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Carvacrol/β-cyclodextrin inclusion complex as a fumigant to control decay caused by *Penicillium digitatum* on *Shatangju* mandarin slices

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ABSTRACT

Preservation and microorganism control of fresh-cut fruit pose a persistent challenge in the food industry. To address this issue, we prepared a β-cyclodextrin (β-CD) inclusion complex containing carvacrol using a coprecipitation method and employed it for the non-contact fumigation of fresh-cut *Shatangju* mandarin slices. This biodegradable and safe preservative offers an effective means to combat spoilage and ensure product quality. We confirmed the formation of the encapsulated structure of the inclusion complex through various characterization methods, including scanning electron microscopy (SEM), Fourier transform-infrared spectroscopy (FTIR), X-ray diffraction (XRD), thermogravimetric analysis (TGA), and differential thermal analysis (DTA). We also demonstrated the inhibitory effect of this preservative on *Penicillium digitatum* and its associated spoilage both *in vitro* and *in vivo*. The incidence and severity were significant lower in the inclusion complex-treated group (75.0% and 46.7%, respectively) compared to the group treated with pure carvacrol (100% and 69.2%, respectively). In addition, fruit freshness parameters and sensory evaluation showed that the inclusion complex treatment effectively maintained the overall quality of the fruit and achieved the highest consumer acceptance.

1. Introduction

Citrus fruits have received much attention due to their high contents of vitamin C and other bioactive compounds including flavonoids and phenolic acids [1]. *Shatangju* is a very popular native citrus fruit in China. During harvesting, handling, packaging, transportation, storage, and marketing, *Shatangju* is easily damaged by both mechanical and microbial factors [2]. Green mold caused by *Penicillium digitatum* is one of the most destructive diseases of citrus fruits after harvest [3,4]. Green mold infection initiates in the citrus peel, however, for salads, cake topping or other fresh-cut fruits that are intended for immediate consumption, madarins are frequently processed or stored as slices. Without the protection of peel, mold can directly contaminate these slices at a rapid pace.

Therefore, more effective and suitable preservation techniques for citrus should be developed to inhibit the growth of fungi while maintaining fruit quality. In the past two decades, various preservation methods including edible coating [5], modified atmosphere

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packaging [6], and irradiation treatment [7] have been developed for fresh fruits. As a food contact material, edible coating is still limited due to its manufacturing cost, packaging performance, and safety concerns [8]. The modified atmosphere packaging can effectively regulate the respiration of fruits and vegetables after harvest, but it is not effective in controlling microorganisms. However, the current protocol of inhibiting microorganisms by high concentration of carbon dioxide often brings about serious cytotoxicity [9]. Radiation treatment may result in significant reduction or disappearance of nutrients, particularly thiamine, vitamin E and vitamin C [10]. Therefore, the preservation of food is eager for a simple, efficient, safe and inexpensive technology. Among them, β-cyclodextrin-based inclusion complexes containing essential oils (EOs) as a noncontact preservative may be a suitable choice for fruit preservation, providing safe, stable, and efficient antimicrobial activity while maintaining the natural texture and original flavor of the fruit.

EOs are a class of highly hydrophobic and volatile multi-component mixtures consisting mainly of low molecular weight compounds (e.g., terpenoid, phenolic-derived compositions, and other aliphatic compositions) [11]. EOs have been shown to be powerful natural antimicrobial agents against a wide variety of foodborne pathogens [12], and many EOs have been granted generally recognized as safe (GRAS) status by the FDA [13]. Carvacrol (5-isopropyl-2-methylphenol), a natural component of plant essential oils extracted from oregano, has been widely used in antimicrobial and antioxidant food preservation applications [14]. However, its unpleasant odor, volatility, thermal instability, and low solubility severely limit its application in food processing and storage [15].

