

Photocatalytic rice bran protein/carboxymethyl cellulose/ZrO₂ fiber produced by microfluidics: Formation mechanism, bacteriostasis and strawberry preservation

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ABSTRACT

Developing cost-effective and environmentally sustainable active packaging materials remains an important challenge. We have developed rice bran protein (RBP)-based fibers incorporating carboxymethyl cellulose (CMC) and ZrO₂ nanoparticles (ZrO₂ NPs, 0 %–7 %, m/m) using microfluidic spinning. The integration of RBP, CMC, and ZrO₂ NPs formed a robust hydrogen bond network that enhanced the fibers' thermal stability and crystallinity, reduced surface hydrophobicity, and aligned the molecular orientation. Under the catalysis of visible light (300 W, 12 h), ZrO₂ NPs in the fiber produced reactive oxygen species, which inhibited the oxidative stress resistance system of *Bacillus subtilis* and destroyed its biofilm and DNA, thus showing excellent antibacterial effect. Additionally, during storage, this fiber also showed the ability to scavenge ethylene, thereby reducing the rate of loss of luminance, hardness and weight of strawberries. This study offers a new idea for RBP fiber in food preservation, antibacterial, and value-added utilization of rice bran by-products.

1. Introduction

Some foodborne substances, such as protein and polysaccharide, have been widely used in the preparation of active packaging materials, extending food shelf life and functional material delivery because of their high safety, good biocompatibility and renewable advantages (Yin et al., 2023). Rice bran protein is a by-product of processing in the preparation of rice and has been shown to have lower sensitization and higher nutritional properties (Görgüç et al., 2020). Moreover, rice bran protein (RBP) also has the advantages of low price, safety and environmental protection. Therefore, the preparation of protein-based active packaging materials using rice bran protein as raw material has great value.

At present, when preparing protein-based materials, some cross-linking agents are often added to improve the film forming property and mechanical strength of the materials (Li et al., 2023). Among them, some foodborne crosslinkers, such as carboxymethyl cellulose (CMC), are increasingly favored by researchers, mainly because of their efficient crosslinking and high biosafety (Thuy et al., 2023). In addition, in the process of preparing active packaging materials, some active substances

will also be added to improve the functionality of the material. Natural active substances such as phenolic acids and flavonoids have good antioxidant and antibacterial activities, so they are often mixed with proteins to prepare protein-based active packaging materials (Li, Feng, et al., 2024; Li, Song, et al., 2024; Wu et al., 2022). However, these active substances are easily oxidized and are more expensive, which also limits their application in food packaging. In addition, the preparation of active packaging materials often involves intense conditions such as high temperature and high pressure, which will further lead to the inactivation of these active substances and affect their load efficiency (Li et al., 2023). Therefore, it is very important to select suitable active substances and preparation methods to prepare protein-based active packaging materials.

In recent years, nanoscale oxides with photocatalytic function have achieved remarkable success in antibacterial and ethylene removal (Böhmer-Maas et al., 2020). ZrO₂ nanoparticles (ZrO₂ NPs) are a cheap, green, and efficient photosensitizer, has played an important role in tissue repair, antibacterial, and antiviral, but has been used less in food engineering (Bannunah, 2023). Additionally, under the catalytic condition of visible light, ZrO₂ NPs can rapidly interact with O₂ and H₂O in

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the environment and produce a large number of reactive oxygen species (ROS), such as $\bullet\text{OH}$ and $\bullet\text{O}_2^-$, to destroy bacteria, which is very favorable for the removal of microorganisms on the surface of the fruit (Wang et al., 2023). *Bacillus subtilis* is believed to be the main bacterial cause of strawberry deterioration, mainly because *Bacillus subtilis* can break down organic matter in strawberries into CO_2 and H_2O (Guo et al., 2024). Notably, strawberries are susceptible to mechanical damage during storage, which is conducive to the invasion of *Bacillus subtilis*, resulting in strawberry deterioration (Guo et al., 2024). Therefore, the use of ZrO_2 NPs to inhibit the growth and reproduction of *Bacillus subtilis* during strawberry storage is of great significance for extending the shelf life of strawberries. In addition, strawberries release a lot of ethylene during storage, which also accelerates the ripening and deterioration of strawberries, so removing ethylene is very important to extend the shelf life of strawberries. Previous studies showed that zein films can absorb ethylene and extend the shelf life of bananas and cherry tomatoes (Böhmer-Maas et al., 2020; Fan et al., 2023). This is mainly due to the fact that active groups in proteins such as sulfhydryl groups can react with ethylene and thus adsorb ethylene (Fan et al., 2023). These results proved that protein-based active packaging materials play an important role in removing ethylene and extending the shelf life of fruits. However, protein-based preservation materials also have the disadvantages of low ethylene removal efficiency and low antibacterial performance. Additionally, few studies have involved the use of rice bran protein as raw material and the addition of nano-oxides to prepare green active packaging materials with photocatalytic antibacterial and ethylene removal

functions. Therefore, preparing an active packaging material that is antibacterial and removes ethylene to extend the shelf life of fruits remains a huge challenge.

Here, we introduced an innovative approach using microfluidic spinning to fabricate RBP/CMC/ ZrO_2 fibers. This study explored the fibers' formation mechanisms, validated their antibacterial properties via photocatalytic experiments, and examined their impact on preserving strawberries. This investigation provides foundational insights into developing protein-based active packaging materials, addressing both antibacterial and ethylene removal challenges, thereby enhancing fruit preservation strategies.

2. Materials and methods

2.1. Materials

Rice bran protein (RBP, 81.24 %) was obtained from Shaanxi Panier Biotechnology Co., LTD, China. Carboxymethyl cellulose (CMC) was provided by Yuanye Biotechnology Co., LTD, Shanghai, China. ZrO_2 (IV) nanoparticles (ZrO_2 NPs, Monoclinic phase, 50 nm) was provided by Macklin Chemical Reagent Co., LTD, Shanghai, China. *Bacillus subtilis* CMCC(B) 63,501 was purchased from Sanyao Science and Technology Co., LTD, Beijing, China.

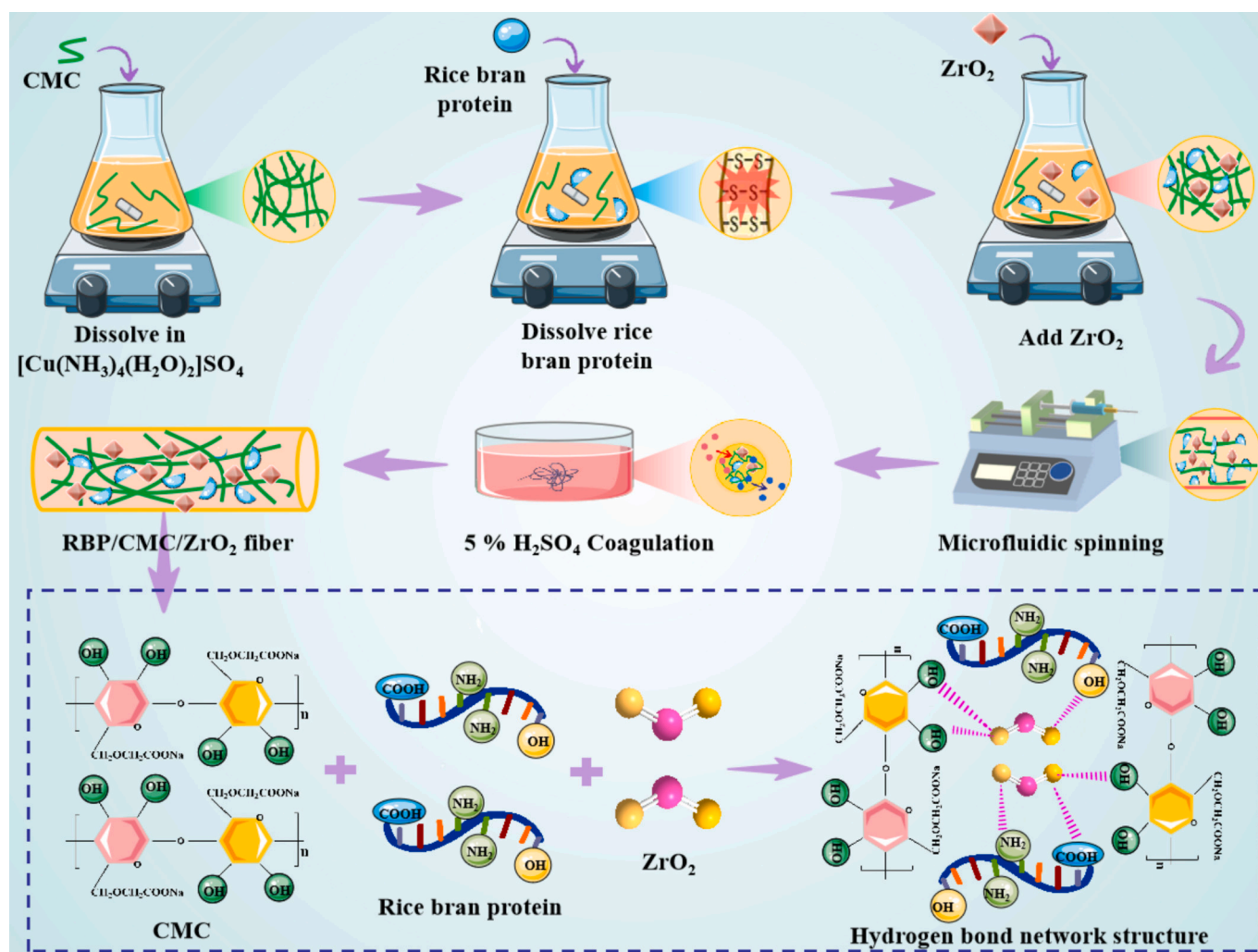


Fig. 1. Preparation process of RBP/CMC/ ZrO_2 fiber and hydrogen bond network structure of fiber.

