

Article

Stabilization of Allyl Isothiocyanate by β -Cyclodextrin: Thermal Robustness and Potent Antimicrobial Activity

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Abstract

Allyl isothiocyanate (AITC) is a potent natural antimicrobial agent; however, its practical application is severely hindered by its extreme volatility and pungent, irritating odor. In this study, AITC inclusion complexes (AITC@ β -CD) were successfully fabricated via a co-precipitation strategy using β -cyclodextrin (β -CD) as the host matrix. Physicochemical characterizations, including FTIR, SEM, and XRD, confirmed the successful integration of AITC into the β -CD framework, inducing a crystalline phase transition from a cage-type to a channel-type structure. TGA demonstrated a substantial enhancement in thermal stability, with the maximum decomposition temperature shifting to 330 °C. This indicates that the spatial confinement within the channel-type lattice acts as a robust molecular shield that minimizes premature volatilization. Notably, water contact angle measurements revealed that the complexes attained a modulated surface wettability (89.0°), attributed to the structural rearrangement of surface hydroxyl groups. This modification ensures that the material remains compatible with aqueous food matrices while notably masking the unpleasant sensory attributes of pure AITC. Antibacterial assays against the standard indicator strain *Escherichia coli* (*E. coli*) confirmed that the encapsulation process preserved the intrinsic bioactivity of the guest, exhibiting comparable inhibitory zones to free AITC. Furthermore, the complexes maintained high inhibitory efficacy against indigenous microbial populations from spoiled fruits. These findings suggest that β -CD encapsulation effectively stabilizes AITC through guest-induced co-crystallization and enhances its consumer acceptability, providing a versatile and efficient strategy for sustainable food preservation.

Keywords: allyl isothiocyanate (AITC); β -cyclodextrin (β -CD); inclusion complex; thermal stability; antimicrobial efficacy; sensory masking



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1. Introduction

Fruits are global dietary staples valued for their nutritional density and unique organoleptic properties [1,2]. However, their perishable nature makes them highly susceptible to physiological disorders and microbial decay postharvest [3,4]. Global postharvest losses for fresh produce are estimated at 20–30% and can exceed 50% in developing regions, translating to annual economic deficits of approximately US \$75 billion [5]. Microbial proliferation not only accelerates spoilage but also poses significant food safety risks through mycotoxin production. Consequently, developing sustainable strategies to extend shelf life while maintaining fruit quality remains a critical objective in food science and agricultural

engineering. Current preservation techniques, including cold chain logistics, controlled atmosphere storage, and synthetic preservatives, face practical limitations [6,7]. Energy-intensive storage methods are often cost-prohibitive for small-scale applications, while synthetic chemicals face increasing regulatory restrictions and consumer resistance due to potential health risks [8–10]. Therefore, there is a paradigm shift towards “green” and “natural” alternatives. Research has increasingly focused on plant-derived bioactive compounds as eco-friendly antimicrobial agents that align with modern food safety standards [11].

Allyl isothiocyanate (AITC), a prominent glucosinolate derivative from Brassicaceae, has emerged as a potent broad-spectrum antimicrobial agent [12–14]. Previous studies have demonstrated its efficacy. Yang et al. [15] reported that 25 µg/mL AITC significantly inhibited *Aspergillus* infection and reduced ochratoxin A levels in grapes by up to 93%. Gao et al. [16] developed AITC-PLA films that achieved a 2–3 log reduction in microbial counts on tomatoes. However, the practical application of AITC is severely hampered by its intrinsic chemical instability. The highly reactive isothiocyanate group is prone to hydrolysis, and its extreme volatility leads to rapid concentration dissipation [9]. Furthermore, high vapor concentrations can induce phytotoxicity and undesirable pungent odors, adversely affecting the sensory attributes of treated fruit [17]. Therefore, stabilizing AITC to maintain its bioactivity while mitigating its volatility is essential for its utilization.

Encapsulation offers a robust strategy to overcome these challenges. By confining active compounds within protective matrices, encapsulation can shield guests from environmental degradation and suppress premature volatilization [18]. Cyclodextrins (CDs), cyclic oligosaccharides with a hydrophobic interior cavity and a hydrophilic exterior, are ideal hosts for forming non-covalent inclusion complexes with hydrophobic molecules [19]. Recent studies have successfully utilized β -CD to encapsulate various bioactives, such as folic acid and allicin, significantly improving their thermal stability and functional retention [20–22]. While some CD-based systems aim for responsive release, their primary advantage for volatile guests like AITC lies in preserving the active structure during processing and ensuring the retention of antimicrobial potency [23]. While high-temperature processes like twin-screw extrusion can incorporate AITC into polymers, the thermal stress often triggers significant AITC loss. In contrast, the co-precipitation method is a mild, aqueous-phase technique that facilitates encapsulation at or below room temperature, thereby minimizing the loss of thermosensitive and volatile compounds. Kong et al. demonstrated the efficacy of this approach by preparing tea tree oil- β -CD complexes using an ethanol–water co-solvent system [24].

In this study, β -cyclodextrin was employed as a functional host to construct AITC@ β -CD inclusion complexes via an optimized co-precipitation strategy. While cyclodextrin encapsulation is a known technique, this work specifically aimed to elucidate the structure–property relationships that govern the stabilization mechanism. We focused on how the guest-induced crystalline phase transition directly correlated with the enhancement of thermal robustness and the modulation of surface wettability. To elucidate the formation mechanism and structural integrity, comprehensive physicochemical characterizations were performed. Furthermore, the practical antimicrobial potency was strictly validated against the standard indicator strain *Escherichia coli* (*E. coli*), alongside an evaluation of its broad-spectrum utility against natural spoilage microflora. This research not only establishes an efficient methodology for stabilizing volatile bioactives but also provides mechanistic insights into the design of high-performance natural preservatives for sustainable food applications.