Beta-cyclodextrin (β-CD) is an oligosaccharide consisting of seven glucose molecules with a hydrophobic conical cavity where guest molecules can be embedded to form inclusion complexes [16]. The inclusion complexes not only improve the guests' solubility and physical chemistry stability but also mask their unpleasant odor [17]. This phenomenon can be attributed to the inherent hydrophobic cavity of cyclodextrin, which forms non-covalent interactions with the hydrophobic small molecules or moieties present in essential oils, consequently retarding the release of essential oil molecules [18]. Moreover, after encapsulation with cyclodextrin, guest molecules have been shown to have the same or better activity as the pure molecules. For example, compared with unencapsulated tea tree oil, the hydroxypropyl-β-cyclodextrin inclusion complexes of tea tree oil showed better stability and longer-lasting antifungal activity, and inhibited the growth of *Monilinia fructicola in vitro* and in peach fruit [19]. Another study found that *trans*-2-hexenal/β-CD inclusion complexes had a considerable inhibitory effect on the mycelial growth of *P. digitatum*, which could significantly reduce the incidence of green mold in citrus fruits inoculated with *P. digitatum* while maintaining fruit quality [20]. In our previous studies on strawberries, *p*-anisaldadyde/β-CD inclusion complexes were prepared and used to provide long-term antifungal activity as a fumigant [21,22]. By using the properties of these inclusion complex to slow release guest active substance, we hope to employ them as a non-contact fumigant for modified atmosphere packaging or controlled atmosphere packaging, providing a continuous and stable antimicrobial environment with low concentration levels to extend the shelf life of food. Thus, the current study aims to provide a β-CD-based inclusion containing carvacrol, which can inhibit the fungi growth and maintain the freshness of *Shatangju* slices. In this study, we prepared and characterized the carvacrol/β-CD inclusion complex, and then determined its antifungal activity on *P. digitatum in vitro* and its effects on controlling the postharvest decay caused by *P. digitatum* in *Shatangju* slices when applied as a fumigant. To further assess its potential as a food preservative, the influence of the complex on fruit quality and consumer acceptance was also evaluated.

2. Materials and methods

2.1. Materials

Carvacrol (98%, CAS Registry Number 123-11-5) was purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). β-cyclodextrin (β-CD, CAS Registry Number 68168-23-0) and absolute ethanol were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All the chemical reagents were of analytical grade. *Shatangju* fruit were harvested from a local orchard in Zhuhai, China. Fruit selected for the experiments were uniform in size, shape, and maturity, and without any physical injuries or disease symptoms. *P. digitatum* was isolated from the harvested diseased *Shatangju* fruit and identified by morphological characterization. Cultures of the microorganisms were maintained on potato dextrose agar (PDA) media.

2.2. Preparation of inclusion complexes

The carvacrol/β-CD inclusion complexes were prepared according to the ultrasound/microwave-assisted co-precipitation method described in a previous study with slight modifications [22]. Firstly, an aqueous solution of β-CD at a concentration of 0.18 mol/L was stirred at 50 °C. Next, 2.5 g of carvacrol was dissolved in ethanol solution and added to the β-CD solution to ensure that the molar ratio of β-CD to carvacrol was approximately 1:1. For control treatments containing β-CD and carvacrol in their non-complexed form, this "physical mixture" was used with no further processing. To produce the inclusion complexes, the blended solution was treated by a microwave-ultrasonic combined synthesis extraction instrument (XH-100A, Xianghu, China) for 3 h. During the treatment, the powers of microwave and ultrasound were set to 200 and 500 W, respectively, and the sample temperature was controlled at 50 ± 5 °C by a fan cooling system. The treated solution was then placed in a refrigerator at 4 °C overnight for precipitation. The precipitated inclusion complexes were obtained by suction filtration and dried in an oven at 50 °C for 24 h.

2.3. Physical properties and characterization of the inclusion complexes

2.3.1. Scanning electron microscopy (SEM)

The solid samples were coated with gold and the micro-morphology was observed using a scanning electron microscope (SEM, JSM-6510, JEOL, Japan). The acceleration voltage was 5 kV and the magnification was 500×.

2.3.2. Particle size

Particle size analysis was determined using a laser diffraction particle size analyzer (LS13320, Beckman Coulter, USA). The average size based on the unit weight of particles ($D_{4,3}$) and average size based on the specific surface per unit volume ($D_{3,2}$) were measured and the particle size distribution of the inclusion complexes was calculated according to the following equation:

$$Span = \frac{D_{90} - D_{10}}{D_{50}} \times 100\% \quad (1)$$

where D_{90}, D_{50}, and D_{10} are the volume diameters at 90%, 50%, and 10% of the cumulative volume, respectively.

