synergistically improve the overall release efficiency of catechins. Further, the concentrations of catechin, CG, GC, GCG, EGC, EGCG, total catechins, and gallic acid in 2.0 group increased significantly compared to the 2.0 + pre ferm. group, with CG showing the largest increase (1.01-fold), followed by EGC (0.58-fold) and catechin (approximately 0.51-fold). In contrast, the concentrations of ECG and caffeine decreased significantly, while EC remained unchanged. The concentration order of the individual components was: EGCG (291.4 μg/mL) > EGC > CG > EC > ECG > GC > GCG (only 6.9 μg/mL), which was in line with the majority reported research (2, 6).

It should be particularly noted that the changes in release degree for each component did not always parallel their concentration changes. Across the different matcha dosage groups, the concentrations of all components were positively correlated with the dosage, but their release efficiencies showed divergent trends. Only the release degree of catechin, CG, and EC were positively correlated with the matcha dosage (Fig. 5C-N). Those of GC and GCG peaked in the 2.0 group, whereas the release degree of EGC, ECG, and EGCG showed a gradual decreasing trend. The release degree of total catechins reached its lowest level (46.3%) in the 2.0 group (Fig. 5K). Compared with the 2.0 group, the 2.0 + Lac group exhibited significantly increased concentrations and release degree of catechin, EGCG, EGC, EC, CG, total catechins, gallic acid, and caffeine, with catechin showing the greatest increase (approximately

2.0-fold), whereas EGCG and EGC increased by only about 11%. In contrast, the concentrations and release degree of GCG and GC decreased significantly, while ECG showed no significant change. These results indicate that lactose significantly enhanced the release or producing of specific catechin components during fermentation, particularly non-ester-type catechins.

3.5. Antioxidant and correlation analysis

The DPPH, hydroxyl (•OH), and superoxide anion ($\bullet O_2^-$) radical scavenging activities, as well as the total antioxidant capacity (T-AOC), of fermented matcha infusions are presented in Fig. 6. Compared with the pre-fermentation group (2.0 + pre-ferm.), the DPPH radical scavenging activity of fermented matcha infusions was significantly enhanced (Fig. 6A). Among all groups, the 2.5 group exhibited the strongest DPPH scavenging capacity. When matcha and lactose were co-utilized as substrates, the scavenging activity was further improved. The trends in •OH and $\bullet O_2^-$ radical scavenging activities of the different fermented matcha infusions were generally consistent with those observed for DPPH. The T-AOC of fermented matcha infusions exhibited a somewhat different trend from that of their radical scavenging activities, primarily manifested as no significant difference in T-AOC between the 2.0 + pre-ferm. (unfermented) and 1.5 (fermented) groups. Moreover, correlation and Mantel test analysis indicated that antioxidant activity showed a significant positive correlation with matcha concentration, with epicatechin-type catechins significantly contributed to antioxidant capacity (Fig. 6E).

4. Discussion

The release efficiency and bioaccessibility of plant polyphenols are influenced by multiple factors, including plant species, processing methods, extraction solvents, and digestive/fermentation environments. In this study, we systematically investigated the effects of solvent type, acidity conditions, and LAB fermentation on the release behaviour of catechins from matcha, and explored the underlying mechanisms.

In the present study, when water was used as the extraction solvent, the content of bound total catechins in matcha was significantly higher than that of the free form (Fig. 2). In contrast, when 70% methanol solution was used as the solvent, the contents of free TPC, EGCG, EC, and CG increased markedly, and the proportion of total free catechins approached nearly 90% (Fig. 2). These results indicate that polyphenols such as EGCG, EC, and CG essentially exist predominantly in the bound form through hydrophobic interactions with dietary fibre [27–29], leads to substantial retention of target compounds in the solid matcha matrix.

Under acidic conditions (pH 4.0–6.0), the detectable levels of free catechins such as GCG, CG, and EGCG were significantly increased compared with neutral conditions (Fig. 3), indicating that an acidic environment per se possesses the capacity to promote the desorption of catechins from the matcha matrix. The underlying mechanisms can be summarised as follows: 1) under acidic conditions, H^+ weakens the donor–acceptor interactions of hydrogen bonds between the phenolic hydroxyl groups of catechins and dietary fibre/proteins, while also altering the surface charge distribution of the matrix,

thereby reducing the binding affinity between catechins and the matrix[27]; 2) H^+ neutralises the negative charges on the surface of matcha particles, weakening electrostatic adsorption of polyphenols onto the particle surface; and 3) the carboxyl groups of organic acids (e.g., lactic acid) can compete with polysaccharides for hydrogen-bonding sites, further displacing catechin molecules bound to dietary fibre. It is noteworthy that phenolic hydroxyl groups are weakly acidic. Under neutral conditions, they exist predominantly in the molecular state and interact with the matrix via hydrogen bonding, hydrophobic interactions, and/or through electrostatic attraction[15, 28]. Therefore, the promoting effect of acidic environments on release primarily originates from hydrogen-bond weakening and protonation competition, as well as desorption of electrostatic and hydrophobic interactions (Fig. 7, pathway 1). Furthermore, epicatechins such as EGCG exhibit relatively high chemical stability under acidic conditions at ambient temperature (< 65°C) and pH 3 ~ 7, and their ester bonds do not undergo significant spontaneous hydrolysis [30–32]. Taken together, the pH-induced increase in release is mainly attributed to physical desorption rather than to secondary formation of degradation products.