2. Results and Discussion

2.1. Preparation and Yield Optimization of AITC@ β -CD Inclusion Complexes

The encapsulation of hydrophobic AITC within the β -CD cavity formed stable inclusion complexes, effectively mitigating the chemical instability of the guest molecule under ambient conditions. As shown in Figure 1, the recovery yield (RY) and encapsulation efficiency (EE) were utilized to determine the feasibility of the co-precipitation process. Crucially, the formation of these solid precipitates was exclusively driven by a guest-induced self-assembly mechanism (illustrated in Scheme 1). This was verified by control experiments (Figure S1, Supplementary Materials), which confirmed that pure β -CD remained completely dissolved in the ethanol-water co-solvent system without the addition of AITC. Therefore, the recovered solids discussed were true inclusion complexes formed by the organization of β -CD into insoluble crystalline structures upon guest recognition rather than self-crystallized host material. Across the tested solvent ratios, RY remained consistent between 49.8% and 56.1%, while EE values ranged from 25.0% to 28.5%. These results confirmed the successful integration of AITC into the β -CD framework with sufficient loading for bioactive applications. The ethanol–water volume ratio exerted a significant influence on the crystallization and recovery of the complexes. An ethanol/water ratio of 1:3 yielded the optimal EE while maintaining a RY above 50%. While a higher ethanol proportion (1:1.5) produced a marginally greater amount of solid powder, it entailed increased solvent costs and a larger environmental footprint. The relative stability of the EE across various ratios demonstrated the robust adaptability of the β -CD host in capturing AITC molecules under the selected co-precipitation parameters. Consequently, the 1:3 ratio was selected as the optimal condition for fabricating AITC@ β -CD inclusion complexes to maximize resource efficiency and guest loading.

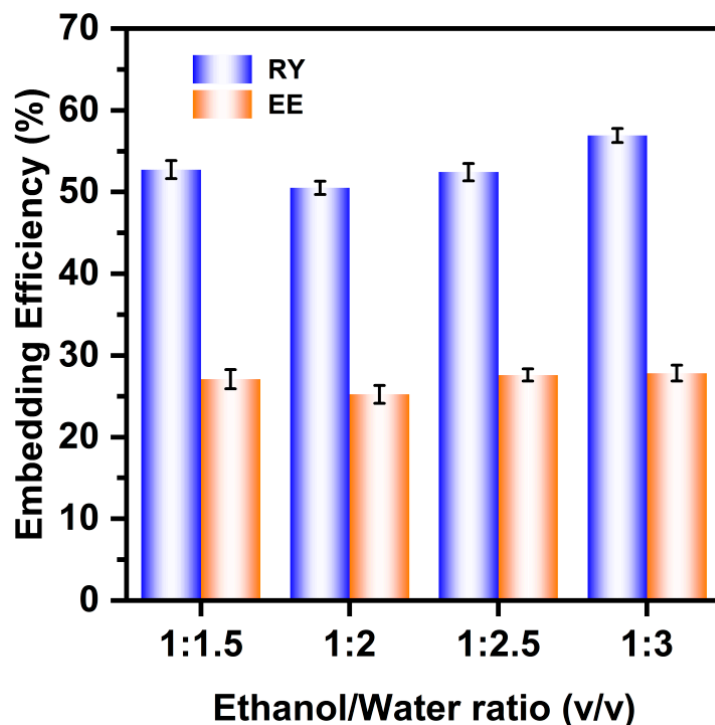
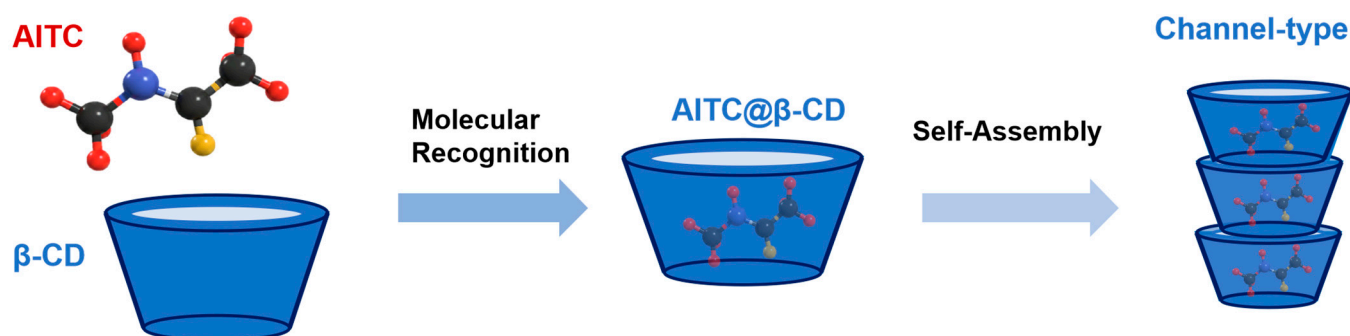


Figure 1. Recovery yield (RY) and encapsulation efficiency (EE) of AITC@ β -CD inclusion complex prepared at different ethanol/water ratios (v/v) ($n = 3$).



Scheme 1. Schematic illustration of the formation mechanism of AITC@ β -CD inclusion complexes. The encapsulation process proceeds through two distinct stages: (i) molecular recognition, where the hydrophobic AITC guest molecules penetrate the hydrophobic cavity of β -CD; and (ii) guest-induced self-assembly, resulting in the longitudinal stacking of inclusion complexes into an ordered channel-type crystalline lattice. In the accompanying ball-and-stick model of Allyl Isothiocyanate (AITC), the atomic constituents are represented by colored spheres: black for carbon (C), red for hydrogen (H), blue for nitrogen (N), and yellow for sulfur (S) atoms.

