





Purification and Antimicrobial Use of Egg-white Lysozyme against *Staphylococcus aureus*

Yuling Wang¹ , Xianmin Xu¹ , Huiting Wu¹ , Wenjing Jia^{1,2}, Jing Luo¹, Wenqian Qiu¹, Jianzhong Huang¹, Zhengyu Shu^{1*} 

1. National and Local United Engineering Research Center of Industrial Microbiology and Fermentation Technology, Ministry of Education, Fujian Normal University, 350117, Fuzhou, P. R. China
2. Department of Biology and Food Engineering, Bozhou University, 236800, Bozhou, China

Article Information

Article history:

Received 16 Sep 2025
Revised 4 Nov 2025
Accepted 1 Dec 2025
Published 1 Jan 2026

* Corresponding author:

Zhengyu Shu

Room 506, Building 13, Science and Engineering Building, Fujian Normal University (Qishan Campus), No. 18 Middle Wulongjiang Avenue, Shangjie, Fuzhou City, Fujian Province, China

Tell: +86-0591-22868193

E-mail: shuzhengyu@fjnu.edu.cn

†Equal contributors

To cite: Wang Y, Xu X, Wu H, Jia W, Luo J, Qiu W, Huang J, Shu Z. Purification and Antimicrobial Use of Egg-white Lysozyme against *Staphylococcus aureus*. *Appl Food Biotechnol*. 2026; 13 (1): e1. <http://dx.doi.org/10.22037/afb.v13i1.50307>

Abstract

Background and Objective: *Staphylococcus aureus* is a significant foodborne and zoonotic pathogen. This study aimed to enhance the anti-*Staphylococcus aureus* activity of egg-white lysozyme through heat treatment and synergistic combinations with natural antimicrobials.

Material and Methods: The lysozyme was purified from egg white via ammonium sulfate precipitation and cation-exchange chromatography, yielding a homogeneous protein. Anti-*Staphylococcus aureus* activity of native lysozyme, heat-treated lysozyme and its combination with ferulic acid or *Mycobacterium smegmatis* acyltransferase was assessed, respectively. All experiments were carried out in triplicate and statistical analysis was carried out using SPSS software.

Results and Conclusion: The specific activity of lysozyme to *Micrococcus lysodeikticus* was 27,407.4 U.mg⁻¹. The lysozyme IC₅₀ against *Staphylococcus aureus* was 300.8 µg.ml⁻¹, with transmission electron microscopy verifying bacteriolytic action. Heat treatment under optimized conditions (90°C, 15 min, pH 6.2) significantly enhanced the lysozyme antibacterial activity by 35.1%, which was correlated with structural changes evidenced by circular dichroism spectroscopy. Furthermore, synergistic effects were observed when heat-treated lysozyme was combined with ferulic acid or *Mycobacterium smegmatis* acyltransferase (MsAcT), leading to prolonged inhibition and decreased viable bacterial counts. The findings of this research demonstrated that structural modifications and combinatorial strategies could effectively improve the efficacy and application potential of lysozyme as a natural antimicrobial agent in food safety.

Keywords: Egg-white lysozyme, Ferulic acid, Heat-induced fibrillar aggregates, *Mycobacterium smegmatis* acyltransferase, *Staphylococcus aureus*

What is “already known” on this topic:

- Native egg-white lysozyme inhibits *S. aureus* by hydrolyzing peptidoglycan bonds in the cell wall and through non-lytic cationic mechanisms.
- Heat treatment can enhance lysozyme's antibacterial activity by inducing structural changes that enable non-lytic, membrane-perforating mechanisms.
- The antibacterial activity of lysozyme can be diminished or broadened by its interactions with other compounds, depending on interaction conditions.

What this article adds:

- A novel synergistic combination of heat-treated lysozyme with MsAcT significantly enhances bacteriolytic efficacy, contrary to typical bacterial resistance mechanisms.
- The developed sequential combination strategy offers a viable solution to overcome biocompatibility issues in multi-agent antimicrobial systems.
- It provides a practical, non-chemical method to significantly enhance lysozyme's efficacy, broadening its application as a natural food preservative.

1. Introduction

Staphylococcus aureus is a Gram-positive bacterium widely spread in nature and a common foodborne pathogen. The enterotoxins (SEs) of the bacteria show significant heat resistance, rendering them difficult to completely eliminate through conventional cooking methods and posing a significant risk of food poisoning. Furthermore, *S. aureus* facilitates cross-infection between humans and animals via the food chain through contamination of animal feed, subsequent infection of animals and transmission to humans, establishing it as an important zoonotic pathogen. The *S. aureus* has been detected in various animal species and a wide range of food products, with its prevalence continuously increasing on a global scale. Therefore, *S. aureus* is still a high-priority target in food safety monitoring [1, 2].

To combat *S. aureus* contamination in food processing, diverse biological control strategies have been investigated. These include inhibition using lactic acid bacteria (LAB) probiotics [3], Lysostaphin [4], bacteriophages [5,6], essential oils (EO) [5] and lysozyme [7,8]. From these, egg-white lysozyme has widely been used due to its high catalytic activity, simple preparation and cost-effectiveness [7,9]. It inhibits *S. aureus* through two primary mechanisms of (1) lytic mechanism as lysozyme hydrolyzes β -1, 4-glycosidic bonds to peptidoglycan, which causes cell wall damage, induces cell lysis and results in bactericidal activity; and (2) the non-lytic mechanism, where under denaturing conditions, it suppresses growth through inherent protein characteristics such as hydrophobicity and cationic effects [8,10]. While the antimicrobial characteristics of native egg-white lysozyme are well-documented, its efficacy under common food processing conditions, particularly those involving heat, needs further investigation. Moreover, strategies to enhance its activity, especially in a heat-treated state, through combination with other natural antimicrobial agents are still under-investigation. Under various reaction conditions, the interactions between other natural antibacterial agents and egg-white lysozyme can lead to various effects on its antimicrobial activity. For example, in an alkaline solution, theaflavin covalently binds to egg-white lysozyme, resulting in significant decrease of its antibacterial activity [11]. However, in an amyloid fibril hydrogel, the interaction between epigallocatechin gallate (EGCG) and egg-white lysozyme significantly broadens the antibacterial spectrum of egg-white lysozyme [12]. Therefore, an in-depth investigation into the interaction conditions between natural antibacterial factors and egg-white lysozyme is greatly important for enhancing the antibacterial efficiency of egg-white lysozyme.

This research aimed to isolate and purify egg-white lysozyme using chromatography and to assess the effect of heat treatment on its anti-*S. aureus* activity. Furthermore, the study prepared a synergistic combination of heat-treated lysozyme with ferulic acid and acyltransferase to develop an enhanced strategy for suppressing *S. aureus* growth. The experimental results demonstrated that heat-induced structural modification (fibrillar aggregation) enhanced lysozyme antimicrobial mechanism beyond native peptidoglycan hydrolysis. Moreover, it has first been reported that a synergistic combination of heat-treated lysozyme with ferulic acid and MsAcT against *S. aureus* significantly improves the efficacy and time of inhibition.

2. Materials and Methods

2.1. Strains, biochemical reagents and chemical reagents

Micrococcus lysodeikticus CGMCC 1.4547 and *S. aureus* CGMCC 1.282 were purchased from China General Microbiological Culture Collection Center, China. Bovine serum albumin (BSA) and standard protein molecular weight marker were purchased from Takara Biomedical Technology, China. Moreover, CM-Sepharose fast flow chromatography column was purchased from GE Healthcare, China. Ferulic acid, caffeic acid, gallic acid and N-acetylglucosamine (NAG) included analytical grade unless otherwise specified and purchased from Shanghai Macklin Biochemical Technology, China.