After fermentation of matcha with *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, the pH decreased significantly from neutral to 4.2–5.6 (Fig. 5A), accompanied by significant increases in the concentrations of TPC, CG, GC, GCG, EGC, EGCG, and gallic acid compared with the unfermented group (Fig. 5C–N). Similarly, fermentation of black tartary buckwheat with *Bacillus* DU-106 significantly increased the free contents of kaempferol, quercetin, and total phenolics, while decreasing epicatechin and chlorogenic acid contents (Ren et al., 2021). Fermentation of green tea with *Lactobacillus paracasei* NTU 101 also significantly elevated the levels of EGCG, EGC, and chlorogenic acid (Wang et al., 2018). Liu et al. (2025) found that fermentation of mango juice with LAB strains (*Lactobacillus casei* NCU215) significantly increased the content of free polyphenols such as gallic acid, and this increase was closely associated with pectin degradation. Valero-Cases et al. (2017) revealed that novel phenolic derivatives (catechin and α-punicalagin) in fermented pomegranate juices are degradation products of ellagitannins, epicatechin, and catechin by *Lactobacillus*. Collectively, these findings indicate that the promoting effect of LAB fermentation on catechin release involves at least three mechanisms (Fig. 7): 1) Acidification effect. The production of organic acids by the strains lowers the system pH, promoting physical desorption of bound catechins through the aforementioned hydrogen-bond weakening, which constitutes a universal basis for fermentation-enhanced release [15]. 2) Matrix degradation effect. LAB can partially degrade soluble dietary fibre in matcha, releasing bound components such as CG, GC, EGC, ECG, and EGCG [29, 33]. The net consumption rate of total soluble sugars in the lactose-free groups was 6.7%–8.2% (Fig. 4C), indicating limited fibre-degrading capacity. This finding is consistent with previous reports that cellulase and pectinase treatment significantly increases the total catechin content of tea infusion [34, 35]. 3) Enzymatic conversion effect. When lactose coexisted with matcha, the rapid proliferation of strains may have led to the secretion of small amounts of tannase and galloyl esterase, which partially catalyse the degradation of EGCG to EGC and gallic acid, and ECG to catechin and gallic acid [36, 37]. The release degree of certain catechins and phenolic acids (e.g., catechin and gallic acid) exceeded 100% (e.g., 127.4% for gallic acid in the 1.5% matcha group) (Fig. 5C, J, M), further illustrating that the enzymatic release of covalently or strongly bound polyphenols contributes to this process. Given

that *Lactobacillus* and *Streptococcus* generally lack the enzyme systems (e.g., flavonoid-cleaving enzymes and phenolic acid decarboxylases) capable of further ring-opening degradation of catechin and gallic acid [38, 39], these degradation products continuously accumulate in the system, ultimately resulting in detected levels of catechin (release efficiency up to 230.8% in the 2.0 + Lac group) and gallic acid (release efficiency 127.4%) far exceeding the theoretical upper release limit (Fig. 5B, J, M).

It should be emphasized that chemical acid treatment (pH 4.0–6.0) in Fig. 3 decreased the free content of catechin, whereas LAB fermentation (especially in the presence of lactose) in Fig. 5 markedly increased the release efficiency of catechin. This seemingly contradictory phenomenon precisely corroborates the superimposed effects of mechanisms 2) and 3) described above—purely chemical acid treatment can only release catechins through physical desorption, whereas in the live fermentation system, enzymatic disruption of dietary fibre and hydrolysis of ester bonds jointly contribute to the additional release of catechin and gallic acid, surpassing the contribution of the pH effect alone [31, 32, 38, 39]. Furthermore, although the concentrations of most catechins increased with increasing matcha dosage, their release degree did not follow the same trend (Fig. 5). The release degree of GC, GCG, EGC, ECG, and EGCG at 2.5% matcha dosage were all lower than those at 2.0% (Fig. 5). This phenomenon suggests that the excessively high solid content in high-concentration matcha matrices may facilitate the re-adsorption of solubilised catechins onto excess insoluble residues, and potentially cause intermolecular aggregation or precipitation of polyphenols at locally high concentrations [35, 40], collectively constraining further improvement in release efficiency at high dosages.

Regarding antioxidant activity, the radical scavenging capacity of matcha fermentation broth showed a significant positive correlation with its total catechins content (Fig. 6). Although the release degree of certain catechins declined at higher matcha dosage, but their absolute leached concentrations still increased significantly with increasing matcha dosage (Fig. 5). Consequently, the 2.5 group displayed the highest radical scavenging capacity. Several facts can account for this phenomenon. On the one hand, the epicatechin-type catechins can more efficiently reduce radicals via hydrogen atom transfer or single electron transfer mechanisms owing to the presence of more ortho-phenolic hydroxyl groups on the B-ring and galloyl moieties on the C-ring (e.g. EGCG) [41, 42]. Coincidentally, the concentrations of EGCG and EGC accounted for over 76% of the total catechins in the fermentation broths (Fig. 5), thereby the 2.5 group manifesting the strongest scavenging capacity toward •OH, $\bullet O_2^-$, and DPPH radicals. On the other hand, the radical scavenging capacity depends on the total number of hydroxyl groups [43, 44]. Although the mechanisms differ—DPPH scavenging occurs mainly via hydrogen atom transfer or single electron transfer, •OH scavenging predominantly involves electrophilic addition or hydrogen abstraction reactions, and $\bullet O_2^-$ scavenging mainly relies on dismutation and electron transfer. However, all these processes depend strongly on the ability of phenolic hydroxyl groups to donate hydrogen atoms or electrons [45, 46]. Therefore, the scavenging activities of the fermented matcha infusions against radicals were closely associated with the absolute total content of EGCG plus EGC, which was further corroborated by reported research [26].