2.2. Characterization of Inclusion Complexes

The SEM morphology of β -CD and the AITC@ β -CD was examined. Figure 2a presents the SEM image of β -CD, which exhibited characteristic layered or irregular block-like structures. These particles were characterized by blunt edges and incomplete crystal faces. Additionally, stacking defects and layered cracks were evident on the surfaces of the β -CD particles, representing the typical morphology of the raw material. In contrast, the AITC@ β -CD displayed a distinct morphological transition toward more regular, rhombohedral structures with sharp, well-defined edges (Figure 2b). The surface of these particles appeared slightly roughened, characterized by thin-film or microgranular attachments, which may be attributed to the precipitation conditions or the presence of surface-adsorbed molecules. The particles remained structurally intact, with no signs of fracture or collapse. These observations indicate that the interaction between AITC and β -CD leads to a significant change in particle shape, from irregular blocks to regular rhombohedra [24].

The crystalline states of the materials were investigated using XRD. Figure 2c shows the XRD patterns of β -CD and the AITC@ β -CD complex, which exhibited distinct differences in peak positions and intensities. β -CD showed characteristic diffraction peaks at 2θ values of 10.6° , 12.4° , 15.5° , and 22.7° . These reflections corresponded to a cage-type crystal structure, a typical arrangement for host molecules where the cavity openings are partially obstructed by neighboring molecules. In contrast, the AITC@ β -CD complex displayed a significantly altered diffraction profile. A new prominent peak emerged at 12.0° , while the intensity of the original peaks at 10.6° and 15.5° was notably weakened or disappeared. Additionally, new diffraction peaks were observed at 17.5° and 18.7° , and the peak originally at 22.7° shifted to 23.7° . These systematic changes in the XRD pattern indicate a fundamental transformation of the β -CD crystal lattice. This structural transition to a more ordered channel-type packing is consistent with the regular rhombohedral morphology observed in the SEM analysis.

Figure 2d shows the FTIR spectra of AITC, β -CD, and the AITC@ β -CD inclusion complex. AITC exhibited sharp characteristic peaks at 2165 cm^{-1} and 2082 cm^{-1} , which corresponded to the stretching vibrations of the N=C=S functional group. Additionally, a peak at 1647 cm^{-1} was observed, attributed to the stretching vibration of the allyl C=C group. For β -CD, a broad band appeared at 3310 cm^{-1} (-OH stretching), along with a peak at 1636 cm^{-1} related to adsorbed water. Characteristic skeletal peaks of β -CD were identified at 2922 cm^{-1} (C-H stretching), as well as 1158 cm^{-1} and 1023 cm^{-1} (C-O-C stretching). In the spectrum of the AITC@ β -CD, the N=C=S stretching peaks at 2165 cm^{-1}

and 2082 cm^{-1} remained essentially unchanged in position. The allyl C=C peak shifted slightly from 1647 cm^{-1} to 1644 cm^{-1} and showed a reduction in intensity. Furthermore, the -OH broad band of β -CD shifted from 3310 cm^{-1} to 3265 cm^{-1} and became broader, while the skeletal peaks at 2922 , 1158 cm^{-1} , and 1023 cm^{-1} showed no significant variations. No new absorption peaks were detected in the complex. These spectroscopic results confirm the successful physical encapsulation of AITC within the β -CD cavity. The preservation of the N=C=S peaks indicates that the chemical structure of AITC remains intact during the inclusion process. The shift and intensity decrease in the allyl C=C peak suggest that the hydrophobic allyl moiety is confined within the host cavity, stabilized by hydrophobic interactions and van der Waals forces. Meanwhile, the broadening and shifting of the -OH band reflect a perturbation of the β -CD hydrogen-bonding network due to the presence of the guest. The absence of new peaks confirms that the inclusion process is dominated by non-covalent interactions rather than chemical bonding.