2.2. Purification of egg-white lysozyme

Egg white was initially diluted by 50-fold using 50 mmol.l⁻¹ pH8.0 tris-HCl buffer and the resulting protein solution was ultrasonicated for 10 min. The supernatant was collected by centrifugation at 12,000 rpm for 5 min at 4 °C. Solid ammonium sulfate was added to the supernatant to achieve 40% (w/v) saturation and the mixture was set to precipitate for 2 h at 4 °C. After centrifugation, the precipitate was removed and the collected supernatant was further loaded onto a CM-Sepharose fast flow column (5 × 20 cm) that was pre-equilibrated with 50 mmol.l⁻¹ tris-HCl buffer (pH 8.5). The lysozyme was eluted with a 5-fold column volume of 50 mmol.l⁻¹ tris-HCl buffer (pH 8.5) with increasing concentrations of NaCl (0.1 and 0.5 mol.l⁻¹) at a flow rate of 20 ml.h⁻¹, respectively. The active fractions were pooled. Protein concentration was assessed using Bradford method with BSA as a standard. The homogeneity of the purified egg-white lysozyme was assessed using sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

2.3. Activity assay of egg-white lysozyme

The cell lysis activity of lysozyme from egg white to *M. lysodeikticus* was quantitatively assessed using



spectrophotometric assay method, which was described by the National Standard of the People's Republic of China (GB/T 30990-2014, Determination of Lysozyme Activity) as well as Naveed et al. [13]. Briefly, after incubation overnight, *M. lysodeikticus* was transferred into the fresh LB liquid media at 1% (v/v) and incubated at 37 °C for 2 h at 220 rpm. The cells were harvested by centrifugation and resuspended in 50 mmol.l⁻¹ Na₂HPO₄-Na₂HPO₄ buffer (pH 6.2) to a final concentration of 5 × 10⁵ CFU.ml⁻¹. The reaction system contained 2.5 ml of cell suspension and 0.5 ml of lysozyme solution, while the control system received 0.5 ml of inactivated lysozyme solution. The reaction was carried out at 50 mmol.l⁻¹ Na₂HPO₄-Na₂HPO₄ buffer (pH 6.2) for 5 min at 25 °C and the absorbance of the reaction system was continuously monitored at 450 nm. One unit of lysozyme activity (U) was defined as the quantity of enzyme necessary to decrease OD₄₅₀ by 0.001 per minute under the standard assay condition.

2.4. Anti-*Staphylococcus aureus* activity of egg-white lysozyme

Briefly, *S. aureus* in the logarithmic growth phase was used as an indicator microorganism to assess antibacterial activity of the purified lysozyme. To set the growth curve, overnight-cultured *S. aureus* was subcultured into the fresh LB liquid media at a 1% (v/v) inoculum and incubated at 37 °C for 14 h at 220 rpm. Samples were collected every 30 min to measure the optical density (OD) of the culture broth at 600 nm. The growth curve was plotted with OD₆₀₀ on the y-axis and incubation time on the x-axis.

The antibacterial assessments were carried out using a method described by Carrillo et al. [14] with slight modification. Following 2 h of subculture, *S. aureus* suspensions were adjusted to 5 × 10⁵ CFU.ml⁻¹ through serial dilution in LB liquid media. Aliquots of *S. aureus* suspensions were mixed with equal volumes of the purified lysozyme at various concentrations and incubated at 37 °C for 6 h at 220 rpm. The OD₆₀₀ of the mixture was recorded after a 6-h subculture. The control group used 50 mmol.l⁻¹ Na₂HPO₄-Na₂HPO₄ filter-sterilized buffer (pH 6.2) instead of lysozyme. Antibacterial activity of the purified lysozyme to *S. aureus* was assessed using the antibacterial ratio. Antibacterial ratio was calculated using the following equation of $R = (A - B) \div A \times 100\%$; in which, R was the antibacterial ratio (%); A was the OD₆₀₀ value of the control group; and B was the OD₆₀₀ value of the experimental group. The antibacterial effect curve was plotted with the antibacterial ratio on the y-axis and the logarithmic of the purified lysozyme concentration on the x-axis. The IC₅₀ was defined as the purified lysozyme concentration, which resulted in a 50% decrease in the level of the antibacterial ratio, compared with untreated groups after a 6-h treatment. The IC₅₀ value was calculated using GraphPad Prism software. The cell morphology of *S. aureus* after a 6-h

lysozyme treatment (IC₅₀ concentration) was reported using transmission electron microscopy (TEM).

2.5. Effects of heat treatment on anti-*Staphylococcus aureus* activity of the purified egg-white lysozyme

To assess the anti-*S. aureus* effects of the heat-treated egg-white lysozyme, a specific concentration of the purified lysozyme solution was incubated at various temperatures for a set duration before assessing its anti-*S. aureus* activity using water bath. In this study, three key parameters were primarily investigated, including heat treatment temperature, time and initial lysozyme concentration. The temperatures were set at 37, 50, 60, 70, 80, 90 and 100 °C, respectively. The treatment times were set at 5, 10, 15, 60, 120 and 240 min, respectively. The initial lysozyme concentration were set at 601.6, 1000, 2000, 4000 and 6000 µg.ml⁻¹, respectively. The three highlighted factors were optimized using one-factor-at-a-time method. After heat treatment, equal volumes of the lysozyme solution and *S. aureus* suspension were thoroughly mixed and co-cultured at 37 °C for 6 h at 220 rpm. The antibacterial ratio was then quantified. Untreated lysozyme was used as control group.

To assess the effect of heat treatment on lysozyme molecular structure, circular dichroism (CD) spectroscopy was used to analyze changes in its secondary structural components before and after thermal exposure. The CD spectra were assessed using Jasco J-1500 spectropolarimeter (Jasco, Japan) with a 1-mm cell in the far-UV region from 190 to 300 nm. The concentration of lysozyme was 0.1 mg.ml⁻¹ in 5 mmol.l⁻¹ phosphate buffer (pH 6.2).

2.6. Inhibitory effects of phenolic acids combined with heat-treated egg-white lysozyme on *Staphylococcus aureus*

2.6.1. Compatibility screening of various phenolic acids with egg-white lysozyme

In this experiment, the inhibitory effects of egg-white lysozyme combined with three phenolic acids (ferulic acid, caffeic acid and gallic acid) at various concentrations on *S. aureus* growth were assessed, respectively. The final concentrations of each phenolic acid were 200, 400, 800 and 1000 µg.ml⁻¹, while the final concentration of egg-white lysozyme was set at 300.8 µg.ml⁻¹. The anti-*S. aureus* activity analysis and antibacterial ratio calculation methods were based on those described in Section 1.4. For the control group, phenolic acids or lysozyme were replaced with 50 mmol.l⁻¹ Na₂HPO₄-Na₂HPO₄ buffer solution (pH 6.2).

2.6.2. Effects of the heat-treated lysozyme and ferulic acid combination on *Staphylococcus aureus* growth

The method for assessing the growth curves of *S. aureus* was based on Section 1.4. The heat treatment procedure for egg-white lysozyme was carried out according to Section 1.5, with the final concentration of heat-treated lysozyme adjusted to 300.8 µg.ml⁻¹. For the control group, ferulic acid



or lysozyme were substituted with 50 mmol.l⁻¹ Na₂HPO₄-Na₂HPO₄ buffer solution (pH 6.2).

2.7. Numberized subsection inhibitory effects of *Mycobacterium smegmatis* acyltransferase combined with heat-treated egg-white lysozyme on *Staphylococcus aureus* growth

The mechanism by which, *M. smegmatis* acyltransferase (MsAcT) combined with heat-treated egg white lysozyme inhibited the growth of *S. aureus* is illustrated in Fig. 6.1. The preparation of the purified MsAcT was based on the methods described by Jia et al. [15]. Briefly, recombinant *Escherichia coli* BL21(DE3) strain was inoculated to Luria-Bertani (LB) broth and grown at 30 °C. The IPTG was added to culture broth to the final concentration of 1 mmol.l⁻¹, when the OD₆₀₀ reached 0.6–0.8. After 14 h interval, cell pellet was collected, resuspended using loading buffer (20 mmol.l⁻¹ pH 7.4 Na₂HPO₄-NaH₂PO₄, 20 mmol.l⁻¹ imidazole and 500 mmol.l⁻¹ NaCl) and then lysed using sonication. The supernatant from the cell lysate was collected and directly loaded on the HisTrap HP affinity chromatography column pre-equilibrated with loading buffer, respectively. Recombinant protein was eluted with a linear gradient of 20 ml of 20–500 mmol.l⁻¹ imidazole in the buffer with a flow rate of 0.8 ml.min⁻¹. The fractions with pure MsAcT were pooled and dialyzed against 20 mmol.l⁻¹ Na₂HPO₄-NaH₂PO₄ (pH 6.2) buffer overnight at 4 °C. The hydrolysis activity of MsAcT to NAG was assessed according to Jiang et al. [16] and Muzzarelli and Rocchetti [17]. In brief, the reaction mixture contained 20 µg.ml⁻¹ NAG in 20 mmol.l⁻¹ Na₂HPO₄-Na₂HPO₄ buffer (pH 6.2). Appropriately diluted MsAcT was added into the reaction mixture to create a linear dependence of the reaction rate to protein concentration. The reaction was carried out at 37 °C and the kinetics was detected for 3 h at 202 nm.

The specific procedure for inhibiting *S. aureus* growth using MsAcT combined with heat-treated egg-white lysozyme was carried out as follows: MsAcT, heat-treated egg white lysozyme and buffer solutions were sterilized using 0.22-µm filters. Then, 200 µl of appropriately diluted log-phase *S. aureus* cells were mixed with 200 µl of 1 mg.ml⁻¹ MsAcT solution, while the control group received 200 µl of 50 mmol.l⁻¹ NaH₂PO₄-Na₂HPO₄ solution (pH 6.2). After incubation at 37 °C for 3 h, 200 µl of the heat-treated egg-white lysozyme (final concentration of 300.8 µg.ml⁻¹) were added to the experimental and control groups and mixed thoroughly, followed by incubation at 37 °C for 3 h. The samples were then centrifuged at 12,000 rpm for 3 min

to separate the supernatant and the bacterial cell pellet. The protein concentration in the supernatant was measured to calculate the increase in protein, while the number of viable bacteria in the pellet was assessed according to the National Food Safety Standard of China (GB4789.2-2022, Food Microbiological Examination: Determination of Total Bacterial Count).