It should be noted that the scavenging percentages of •OH and $•O_2^-$ radicals for all fermentation groups did not exceed 50%, indicating that, under the fermentation broth concentrations employed in this study, the absolute dose of effective antioxidant components was limited, and the overall scavenging efficiency was not particularly prominent. This is primarily attributable to the relatively low final molar concentrations of total catechins, especially epicatechins, derived from the matcha matrix in the reaction system [5, 43].

Nevertheless, although the present study indirectly assessed the metabolic activity of the strains through total soluble sugar consumption and colony counts, it did not directly determine the content changes of specific dietary fibre components such as pectin and hemicellulose, nor did it measure the activities of key enzymes such as tannase and galloyl esterase in the fermentation system. Future work may further verify and quantify the relative contributions of the above enzymatic pathways to catechin release through enzyme activity assays, metabolomics analyses, and control experiments with purified enzyme additions. In addition, the in vitro fermentation model used in this study cannot fully simulate the complex environment of human gastrointestinal digestion and gut microbiota interactions. Therefore, the release efficiency data obtained herein can only serve as in vitro reference indicators for bioaccessibility, and their actual absorption and utilisation in vivo require validation through digestion models or animal experiments. Overall, the release of matcha catechins during LAB fermentation is synergistically regulated by acid chemical desorption, dietary fibre degradation, and enzymatic conversion. The matcha dosage, carbon source type (lactose), and metabolic activity of the strains collectively determine the final concentrations and release degree of individual catechin components in the system. This study provides a theoretical basis and data support for the formulation design of fermented matcha-based products, particularly regarding the optimization of matcha dosage and carbon source ratio.

5. Conclusion

This study investigated the effects of solvent type, acidity conditions, and LAB fermentation on the release fate of catechins from matcha, and elucidated the relationship between the release mechanisms and antioxidant activity. Aqueous extraction leaves a considerable amount of the target compounds in the solid matrix owing to its insufficient extraction efficiency. In contrast, a 70% methanol solution increased the proportion of total free catechins from 43% with aqueous extraction to nearly 90%. The promoting effect of LAB fermentation on catechin release is synergistically regulated by acid-induced chemical desorption, dietary fibre degradation, and enzymatic conversion. The radical scavenging capacity of fermented matcha infusions is positively correlated with the absolute leached concentration of epicatechin-type catchins. Lactose addition indirectly enhances catechin release by boosting microbial metabolism, thereby improving antioxidant activity. Although higher matcha concentrations increase the absolute catechin concentrations, the release degree generally declines and bacterial proliferation is inhibited, suggesting that the matcha dosage should be carefully balanced between the catechins release effect and the antibacterial effect. This study provides a theoretical basis for the formulation design and processing optimization of fermented matcha-based products. Future work may

further quantify the relative contributions of each mechanism through enzyme activity assays, epimerization kinetics, and metabolomics analyses.

Abbreviations

LAB
lactic acid bacteria
CG
Catechin gallate
GC
Gallocatechin
GCG
Gallocatechin gallate
EC
Epicatechin
ECG
Epicatechin 3-O-gallate
EGC
Epigallocatechin
EGCG
Epigallocatechin gallate
TPC
total polyphenol content
DPPH
2,2-Diphenyl-1-picrylhydrazyl
HPLC
High-Performance Liquid Chromatography.

Declarations

Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest.

Author Contribution

Yong Xie, Writing– review & editing, Visualization, Methodology, Investigation, Conceptualization, Supervision, Funding acquisition. Feifeng Jian: writing, Methodology, Investigation,data curation. Yanan Wang: Investigation, data curation. Mingxing Li: Investigation, Data curation.