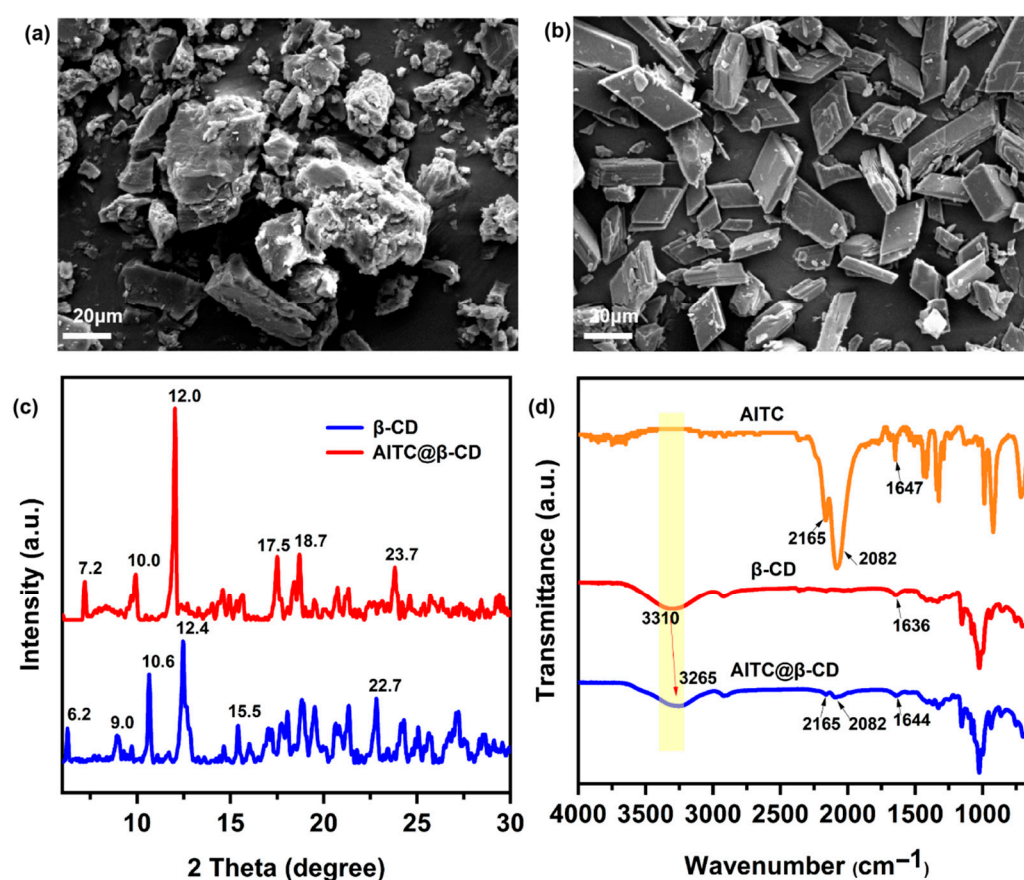


Figure 2. Characterization of AITC, β -CD and AITC@ β -CD inclusion complex: (a) SEM image of β -CD; (b) SEM image of AITC@ β -CD; (c) XRD patterns; (d) FTIR spectra.

The characteristic shifts and intensity variations observed in the FTIR spectra demonstrated the effective encapsulation of AITC within the host matrix. These findings were consistent with the morphological changes observed via SEM and the structural transitions revealed by XRD, collectively confirming the successful fabrication of AITC@ β -CD inclusion complex. This multi-analytical approach verified that the AITC guest was securely accommodated within the β -CD cavity through non-covalent interactions, maintaining its chemical integrity while forming a stable crystalline system.

2.3. Thermal Analysis of the AITC@ β -CD Inclusion Complexes

Figure 3 presents the thermogravimetric (TGA) and derivative thermogravimetric (DTG) curves of AITC, β -CD, and the AITC@ β -CD inclusion complex. AITC underwent a rapid and complete mass loss starting from 50 °C, reflecting its inherent high volatility. β -CD exhibited an initial weight loss of approximately 10% near 101 °C due to water desorption, followed by a major decomposition stage beginning at 290 °C, with the maximum decomposition rate occurring at 313 °C. For the AITC@ β -CD complex, three distinct weight loss stages were recorded. An initial loss of 6% was observed below 100 °C, primarily attributed to the desorption of physically adsorbed water. This was followed by a gradual and significant mass reduction of 17% between 100 °C and 305 °C. The final decomposition stage occurred from 305 °C to 372 °C, during which the maximum decomposition rate (DTG peak) shifted from 313 °C to 330 °C. These TGA results indicated that the thermal stability of AITC was significantly enhanced upon encapsulation. The fact that this characteristic weight loss occurred well above the boiling point of pure AITC indicates that the stabilization is not merely due to weak surface interactions. Instead, it is a consequence of the spatial confinement effect within the channel-type crystalline lattice. As AITC molecules act as templates to organize β -CD into continuous hydrophobic channels, they become physically entrapped deep within this rigid framework via a near-perfect geometric fit. Consequently, the encapsulated guest molecules face a substantial kinetic barrier to diffusion and are effectively locked in place, unable to escape until the thermal collapse of the host matrix itself. Furthermore, the TGA data provided a quantitative validation of the guest loading. Based on the aforementioned mass loss (17%) and the recorded recovery yield (56.1%), the encapsulation efficiency (EE) was calculated to be approximately 38.2%. This result was consistent with the EE of 25.8% previously determined by UV-Vis spectrophotometric analysis. The higher EE value estimated by TGA might be attributed to the loss of bound water and structural changes within the complex during heating, whereas the UV-Vis result reflects the actual amount of AITC extracted from the host matrix. This cross-verification further confirmed that AITC was securely immobilized within the host matrix rather than being merely surface-adsorbed, demonstrating the improved thermal robustness conferred by the inclusion complex structure.

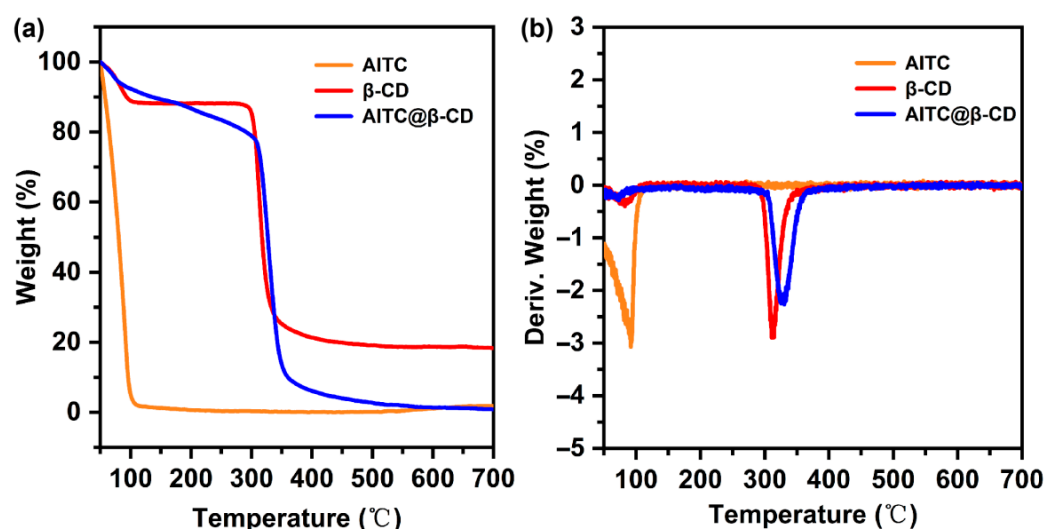


Figure 3. (a) TGA and (b) DTG curves of AITC, β -CD, and AITC@ β -CD inclusion complex.