2.3.3. Entrapment efficiency (EE)

The amount of carvacrol entrapped in the inclusion complexes was determined according to a previous study with minor modifications [23]. First, 0.1 g of the obtained inclusion complexes was dissolved in 25 mL of ethanol and homogenized for 30 min. Then, an appropriate amount of supernatant was transferred from the centrifuge tube into a 50 mL volumetric flask, filtered, and diluted to the mark with absolute ethanol. Ethanol was used as a blank sample. The absorbance of the solutions was measured at 275.8 nm using an ultraviolet spectrophotometer (UV-2450, Shimadzu, Japan) and the values were converted to concentrations by reference to a carvacrol standard curve. The *EE* of the inclusion complexes was calculated using the following equation:

$$EE = \frac{\text{Amount of active compound entrapped}}{\text{Initial amount of active compound}} \times 100\% \quad (2)$$

2.3.4. Fourier transform-infrared spectroscopy (FTIR)

FTIR spectroscopy of the inclusion complexes was conducted using a spectrometer (Prestige-21, Shimadzu, Japan) in the wavenumber range of 4000 to 400 cm^{-1} at 4 cm^{-1} resolutions.

2.3.5. X-ray diffractometry (XRD)

The XRD patterns of the samples (β-CD, physical mixture, and the inclusion complexes) were collected using an X-ray diffractometer (PANalytical, Philips, Netherlands). The samples were scanned at the range of (2θ) 5–35° with a scanning rate of 1.5° min^{-1} operating at 40 kV and 30 mA.

2.3.6. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA)

A TA instrument (DTG-60H, Shimadzu, Japan) was used to carry out TGA and DTA. Operating under a dynamic nitrogen atmosphere (40 mL min^{-1}), the heating rate was 10 °C min^{-1} and the temperature range was 30–800 °C.

2.4. In vitro antifungal activity

The *in vitro* antifungal activity of the inclusion complexes was determined by observing the growth of fungal colonies on potato dextrose agar (PDA) media under different conditions. Firstly, 0.5 g of β-CD, 0.07g of carvacrol, or 0.5 g of inclusion complex (containing 0.07 g of carvacrol) was added into the unsolidified PDA media. In addition, a media-only control group was set up. After being gently homogenized, the media were left standing at approximately 25 °C until solidified. Then, three 9 mm-diameter PDA plugs with mycelia cut randomly from the edge of a vigorous *P. digitatum* colony were placed upside down on each PDA medium. The inoculated media were incubated at 25 °C for 48 h before further observation.

2.5. Decay assessment

The *in vivo* antifungal activity of the inclusion complexes was evaluated by investigating the incidence and severity of rot induced by *P. digitatum*. *Shatangju* fruits were peeled in a sterile environment and separated into slices for use. For each treatment, 30 slices were randomly divided into five replicates, thus providing six slices for each replicate, which were placed in a 0.75 L sealed container. For different treatments, semi-permeable sachets (3 × 3 cm) containing 1.0 g of β-CD, 0.13 g of carvacrol, or 1.0 g of inclusion complex (containing 0.13 g of carvacrol) were placed in the center of the container. For the control group, the sachet was excluded. All containers were incubated at room temperature (25 °C) to allow fungal growth. Both the incidence and severity were assessed daily during the following seven days. The severity of citrus slice decay was assessed using a 5-point scale according to a previous study with some modifications [24], which defined 0–5 as 0%, 1–25%, 26–50%, 51–75%, and 76–100% of the surface covered with mycelia, respectively. The incidence and severity were calculated by equations (3) and (4), respectively [25]:

