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

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S1 Supplementary Methods

S1.1 Control Experiments for β-CD

To investigate the potential self-crystallization of the host matrix, control experiments were performed using pure β-cyclodextrin (β-CD) without the addition of the guest molecule (AITC). Ethanol–water mixtures were prepared at volume ratios of 1:1.5, 1:2.0, 1:2.5, and 1:3.0 (total volume: 50 mL). β-CD (3.0 g) was added to each solvent system and stirred at 60 °C (700 rpm) until complete dissolution. The solutions were then cooled to 30 °C and maintained under stirring for 2 h, matching the synthesis conditions of the inclusion complexes. The mixtures were subsequently left to stand for 24 h at room temperature, and the visual appearance of the solutions was recorded to detect any precipitation or turbidity.

S1.2 Antimicrobial Assay against Natural Spoilage Microorganisms

The antimicrobial potency of the AITC@β-CD inclusion complexes was evaluated using a natural microbial suspension derived from spoiled fruits to assess their functional performance in a simulated food spoilage environment. Four experimental groups were established: a blank control (CK), pristine β-cyclodextrin (CD), free AITC (AITC), and (4) AITC@β-CD inclusion complexes (AITC@β-CD). The antimicrobial efficacy was determined using the plate counting method. Briefly, 15 mL of sterile Tryptic Soy Agar (TSA) was poured into 96 mm Petri dishes. Upon solidification, the natural microbial suspension was uniformly spread onto the agar surface. The treatments were applied as follows: 20 μL of PBS buffer for the CK group, 0.03 g of β-CD for the CD group, 20 μL of pure AITC for the AITC group, and 0.03 g of AITC@β-CD complexes for the AITC@β-CD group. To prevent the escape of volatile components, all dishes were sealed with parafilm and subsequently incubated at 37 °C for 24 h. Following incubation, the microorganisms from each plate were collected, serially diluted, and inoculated onto fresh agar plates. The colony-forming units (CFU/mL) were recorded after further incubation to quantify the inhibitory effect of each treatment. All operations were performed in a laminar flow hood (CCV-811, Hitachi, Japan) to ensure aseptic conditions.

S2 Supplementary Results

S2.1 Control Experiments: Solubility of Pure β-CD

To verify that the formation of the solid precipitate was exclusively driven by guest-induced co-crystallization rather than the self-crystallization of the host, control experiments were conducted. Pure β-CD (3.0 g) was dissolved in ethanol-water mixtures at the same ratios used for complex

preparation (1:1.5, 1:2.0, 1:2.5, and 1:3.0) under identical conditions (stirring at 60 °C, then cooling to 30 °C), but **without the addition of AITC**.

As shown in **Figure S1**, pure β-CD remained completely dissolved in the co-solvent systems across all tested ratios. The solutions appeared clear and transparent, with no visible precipitation or turbidity observed even after standing for 24 h. This result confirms that the rhombohedral particles obtained in the experimental groups are true inclusion complexes formed by the interaction between AITC and β-CD.

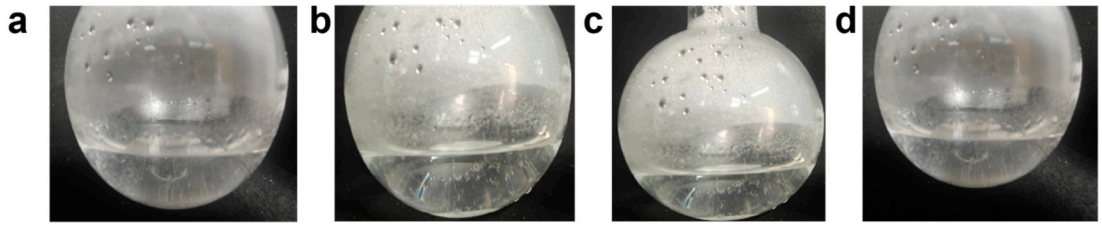


Figure S1. Digital photographs of control experiments showing pure β-CD dissolved in ethanol-water mixtures at different volume ratios: (a) 1:1.5, (b) 1:2.0, (c) 1:2.5, and (d) 1:3.0. All solutions remained clear and transparent without the addition of AITC, confirming that no host self-crystallization occurred under the experimental conditions used for complex preparation.

S2.2 Antimicrobial Activity against Natural Spoilage Microorganisms

To evaluate the broad-spectrum applicability of the inclusion complexes in a simulated practical environment, antibacterial assays were conducted against a natural microbial suspension derived from spoiled fruits.

Table S1 and **Figure S2** summarize the quantitative inhibitory effects. The blank control (CK) exhibited a high total plate count of 4.4×10^6 CFU/mL after 24 h of incubation. The pure β-CD group (CD) showed a slightly higher count (7.6×10^6 CFU/mL), suggesting the host matrix itself lacks antimicrobial activity. In contrast, both the free AITC group and the AITC@β-CD inclusion complexes group showed a sharp reduction in microbial growth, with counts decreasing to 2.2×10^3 CFU/mL and 2.8×10^3 CFU/mL, respectively. This represents an inhibition rate of over 99.9%.

These results demonstrate that the AITC@β-CD inclusion complexes retain strong broad-spectrum bioactivity against indigenous food spoilage flora, comparable to that of free AITC.

Table S1. Total Plate Count under different treatments.

Treatments	Total Plate Count (CFU/ml)
CK Group	4.4×10^6
CD Group	7.6×10^6
AITC Group	2.2×10^3
AITC@β-CD Group	2.8×10^3

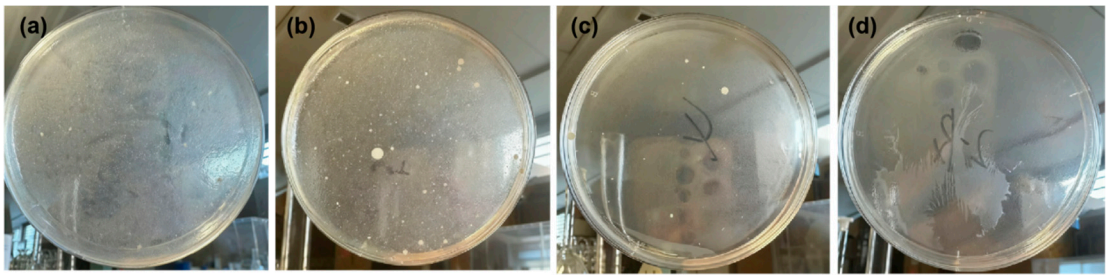


Figure S2. Growth inhibitory effects of blank control (CK) (a), β-CD (b), AITC (c), and AITC@β-CD inclusion complex (d) against natural fruit spoilage microorganisms after incubation at 37 °C for 24 h.