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Figures

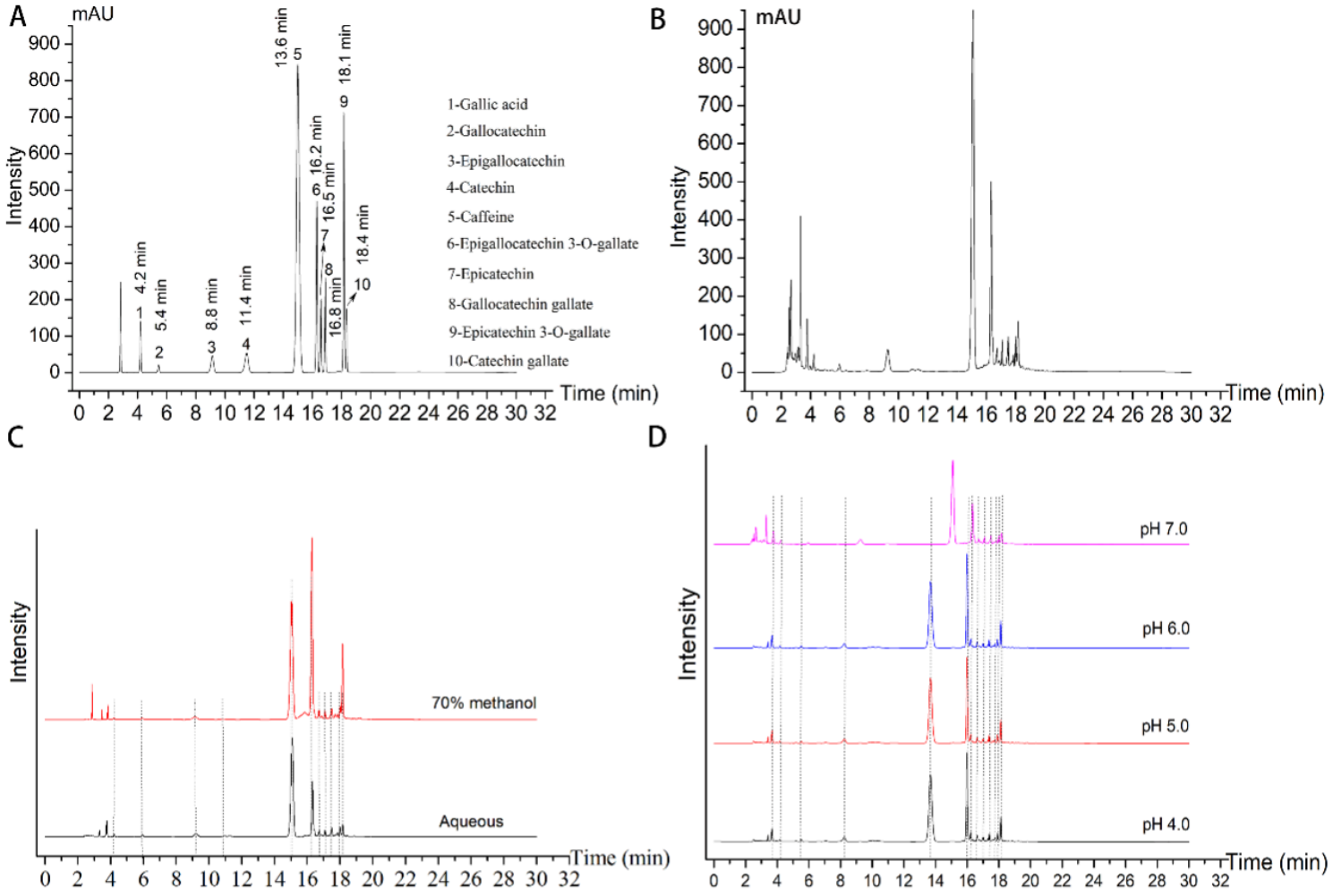


Figure 1

HPLC chromatograms of catechin standards (A) and matcha samples (B), as well as the effects of solvent (C) and pH (D)