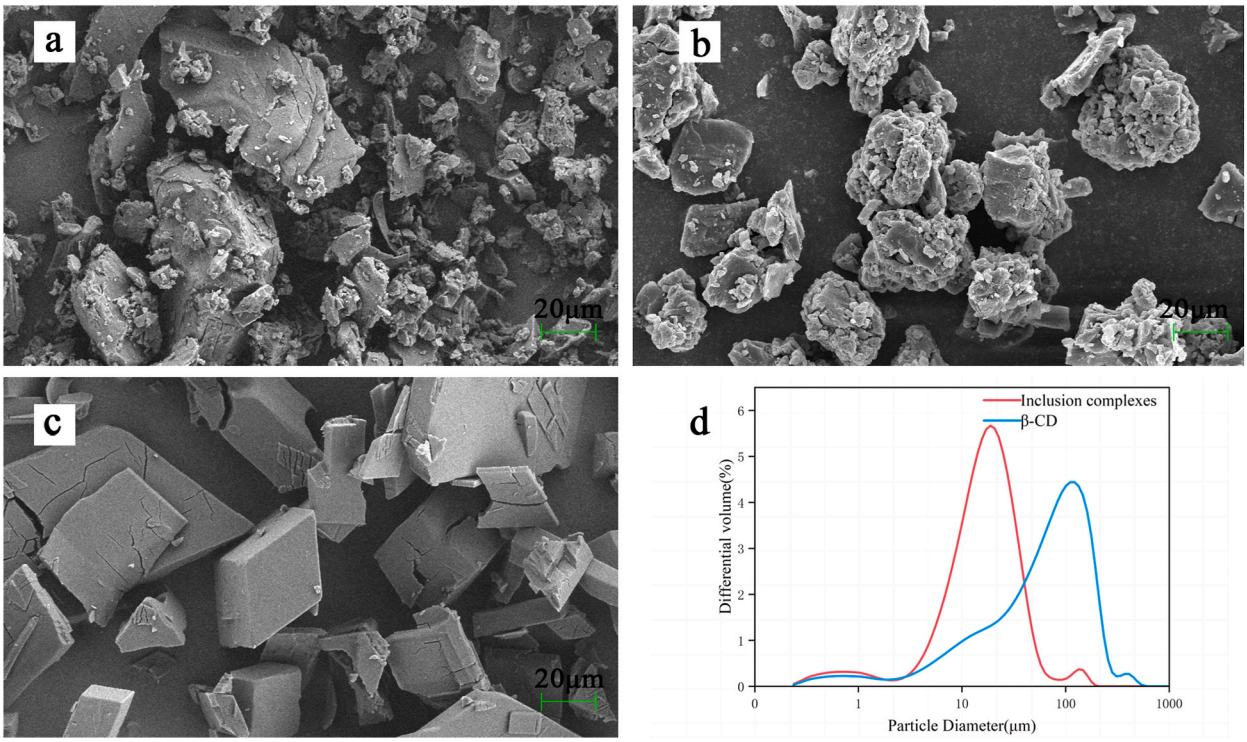

Fig. 1. Scanning Electron Microscopy (SEM) images of raw β-CD particles (a), carvacrol/β-CD physical mixture (b), carvacrol/β-CD inclusion complexes (c), and particle sizes of β-CD and inclusion complexes (d).

$$\text{Incidence } (\%) = \frac{n_1 + n_2 + n_3 + n_4}{n} \times 100 \tag{3}$$

$$\text{Severity } (\%) = \frac{1n_1 + 2n_2 + 3n_3 + 4n_4}{4n} \times 100 \tag{4}$$

where n_1–n_4 are the numbers of fruit in categories 1 to 4 of the disease scale and n is the total number assessed in each replicate.

2.6. Quality evaluation

For the fruit quality assessment, 504 *Shatangju* slices without any decay lesions were randomly divided into four groups with 126 slices in each group and were subjected to different treatments (control, β-CD, carvacrol, or inclusion complexes). For each treatment, the *Shatangju* slices were arranged into 21 0.75 L sealed containers with 6 slices in each. The arrangement of sachets was the same as that in the decay assessment described above. The weight loss and TSS analysis were measured at 25 °C for six consecutive days. The weight loss was determined by weighing the total weight of the same 18 orange slices selected from each group at each time interval and expressed as a percentage compared with the initial total weight. After the weight loss test, the slices were pressed and homogenized into juice for TSS analysis. The TSS values were measured using a PAL-1 pocket refractometer (Atago, Japan) and expressed as a percentage.

2.7. Sensory analysis

The sensory analysis was carried out based on ISO 11035:1994.432 *Shatangju* slices were divided into four groups and treated with different treatments (control, β-CD, carvacrol, or inclusion complexes) using the method described in the previous section. Samples from each group were tasted and scored daily by six assessors during a three-day evaluation period. Sensory quality indicators—appearance, smell, flavor, mouthfeel and texture—were rated on a 10-point scale. The overall acceptance of each treatment was calculated by averaging the scores of all the indicators.

2.8. Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) using SPSS software (ver. 17.0; Experian QAS, Boston, MA, USA). Duncan's multiple range test was used to determine the differences in treatment means ($p = 0.05$). For each sample, at least

Table 1
Encapsulation efficiency (EE) and particle size of β-CD and inclusion complexes.

	EE (%)	Span	$D_{4,3}$ (μm)	$D_{3,2}$ (μm)
β-CD	/	2.33 ± 0.01[a]	83.66 ± 3.03[a]	14.69 ± 0.19[a]
Inclusion complexes	83.71 ± 3.63	1.87 ± 0.10[b]	22.05 ± 0.44[b]	8.70 ± 0.46[b]

[a,b] different letters indicate a significant difference between the values in each column.