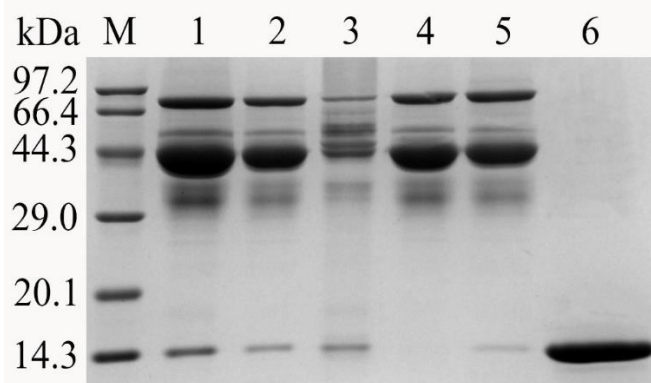


Figure 1. The SDS-PAGE analysis of the purified egg-white lysozyme with 5% stacking gel and 15% resolving gel. M, Standard protein molecular weight marker; Lane 1, diluted egg-white solution; Lane 2, supernatant of diluted egg-white after ammonium sulfate precipitation; Lane 3, pellet of diluted egg-white after ammonium sulfate precipitation; Lane 4, unbound protein fraction using CM-sepharose fast flow chromatography column; Lane 5, eluate from the CM-sepharose fast flow chromatography column after elution with 0.1 mol.l⁻¹ sodium chloride; and Lane 6, eluate from the CM-sepharose fast flow chromatography column after elution with 0.5 mol.l⁻¹ sodium chloride.

3. Results and Discussion

3.1. Purification and characterization of egg-white lysozyme

The purification of egg-white lysozyme was achieved through ammonium sulfate precipitation and CM-Sepharose fast flow column chromatography, resulting in 8.7-fold purification and yield of 41.0% (Table 1). Cation exchange chromatography verified particularly effective, accounting for 5.8-fold increase in specific activity. The purified enzyme was verified as homogenous using SDS-PAGE, showing a single band at 14.3 kDa (Fig. 1, Lane 6), which was similar to the molecular mass of egg-white lysozyme [18]. Its specific activity was assessed as 27,407.4 U.mg⁻¹ using *M. lysodeikticus* cells as substrate. This value was less than that reported by Chen et al. [18], a discrepancy likely attributable to the use of whole cells in this assay instead of the isolated cell walls used in the highlighted study.

Table 1. Isolation and purification of lysozyme from egg white

Step	Total activity (U)	Total protein (mg)	Specific activity (U.mg ⁻¹)	Yield (%)	Purification (fold)
Crude lysozyme	154000	49.2	3133.3	100	1
(NH ₄) ₂ SO ₄ precipitation	127325.9	26.9	4729.2	82.7	1.5
CM Sepharose F. F.	63136.8	2.3	27407.4	41.0	8.7



A gradual loss of total activity was observed throughout purification. Particularly, lysozyme was detected in pellets at a relatively low ammonium sulfate saturation (40%), suggesting the potential formation of insoluble aggregates under these conditions, as previously documented [19, 20]. For practical uses, it is critical to state that the growth state of *M. lysodeikticus*, affected by culture conditions and equipment, significantly affects the assessed specific activity [11, 13, 21]. Therefore, standardizing the substrate by setting the growth curve and harvesting log-phase cells under local laboratory conditions is essential for accurate activity assessment. In this study, the buffer solution for the assessment of the egg-white lysozyme activity according to the National Standard of the People's Republic of China (GB/T 30990-2014) requirements included 50 mmol.l^{-1} $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$ buffer (pH 6.2). In practical fields, particularly in the food processing industry, buffer solutions with pH 6.2 are rarely used.

Therefore, it is essential to assess optimal pH and temperature under the specific conditions necessary for the target uses, when assessing using effectiveness of egg-white lysozyme.

3.2. Anti-*Staphylococcus aureus* activity and mechanism of the purified egg-white lysozyme

The growth curve of *S. aureus* was characterized, identifying a log phase from 1 to 8 h (Fig. 2A), similar to the previous reports [22]. Cells from the exponential phase (2 h) were used for the assays. The IC_{50} of native lysozyme against *S. aureus* was $300.8 \text{ }\mu\text{g.ml}^{-1}$ (Fig. 2B). The antibacterial efficacy of lysozyme is affected by the composition and sequence of bacterial cell wall peptidoglycan, as well as the physiological state of the enzyme [23, 24]. Lysozyme fights microbes through bacteriostatic, bactericidal and bacteriolytic mechanisms [25]. The transmission electron microscopy (TEM) images provided direct evidence of the bacteriolytic action, showing damaged *S. aureus* cell walls with distinct light/dark contrast and the collapse of cells, leading to leakage of intracellular contents (Fig. 2C, arrows). Despite this significant effect, the inhibitory activity of the native lysozyme is difficult to sustain over extended times (Figs. 2A, 5A), highlighting a limitation for its use as a standalone antimicrobial agent.

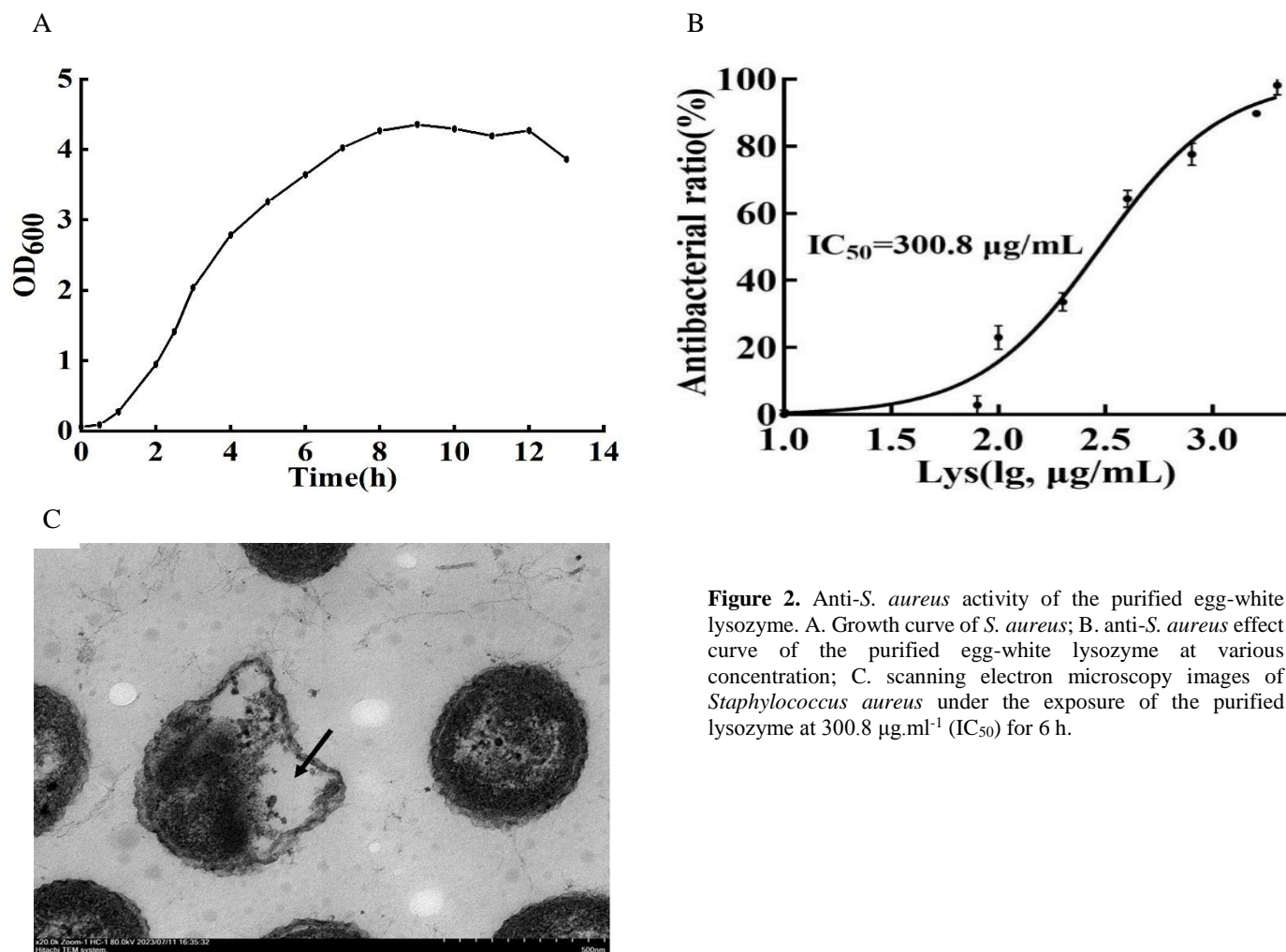


Figure 2. Anti-*S. aureus* activity of the purified egg-white lysozyme. A. Growth curve of *S. aureus*; B. anti-*S. aureus* effect curve of the purified egg-white lysozyme at various concentration; C. scanning electron microscopy images of *Staphylococcus aureus* under the exposure of the purified lysozyme at $300.8 \text{ }\mu\text{g.ml}^{-1}$ (IC_{50}) for 6 h.