2.4. Hydrophilicity of AITC@ β -CD Inclusion Complexes

The surface wettability of the materials was evaluated via water contact angle measurements to understand their interfacial properties. As shown in Figure 4, β -CD exhibited

a strong hydrophilic character with a contact angle of 11.3° . With the molecular assembly of the AITC@ β -CD complex, the contact angle increased significantly to 89.0° . This shift confirms that the hydrophobic AITC molecules have been successfully hosted within the β -CD cavities, thereby modulating the surface energy of the host. Crucially, this drastic change in surface wettability was not merely a result of guest encapsulation but is attributed to the fundamental crystalline phase transition from the native cage-type to the channel-type lattice. As indicated by the XRD results, this structural rearrangement significantly altered the surface topology and effectively reduces the accessibility and density of the hydrophilic hydroxyl groups on the crystal facets. Notably, the contact angle remains below 90° , indicating that the AITC@ β -CD complex maintains a degree of surface wettability. This interfacial property ensures that the complexes can be effectively dispersed in aqueous food systems while providing a stable environment for the hydrophobic guest.

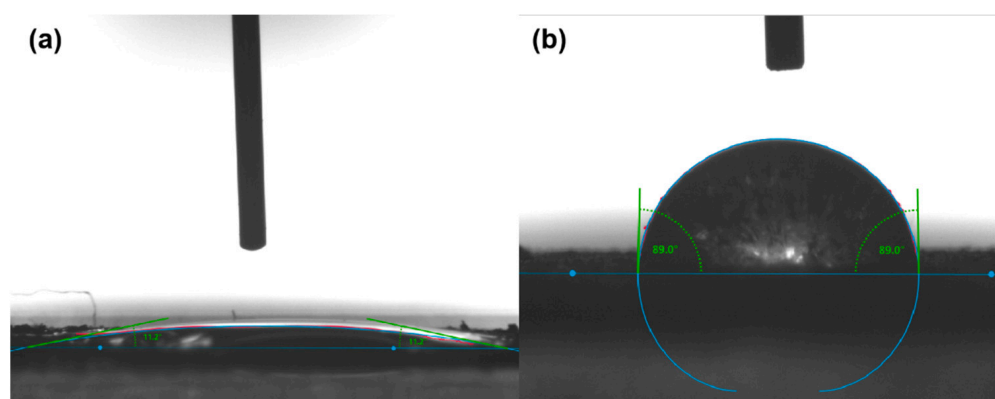


Figure 4. Water contact angles of β -CD (a) and AITC@ β -CD inclusion complex (b).

2.5. Antibacterial Activity of AITC@ β -CD Inclusion Complexes

The antibacterial efficacy of the AITC@ β -CD inclusion complexes was evaluated using the disk diffusion assay against the standard indicator strain *E. coli*. As shown in Figure 5, the blank control (CK) and pure β -CD group showed no inhibition zones, confirming that the host matrix itself lacks antimicrobial activity. In contrast, both free AITC and the inclusion complexes exhibited distinct clear zones, indicating potent bacterial inhibition. Specifically, the AITC@ β -CD inclusion complexes produced a well-defined inhibition zone with a mean diameter of 11 mm. This result confirmed that the encapsulation process successfully preserves the intrinsic biological potency of the guest molecule. It was worth noting that this zone was slightly smaller than that of free AITC (approximately 16 mm). This reduction was not interpreted as a loss of potency, but rather as positive evidence of the volatility suppression capability of the complex. Unlike free AITC, which volatilized and diffused rapidly (generating intense pungent odors), the encapsulated AITC was spatially confined within the crystalline lattice. This confinement restricted the premature volatilization of the guest, thereby effectively masking the pungent odor while maintaining sufficient bioavailability to inhibit microbial growth.

Additionally, the broad-spectrum inhibitory effected against natural spoilage microorganisms (derived from decayed fruits) are presented in Figure S2 (Supplementary Materials). These results further demonstrated that the complex retains significant bioactivity in simulated practical environments, providing a green solution for food preservation.

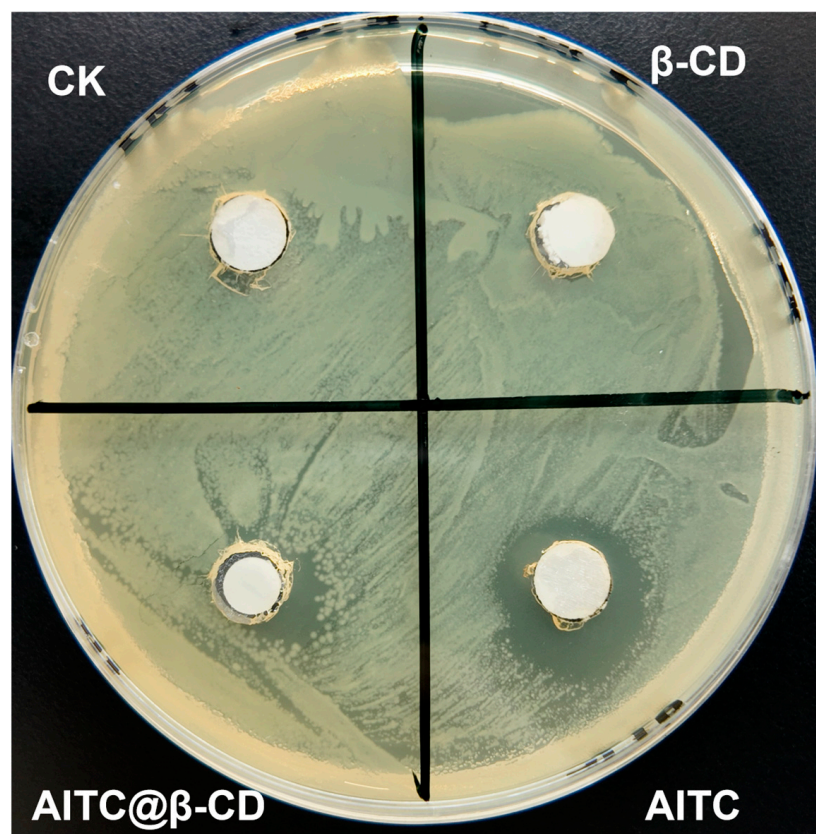


Figure 5. Antibacterial activity of AITC@ β -CD inclusion complexes against *E. coli* compared to controls and free AITC using the disk diffusion method. The plate is divided into four quadrants: CK: Blank control (PBS); β -CD: Pure β -cyclodextrin host; AITC: Free allyl isothiocyanate; AITC@ β -CD: AITC inclusion complexes.