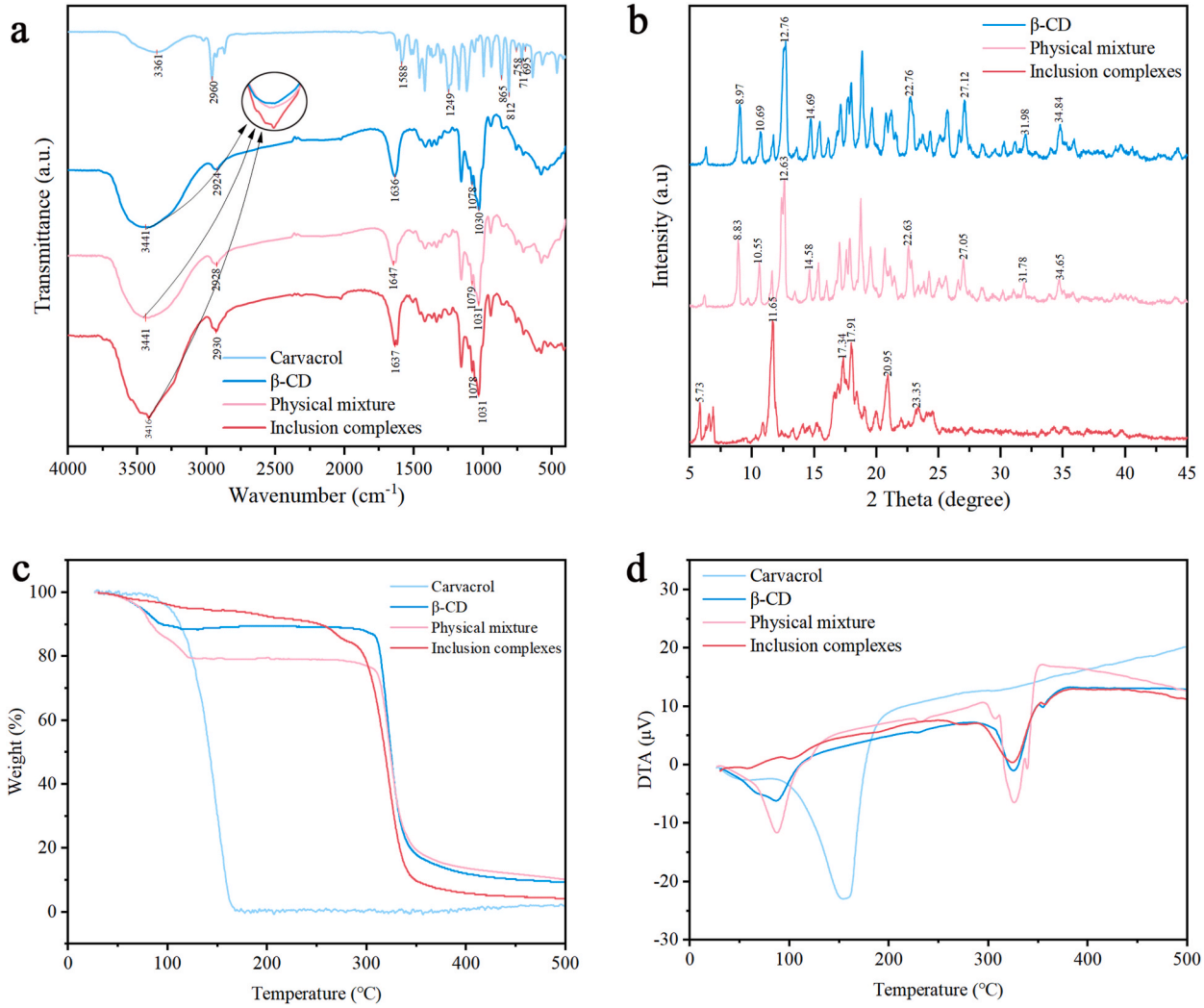

Fig. 2. FT-IR (a), XRD (b), TGA (c), and DTA (d) curves of β-CD, carvacrol, physical mixture, and inclusion complexes.

three replicates were used.

3. Results and discussion

3.1. Physical properties and characterization of the inclusion complexes

3.1.1. Scanning electron microscopy (SEM)

SEM images of raw β-CD, a physical mixture of carvacrol and β-CD, and carvacrol/β-CD inclusion complexes are shown in Fig. 1a–c, respectively. The images of β-CD and the physical mixture are similar, with rough surfaces and irregular shapes. This indicated that the simple mixture of carvacrol and β-CD did not form inclusion complexes, and the free carvacrol evaporates in the vacuum under the scanning electron microscope. However, the morphology of inclusion complexes is completely different from that of β-CD and the physical mixture; the shape changes from irregular to a square diamond with a smooth surface, suggesting that an interaction between

the host and guest molecules has occurred, changing the polymerization state of the particles. The same phenomenon was reported in previous studies, in which carvacrol was inserted into the cyclodextrin cavity to form inclusion complexes by a co-precipitation method, and similar micromorphology was observed by SEM [26]. Similar rhomboid-shaped crystals were also observed in SEM images of β-CD inclusion complexes containing clove essential oils [27].