3.3. Enhancement of anti-*Staphylococcus aureus* activity using heat treatment and structural changes

Heat treatment under optimized conditions (600 $\mu\text{g}\cdot\text{mL}^{-1}$, 90°C, 15 min, pH 6.2) enhanced the anti-*S. aureus* activity of lysozyme by 35.1%, achieving 82.5% inhibition compared to the native enzyme (Fig. 3A–C). This increase in activity after thermal denaturation was similar to that against other microbes such as SARS-CoV-2 and *Bacillus subtilis* [26, 27, 28]. Structural analysis revealed the reason behind this enhancement as a significant rearrangement of secondary structure occurred, with α -helix content decreasing from 35.59 to 23.60% and β -sheet, β -turn and random coil structures increasing (Fig. 3D, Table 2). This unfolding and proliferation of β -sheets drove the formation

of fibrillar aggregates [30,29], which were postulated to perforate microbial membranes, a mechanism distinct from the native enzyme peptidoglycan hydrolysis [27]. This suggested that structural modification was a viable strategy to improve the efficacy of lysozyme.

Table 2. Secondary structure composition of the native and modified lysozymes using heat treatment

Types of lysozyme	α -helix (%)	β -sheet (%)	β -turn (%)	Random coil (%)
Native lysozyme	35.50	14.63	22.66	27.10
Modified lysozyme	23.60	19.40	25.96	31.23

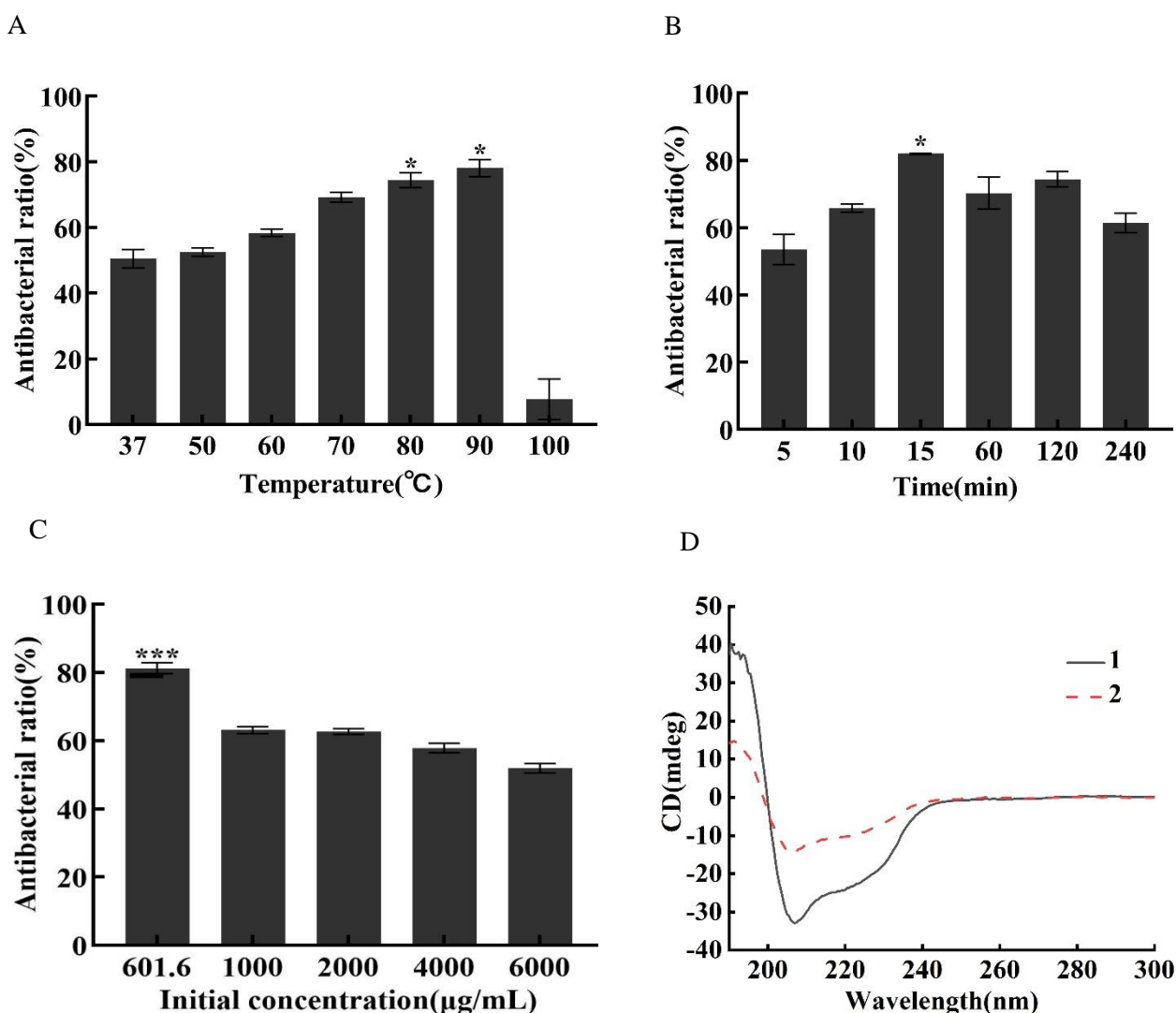


Figure 3. Antibacterial activity and changes in the secondary structure composition of the modified egg-white lysozyme using heat treatment. A. Effects of temperature on thermal modification efficiency of the egg-white lysozyme; B. effects of heat treatment time on thermal modification efficiency of egg-white lysozyme; C. effects of the primary protein concentration on thermal modification efficiency of the egg-white lysozyme; D. changes in the secondary structure composition of the egg-white lysozyme on heat treatment. 1. Circular dichroism spectrum of native egg-white lysozyme; 2, circular dichroism spectrum of heat-treated egg-white lysozyme.



3.4. Synergistic anti-*Staphylococcus aureus* effects of heat-treated egg-white lysozyme and phenolic acids

From the phenolic acids (ferulic, caffeic and gallic acids), ferulic acid showed the highest inhibition (52.2%) at 200 $\mu\text{g}\cdot\text{mL}^{-1}$, though differences diminished at higher concentrations (1000 $\mu\text{g}\cdot\text{mL}^{-1}$), where all acids reached ~99% inhibition (Fig. 4A–C). Ferulic acid is reported to inhibit *S. aureus* by suppressing *tetK* and *MsrA* efflux pumps on the bacterial membrane [31,32].

A combination of 400 $\mu\text{g}\cdot\text{mL}^{-1}$ ferulic acid with native lysozyme (300.8 $\mu\text{g}\cdot\text{mL}^{-1}$) showed a synergistic effect, increasing inhibition by respectively 12.3 and 29%, compared to either compound alone (Fig. 4A). This synergy was further increased, when ferulic acid was combined with heat-treated lysozyme, reducing bacterial biomass (OD_{600}) by additional 18.7%, compared to the combination with native enzyme (Fig. 5A). The optimal protocol involved sequential addition of heat-treated lysozyme added at time

zero, followed by ferulic acid after 6 h. This not only delayed the entry into the log phase by an additional hour but also decreased final biomass by 48.8% (Fig. 5B). This demonstrated that combining lysozyme with other antibacterial compounds, particularly after structural modification, could significantly enhance and prolong its inhibitory effect, potentially broadening its antibacterial spectrum [12,33]. However, when designing such combinations, concentration and addition sequence of egg-white lysozyme and phenolic acid, biocompatibility, reaction condition and differences in the mechanisms of action must carefully be addressed. Otherwise, adverse effects may occur. For example, in a solution with pH 9.0, phenolic acids (e.g., theaflavin) form covalent bonds with egg-white lysozyme, resulting in a 20% decrease in the antibacterial activity of egg-white lysozyme [12,33].