3. Materials and Methods

3.1. Materials

Allyl isothiocyanate (AITC, 98%) was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). β -cyclodextrin (β -CD, 98%), anhydrous ethanol, plate count agar (PCA), and sodium chloride were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Tryptic soy agar (TSA) was supplied by Shanghai Titan Scientific Co., Ltd. (Shanghai, China). All chemical reagents used in this study were of analytical grade and were used as received without further purification.

3.2. Preparation of AITC@ β -CD Inclusion Complexes

AITC@ β -CD inclusion complexes were prepared via a co-precipitation method. Ethanol–water mixtures were prepared at volume ratios of 1:1.5, 1:2.0, 1:2.5, and 1:3.0, each with a total volume of 50 mL. β -CD (3.0 g) was dissolved in the mixed solvent at 60 °C under magnetic stirring (700 rpm), with the solution covered by aluminum foil to minimize solvent evaporation. After complete dissolution, AITC (3.0 g) was added, followed by stirring at 30 °C for 2 h. The resulting mixture was maintained at room temperature for 24 h to reach equilibrium and facilitate the formation of inclusion complexes. The precipitated inclusion complexes were washed with 40 mL of 60% (v/v) ethanol to remove unencapsulated AITC, and subsequently dried in a vacuum oven (Thermo Scientific, Shanghai, China) at 30 °C for 24 h. The dried inclusion complexes were stored in a sealed container until further use.

3.3. Evaluation of AITC Encapsulation

The recovery yield (RY) and AITC encapsulation efficiency (EE) were calculated to evaluate the effectiveness of the co-precipitation procedure according to the following equations:

$$\text{RY (\%)} = \frac{\text{Total weight of recovered AITC@}\beta\text{-CD}}{\text{Initial } \beta\text{-CD} + \text{Initial AITC}} \times 100\% \quad (1)$$

$$\text{EE (\%)} = \frac{\text{Weight of encapsulated AITC}}{\text{Initial AITC}} \times 100\% \quad (2)$$

The AITC content within the complexes was determined via UV–visible spectrophotometry. To establish the calibration curve, an AITC stock solution (10 g/L) was prepared by dissolving 0.5 g of AITC in anhydrous ethanol and diluting it to 50 mL. This solution was further diluted to obtain a series of standard solutions with concentrations ranging from 10 to 50 mg/L. The absorbance of each solution was measured at 244 nm, yielding a linear regression equation, $y = 0.0106x + 0.0706$, $R^2 = 0.9985$, where y represents the absorbance and x is the AITC concentration (mg/L). For the analysis of the inclusion complexes, 0.04 g of the sample was dispersed in 40 mL of anhydrous ethanol. The mixture was subjected to ultrasonic treatment for 30 min followed by vortexing for 10 min to ensure the complete dissociation of the inclusion complexes and full release of the guest molecules. After centrifugation at 5000 rpm for 25 min, the supernatant was collected and its absorbance was recorded at 244 nm. The concentration of encapsulated AITC was then derived from the calibration curve to calculate the EE using Equation (2).

3.4. Physicochemical Characterization of AITC@ β -CD Inclusion Complexes

The morphology of the inclusion complexes was characterized using scanning electron microscopy (SEM; MAIA3 LMH, TESCAN, Brno, Czech Republic). To minimize the volatilization of encapsulated compounds, observations were performed under low-vacuum conditions using backscattered electron mode. Prior to imaging, the inclusion complexes surfaces were sputter-coated with a thin layer of platinum to enhance conductivity, and images were acquired at an accelerating voltage of 10 kV. Pure β -CD was observed under the same conditions as a control to compare morphological differences.

The crystalline structures of the samples before and after encapsulation were analyzed by X-ray diffraction (XRD-6100, Shimadzu, Japan). The XRD patterns of β -CD and AITC@ β -CD inclusion complexes were obtained using $\text{CuK}\alpha$ radiation at 40 kV and 40 mA. All scans were performed over a 2θ range of $6\text{--}30^\circ$ with a step size of 0.02° .

Molecular interactions between AITC and β -CD within the inclusion complexes were characterized by Fourier-transform infrared spectroscopy (FTIR, Nicolet iS50, Thermo Scientific, Waltham, MA, USA). Spectra were collected over the range of $4000\text{--}650\text{ cm}^{-1}$ at a resolution of 4 cm^{-1} , with 32 scans averaged for each sample. For sample preparation, pure AITC was placed directly on a potassium bromide (KBr) plate, while β -CD and AITC@ β -CD inclusion complexes were ground with dried KBr powder at a mass ratio of 1:50 and pressed into approximately 1 mm thick transparent pellets. Raw spectral data were smoothed and baseline-corrected using the instrument software to ensure accuracy and comparability.

The thermal stability of the inclusion complexes was assessed by thermogravimetric analysis (TGA; BCSY, Baichuan Analytical Instruments, Tianjin, China). An appropriate amount of sample was placed in the instrument pan and heated from room temperature to 700°C at a rate of $10^\circ\text{C}/\text{min}$ under a nitrogen atmosphere, while recording the sample mass change with temperature.