3.1.2. Particle size

The curves and data of particle size distribution for β-CD and inclusion complexes are presented in Fig. 1d and Table 1. Compared with the original β-CD particles, the values of $D_{4,3}$ and $D_{3,2}$ of inclusion complexes decreased significantly after encapsulation, which was caused by the interaction between carvacrol and cyclodextrin molecules [28]. In the process of preparing inclusion complexes by co-precipitation, dissolution, encapsulation, and recrystallization all lead to the reduction of particle size. Moreover, the span index of the inclusion complexes (1.87) is significantly smaller than that of β-CD (2.33), reflecting the more regular shape and more concentrated size distribution of the inclusion complexes. A similar span index of the inclusion complexes (1.83) was reported in a previous study on β-CD containing encapsulated lemongrass oil [29].

3.1.3. Entrapment efficiency (EE)

EE is a quantitative parameter that indicates the content of active compounds in inclusion complexes and is a precise method to evaluate the solubilization effect of cyclodextrin. As shown in Table 1, the *EE* of carvacrol/β-CD inclusion complexes in this study was 83.71%, which was consistent with most reported values. A previous study reported that the *EE* of carvacrol/β-CD inclusion complexes obtained by a freeze-drying method is 81.20% [30].

3.1.4. Fourier transform-infrared spectroscopy (FTIR)

The chemical structure of β-CD and its changes after encapsulation can be represented by FTIR spectra [31]. As shown in Fig. 2a, the spectrum of carvacrol presents all the characteristic peaks of carvacrol including the vibration of C═C stretching from the aromatic ring (peaks around 1588 cm^{-1}), the stretching vibration of O–H (3361 cm^{-1}), the stretching vibration of the aromatic hydroxyl group (1249 cm^{-1}), the asymmetric stretching vibration of C–H (2960 cm^{-1}), and the ring C–H bending vibration (900–650 cm^{-1}) [32,33]. The spectrum of β-CD was characterized by absorption bands of symmetrical and asymmetrical stretching of –OH groups (around 3441 cm^{-1}), C–H stretching (2924 cm^{-1}), H–O–H bending (1630 cm^{-1}), and asymmetric and symmetric stretching of the C–O–C (1079 cm^{-1} and 1031 cm^{-1}) [34–36]. The spectra of the physical mixture showed only a simple superposition of the β-CD and carvacrol molecular characteristics without any change in peaks, indicating no interaction between these two molecules. In contrast, due to the influence of guest molecules, the spectrum of the inclusion complex showed obvious changes compared with empty β-CD: the stretching vibration of O–H shifted from 3441 to 3416, and the intensity also weakened.

3.1.5. X-ray diffractometry (XRD)

XRD is widely used to study cyclodextrin complexation in powder or microcrystalline states [37]. In the XRD patterns of the β-CD sample (Fig. 2b), the characteristic peaks at 8.97°, 10.69°, 12.76°, 14.69°, 22.76°, 27.12°, 31.98°, and 34.84° demonstrate the crystalline state of the β-CD particles [38]. The characteristic peaks of the physical mixture did not change much compared to those of β-CD, suggesting that no new crystal structure was formed. However, compared with β-CD, the inclusion complexes lost the characteristic peaks at 8.97°, 12.76°, 31.98°, and 34.84°, and formed new peaks at 5.73°, 11.65°, 17.34°, 17.91°, 20.95°, and 23.35°. It is well known that the formation of diffuse diffraction patterns, appearance of new peaks, and disappearance of characteristic peaks are all evidence of the formation of inclusion complexes [39]. These peak changes can also be attributed to the interactions between the host and guest components forming new solid crystalline phases in inclusion complexes.