2.2. Preparation of RBP/CMC/ZrO₂ fibers

The fabrication process of RBP/CMC/ZrO₂ fibers were presented in Fig. 1. CMC (0.3 g) was dissolved in [Cu(NH₃)₄(H₂O)₂]SO₄ solution (10 mL), and RBP powder (1.0 g) was added to the mixed solution to dissolve fully. Then, ZrO₂ NPs (0 %, 1 %, 3 %, 5 %, and 7 %, m/m) were added and fully stirred so that they were evenly dispersed in the mixed solution. The prepared spinning solution (5 mL) was slowly sucked into the syringe and then placed on the microfluidic pump. The syringe and chip were connected with a hose, and the chip outlet was placed in a 5 % H₂SO₄ solution. The spinning solution was slowly injected into the chip and solidified into fibers at the outlet under the thrust of the pump (1.5 mL/min). The prepared fibers were rinsed in deionized water and then freeze-dried (−80 °C) for experimental analysis. The information about the microfluidic spinning equipment used in this experiment can be found in Supplementary Material. The RBP/CMC/ZrO₂ fibers with different concentrations of ZrO₂ NPs (0 %, 1 %, 3 %, 5 % and 7 %, m/m) were named RC, RCZ-1, RCZ-3, RCZ-5, and RCZ-7.

2.3. Microstructure and intermolecular structure

The microstructure of RBP/CMC/ZrO₂ fibers was measured by an S-4800 scanning electron microscope. A small amount of fiber was attached to a metal tray, and then gold was sprayed. The tray was then placed in the machine, and the microstructure of the fiber was determined at the 20 kV voltage.

The intermolecular structure of RBP/CMC/ZrO₂ fibers was measured by Nicolet iS10 Fourier transform infrared spectroscopy (FTIR). The fibers were placed into the instrument, and a probe of the instrument was pressed tightly against the fiber surface. In the test, the resolution was 4 cm^{−1}, the wavelength was set at 4000–400 cm^{−1}.

The disulfide bond, free, and total sulfhydryl group of RBP/CMC/ZrO₂ fibers were measured by the previously reported method (Feng et al., 2021). The 40 mg freeze-dried sample was added with 6 mL Ellman's reagent, oscillated away from light for 2 h, centrifuged at 4000 r/min for 15 min, and then determined the absorbance value (412 nm). Then, the 40 mg freeze-dried sample was added to 5 mL dithiothreitol solution and reacted at 60 °C for 2 h. Then, 3 mL of 100 mM acetone glacial acetate solution was added to the mixture and centrifuged at 4 °C at 4000 r/min for 15 min to obtain the precipitate. The precipitations were dissolved with Ellman's solution, and the OD values at 412 nm was measured. According to the following two formulas, the contents of sulfhydryl groups and disulfide bonds in fiber were obtained. In the formula: $b = 1 \text{ cm}$; $\epsilon = 13,600 \text{ M}^{-1} \text{ cm}^{-1}$; S is OD values (412 nm); m indicate the fiber weight (g); C_b and C_a indicate the contents of free and total sulfhydryl (mol/L), respectively; V_b and V_a indicate the solution volume of total and free sulfhydryl (L), respectively.

$$C = \frac{S}{\epsilon \cdot b}$$

$$S - S = \frac{C_a V_a - C_b V_b}{2m}$$

2.4. Thermal stability, zeta potential and surface hydrophobicity

The thermal stability of RBP/CMC/ZrO₂ fibers was measured by DSC-60 differential scanning calorimetry (Li, Feng, et al., 2024). The 10 mg of fiber was weighed into the crucible, covered with a crucible lid and pressed tightly. The crucible was put into the instrument, the temperature rise rate of the instrument was set to 10 °C/min, and the temperature rise range was 30 °C ~ 300 °C, and then the measurement was carried out.

The Zeta potential of RBP/CMC/ZrO₂ fibers was measured by Mastersizer 3000 Zetasizer Nano equipment (Zhang, Lv, et al., 2023). Briefly, RBP/CMC/ZrO₂ fibers (3 mg) were dissolved in phosphate

buffer (6 mL) of pH 7. The fiber solution was then diluted to different concentrations and tested on the machine.

The surface hydrophobicity of RBP/CMC/ZrO₂ fiber was determined by the previously described method with minor modification (Zi et al., 2024). The RBP/CMC/ZrO₂ fibers was dissolved in 0.01 mol /L phosphate buffer and diluted to 0.05–0.5 mg/mL. The 200 μ L sample was mixed with 8 mmol/L 1-aniline-8-naphthalene sulfonate solution (10 μ L) for 10 min, respectively. Then the fluorescence intensity of solution was determined at the emission and excitation wavelengths of 480 nm and 390 nm, respectively.

2.5. Crystal structure

The crystal structure of RBP/CMC/ZrO₂ fibers was measured by Bruker AXS X-Ray Diffraction (XRD). A 2θ range of test was from 5° to 90°. Additionally, the crystal structures of RC and RCZ-5 were also determined by small-angle X-ray scattering (SAXS).

2.6. X-ray photoelectron spectroscopy (XPS) analysis

The XPS analysis of RC and RCZ-5 was measured by XPS (Thermo Scientific K-Alpha, USA). Full spectrum scanning energy was 150 eV. Narrow-spectrum scanning energy was 50 eV. The working voltage and filament current were 12 kV and 6 mA, respectively.

2.7. Antibacterial experiment

2.7.1. OD value and antibacterial rate

The OD value and inhibition ratio of *Bacillus subtilis* was determined by the previously described turbidimetric method with minor modification (Wang et al., 2017). The *Bacillus subtilis* solution (0.1 mL, 1×10^4 CFU/mL) was cultured in 30 mL tryptone soybean broth culture medium. Then, fibers (30 mg) were added to a culture medium, and the *Bacillus subtilis* solution without fiber was used as a control. The OD (600 nm) value of the culture medium was measured every 2 h. The inhibition rates of RBP/CMC/ZrO₂ fibers were calculated as follows:

$$\text{Inhibition ratio} = \frac{OD_1 - OD_2}{OD_1} \times 100\%$$

where OD₂ and OD₁ are OD value of fiber group and control group, respectively.

2.7.2. Photocatalysis experiment

The photocatalytic experiments of fiber antibacterial were carried out according to the previously described method with minor modifications (Zhang et al., 2024). In the experiment of 2.7.1, the bacteriostatic rate of RCZ-5 and RCZ-7 was not significant, so RCZ-5 was selected for the subsequent photocatalytic experiment. In the photocatalytic experiment, the simulated visible light provided by Germany Osram Corporation (Ultra-Vitalux 300 W) was selected as the light source. The RCZ-5 group was placed under a light source, and the OD values of the culture medium were determined every 2 h, and compared with the RCZ-5 group without light. The photocatalytic group of RCZ-5 was named RCZ-5 + P.

2.7.3. Live/dead bacteria fluorescence imaging

Briefly, the bacterial solution (12 h, 2 mL) was incubated with SYTO-9 (1 mL) and PI (1 mL) at 25 °C for 20 min. The bacterial solutions were then applied to a slide and the bacteria were fluorescently detected using a Leica confocal laser scanning microscopy (CLSM).

2.8. Antibacterial mechanism

2.8.1. Antibacterial process

The antibacterial process of fiber antibacterial were performed

following a previously described method with minor modification(Liu et al., 2023). *Bacillus subtilis* was cultured to the logarithmic growth stage (1×10^7 CFU/mL), centrifuged for 15 min at 8500 r/min, supernatant was discarded to obtain bacterial pellets. After suspension with normal saline, the bacteria were centrifuged for 15 min at 8500 r/min, then supernatant was discarded. The bacteria pellets were suspended again in saline solution (40 mL), then divided into four test tubes in equal volume. Then, RC and RCZ-5 fiber (10 mg) were added in test tubes, and the control group without fiber was used. Then, the same mass of RCZ-5 fiber was added to another test tube and used as a photocatalytic experiment (RCZ-5 + P). Samples was cultured at 12 h for 37 °C, then centrifuged for 15 min at 8500 r/min to obtain bacteria pellets.