The hydrophobicity of the inclusion complexes was evaluated by water contact angle measurement (DSA100, KRÜSS, Hamburg, Germany). Inclusion complexes powders were

pressed into small disks, and 5 μ L droplets of deionized water were applied to the surface. Static contact angles were measured at three different positions, and the average value was used to represent surface wettability.

3.5. Antimicrobial Activity of AITC@ β -CD Inclusion Complexes

The antimicrobial efficacy of the AITC@ β -CD inclusion complexes was evaluated using the disk diffusion method (Oxford cup method) against the standard indicator strain *Escherichia coli* (*E. coli*). The bacteria were cultured in Tryptic Soy Broth (TSB) to the logarithmic growth phase. The bacterial suspension was serially diluted (10-fold) using 0.01 M phosphate-buffered saline (PBS, pH 7.4) to achieve a final concentration of approximately 10^7 – 10^8 CFU/mL. For the assay, 100 μ L of the diluted *E. coli* suspension was spread uniformly onto Tryptic Soy Agar (TSA) plates. Sterile Oxford cups were placed equidistantly on the agar surface. The experimental groups were designed as follows: (1) Blank control (CK, 20 μ L PBS); (2) β -CD (0.03 g pure β -CD); (3) AITC (20 μ L); and (4) AITC@ β -CD (0.03 g AITC@ β -CD inclusion complexes). The plates were incubated at 37 °C for 24 h. The diameter of the inhibition zone was measured using the cross method to quantify the antimicrobial activity. Additionally, to assess the broad-spectrum applicability in a simulated spoilage environment, the inhibitory effect against a natural microbial suspension derived from spoiled fruits was also evaluated. The detailed methodology and results for this supplementary test are provided in the Supplementary Materials (Figure S2).

4. Conclusions

This study demonstrated the successful development of a stable and bioactive antimicrobial system by encapsulating Allyl Isothiocyanate (AITC) within β -cyclodextrin (β -CD) via a co-precipitation strategy. Physicochemical characterizations revealed that the formation of crystalline inclusion complexes transformed the β -CD matrix from a cage-type to a channel-type structure. This transformation created a robust spatial confinement environment that effectively shielded the guest molecules. Consequently, the maximum decomposition temperature of AITC was elevated to 330 °C, as the guest molecules were “locked” within the lattice, facing a substantial kinetic barrier to premature volatilization. Beyond structural stabilization, the encapsulation strategy achieved a critical balance between hydrophobicity and dispersibility. The AITC@ β -CD inclusion complexes exhibited a modulated surface wettability (contact angle of 89.0°), attributed to the reduced exposure of hydroxyl groups following the crystalline phase transition. This ensured excellent compatibility with aqueous food matrices while notably masking the pungent, irritating odor of pure AITC. Most importantly, the inclusion process preserved the intrinsic antimicrobial potency of AITC. The complexes demonstrated a clear and well-defined inhibition zone against the standard indicator strain *E. coli*, comparable to that of free AITC, while also showing broad-spectrum efficacy against indigenous spoilage microorganisms.

In summary, this research established AITC@ β -CD inclusion complexes as a high-performance, food-safe, and environmentally sustainable alternative to synthetic preservatives, offering a versatile solution for postharvest preservation driven by superior thermal stability and controlled volatility.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules31050780/s1>, Figure S1: Digital photographs of control experiments showing pure β -CD dissolved in ethanol-water mixtures at different volume ratios; Table S1: Total Plate Count under different treatments; Figure S2: Growth inhibitory effects of different groups against natural fruit spoilage microorganisms.