3.1.6. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA)

TGA and DTA have been frequently used to verify the formation of inclusion complexes. In the TGA curves (Fig. 2c), the weight of the samples began to decrease to different degrees above 103.5 °C. Among these samples, the weight loss rates of β-CD (10.83%) and the physical mixture (14.79%) were much greater than that of the inclusion complexes (3.89%), due to the higher water content of β-CD and the physical mixture. Some studies have revealed that the cavity of β-CD is occupied by water molecules before the new hydrophobic molecules are encapsulated, and the temperature of 103.5 °C is sufficient to vaporize this portion of water [40], resulting in a significant decrease in overall weight [26]. This is also confirmed by the DTA results (Fig. 2d), which showed an endothermic peak near 103.5 °C in both the β-CD and physical mixture curves, but not in the inclusion complexes curve. When examining cyclodextrin complexes using DTA, the absence of characteristic guest molecule thermal events is generally taken as proof of real inclusion [26]. Due to the natural volatility of carvacrol, its TGA curve presents a particular process, with a sharp decline of 100% before 168.8 °C, while its DTA curve demonstrates a corresponding endothermal peak at 155 °C, which can be regarded as its boiling point. In the range of 120–300 °C, the weight loss rate of inclusion complexes is considerably slower than that of β-CD and the physical mixture. This is because encapsulation significantly improves the thermal stability of carvacrol, which makes it less likely to evaporate or decompose due to heat. The three samples containing β-CD exhibited an acute mass loss of approximately 70% below 335°C—the stage at which β-CD decomposition occurs [41]. This feature is also demonstrated by the endothermic peak near 335 °C in the DTA curve (Fig. 2d). Above 400 °C, the three β-CD-containing samples entered the final stage of cyclodextrin carbonization, and the elemental carbon was slowly released [40]. All these results proved the formation of the inclusion complex and the improvement of thermal stability of volatile compounds by encapsulation.

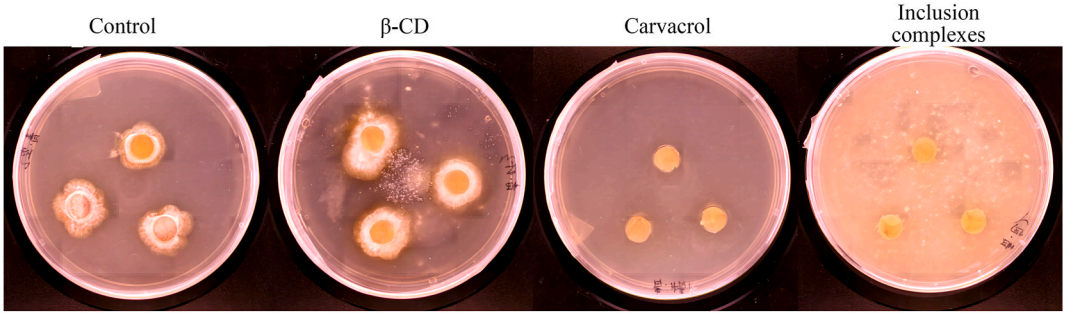

Fig. 3. Growth of *P. digitatum* mycelia on potato dextrose agar (PDA) media with different additives after incubation at 25 °C for 48 h.

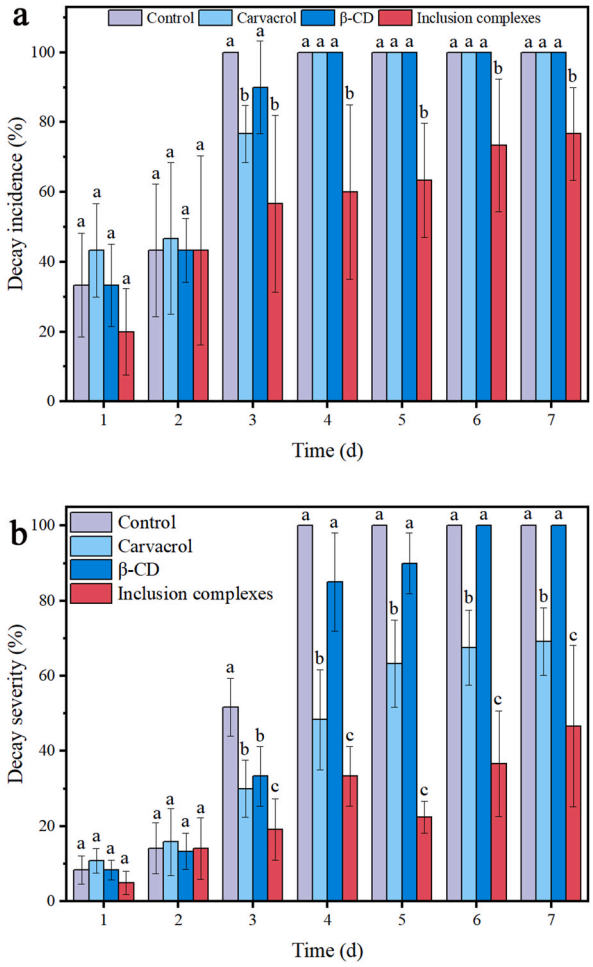

Fig. 4. The impact of different treatments on the incidence (a) and severity (b) of the decay of *Shatangju* slices during storage at room temperature (25 °C). Different letters (a–c) above the columns indicate significant differences among the treatments based on Duncan's multiple range test ($p < 0.05$).