2.8.2. Microstructure and fluorescein diacetate (FDA) staining

The bacteria pellet was fixed with glutaraldehyde (2.5 %) for 24 h. The bacteria morphology was observed by transmission electron microscope (TEM). The bacteria pellets were suspended with normal saline (5 mL) and then added to FDA (50 μ L, 2 mg/mL), incubated for 25 min at 25 °C. Then the supernatant was discarded and washed with normal saline to determine its fluorescence intensity. The excitation wavelength was 297 nm, and the emission wavelength was 527 nm. The excitation slit and emission slit were 5 nm.

2.8.3. Oxidation reduction potential (ORP) and ROS

The ORP value of bacterial solution cultured for 12 h was determined by ORP potentiometer (Jinan Huiquan Electronics Co., LTD, China). The 2',7'-dichlorofluorescent yellow diacetate (10 μ M, 10 μ L) was added to the bacterial solution and incubated for 30 min at 37 °C. After washing with normal saline, the bacterial solution was collected and the ROS production was measured by fluorescence spectrophotometer with 488 nm excitation wavelength and 525 nm emission wavelength.

2.8.4. Intracellular enzyme activity analysis

The superoxide dismutase (SOD) and catalase (CAT) activity in bacterial were measured by the method of ELISA kit provided by Jiangsu Meimian Industrial Co., Ltd., Yancheng, China. The alkline phosphatase (AKP) activity was measured using a method of AKP assay kit (Beijing Boxbio Science & Technology Co., Ltd., China).

2.8.5. Intracellular protein and DNA content

The bacteria pellets were suspended with normal saline, and the supernatant was discarded. Then, normal saline was added to the test tube, the bacteria were broken by ultrasound (2 min, 100 W). After centrifugation (3500 r/min, 15 min), supernatant was taken. The intracellular proteins of supernatant were measured using a method of BCA assay kit (Beijing Boxbio Science & Technology Co., Ltd., China). The extraction and determination of DNA in bacterial were measured by the method of kit obtained from Beijing Tiangen Biochemical Technology Co., LTD, China.

2.9. Strawberry preservation

2.9.1. Strawberry storage process

The strawberries (Dandong No.99) in the experiment were provided by Yonghui Superstores Co., Ltd., China. The strawberry storage process were carried out according to a previously described method with minor modification(Böhmer-Maas et al., 2020). Strawberry with no rot disease, no mechanical damage, uniform size and maturity were selected for experiment. After the strawberries were cleaned and dried, they were randomly divided into 4 groups (Control, RC, RCZ-5 and RCZ-5 + P) with 4 strawberries in each group. The prepared fibers were filled with petri dishes and were freeze-dried to form fiber pads (Supplementary Material, Fig. S2). The fiber pad was placed at the bottom of the food storage box, and strawberries were placed on the fiber pad for storage experiments. Strawberries in the photocatalytic group (RCZ-5 + P) were

irradiated every 24 h for 1 h. The storage temperature of strawberries was 25 °C, and the quality index of strawberries was measured every 1 day.

2.9.2. Luminance and hardness

The luminance of strawberry was measured by a colorimeter (CM-700d, KonicaMinolta, Japan). Strawberry hardness was determined using the hardness tester (GY-2, Airuipu, China).

2.9.3. Ethylene concentration and weight loss rate

The ethylene concentration released by strawberries was determined by an ethylene detector provided by Shenzhen Ruian electronic Technology Co., LTD. The weight loss rate of strawberry were measured according to the previously reported method(Nguyen Thi Hang et al., 2024).

2.10. Statistical analysis

Test result was derived from at least 3 replicates and expressed as mean $\pm$ standard deviation (SD). Duncan test and ANOVA were performed by SPSS 25 (SPSS Inc., USA). $P < 0.05$ presented a statistically significant.

3. Results and discussions

3.1. Microstructure and intermolecular structure analysis

Fig. 2 A and B shows the microstructure and intermolecular structure of the fibers. According to Fig. 2A, it can be seen that RC shows a smooth and dense surface, and the fiber surface structure becomes more and more rough with the increase of the addition of ZrO₂ NPs. The average diameter of the fibers ranges from 12.01 μ m to 14.45 μ m. Fig. 2B shows that with the increase of the addition of ZrO₂ NPs, the characteristic peaks of fibers are enhanced in the range of 3200–3400 cm^{-1} , 2800–3000 cm^{-1} , 1600–1700 cm^{-1} , and 1450–1550 cm^{-1} . Additionally, Table 1 shows the changes of disulfide bonds and sulfhydryl groups in the fibers. The contents of disulfide bond, free and total sulfhydryl group in fiber reduced significantly ($p < 0.05$) with an increase of addition of ZrO₂ NPs. Compared with RC, the content of free and total sulfhydryl, and disulfide bond in RCZ-7 decreased by 0.59, 1.89 and 0.65 $\mu\text{mol/g}$, respectively.

With the increase of ZrO₂ NPs addition, more hydrogen bonds was formed between RBP, CMC and ZrO₂ NPs, which was easy to make the three aggregated and form a denser network structure, resulting in the fiber becoming more and more rough(Wang et al., 2015). The range from 1450 cm^{-1} to 1550 cm^{-1} , and 2800 cm^{-1} to 3000 cm^{-1} , correspond to C—H bending vibration and stretching vibration, respectively (He et al., 2020). The enhancement of these two characteristic peaks may be due to the addition of ZrO₂ NPs to promote the polymerization between RBP and CMC(Feng et al., 2024). A range from 1600 cm^{-1} to 1700 cm^{-1} corresponds to the secondary structure of proteins, and the enhancement of this characteristic peak may be due to the addition of ZrO₂ NPs promoting the increase of β -sheet structure in proteins(Shan et al., 2023). The decrease of disulfide bond and sulfhydryl group in fiber may be attributed to the addition of ZrO₂ NPs to destroy the disulfide bond and sulfhydryl group in RBP(Xu et al., 2022).

3.2. Zeta potential, surface hydrophobicity and thermal property

Fig. 2C-D show the Zeta potential, surface hydrophobicity and thermal properties of the fibers, respectively. Fig. 2C showed that Zeta potential value of the fiber enhanced significantly ($p < 0.05$) with the increase of the addition of ZrO₂ NPs. Fig. 2D presented that the surface hydrophobicity of fibers reduced significantly ($p < 0.05$) with the increase of the addition of ZrO₂ NPs. It can be seen from Fig. 2E that the peak temperature and enthalpy of the fiber enhanced obviously with an