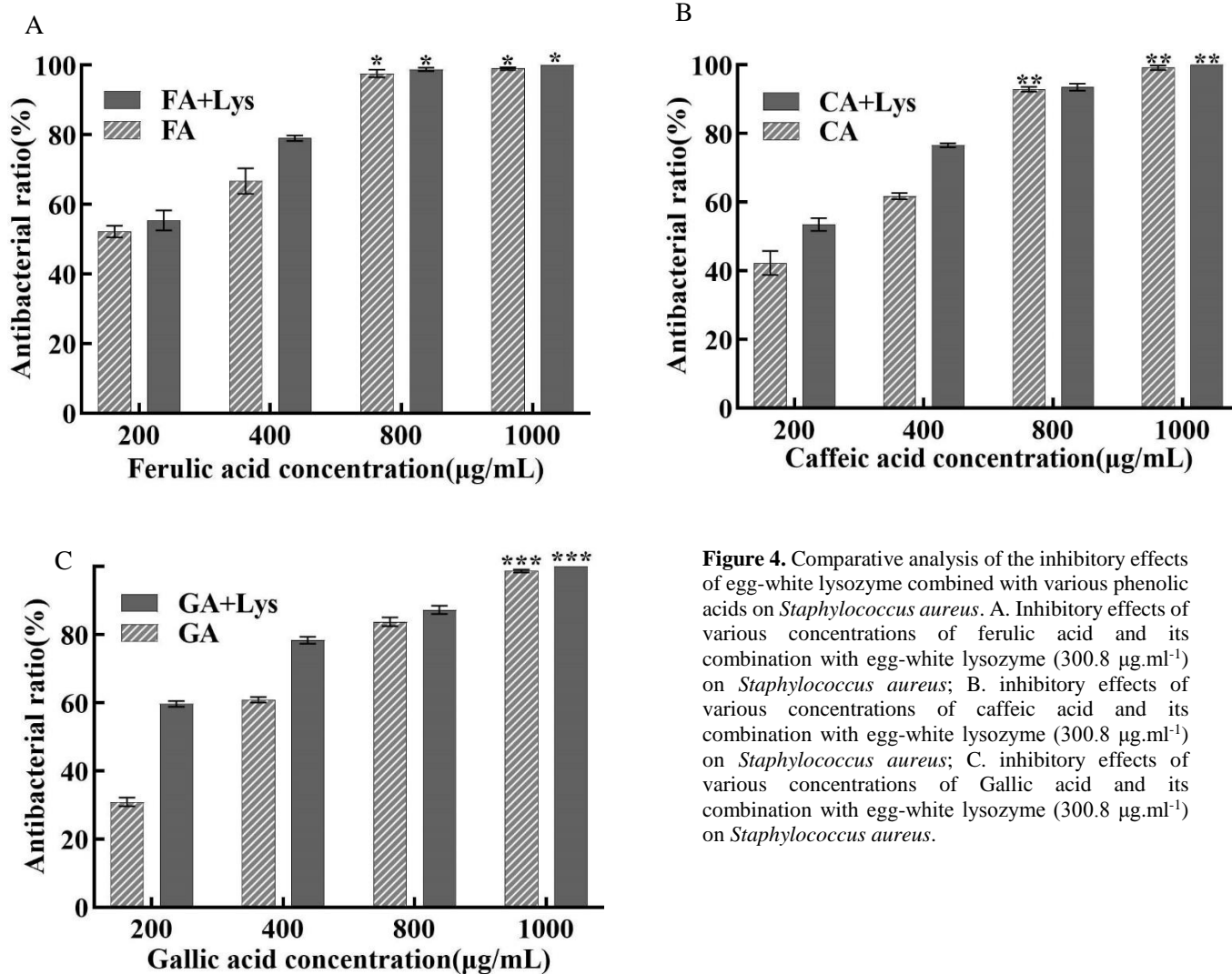


Figure 4. Comparative analysis of the inhibitory effects of egg-white lysozyme combined with various phenolic acids on *Staphylococcus aureus*. A. Inhibitory effects of various concentrations of ferulic acid and its combination with egg-white lysozyme (300.8 $\mu\text{g}\cdot\text{mL}^{-1}$) on *Staphylococcus aureus*; B. inhibitory effects of various concentrations of caffeic acid and its combination with egg-white lysozyme (300.8 $\mu\text{g}\cdot\text{mL}^{-1}$) on *Staphylococcus aureus*; C. inhibitory effects of various concentrations of Gallic acid and its combination with egg-white lysozyme (300.8 $\mu\text{g}\cdot\text{mL}^{-1}$) on *Staphylococcus aureus*.



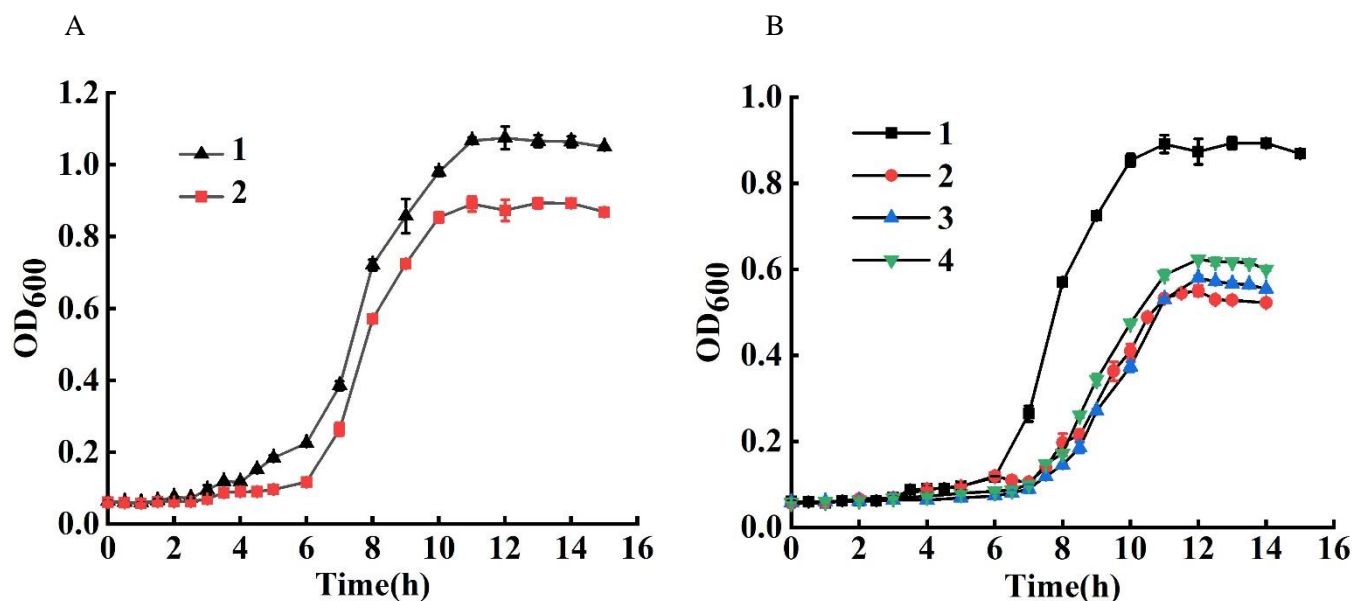


Figure 5. Inhibitory effects of the combination of heat-treated egg-white lysozyme and ferulic acid on *Staphylococcus aureus* growth. A. Effects of native and heat-treated egg-white lysozymes on the growth curves of *Staphylococcus aureus*; 1. growth curves of *Staphylococcus aureus* in culture media supplemented with 300.8 $\mu\text{g}\cdot\text{ml}^{-1}$ native egg-white lysozyme at 37 °C; 2. growth curves of *Staphylococcus aureus* in culture media supplemented with 300.8 $\mu\text{g}\cdot\text{ml}^{-1}$ heat-treated egg-white lysozyme at 37 °C; B. effects of the combination of heat-treated egg-white lysozyme and ferulic acid on *Staphylococcus aureus* growth; 1. growth curves of *S. aureus* in culture media supplemented with 300.8 $\mu\text{g}\cdot\text{ml}^{-1}$ heat-treated egg-white lysozyme at 37 °C; 2. growth curves of *Staphylococcus aureus* in culture media with sequential addition; first, 300.8 $\mu\text{g}\cdot\text{ml}^{-1}$ heat-treated egg-white lysozyme (6-h incubation) and then 400 $\mu\text{g}\cdot\text{ml}^{-1}$ ferulic acid at 37 °C; 3., growth curves of *Staphylococcus aureus* in culture media co-supplemented with 400 $\mu\text{g}\cdot\text{ml}^{-1}$ ferulic acid and 300.8 $\mu\text{g}\cdot\text{ml}^{-1}$ heat-treated egg-white lysozyme at 37 °C; 4. growth curves of *Staphylococcus aureus* in culture media co-supplemented with 200 $\mu\text{g}\cdot\text{ml}^{-1}$ ferulic acid and 300.8 $\mu\text{g}\cdot\text{ml}^{-1}$ heat-treated egg-white lysozyme at 37 °C.