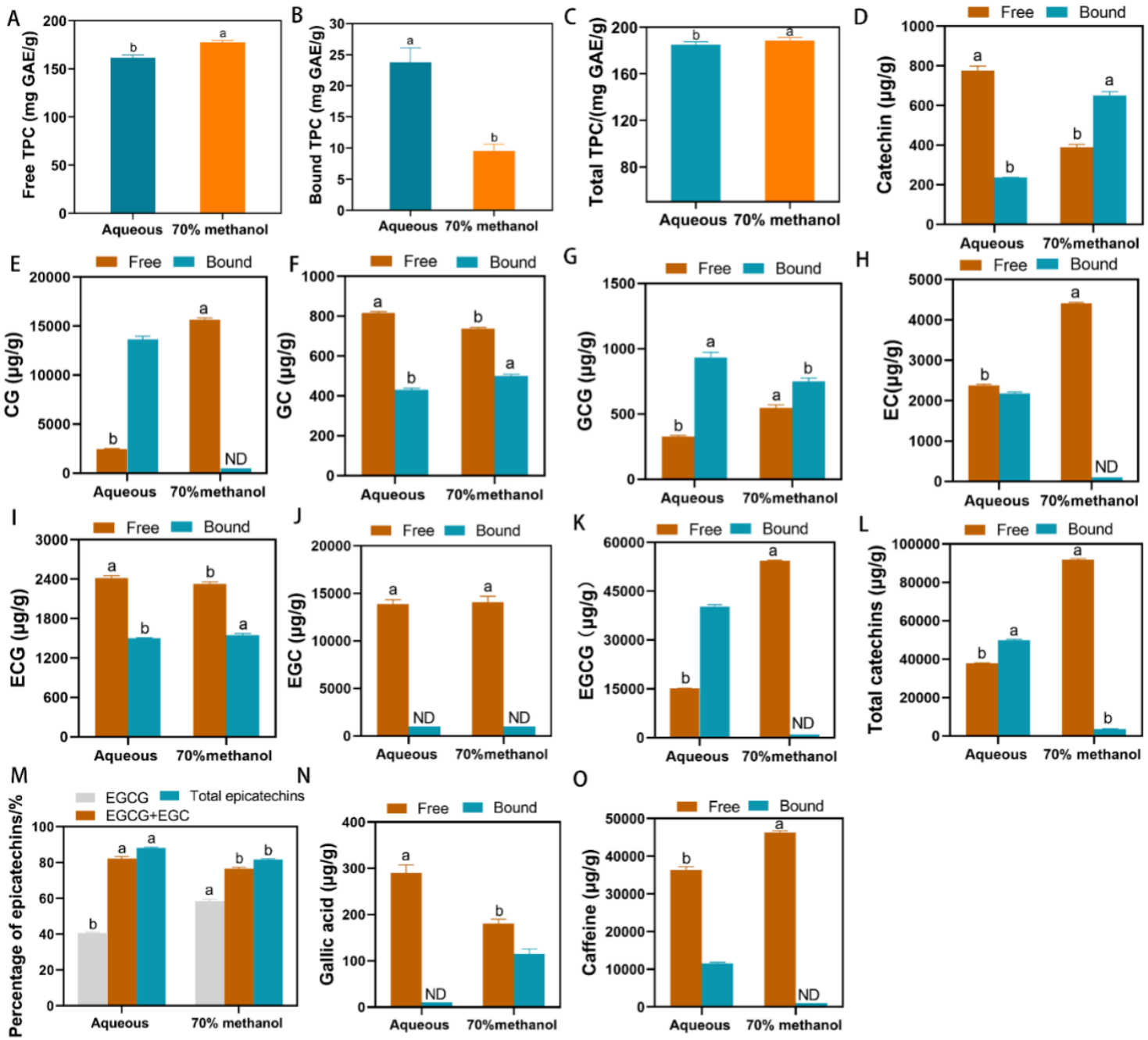


Figure 2

Effects of solvent type on the content of TPC (A, B, C), catechins (D-L), percentage of epicatechins in total catechins (M), gallic acid (N), and caffeine (O) in matcha. CG, Catechin gallate; GC, Gallocatechin; GCG, Gallocatechin gallate; EC, Epicatechin; ECG, Epicatechin 3-O-gallate; EGC, Epigallocatechin; EGCG, Epigallocatechin gallate

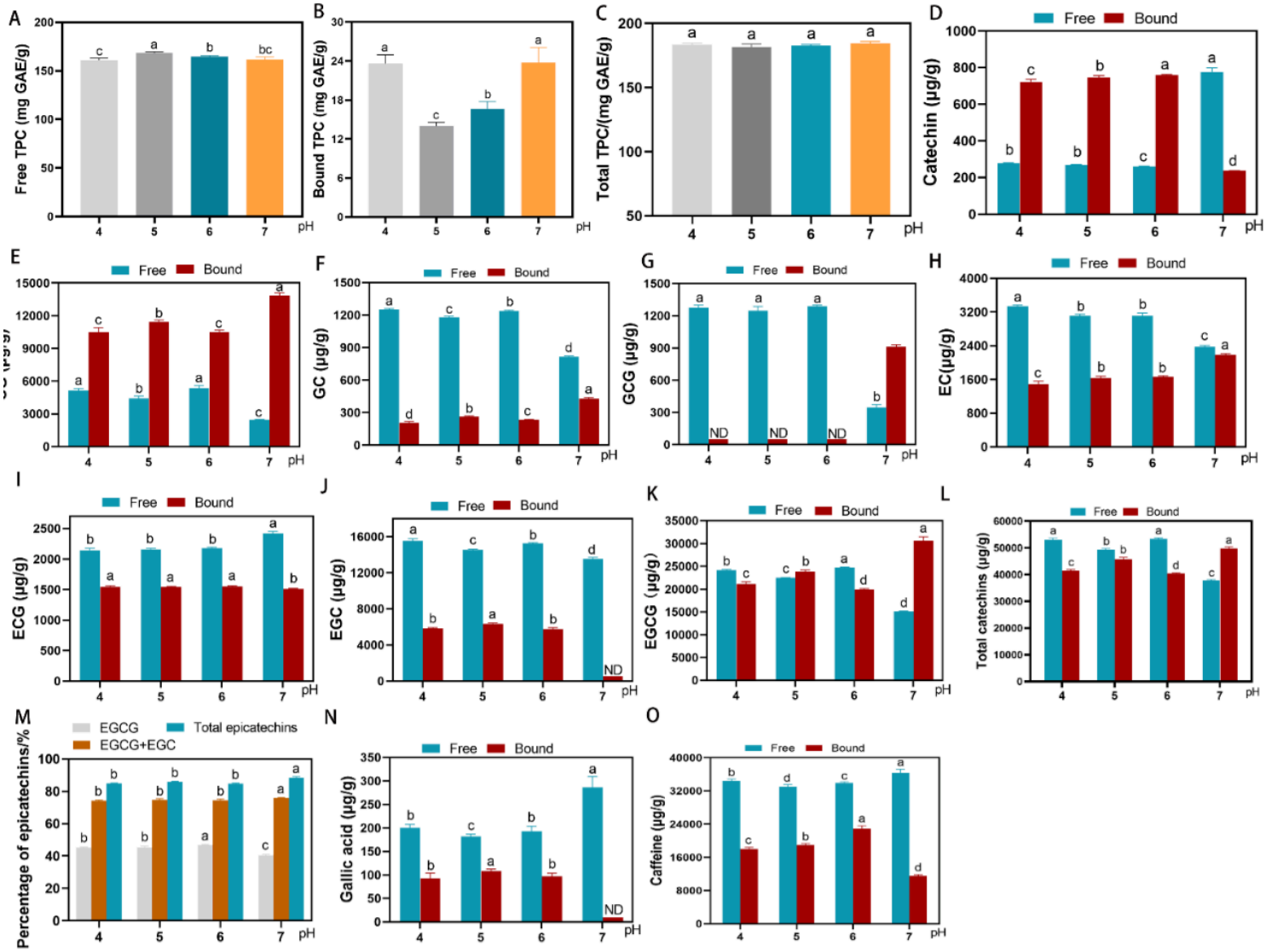


Figure 3

Effects of pH on the content of TPC (A, B, C), catechins (D-L), percentage of epicatechins in total catechins (M), gallic acid (N), and caffeine (O) in matcha