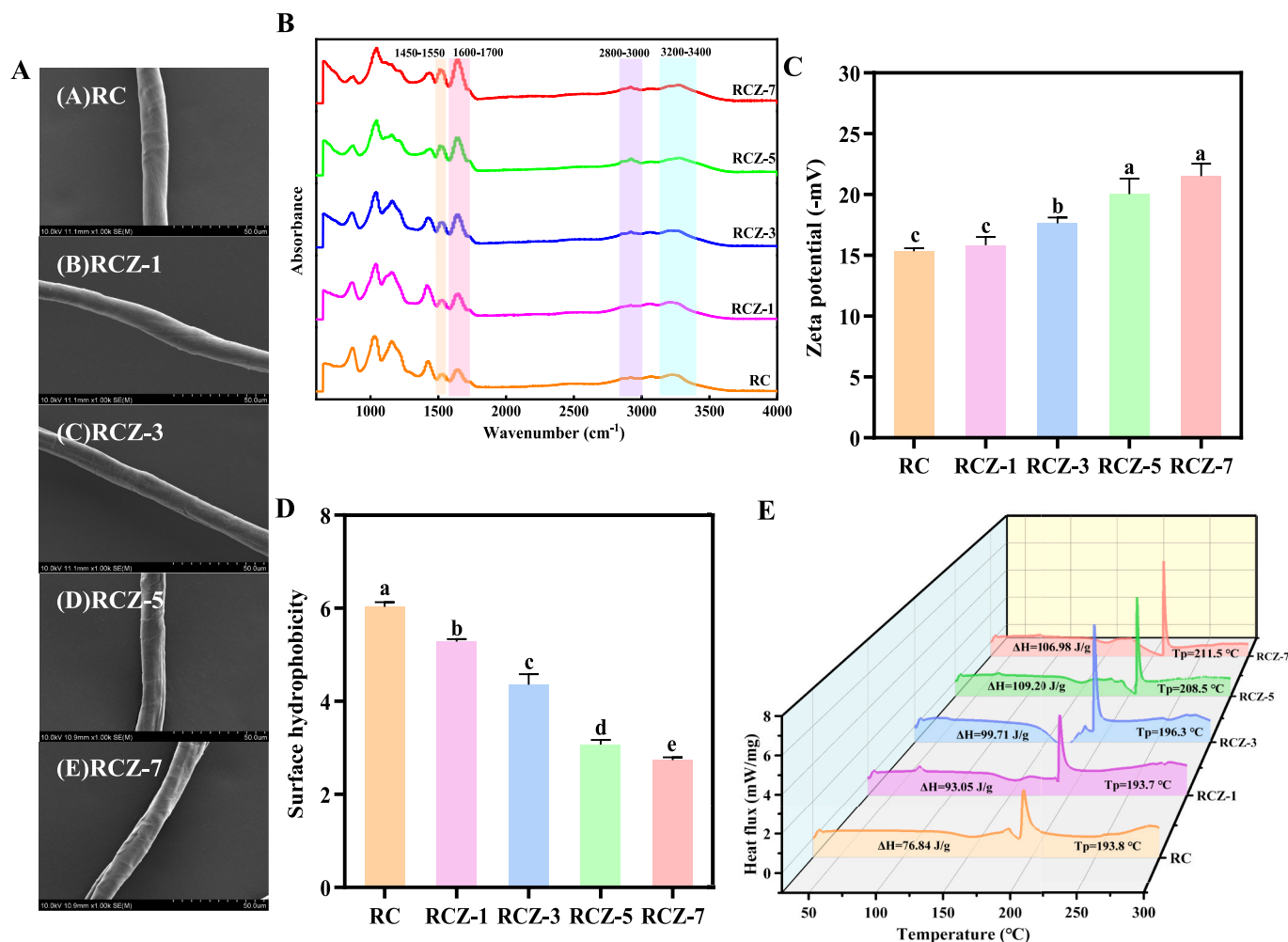


Fig. 2. Microstructure, intermolecular structure and stability of RBP/CMC/ZrO₂ fibers. A: SEM image of fiber; B: FTIR image of fiber; C: Zeta potential of fiber; D: Surface hydrophobicity of fiber; E: DSC image of fiber. Different letters at the top of the columns indicate significant differences ($p < 0.05$) in the same group.

Table 1

Content of free sulfhydryl, total sulfhydryl and disulfide bonds in fiber.

Samples	Free sulfhydryl (μmol/g)	Total sulfhydryl (μmol/g)	Disulfide bond (μmol/g)
RC	1.25 ± 0.07a	7.67 ± 0.48a	3.21 ± 0.19a
RCZ-1	1.20 ± 0.03ab	7.53 ± 0.17a	3.16 ± 0.08a
RCZ-3	1.07 ± 0.06b	6.38 ± 0.53b	2.66 ± 0.22b
RCZ-5	0.92 ± 0.05c	6.07 ± 0.52b	2.58 ± 0.21b
RCZ-7	0.66 ± 0.09d	5.78 ± 0.42b	2.56 ± 0.15b

Note: Different letters in the same column indicate significant differences ($p < 0.05$).

increase of the addition of ZrO₂ NPs, indicating that the addition of ZrO₂ NPs enhances thermal stability of fiber. Compared with RC, the peak temperature and enthalpy of RCZ-7 increased by 11.7 °C and 30.14 J/g, respectively.

The increase of Zeta potential indicated that the fiber stability was enhanced. This may be related to the addition of ZrO₂ NPs to enhance the electrostatic repulsion of the fiber system (Zhang, Saravanakumar, et al., 2023). A decrease of surface hydrophobicity of fiber may be owing to addition of ZrO₂ NPs destroying the hydrophobic groups of protein, thus exposing more hydrophilic groups (Sun et al., 2014). Additionally, the decrease of surface hydrophobicity of fiber may also be due to the presence of ZrO₂ NPs in RCZ, and the oxygen atom in ZrO₂ NPs is hydrophilic (Ling et al., 2022). The increase of enthalpy value and peak

temperature of fiber may be attributed to the formation of a lot of strong hydrogen bonds between RBP, CMC and ZrO₂ NPs, resulting in higher heat energy and temperature required for fiber disintegration (Mallakpour & Hajjari, 2018). Therefore, the fiber has good thermal stability and low hydrophobicity, which provides a research basis for the preparation of food insulation materials and food dehumidification materials.

3.3. Crystal structure analysis

The results of crystal structure analysis of the fibers were presented in Fig. 3. According to XRD image (Fig. 3A), RC had an obvious characteristic peak at $2\theta = 27.84^\circ$ indicated that RC contained CMC crystals (Khalil et al., 2024). With the addition of ZrO₂ NPs, the XRD image of the fiber indicated four characteristic peaks, corresponding to the four crystal faces of ZrO₂ NPs (110), ($\bar{1}\bar{1}\bar{1}$), (002) and (022), and the diffraction intensity of the four characteristic peaks also enhanced with the increase of addition of ZrO₂ (Lee et al., 2022). Fig. 3B-H show the SAXS analysis results of RC and RCZ-5 of fibers. According to the 1D image (Fig. 3B), the diffraction intensity of RCZ-5 was significantly higher than that of RC. Similarly, the 2D image (Fig. 3C) indicated that the diffraction dispersion intensity of RCZ-5 was lower than that of RC, indicated that RCZ-5 had a higher crystallinity. According to 1D image, we drew the $I(q)q^2-q^{-2}$ (Fig. 3D), $\ln[I(q)q^4]-q^2$ (Fig. 3E), and $B_{\text{obs}}-q^{-2}$ (Fig. 3F) curves of RC and RCZ-5, and calculated the interface factor (σ), interface zone thickness (ΔR), integral breadth (B_{p}), and average fibril

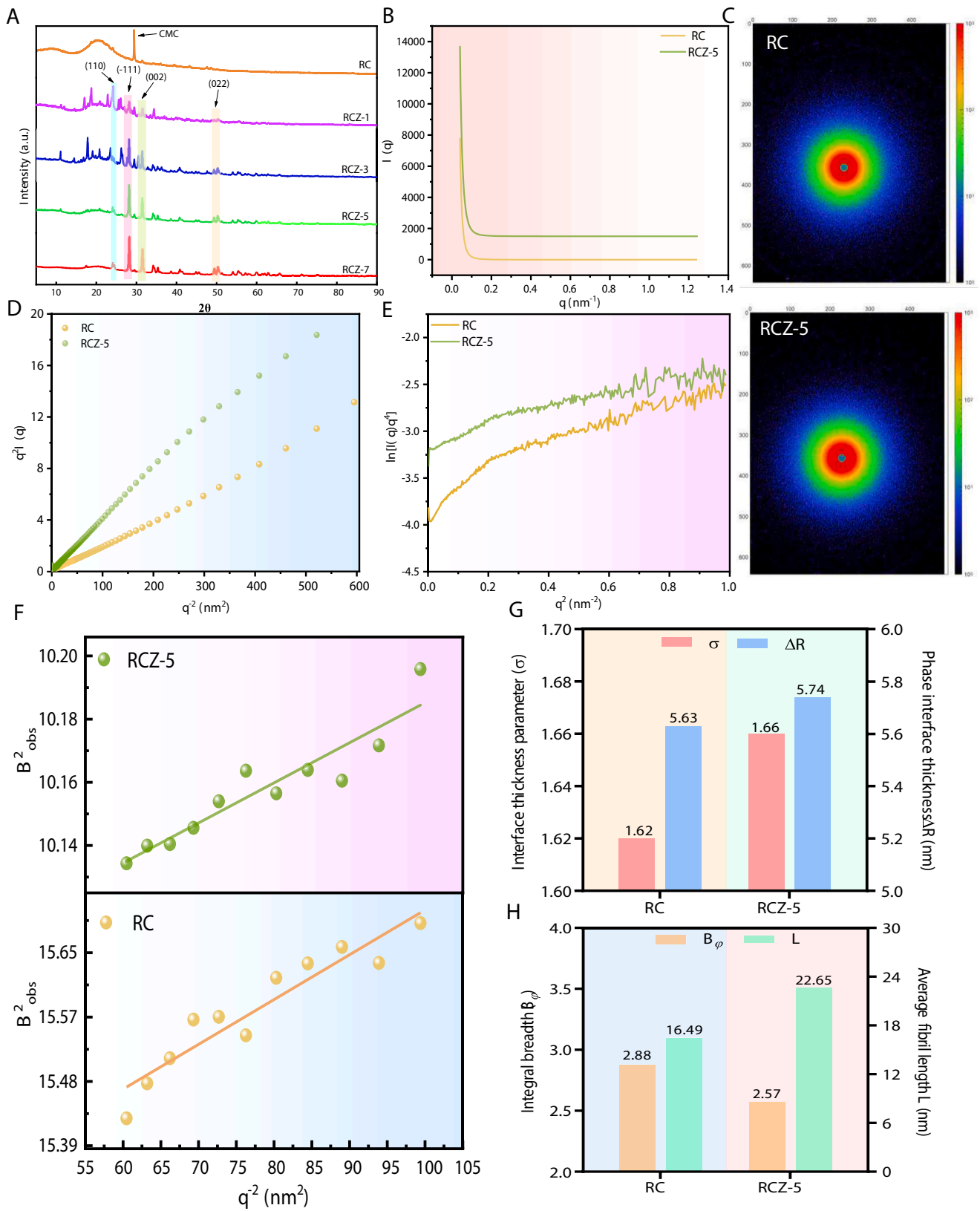


Fig. 3. Crystal structure of RBP/CMC/ZrO₂ fibers. A: XRD image of fiber; B: 1D image of fiber; C: 2D image of fiber; D: $I(q)q^2$ - q^2 image of fiber; E: $\ln[I(q)q^4]$ - q^2 curve of fiber; F: B^2_{obs} - q^2 image of fiber; G: Interface thickness parameter and phase interface thickness of fiber; H: Integral breadth and average fibril length of fiber.