3.5. Synergistic inhibition using MsAcT and heat-treated lysozyme

The enzyme MsAcT hydrolyzes acyl groups on peptidoglycan structural units, theoretically facilitating subsequent hydrolysis by lysozyme (Fig. 6A). Experimental data verified this synergy. Treatment with heat-treated lysozyme alone increased the extracellular protein concentration by 0.115 $\text{mg}\cdot\text{ml}^{-1}$, indicating cell lysis. When combined with MsAcT, the protein release increased further substantially by 0.208 $\text{mg}\cdot\text{ml}^{-1}$ (Fig. 6B). This was verified by colony counts, which showed a decrease in viable cells from 1.69×10^8 CFU. ml^{-1} (lysozyme alone) to 1.41×10^8 CFU. ml^{-1} (combination treatment) (Fig. 6C). This is a significant finding, as bacterial deacylation of peptidoglycan is typically a resistance mechanism that decreases hydrophobic interaction with lysozyme [10]. However, the present results demonstrated that external enzymatic deacylation by MsAcT could instead sensitize cells to lysozyme. It is challenging to explain this novel result at the molecular level. Since the lysozyme used in this research was achieved through separation and purification from egg-white and its 3-dimensional (3D) structure has not been resolved, molecular docking techniques cannot be used to investigate the differential interactions between NAM/NAG and deacetylated NAM/NAG with the lysozyme. Additionally, MsAcT is a promiscuous biocatalyst possessing multiple catalytic activities such as

hydrolytic activity and perhydrolysis activity [15]. Further in-depth investigation is needed to assess which catalytic activity dominates during its synergistic action with egg-white lysozyme in inhibiting *S. aureus*.

When egg-white lysozyme acts synergistically with other natural antibacterial agents to inhibit the growth of *S. aureus*, biocompatibility is one of the primary key parameters. Environmental conditions such as buffer pH [10,24], ionic strength [10,20,24] and temperature [14,27] of the egg-white lysozyme solution can alter its conformational state to various degrees; thereby, decreasing its antibacterial activity. Polyphenolic compounds may modify and disrupt the conformation of egg-white lysozyme, diminishing or eliminating its antibacterial activity [11, 12, 21, 31]. When other antibacterial proteins (e.g., proteases) act synergistically with egg-white lysozyme to inhibit the growth of *S. aureus*, the degradation of lysozyme by proteases can directly lead to its inactivation [14]. This study investigated the synergistic inhibitory effect of egg-white lysozyme and ferulic acid on growth of *S. aureus* under a sequential interaction mechanism, where two antibacterial agents were sequentially added to the reaction system to inhibit the bacterial growth. The experimental results indicated that the sequential interaction mechanism could partially overcome the issue of biocompatibility and maximize the antibacterial efficacy [Fig. 5B].



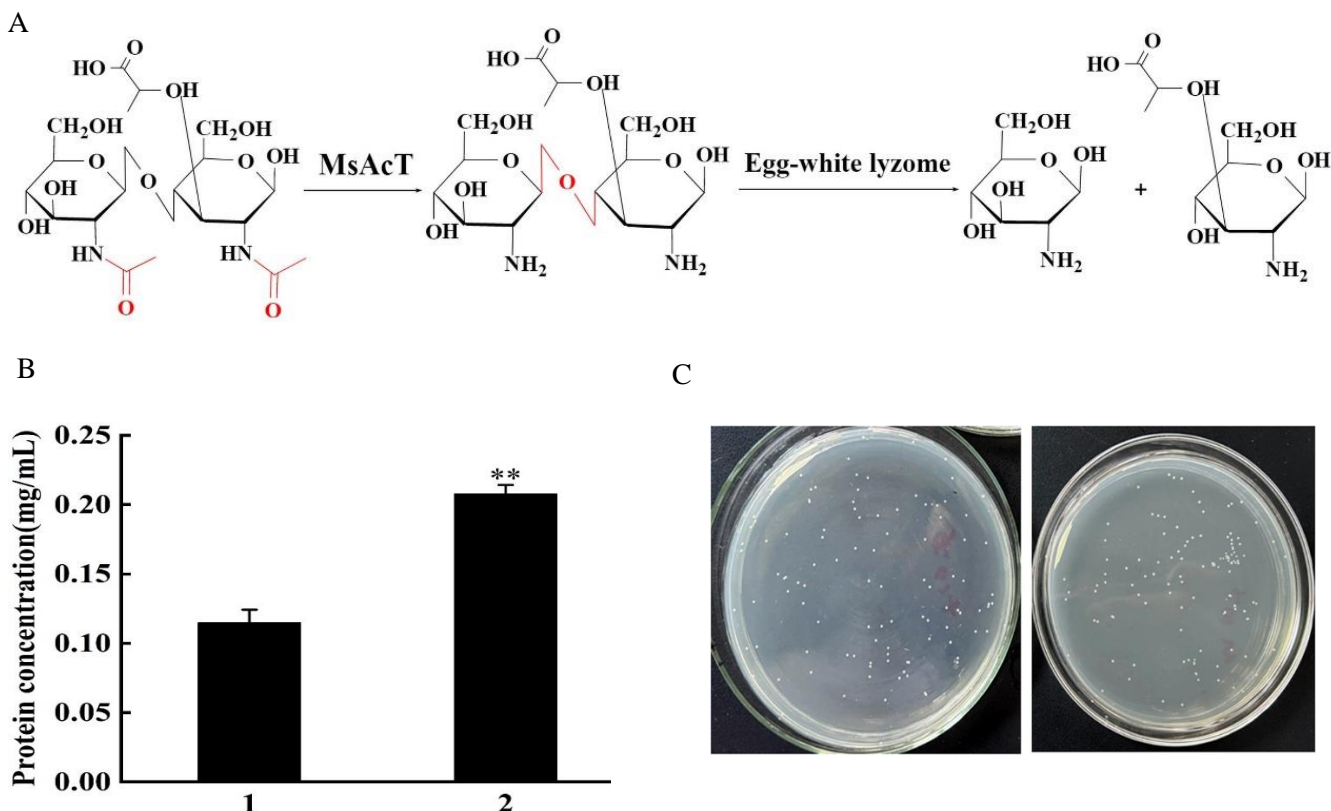


Figure 6. Inhibitory effects of MsAcT combined with heat-treated egg-white lysozyme on *Staphylococcus aureus* growth. A, schematic diagram of the mechanism; by which, MsAcT and heat-treated egg white lysozyme degrade peptidoglycan through a cascade reaction; B, changes in extracellular protein concentration in the reaction system after treatment of *Staphylococcus aureus* with heat-treated egg white lysozyme alone or in combination with MsAcT. Column 1 represents the system treated with heat-treated egg white lysozyme alone at 37 °C for 3 h in 20 mmol.l⁻¹ Na₂HPO₄-NaH₂PO₄ buffer (pH 6.2); Column 2 represents the system treated with heat-treated egg-white lysozyme in combination with MsAcT at 37 °C for 3 h in 20 mmol.l⁻¹ Na₂HPO₄-NaH₂PO₄ buffer (pH 6.2); C, changes in the number of culturable colonies of *Staphylococcus aureus* after treatment with heat-treated egg-white lysozyme alone or in combination with MsAcT. The left panel shows the result for treatment with heat-treated egg-white lysozyme alone at 37 °C for 3 h in 20 mmol.l⁻¹ Na₂HPO₄-NaH₂PO₄ buffer (pH 6.2); the right panel shows the result for treatment with heat-treated egg-white lysozyme in combination with MsAcT at 37 °C for 3 h in 20 mmol.l⁻¹ Na₂HPO₄-NaH₂PO₄ buffer (pH 6.2).

With the public in-depth understanding of the bacterial resistance mechanisms, growing awareness of environmental hazards caused by antibiotic misuse and heightened self-health consciousness, prohibition of antibiotics in animal feed and breeding processes has become a global interest. Therefore, it is vital to actively search for various alternatives to antibiotics [34, 35, 36]. From various antibiotic alternatives, egg-white lysozyme is a promising candidate due to its excellent biosafety. This study demonstrated that structural modification through heat treatment and strategic combination with other antimicrobials such as ferulic acid or enzymes such as MsAcT could significantly enhance its efficacy and time of action against *S. aureus*. Further studies should focus on clarifying the detailed mechanisms of these synergistic interactions and investigating other modification strategies such as glycosylation and lipophilization [10,24] to fully realize the potential of lysozyme as an effective antibiotic alternative.