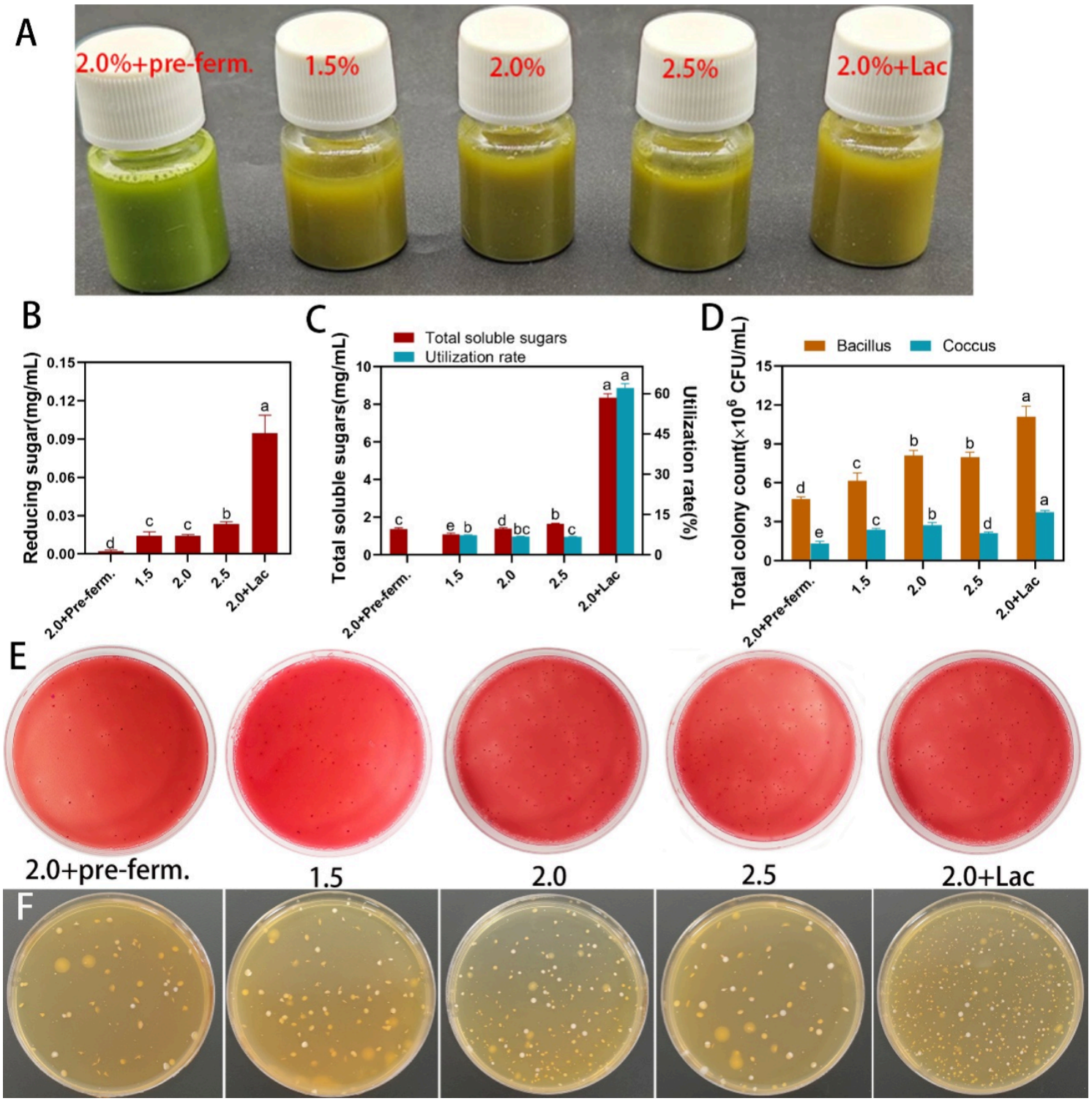


Figure 4

Visual appearance (A), reducing sugar content (B), total soluble sugars and sugar utilization rate (C), bacterial colony counts (D), and colony morphology (E–F) of matcha matrices after fermentation with LAB