length (L) from these curves. It can be seen from Figs. 3G and H that the σ , ΔR , and L of RCZ-5 were higher than RC, and B_ϕ was lower than RC. These results showed that the crystal orientation of RCZ-5 was significantly greater than that of RC, so the addition of ZrO₂ NPs promoted the orientation of the fiber.

With the addition of ZrO₂ NPs, the intensity and position of the characteristic diffraction peaks of the fibers changed. It is speculated that ZrO₂ NPs is not simply superimposed in the fiber, but forms hydrogen bonds with RBP and CMC through interaction, which is consistent with the results of FTIR analysis (Sani et al., 2021). The B_ϕ is a key factor to judge the orientation of fiber structure, and the orientation of fiber is inversely proportional to the B_ϕ value (Xiao et al., 2023). The B_ϕ of RCZ-5 was lower than RC, which might be attributed to the strong shear forces during microfluidic spinning that promoted cross-linking between RBP, CMC, and ZrO₂ NPs (Lu et al., 2019). The σ , ΔR , and L of RCZ-5 were higher than RC, which might be due to the addition of ZrO₂ NPs to promote the aggregation of RBP and CMC (Lu et al., 2019). The oxygen atoms in ZrO₂ NPs, the groups in RBP (amino and carboxyl groups) and the hydroxyl groups in CMC formed a network structure of intermolecular hydrogen bonds, which further enhanced the molecular orientation of the fiber phase interface, so that the fiber could obtain higher crystallinity and crystallization orientation (Lu et al., 2021). Therefore, the improvement of crystallinity and structural orientation of fiber also enhances the fiber stability, which corresponds to the findings of fiber stability studies in this work.

3.4. XPS analysis

The results of XPS analysis of the fibers were presented in Fig. 4. According to the full spectrum analysis of XPS (Fig. 4A), RC and RCZ-5 both contained C, N and O, and RCZ-5 contained Zr at 182.25 eV, indicating that ZrO₂ NPs had been successfully added to RCZ-5. The Zr3d fine spectrum (Fig. 4B) showed that the spin orbit of Zr3d had two splitting peaks (Zr3d5 and Zr3d3) with an energy difference of about 2.43 eV and a peak-to-area ratio of about 3:2. The fine spectra of C1s (Fig. 4C and D) and N1s (Fig. 4E and F) showed that the addition of ZrO₂ NPs did not promote the formation of new chemical bonds in the fibers, but increased the peak strength of C1s- and N1s-related bonds. From the fine spectra of O1s (Fig. 4G and H), it can be seen that both RC and RCZ-5 contained C=O and C—O. Notably, the addition of ZrO₂ NPs reduced the peak strength of C—O, increased the peak strength of C=O, peak area ratio of C=O to C—O changed from about 4:6 to 6:4. These results showed that although the addition of ZrO₂ NPs did not promote the formation of new chemical bonds, it changed the distribution of chemical bonds in the fiber and promoted the formation of hydrogen bond network structure between RBP, CMC and ZrO₂ NPs (Wang et al., 2015). This was similar to the FTIR analysis results. Additionally, the S2p fine spectrum (Fig. 4I and J) showed that the spin orbit of S2p had two splitting peaks (S2p3 and S2p1) with an energy difference of about 1.16 eV and a peak-to-area ratio of about 2:1. According to the position of S2p peak energy (168.6 eV), it was speculated that S mainly existed in a form of S—O bond in the fiber, and the S—O bond peak intensity of RCZ-5 was higher than that of RC. Therefore, the addition of ZrO₂ NPs destroyed the spatial structure of the RBP, so that the disulfide bond and the sulfhydryl group were oxidized into the S—O bond (Shen et al., 2023). This corresponded to the results of the changes of disulfide bonds and sulfhydryl groups measured earlier (Table 1).

According to these results of the characterization analysis, we obtained the potential mechanism of fiber formation (Fig. 1). During the preparation of the spinning solution, the structure of CMC became stretched after it was dissolved in [Cu(NH₃)₄(H₂O)₂]SO₄ solution (Lu et al., 2019). Disulfide bonds and sulfhydryl groups were destroyed after rice bran protein was dissolved, resulting in more chemical groups in the protein being exposed (Li et al., 2023). Then, ZrO₂ NPs were added and evenly dispersed into the mixture to obtain a spinning solution. At the spinning stage, the microchannel of the microfluidic chip provided a

strong shear force to polymerize CMC, rice bran protein and ZrO₂ NPs in the form of hydrogen bonds (Håkansson et al., 2014). Then, during the solidification process, a solvent exchange reaction occurred between the polymer and the solidification bath, and then the fiber was formed (Li et al., 2023).

3.5. Antibacterial activity

Fig. 5 shows the inhibitory activity of fiber against *Bacillus subtilis*. According to Fig. 5A, in the culture process, the OD value of the culture medium showed an increasing trend, and *Bacillus subtilis* entered the logarithmic stage after 4 h and the stable stage after 12 h. The more ZrO₂ NPs in the fiber, the smaller OD value of the culture medium during the antibacterial process of fiber. However, during the antibacterial process, OD values of RC and control group showed the same trend, indicating that RC had no obvious inhibitory effect on *Bacillus subtilis*. Fig. 5B shows the inhibition rate of the fiber loaded with ZrO₂ NPs against *Bacillus subtilis*. The inhibition rate of fiber to *Bacillus subtilis* showed an increasing trend with the increase of culture time. The more ZrO₂ NPs loaded in the fiber, the more obvious the inhibitory rate of the fiber against *Bacillus subtilis*. Notably, during the antibacterial process, the change trend of the antibacterial inhibition rate of RCZ-5 and RCZ-7 was similar, and the final antibacterial rate value was not significant. Therefore, we chose RCZ-5 to carry out the photocatalytic antibacterial experiment in the subsequent experiment. According to Fig. 5C, the inhibition rate of RCZ-5 and RCZ-5 + P against *Bacillus subtilis* showed an increasing trend with the increase of culture time. At 8–18 h, the antibacterial rates of the RCZ-5 + P were significantly higher ($p < 0.05$) than that of RCZ-5, suggesting that the fiber loaded ZrO₂ NPs had higher antibacterial ability under the condition of photocatalysis. A large amount of ROS produced under photocatalytic conditions may be the main reason for the inhibition of growth and reproduction of *Bacillus subtilis* (Zhu et al., 2020). An inhibitory effect of fiber on *Bacillus subtilis* was further evaluated by live/dead fluorescence staining (Fig. 5D). PI and SYTO-9 can stain dead and live bacteria with red and green fluorescence, respectively (Ge et al., 2023). Both RC and control group had a large amount of green fluorescence, and the red fluorescence level was the lowest, indicating that the antibacterial effect of RC was not significant. The RCZ-5 treatment group showed obvious red fluorescence, and the level of green fluorescence was reduced, indicating that the fiber loaded with ZrO₂ NPs had obvious bactericidal effect. Additionally, compared with RCZ-5 group, the green fluorescence of RCZ-5 + P group was further reduced, the red fluorescence was significantly enhanced, and the ratio of dead bacteria to live bacteria was increased.

A previous study showed that adding 30 % curcumin to zein could increase the antibacterial capacity of zein fiber to about 70 % (Wang et al., 2017). Another study showed that the rice protein-based film prepared with rice protein as raw material and essential oil as antioxidant had good anti-*Bacillus subtilis* effect, and the antibacterial zone reached 7.30 mm (Kurtfaki & Yildirim-Yalcin, 2023). These studies have shown that adding antioxidants to the preparation of protein-based active packaging materials can achieve good antibacterial effects, but the amount of antioxidants added, the cost of proteins and antioxidants, and the antibacterial effect are issues that we need to consider at present (Liu et al., 2024). In this work, by-product rice bran protein was used as raw material and cheap ZrO₂ NPs were added to prepare fiber. The photocatalytic antibacterial capacity of the fiber reached 84.27 %, which indicated that the fiber had good antibacterial effect and was in line with the concept of green sustainable preservation material development. Consequently, the fiber has strong antibacterial properties, and the antibacterial properties are further enhanced under photocatalytic conditions, which indicates that the fiber has potential value in the application of food preservation, environmental purification and wound healing.