Author Contributions: Z.C.: investigation, methodology, formal analysis, conceptualization, and writing—original draft; G.H.: investigation, methodology, and writing—review and editing; L.C.: investigation, methodology, and writing—review and editing; R.Z.: investigation, methodology, and writing—review and editing; S.C.: conceptualization, writing—review and editing, and supervision. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Sivakumar, D.; Bautista-Baños, S. A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. *Crop Prot.* **2014**, *64*, 27–37. [\[CrossRef\]](#)
2. Meng, Y.; Zhao, H.; Dong, C.; He, Z.; Long, Z. Eco-friendly and flexible polysaccharide-based packaging films for fruit preservation. *Int. J. Biol. Macromol.* **2024**, *281*, 136132.
3. Maqbool, M.; Ali, A.; Alderson, P.G.; Mohamed, M.T.M.; Siddiqui, Y.; Zahid, N. Postharvest application of gum arabic and essential oils for controlling anthracnose and quality of banana and papaya during cold storage. *Postharvest Biol. Technol.* **2011**, *62*, 71–76. [\[CrossRef\]](#)
4. Zhang, W.; Pan, Y.; Jiang, Y.; Zhang, Z. Advances in gas fumigation technologies for postharvest fruit preservation. *Crit. Rev. Food Sci. Nutr.* **2024**, *64*, 8689–8708. [\[CrossRef\]](#)
5. Liu, J.; Sui, Y.; Wisniewski, M.; Droby, S.; Liu, Y. Review: Utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. *Int. J. Food Microbiol.* **2013**, *167*, 153–160. [\[CrossRef\]](#)
6. Wang, J.; Yuan, Y.; Liu, Y.; Li, X.; Wu, S. Application of chitosan in fruit preservation: A review. *Food Chem. X* **2024**, *23*, 101589. [\[CrossRef\]](#)
7. Cui, Y.; Cheng, Y.; Xu, Z.; Li, B.; Tian, W.; Zhang, J. Cellulose-Based Transparent Edible Antibacterial Oxygen-Barrier Coating for Long-Term Fruit Preservation. *Adv. Sci.* **2024**, *11*, e2409560.
8. Taban, A.; Haghighi, T.M.; Mousavi, S.S.; Sadeghi, H. Are edible coatings (with or without essential oil/extract) game changers for maintaining the postharvest quality of strawberries? A meta-analysis. *Postharvest Biol. Technol.* **2024**, *216*, 113082. [\[CrossRef\]](#)
9. Jiang, L.; Li, Y.; Xue, C.; He, Z.; Wang, Z.; Qin, F.; Chen, J.; Zeng, M. The inhibitory effects of yellow mustard (*Brassica juncea*) and its characteristic pungent ingredient allyl isothiocyanate (AITC) on PhIP formation: Focused on the inhibitory pathways of AITC. *Food Chem.* **2022**, *373*, 131398. [\[CrossRef\]](#)
10. Fan, X.; Rong, L.; Li, Y.; Cao, Y.; Kong, L.; Zhu, Z.; Huang, J. Fabrication of bio-based hierarchically structured ethylene scavenger films via electrospraying for fruit preservation. *Food Hydrocoll.* **2022**, *133*, 107837. [\[CrossRef\]](#)
11. Xu, W.; Jia, X.; Yang, M.; Peng, Z.; Chen, H.; Zhang, X.; Wei, B. Tea Polyphenol Self-Assembly Nanocomposite Coating for Fruit Preservation. *ACS Nano* **2025**, *19*, 28146–28159. [\[CrossRef\]](#)
12. Baggio, J.S.; Chamorro, M.; Cordova, L.G.; Noling, J.W.; Vallad, G.E.; Peres, N.A. Effect of Formulations of Allyl Isothiocyanate on Survival of *Macrophomina phaseolina* from Strawberry. *Plant Dis.* **2018**, *102*, 2212–2219. [\[CrossRef\]](#)
13. El Khetabi, A.; Lahlali, R.; Ezrari, S.; Radouane, N.; Lyousfi, N.; Banani, H.; Askarne, L.; Tahiri, A.; El Ghadraoui, L.; Belmalha, S.; et al. Role of plant extracts and essential oils in fighting against postharvest fruit pathogens and extending fruit shelf life: A review. *Trends Food Sci. Technol.* **2022**, *120*, 402–417. [\[CrossRef\]](#)
14. Nikkhah, M.; Hashemi, M. Boosting antifungal effect of essential oils using combination approach as an efficient strategy to control postharvest spoilage and preserving the jujube fruit quality. *Postharvest Biol. Technol.* **2020**, *164*, 111159. [\[CrossRef\]](#)
15. Yang, B.; Li, L.; Geng, H.; Zhang, C.; Wang, G.; Yang, S.; Gao, S.; Zhao, Y.; Xing, F. Inhibitory effect of allyl and benzyl isothiocyanates on ochratoxin A producing fungi in grape and maize. *Food Microbiol.* **2021**, *100*, 103865. [\[CrossRef\]](#)
16. Gao, H.; Wu, W.; Chen, H.; Qin, Y.; Fang, X.; Jin, T.Z. Microbial inactivation and quality improvement of tomatoes treated by package film with allyl isothiocyanate vapour. *Int. J. Food Sci. Technol.* **2018**, *53*, 1983–1991. [\[CrossRef\]](#)
17. Kramer, B.; Wunderlich, J.; Muranyi, P. Impact of volatile allyl isothiocyanate on fresh produce. *Food Packag. Shelf Life* **2018**, *16*, 220–224. [\[CrossRef\]](#)
18. Wang, C.; Wu, L.; Zhang, Y.; Xu, M.; Saldaña, M.D.A.; Fan, X.; Sun, W. A Water-absorbent Mat Incorporating β -cyclodextrin/eugenol Inclusion Complex for Preservation of Cold Fresh Mutton. *Food Biophys.* **2022**, *17*, 437–447. [\[CrossRef\]](#)
19. Celebioglu, A.; Ipek, S.; Durgun, E.; Uyar, T. Selective and Efficient Removal of Volatile Organic Compounds by Channel-type Gamma-Cyclodextrin Assembly through Inclusion Complexation. *Ind. Eng. Chem. Res.* **2017**, *56*, 7345–7354. [\[CrossRef\]](#)

20. Zhang, Y.M.; Liu, Y.H.; Liu, Y. Cyclodextrin-Based Multistimuli-Responsive Supramolecular Assemblies and Their Biological Functions. *Adv. Mater.* **2020**, *32*, e1806158. [[CrossRef](#)] [[PubMed](#)]
21. Shi, C.; Fang, D.; Xia, S.; Wang, J.; Guo, Y.; Lyu, L.; Wu, W.; Li, W. Preparation and characterization of lemon essential oil@ β -cyclodextrin inclusion for blackberry postharvest preservation. *Food Control* **2023**, *154*, 109979. [[CrossRef](#)]
22. Wang, Y.-F.; Shao, J.-J.; Wang, Z.-L.; Lu, Z.-X. Study of allicin microcapsules in β -cyclodextrin and porous starch mixture. *Food Res. Int.* **2012**, *49*, 641–647. [[CrossRef](#)]
23. Wang, J.; Qiu, C.; Narsimhan, G.; Jin, Z. Preparation and Characterization of Ternary Antimicrobial Films of beta-Cyclodextrin/Allyl Isothiocyanate/Poly(lactic Acid) for the Enhancement of Long-Term Controlled Release. *Materials* **2017**, *10*, 1210. [[CrossRef](#)] [[PubMed](#)]
24. Kong, P.; Abe, J.P.; Masuo, S.; Enomae, T. Preparation and characterization of tea tree oil- β -cyclodextrin microcapsules with super-high encapsulation efficiency. *J. Bioresour. Bioprod.* **2023**, *8*, 224–234. [[CrossRef](#)]

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