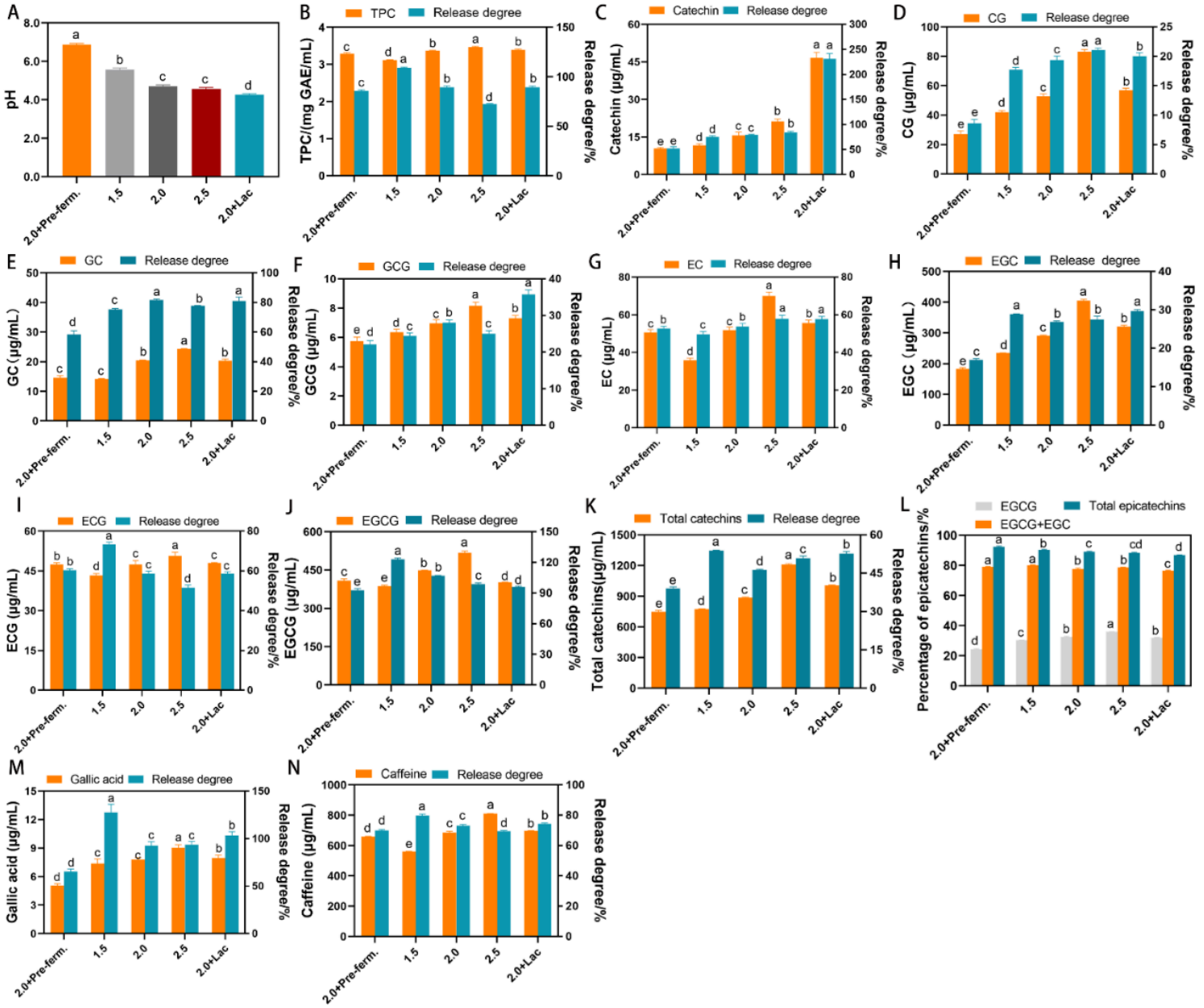


Figure 5

pH (A) and concentration and release degree of TPC (B), catechins (C-J), total catechins (K), percentages of epicatechins in total catechins (L), gallic acid (M), and caffeine (N) from matcha before and after in vitro fermentation