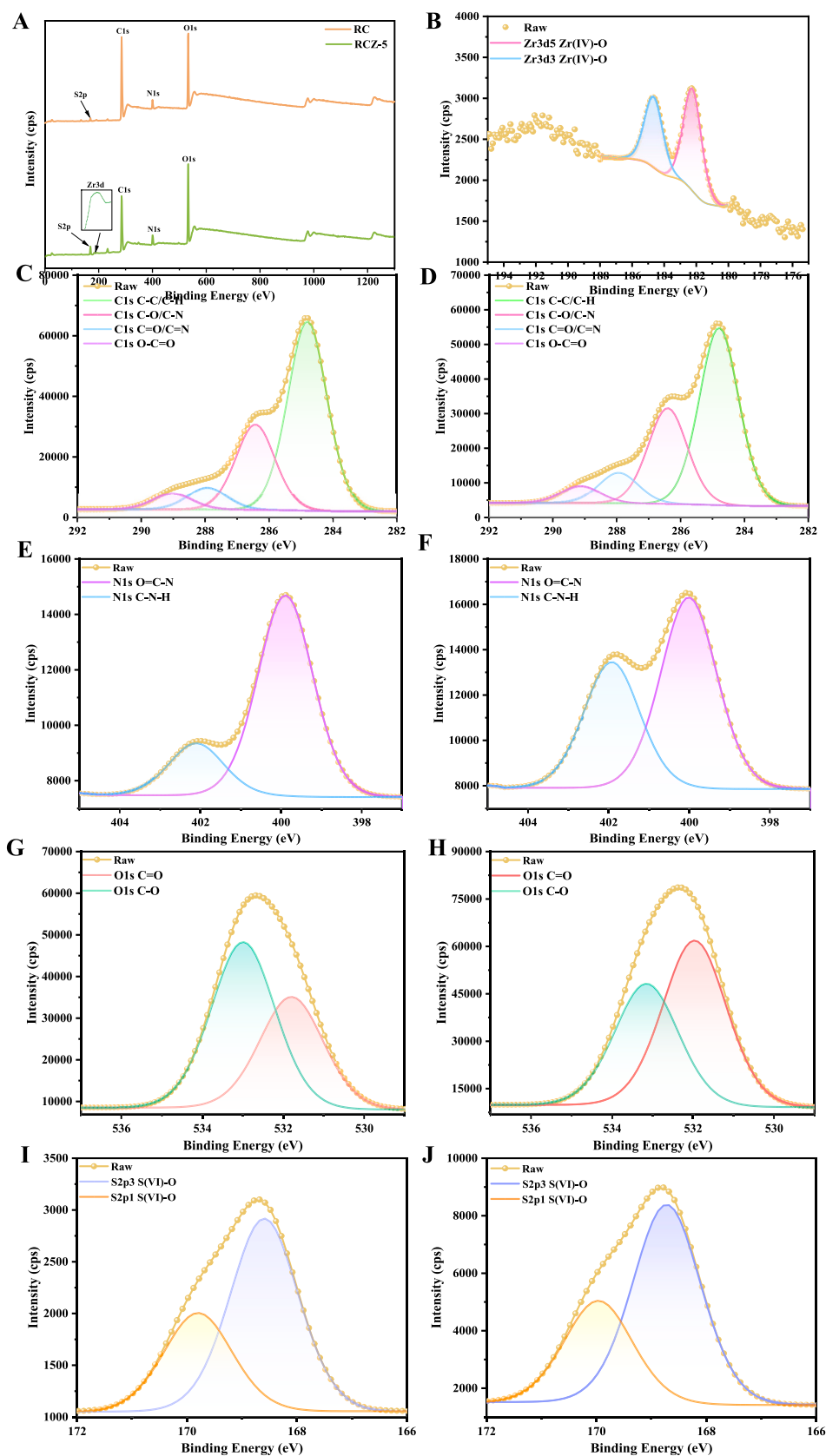


Fig. 4. XPS analysis of RBP/CMC/ZrO₂ fibers. A: Full spectrum image of fiber; B: Fine spectrum of Zr3d in RBP/CMC/ZrO₂ fiber; C, E, G and I: Fine spectrum of C1s, N1s, O1s and S2p3 of RC fiber; D, F, H and J: Fine spectrum of C1s, N1s, O1s and S2p3 of RBP/CMC/ZrO₂ fiber.

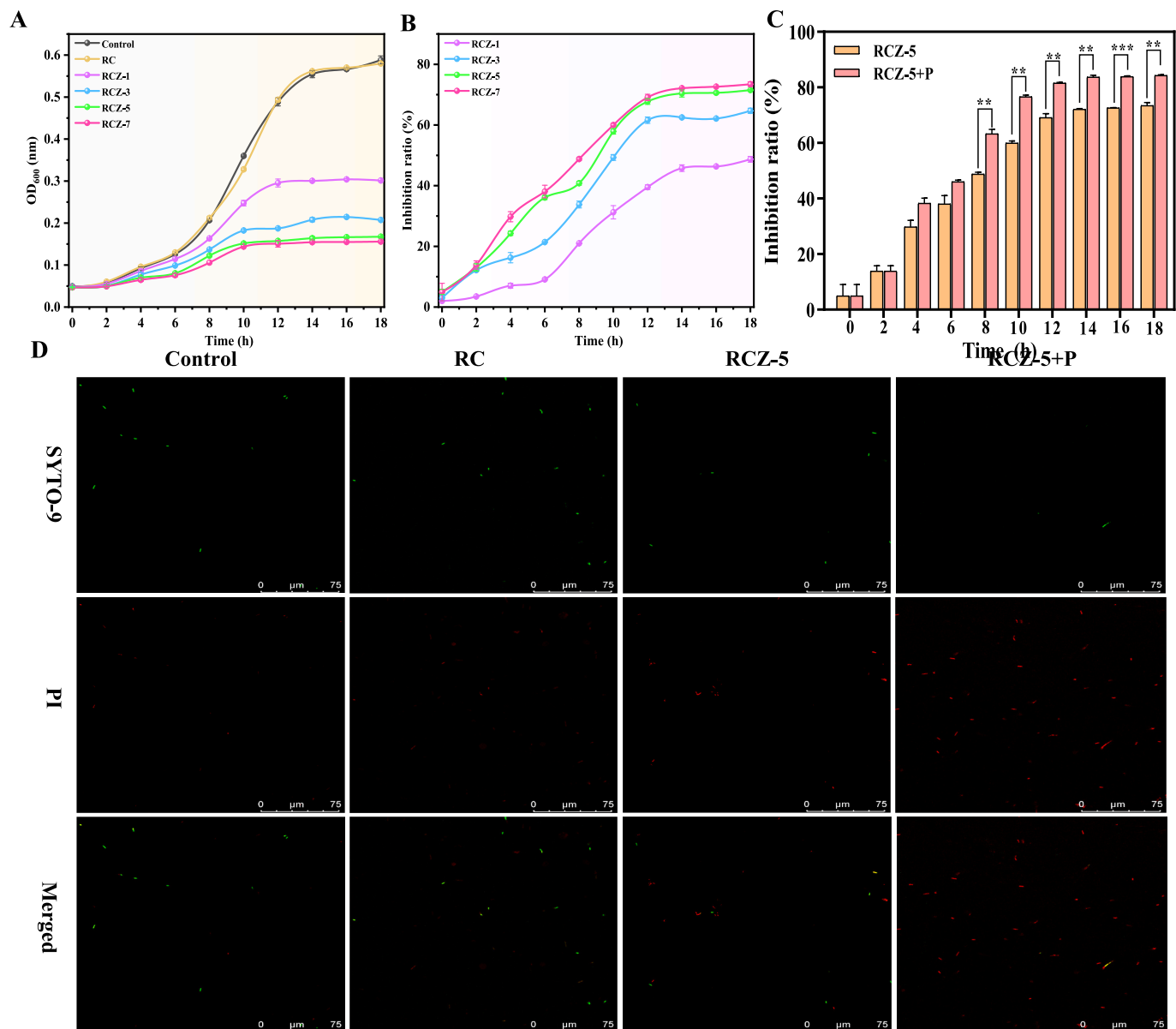


Fig. 5. Antibacterial activity of RBP/CMC/ZrO₂ fiber against *Bacillus subtilis*. A: OD value of bacterial solution; B: Bacteriostatic rate of fiber; C: Photocatalytic bacteriostatic rate of fiber; D: SYTO-9 and PI staining of live and dead bacteria. $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p < 0.0001$ (****).