4. Conclusion

In conclusion, this study successfully purified and characterized egg-white lysozyme and demonstrated that its anti-*S. aureus* activity could significantly be enhanced through thermal denaturation and synergistic combinations. Heat treatment induced structural reorganization of lysozyme, increasing its β -sheet content and enabling membrane-perforating antibacterial mechanisms distinct from its native lytic activity. Furthermore, combining heat-treated lysozyme with ferulic acid or MsAcT resulted in the synergistic effects, delaying bacterial growth and improving bacteriolytic efficacy. The results highlighted the potential of modified lysozyme as part of integrated natural preservation systems to improve food safety. Further studies should focus on explaining detailed mechanisms of synergy and investigating other modification methods to further broaden the applicability and effectiveness of lysozyme-based antimicrobial interventions. To better promote this process in the field of food processing, follow-up research



should thoroughly investigate its use effects, affecting factors and long-term stability in real food matrices (e.g., meat and dairy products). Additionally, a comparison of production costs and profitability with other antibacterial processes is necessary.

5. Acknowledgements

This research was supported financially by the National Natural Science Foundation of China (nos. 31370802 and 31870787) and Fujian Province Natural Science Foundation (no. 2021J01169).

6. Declaration of competing interest

The authors declare no conflict of interest.

7. Authors' Contributions

Conceptualization, W.H. and X.X.; methodology, W.H.; software, Q.W.; validation, L.J., J.W. and Q.W.; formal analysis, W.H. and S.Z.; investigation, X.X.; resources, J.W.; data curation, L.J.; writing—original draft preparation, W.Y.; writing—review and editing, S.Z.; supervision, S.Z.; project administration, H.J.; funding acquisition, S.Z.

8. Using Artificial Intelligent Chatbots

No artificial intelligent chatbots have been used in sections of this manuscript.

9. Ethical Consideration

The subjects of this study are egg white lysozyme and *Staphylococcus aureus*. As no animal or human trials are involved, no ethical review documentation is required.

References

- González-Machado C, Alonso-Calleja C, Capita R. Prevalence and types of methicillin-resistant *Staphylococcus aureus* (MRSA) in meat and meat products from retail outlets and in samples of animal origin collected in farms, slaughterhouses and meat processing facilities. A review. *Food Microbiol.* 2024; 123: 104580. <https://doi.org/10.1016/j.fm.2024.104580>
- Nunes Nascimento JC, Alvarenga VO, Paulino BN, de Cerqueira E Silva AB, Matos JR, Rosário ILDS andrade IHP, Silva JG, Costa M. *Staphylococcus aureus* in food safety: antimicrobial resistance, detection technologies and future perspectives. *Crit Rev Food Sci Nutr.* 2025; 1-24. <https://doi.org/10.1080/10408398.2025.2528165>
- Emiliano JVDS, Fusieger A, Camargo AC, Emiliano JVDS, Fusieger A, Camargo AC, Rodrigues FFDC, Nero L A, Perrone ÍT, Carvalho AF. *Staphylococcus aureus* in dairy industry: enterotoxin production, biofilm formation and use of lactic acid bacteria for its biocontrol. *Foodborne Pathog Dis.* 2024; 21(10): 601-616. <https://doi.org/10.1089/fpd.2023.0170>
- Zha J, Li J, Su Z, Akimbekov N, Wu X. Lysozyme: engineering and potentiation toward better applications. *J Agric Food Chem.* 2022; 70(37): 11441-11457. <https://doi.org/10.1021/acs.jafc.2c03459>
- Fokas R, Giormezis N, Vantarakis A. Synergistic approaches to foodborne pathogen control: a narrative review of essential oils and bacteriophages. *Foods.* 2025; 14(9): 1508. <https://doi.org/10.3390/foods14091508>
- Ge H, Fu S, Guo H, Hu M, Xu Z, Zhou X, Chen X, Jiao X. Application and challenge of bacteriophage in the food protection. *Int J Food Microbiol.* 2022; 380: 109872. <https://doi.org/10.1016/j.ijfoodmicro.2022.109872>
- Ferraboschi P, Ciceri S, Grisenti P. Applications of lysozyme, an innate immune defense factor, as an alternative antibiotic. *Antibiotics.* 2021; 10(12): 1534. <https://doi.org/10.3390/antibiotics10121534>
- Wu T, Jiang Q, Wu D, Hu Y, Chen S, Ding T, Ye X, Liu D, Chen J. What is new in lysozyme research and its application in food industry? A review. *Food Chem.* 2019; 274: 698-709. <https://doi.org/10.1016/j.foodchem.2018.09.017>
- Zhang Q, Zhao Y, Yao Y, Wu N, Chen S, Xu L, Tu Y. Characteristics of hen egg white lysozyme, strategies to break through antibacterial limitation and its application in food preservation: A review. *Food Res Int.* 2024a; 181: 114114. <https://doi.org/10.1016/j.foodres.2024.114114>
- Nawaz N, Wen S, Wang F, Nawaz S, Raza J, Iftikhar M, Usman M. Lysozyme and its application as antibacterial agent in food industry. *Molecules.* 2022; 27(19): 6305. <https://doi.org/10.3390/molecules27196305>
- Li Y, Li Q, Li M, Geng F, Wang Q, Gan N, Li S, Wu D. Effects of conjugation of egg lysozyme to theaflavins on their structure and function. *J Mol Liq.* 2024; 407: 125288-125288. <https://doi.org/10.1016/j.molliq.2024.125288>
- Hu B, Shen Y, Adamcik J, Fischer P, Schneider M, Loessner MJ, Mezzenga R. Polyphenol-binding amyloid fibrils self-assemble into reversible hydrogels with antibacterial activity. *ACS Nano.* 2018; 12(4): 3385-3396. <https://doi.org/10.1021/acsnano.7b08969>
- Naveed M, Wang Y, Yin X, Chan MWH, Aslam S, Wang F, Xu B, Ullah A. Purification, characterization and bactericidal action of lysozyme, isolated from *Bacillus subtilis* BSN314: a disintegrating effect of lysozyme on gram-positive and gram-negative bacteria. *Molecules.* 2023; 28(3): 1058. <https://doi.org/10.3390/molecules28031058>
- Carrillo W, García-Ruiz A, Recio I, Moreno-Arribas MV. Antibacterial activity of hen egg white lysozyme modified by heat and enzymatic treatments against oenological lactic acid bacteria and acetic acid bacteria. *J Food Prot.* 2014; 77(10): 1732-1739. <https://doi.org/10.4315/0362-028X.JFP-14-009>