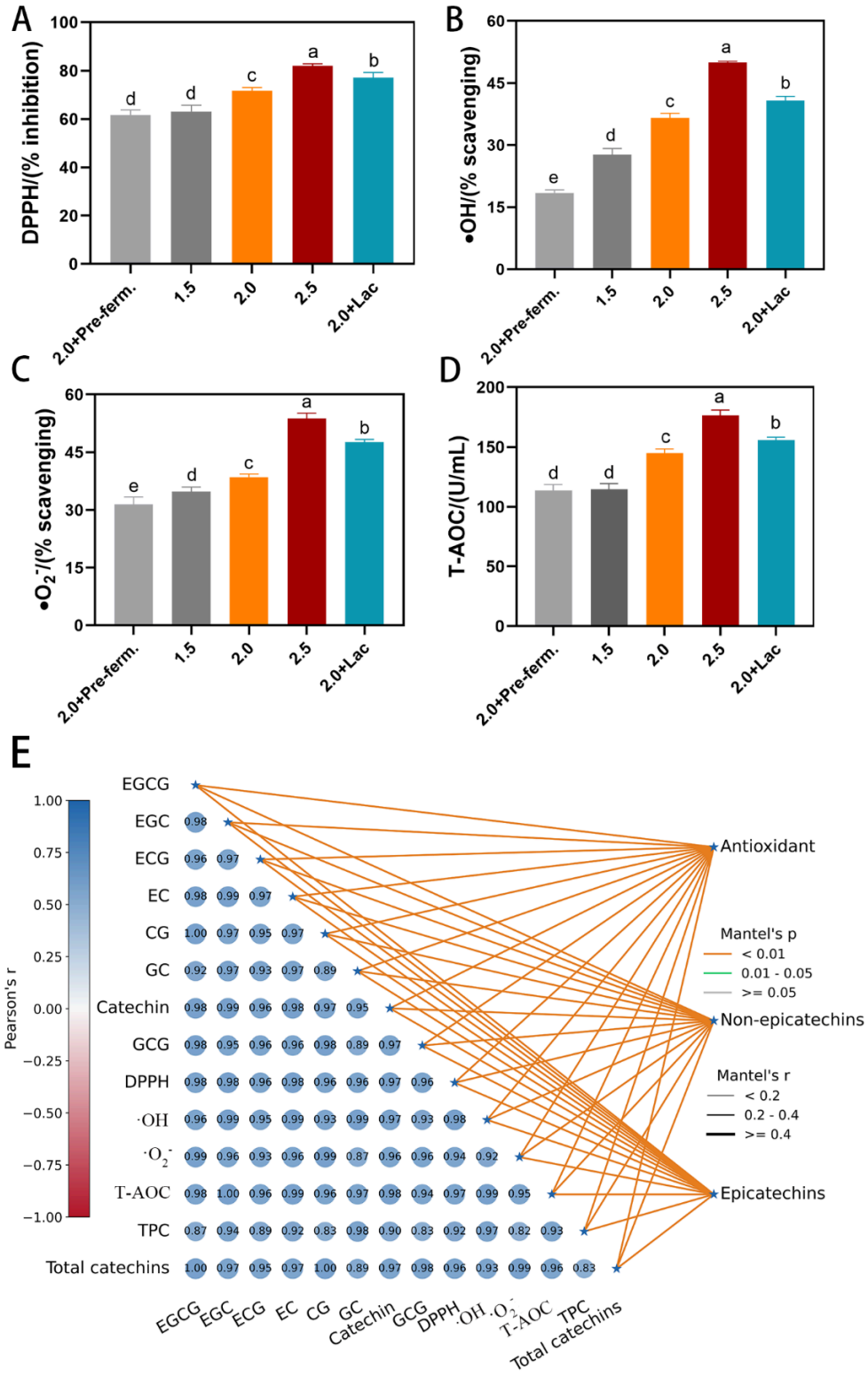


Figure 6

Antioxidant activities (DPPH, ·OH, ·O_2^-, T-AOC) of matcha matrices (A–D), and Pearson correlation coupled with Mantel test showing relationships between catechins and antioxidant properties (E).

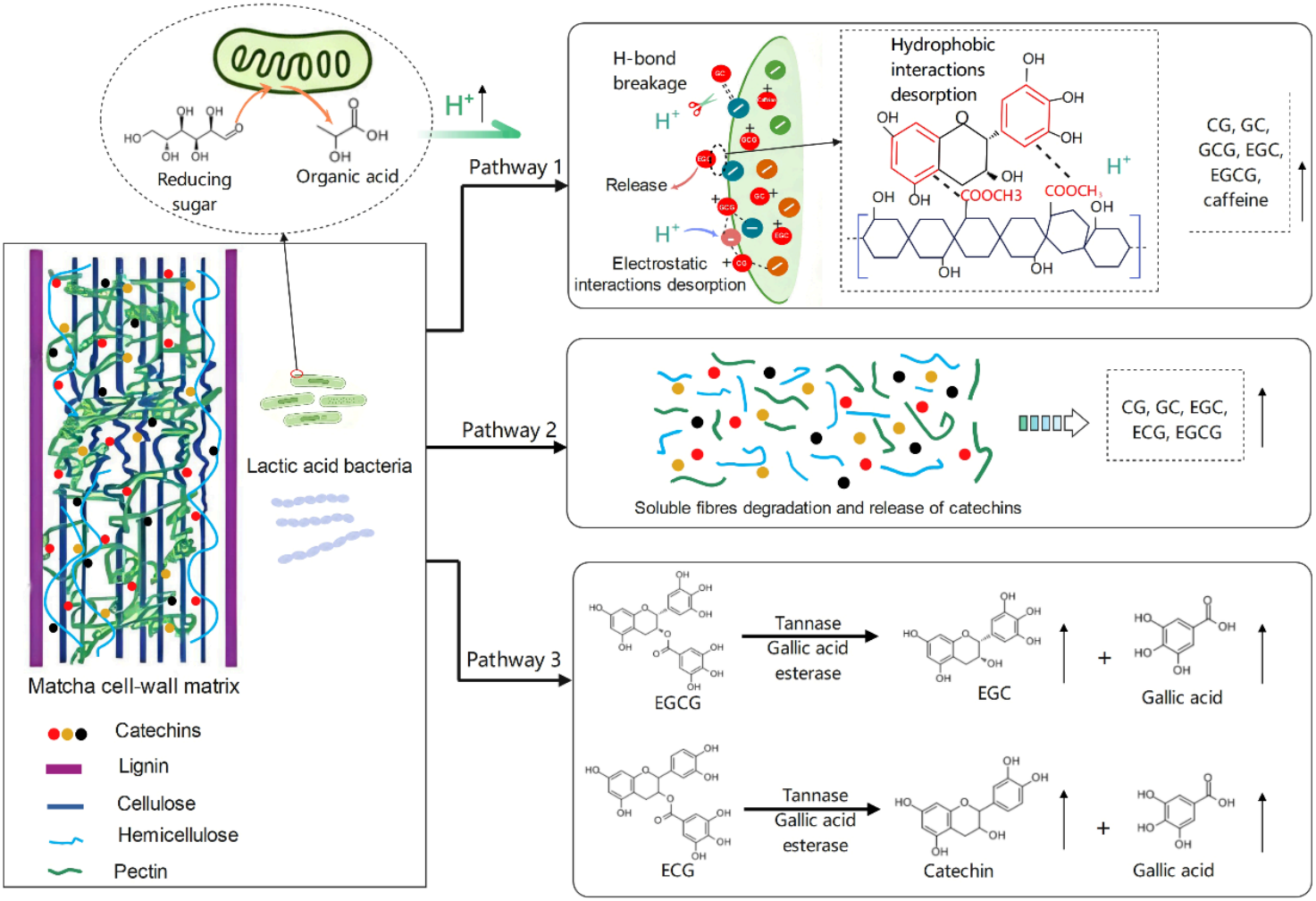


Figure 7

Proposed mechanism of catechins release from matcha during fermentation by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Three main pathways are involved: (1) Organic acids produced by lactic acid bacteria create an acidic environment to improve the release of CG, GC, GCG, EGC, EGCG and caffeine. (2) Degradation of water-soluble dietary fibres releases bound-state catechins into free-state. (3) Secreted tannase and gallic acid estserase hydrolyze EGCG and ECG to produce EGC and gallic acid, catechin and gallic acid, respectively, resulting in the release degree of catechin and gallic acid exceeding 100%.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1S.docx