3.6. Antibacterial mechanism

The antibacterial mechanism of the fibers were presented in Fig. 6. According to Fig. 6A, the surfaces of *Bacillus subtilis* in RC and the control group were clearly contoured with sufficient intracellular matter, showing a complete cellular structure. However, the surface of *Bacillus subtilis* in the RCZ-5 group was destroyed and intracellular matter flowed out, and the surface outline of *Bacillus subtilis* in the RCZ-5 + P group was very blurred and intracellular matter flowed out in large quantities. Fig. 6B showed that there was no obvious difference ($p > 0.05$) in ORP between RC and control groups, while the ORP of RCZ-5 group was higher and significantly different ($p < 0.01$) from that of RC group, and the ORP after photocatalysis was the highest and significantly different ($p < 0.01$) from that of RCZ-5 group. Similarly, according to Fig. 6C, the ROS contents of *Bacillus subtilis* in RCZ-5 group were significantly higher than those in control group and RC group ($p < 0.0001$), and photocatalysis further significantly enhanced ($p < 0.001$) the ROS content in *Bacillus subtilis*. Correspondingly, compared with RC group and control group, SOD ($p < 0.0001$) and CAT ($p < 0.001$) activities in RCZ-5 group

were significantly reduced, and SOD ($p < 0.001$) and CAT ($p < 0.01$) in RCZ-5 + P group were further significantly decreased, indicating that a large number of ROS produced by photocatalysis could significantly inhibit the activities of SOD and CAT (Fig. 6D and E). The significant decrease of AKP activity and the FDA staining fluorescence intensity in RCZ-5 ($p < 0.01$) and RCZ-5 + P ($p < 0.05$) indicated that the ZrO₂ NPs-loaded fibers could damage the cell membrane and wall of *Bacillus subtilis*, and the degree of destruction was aggravated by photocatalysis (Fig. 6F and G). Additionally, the significant decrease of intracellular protein ($p < 0.05$) and DNA ($p < 0.0001$) contents in *Bacillus subtilis* also indicated that the cell wall and membrane in *Bacillus subtilis* were seriously damaged under the condition of photocatalysis, resulting in the intracellular matter drain (Fig. 6H and I).

Under photocatalytic conditions, positively charged holes (h^+) and negatively charged electrons (e^-) will be generated on the surface of ZrO₂ NPs, and e^- can reduce O₂ to $\bullet O_2^-$, while h^+ can H₂O to $\bullet OH$, resulting in a production of a lot of ROS (Wang et al., 2023). These ROS can effectively stimulate microorganisms, inhibit their growth and reproduction, and induce their death. The SOD can clear the $\bullet O_2^-$ in cells

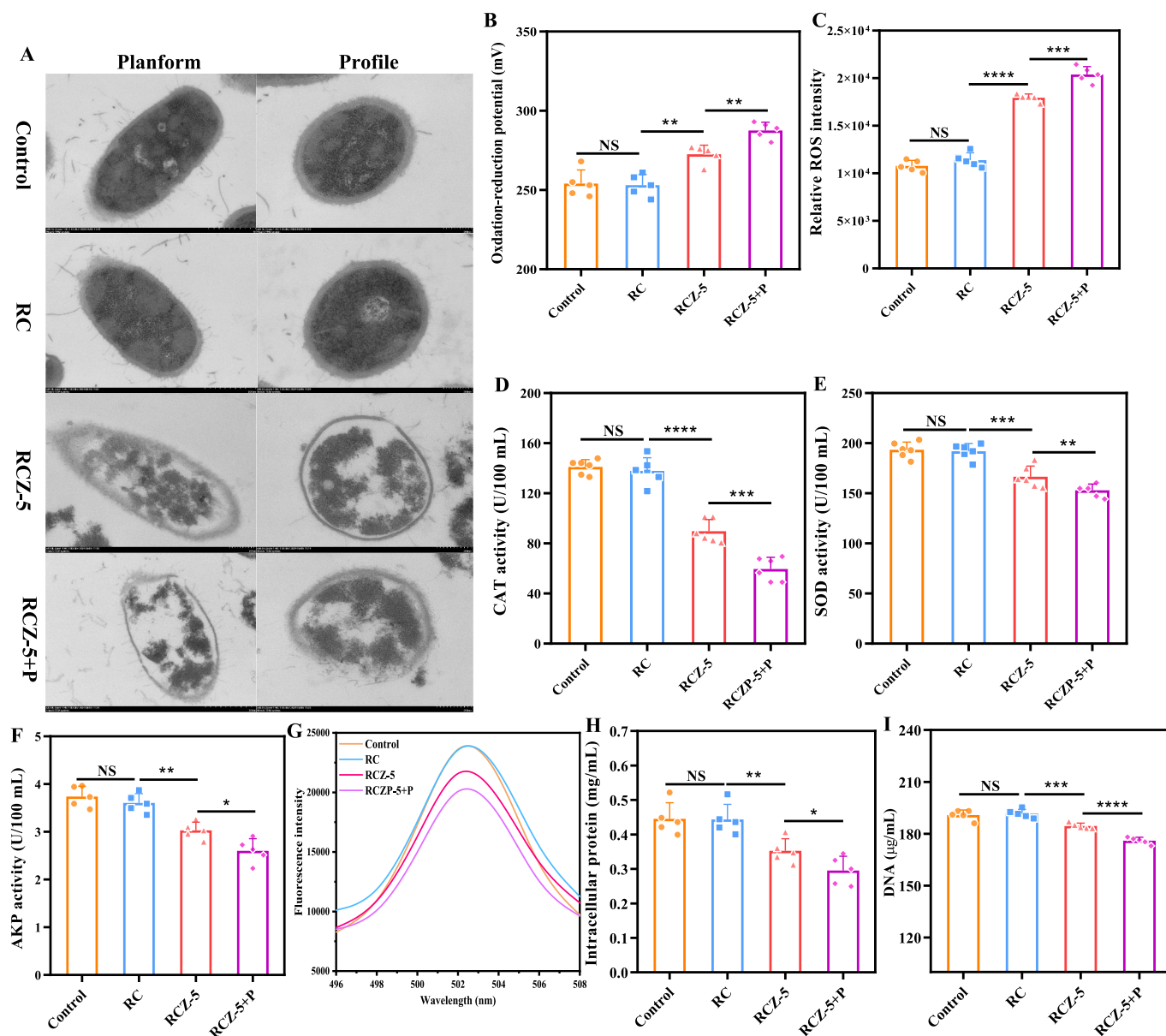


Fig. 6. Antibacterial mechanism of RBP/CMC/ZrO₂ fiber. A: TEM image of bacteria; B: ORP of bacterial solution; C: Relative ROS intensity in bacteria; D-F: CAT, SOD, and AKP activities in bacteria; G: Fluorescence intensity of FDA staining in bacteria; H and I: Intracellular protein and DNA content in bacteria. $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p < 0.0001$ (****).

and convert them into H₂O₂, then H₂O₂ is catalyzed into O₂ and H₂O under the action of CAT, so SOD and CAT are key enzymes to relieve cellular oxidative stress (Yu et al., 2024). A decline in the SOD and CAT activities suggested a diminished cellular capacity to counteract ROS stress. AKP typically resides between the cell wall and cell membrane, and is difficult to detect on the outside of the cell (Diao et al., 2018). However, cell wall damage can result in the leakage of AKP into the extracellular space, leading to reduced AKP activity within the cells. This reduction indicates that ROS has compromised the cell wall's integrity. Furthermore, when FDA is hydrolyzed by intracellular enzymes, it produces significant amounts of fluorescein (Tanmayee et al., 2023). Normally, fluorescein is retained within the cell; however, damage to the cell membrane allows fluorescein to rapidly exit, significantly reducing intracellular fluorescence intensity. This observation suggested that ROS disrupted both the permeability and integrity of cell membranes, as confirmed by a decrease in fluorescein intensity (Trzcinska-Wencel et al., 2023). Correspondingly, with the destruction of the cell membrane and cell wall, important substances such as nucleic

acids and proteins that maintain the normal metabolism of cells flow out of the cell, thereby affecting the reproduction and growth of cells (Liu et al., 2023). Additionally, ROS in cells also cause DNA damage, inhibit gene expression, hinder the synthesis of enzymes and proteins in cells, and ultimately lead to cell death (Maldonado et al., 2023).