15. Jia W, Li H, Wang Q, Zheng K, Lin H, Li X, Huang J, Xu L, Dong W, Shu Z. Screening of perhydrolases to optimize glucose oxidase-perhydrolase-in situ chemical oxidation cascade reaction system and its application in melanin decolorization. *J Biotechnol.* 2021; 328: 106-114. <https://doi.org/10.1016/j.jbiotec.2021.01.013>
16. Jiang Y, Fu C, Wu S, Liu G, Guo J, Su Z. Determination of the deacetylation degree of chitooligosaccharides. *Mar Drugs.* 2017; 15(11): 332. <https://doi.org/10.3390/md15110332>
17. Muzzarelli RAA, Rocchetti R. The determination of the degree of acetylation of chitosans by spectrophotometry. *Chitin in Nature and Technology.* 1986; 385-388. https://doi.org/10.1007/978-1-4613-2167-5_47
18. Chen Y, Liu Q, Yang F, Yu H, Xie Y, Yao W. Lysozyme amyloid fibril: Regulation, application, hazard analysis and future perspectives. *Int J Biol Macromol.* 2022; 200: 151-161. <https://doi.org/10.1016/j.ijbiomac.2021.12.163>
19. Chen C, Li X, Yue L, Jing X, Yang Y, Xu Y, Wu S, Liang Y, Liu X, Zhang X. Purification and characterization of lysozyme from Chinese Lueyang black-bone silky fowl egg white. *Prep Biochem Biotechnol.* 2019; 49(3): 215-221. <https://doi.org/10.1080/10826068.2018.1476887>
20. Wawer J, Szociński M, Olszewski M, Piątek R, Naczka M, Krakowiak J. Influence of the ionic strength on the amyloid fibrillogenesis of hen egg white lysozyme. *Int J Biol Macromol.* 2019; 121: 63-70. <https://doi.org/10.1016/j.ijbiomac.2018.09.165>
21. Wang SW, Wang TY. Study on antibacterial activity and structure of chemically modified lysozyme. *Molecules.* 2022; 28(1): 95. <https://doi.org/10.3390/molecules28010095>
22. Guo L, Wang Y, Bi X, Duo K, Sun Q, Yun X, Zhang Y, Fei P, Han J. Antimicrobial activity and mechanism of action of the *Amaranthus tricolor* crude extract against *Staphylococcus aureus* and potential application in cooked meat. *Foods.* 2020; 9(3): 359. <https://doi.org/10.3390/foods9030359>
23. Khorshidian N, Khanniri E, Koushki MR, Sohrabvandi S, Yousefi M. An overview of antimicrobial activity of lysozyme and its functionality in cheese. *Front Nutr.* 2022; 9: 833618. <https://doi.org/10.3389/fnut.2022.833618>
24. Yang T, Yan W. Strategies for enhancing the antibacterial efficacy of lysozyme and the resulting outcome. *Int J Biol Macromol.* 2025; 310(Pt 1): 143137. <https://doi.org/10.1016/j.ijbiomac.2025.143137>
25. Leśnierowski G, Yang T. Lysozyme and its modified forms: a critical appraisal of selected properties and potential. *Trends Food Sci Tech.* 2021; 107: 333-342. <https://doi.org/10.1016/J.TIFS.2020.11.004>
26. Huang S, Wu Z, Zhou B, Jiang X, Lavillette D, Fan G. Heat-denatured lysozyme is a novel potential non-alcoholic disinfectant against respiratory virus. *Food Environ Virol.* 2023; 15(3): 212-223. <https://doi.org/10.1007/s12560-023-09556-1>
27. Wei Z, Wu S, Xia J, Shao P, Sun P, Xiang N. Enhanced antibacterial activity of hen egg-white lysozyme against *Staphylococcus aureus* and *Escherichia coli* due to protein fibrillation. *Biomacromolecules.* 2021; 22(2): 890-897. <https://doi.org/10.1021/acs.biomac.0c01599>
28. Xin WS, Liu Y, Li JJ, Wu SR, Wang XJ, Wang CF, Zhang Z. Inactivation of high-pressure thermal sterilization combining with egg white lysozyme on *Bacillus subtilis* spores. *J Chin Inst Food Sci Technol.* 2024; 24: 112-121. <https://doi.org/10.16429/j.1009-7848.2024.03.011>
29. Lee S, Jo K, Jeong SK, Jeon H, Kim YJ, Choi YS, Jung S. Heat-induced gelation of egg white proteins depending on heating temperature: insights into protein structure and digestive behaviors in the elderly in vitro digestion model. *Int J Biol Macromol.* 2024; 262(Pt 2): 130053. <https://doi.org/10.1016/j.ijbiomac.2024.130053>
30. Chen X, Xing L, Li X, Chen N, Liu L, Wang J, Zhou X, Liu S. Manganese ion-induced amyloid fibrillation kinetics of hen egg white-lysozyme in thermal and acidic conditions. *ACS Omega.* 2023; 8(18): 16439-16449. <https://doi.org/10.1021/acsomega.3c01531>
31. Pinheiro PG, Santiago GMP, da Silva FEF, de Araújo ACJ., de Oliveira CRT, Freitas PR, Rocha JE, Neto JBA, da Silva MMC, Tintino SR, Siyatatanah A, Norouzi R, Dashti S, Wilairatana P, Coutinho HDM, da Costa JGM. Ferulic acid derivatives inhibiting *Staphylococcus aureus* tetK and MsrA efflux pumps. *Biotechnol Rep.* 2022; 34: e00717. <https://doi.org/10.1016/j.btre.2022.e00717>
32. Zhang X, Huang Z, Liu W, Yang X, Yin L, Jia X. Ferulic acid-arabinoxylan conjugates: synthesis, characterization and applications in antibacterial film formation. *Food Chem.* 2024b; 460(Pt 2): 140544. <https://doi.org/10.1016/j.foodchem.2024.140544>
33. Nassarawa SS, Nayik GA, Gupta SD, Areche FO, Jagdale YD, Ansari MJ, Hemeg HA, Al-Farga A, Alotaibi SS. Chemical aspects of polyphenol-protein interactions and their antibacterial activity. *Crit Rev Food Sci Nutr.* 2023; 63(28): 9482-9505. <https://doi.org/10.1080/10408398.2022.2067830>
34. Singh B, Bhat A, Ravi K. Antibiotics misuse and antimicrobial resistance development in agriculture: A global challenge. *Environ Health (Wash).* 2024; 2(9): 618-622. <https://doi.org/10.1021/envhealth.4c00094>
35. Yang W, Li J, Yao Z, Li M. A review on the alternatives to antibiotics and the treatment of antibiotic pollution: Current development and future prospects. *Sci Total Environ.* 2024; 926: 171757. <https://doi.org/10.1016/j.scitotenv.2024.171757>
36. Adebisi YA. Balancing the risks and benefits of antibiotic use in a globalized world: The ethics of antimicrobial resistance. *Global Health.* 2023; 19(1): 27. <https://doi.org/10.1186/s12992-023-00930-z>



خالص سازی و کاربرد ضد میکروبی لیزوزیم سفیده تخم مرغ در برابر استافیلوکوکوس اورئوس

یولینگ وانگ^۱، شیانمین شو^۱، هوی تینگ وو^۱، ون جینگ جیا^۱، جینگ لوئو^۱، ون چیان کیو^۱، جیان ژونگ هوانگ^۱، ژن یو شو^{۱*}

۱. مرکز تحقیقات مهندسی مشترک ملی و محلی فناوری میکروبیولوژی و تخمیر صنعتی، وزارت آموزش، دانشگاه نرمال فوجیان، ۳۵۰۱۱۷، فوژو، جمهوری خلق چین

۲. دپارتمان زیست شناسی و مهندسی مواد غذایی، دانشگاه بوژو، ۲۳۶۸۰۰، بوژو، چین

تاریخچه مقاله

دریافت ۱۶ سپتامبر ۲۰۲۵

داوری ۴ نوامبر ۲۰۲۵

پذیرش ۱ دسامبر ۲۰۲۵

چاپ ۱ ژانویه ۲۰۲۶

*نویسنده مسئول

ژن یو شو

اتاق ۵۰۶، ساختمان ۱۳، ساختمان علوم و مهندسی، دانشگاه نرمال فوجیان (پردیس چی شان)، شماره ۱۸، خیابان میانی وولونگ جیانگ، منطقه شانگ جیه، شهر فوژو، استان فوجیان، چین

تلفن: ۸۶-۰۵۹۱-۲۲۸۶۸۱۹۳

پست الکترونیک:

shuzhengyu@fjnu.edu.cn

† نویسندگان با سهم مساوی

چکیده

سابقه و هدف: استافیلوکوکوس اورئوس یکی از پاتوژن های مهم منتقله از غذا و دام است. هدف این پژوهش، بهبود فعالیت ضد استافیلوکوکوس اورئوس لیزوزیم سفیده تخم مرغ از طریق تیمار حرارتی و ترکیب هم افزا با ترکیبات ضد میکروبی طبیعی بود.

مواد و روش ها: لیزوزیم از سفیده تخم مرغ با استفاده از رسوب دهی سولفات آمونیوم و کروماتوگرافی تبادل کاتیونی خالص سازی شد و یک پروتئین یکنواخت به دست آمد. فعالیت ضد استافیلوکوکوس اورئوس برای لیزوزیم طبیعی، لیزوزیم حرارت دیده و ترکیب آن با اسید فرولیک یا آنزیم استیل ترانسفراز مایکوباکتریوم اسمگماتیس اندازه گیری شد. تمام آزمایش ها در سه تکرار انجام شد و تجزیه و تحلیل آماری با نرم افزار SPSS صورت گرفت.

یافته ها و نتیجه گیری: فعالیت ویژه لیزوزیم در برابر میکروکوکوس لیزودکتیکوس برابر با $27/407/4 \text{ U} \cdot \text{mg}^{-1}$ بود. مقدار IC_{50} لیزوزیم علیه استافیلوکوکوس اورئوس برابر $300/8 \mu\text{g} \cdot \text{ml}^{-1}$ تعیین شد و تصاویر میکروسکوپ الکترونی عبوری، تخریب دیواره ی باکتری را تأیید کرد.

تیمار حرارتی در شرایط بهینه (۹۰ درجه سانتی گراد، ۱۵ دقیقه، pH ۶/۲) موجب افزایش معنی دار ۳۵/۱ درصدی در فعالیت ضد میکروبی لیزوزیم شد که این تغییر با دگرگونی های ساختاری مشاهده شده در طیف سنجی دوشکستی دورانی مرتبط بود. همچنین، ترکیب لیزوزیم حرارت دیده با اسید فرولیک یا آنزیم MsAct اثرات هم افزایی قابل توجهی نشان داد که باعث مهار طولانی تر رشد باکتری و کاهش تعداد سلول های زنده شد. یافته های این پژوهش نشان می دهد که اعمال تغییرات ساختاری و استفاده از راهبردهای ترکیبی می تواند به طور مؤثری کارایی و قابلیت کاربرد لیزوزیم را به عنوان یک عامل ضد میکروبی طبیعی در ایمنی مواد غذایی افزایش دهد.

واژگان کلیدی: لیزوزیم سفیده تخم مرغ، اسید فرولیک، تجمعات فیبری القاشده با حرارت، آنزیم استیل ترانسفراز مایکوباکتریوم اسمگماتیس، استافیلوکوکوس اورئوس