3.7. Strawberry preservation

The result of strawberry preservation was presented in Fig. 7. Fig. 7A presented that in the process of storage (0–5 d), the strawberry surface appeared different degrees of rot. Among them, the strawberries of control group and RC group showed rot on the third day of storage, and the rot became more and more serious with the increase of storage time (4–5 d). The strawberries in the RCZ-5 group showed slight rot on the 5th day of storage, while strawberries in the RCZ-5 + P group showed a relatively intact surface. Correspondingly, the luminance and hardness of strawberries were reduced to varying degrees (Fig. 7B and C), and the weight loss rate enhanced (Fig. 7D) during storage. The luminance and

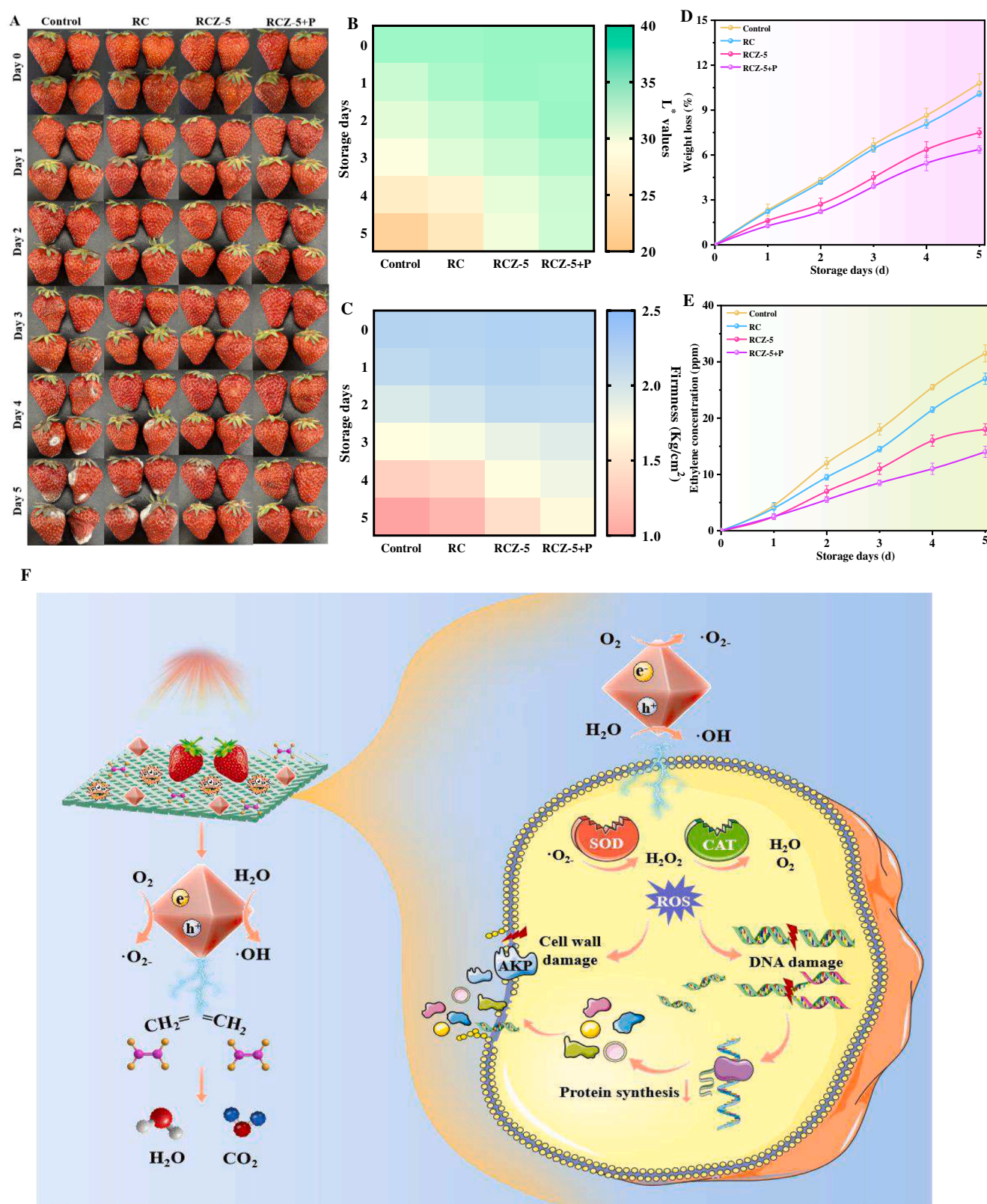


Fig. 7. Effect of RBP/CMC/ZrO₂ fiber on storage quality of strawberry. A: A picture of strawberries in storage. B-D: Luminance, hardness and weight loss rate of strawberry; E: Ethylene concentration during strawberry storage; F: Potential mechanism of antibacterial and ethylene removal of RBP/CMC/ZrO₂ fiber during strawberry storage.

hardness of strawberries in control group and RC group decreased rapidly, and the luminance and hardness of strawberries in RCZ-5 and RCZ-5 + P group decreased slowly. According to Fig. 7D, during storage, weight loss rate of strawberries in RC group and control group indicated a similar trend. The strawberries in the RCZ-5 and RCZ-5 + P group showed lower weight loss rate, and the RCZ-5 + P group had the lowest rate of weight loss. Additionally, it can be seen from Fig. 7E that the ethylene concentration released by strawberries increased during storage. Control group showed a highest ethylene concentration, and the ethylene concentrations in RC group were slightly lower than that in control group. The ethylene concentration of RCZ-5 and RCZ-5 + P group was lower, and the ethylene concentration of RCZ-5 + P group was the lowest, indicating that the fiber could scavenge the ethylene released by strawberry well under the condition of photocatalysis.

Based on the above findings, we proposed a preservation mechanism of RBP/CMC/ZrO₂ fiber for strawberry (Fig. 7F). Under the condition of photocatalysis, a large amount of ROS was produced on the surface of ZrO₂ NPs in the fiber (Li et al., 2022). These ROS stimulated bacteria on the surface of strawberries, causing them to develop oxidative stress. As ROS accumulate in bacteria, key enzymes (SOD and CAT) that relieved oxidative stress were destroyed. Meanwhile, a lot of ROS also damaged the cell membrane, cell wall and DNA of the bacteria, resulting in the bacteria being unable to maintain normal physiological activities, and even death (Liang et al., 2021). Additionally, the photocatalysis induced the production of a large amount of •OH on the surface of ZrO₂ NPs in the fiber, and •OH continuously attacked C=C in the ethylene molecule, converting ethylene successively to coordinatively bonded formaldehyde, bidentate formaldehyde, formic acid, and finally to CO₂ and H₂O (Hauchecorne et al., 2011). Interestingly, the ethylene concentration in RC group was marginally lower than that in control group. This observation led to the speculation that RBP harbors numerous active groups, such as sulfhydryl groups, capable of reacting with ethylene and consequently reducing its concentration within the storage environment (Fan et al., 2023). A previous study reported that zein/TiO₂ fiber prepared by electrospinning had a good ability to remove ethylene, and had a positive effect on extending the shelf life of cherry tomatoes (Böhmer-Maas et al., 2020). Another study showed that zein film prepared by electrospinning showed a stronger ability to adsorb ethylene with an adsorption efficiency of 7.61 mg/m³/h, and the film could be used to extend the storage time of bananas (Fan et al., 2023). These studies have proved that protein-based active preservation materials can extend the shelf life of fruits by removing ethylene, but the development of protein-based fibers with ethylene removal and antibacterial function may be more conducive to extending the storage time of fruits. In this work, RBP/CMC/ZrO₂ fiber not only inhibited bacterial growth and reproduction but also effectively scavenged ethylene, thereby prolonging the storage life of strawberries. Thus, the fiber has great application potential and commercial value in extending the shelf life of fruits.

4. Conclusions

In this investigation, RBP/CMC/ZrO₂ fibers were synthesized using microfluidic spinning. This study thoroughly examined the fiber formation mechanism, the antibacterial properties via photocatalysis, and its application in extending the shelf life of strawberries. The incorporation of ZrO₂ NPs not only enhanced the thermal stability and crystallinity of the fibers but also reduced their surface hydrophobicity. Under the condition of visible light catalysis, the fiber with 5 % ZrO₂ NPs had good resistance to *Bacillus subtilis*, and the inhibitory rate reached 84.27 %. The antibacterial mechanism analysis showed that photocatalysis promoted the production of ROS on the surface of ZrO₂ NPs, which reduced the antioxidant enzyme activity of bacteria, destroyed the biofilm and DNA of bacteria, and led to the death of bacteria. In addition, the fiber also had the ability to absorb ethylene, thus extending the shelf life of strawberries. Therefore, this study developed a low-cost, renewable, sustainable, and effective plant protein-based active

packaging material, which provides a new idea for fruit preservation. Additionally, in order to ensure the practical applicability and application of the fiber, the long-term stability of the fiber under different storage conditions, such as temperature and humidity, should also be the focus of future research.

CRedit authorship contribution statement

Ren Li: Writing – review & editing, Methodology, Conceptualization. **Wenhao Ma:** Methodology. **Yulin Feng:** Methodology. **Meng-meng Zhang:** Writing – review & editing. **Huijuan Zhang:** Writing – review & editing, Funding acquisition. **Jing Wang:** Resources.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2024.142022>.

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