3.2. *In vitro antifungal activity*

As illustrated in Fig. 3, mycelium growth was observed surrounding the plugs in both the β-CD and control groups, whereas no mycelium was detected in either carvacrol or inclusion complex groups. Several studies have demonstrated that β-CD may serve as a potential carbon source, providing nutrients for fungi, rather than inhibiting their growth [31]. PDAs supplemented with carvacrol and inclusion complexes exhibited favorable antifungal properties and no new mycelium was found on them. This result suggests that the inclusion complex exhibits inhibitory effects against *P. digitatum* mycelial growth that are equivalent to pure carvacrol. As an active

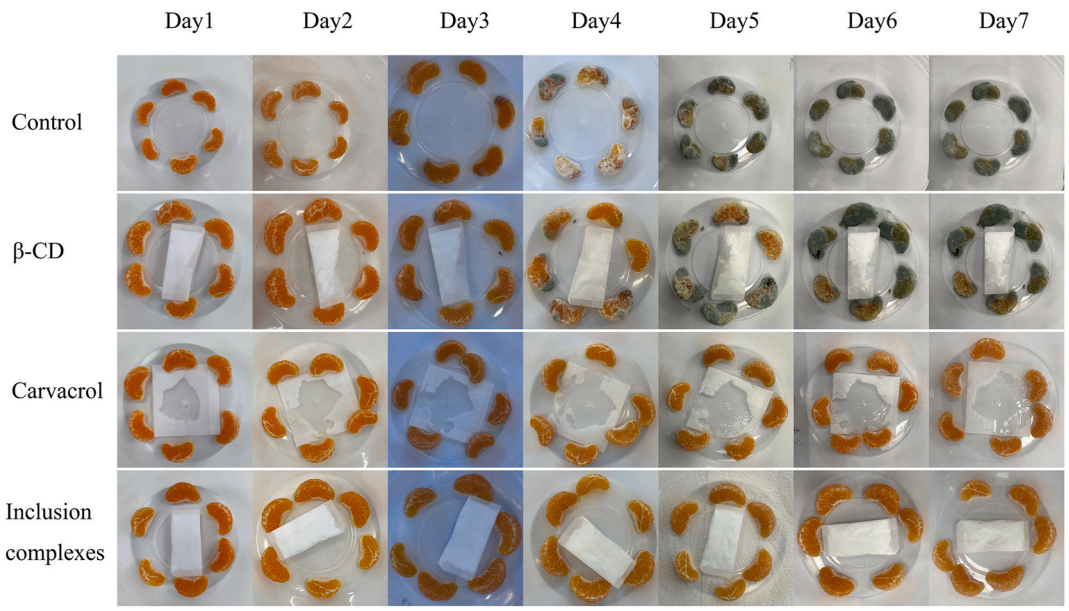

Fig. 5. Time-lapse images of *Shatangju* slices under different treatment conditions at 25 °C.

compound in essential oils, carvacrol can inhibit fungal growth by damaging the fungal envelope, disrupting membrane integrity, and blocking ergosterol biosynthesis [42]. Encapsulation by β-CD not only did not affect the antifungal activity but also improved the solubility of hydrophobic guest molecules. A study on β-CD inclusion complexes of *Litsea cubeba* essential oil also reported that the diffusion of the inclusion in PDA inhibited the mycelial growth of *P. digitatum* to some extent [43].

3.3. Decay assessment

The incidence and severity of citrus spoilage were investigated daily during the seven-day storage period. As shown in Fig. 4, there was no significant difference in incidence and severity in the first two days among the four treatment groups. However, the incidence in the control, cyclodextrin-treated and carvacrol-treated groups reached 100% by day 4, and the incidence in the inclusion complex group was significantly lower ($p < 0.05$), reaching only 75% by day 7. In their degree of disease severity, the control and β-CD-treated groups were higher at each time point than those of the carvacrol-treated and inclusion complex-treated groups. In these latter two groups, a significant difference in severity began to emerge from day 3 ($p < 0.05$). Among the four groups, the inclusion complex-treated group maintained the lowest level of severity within the storage duration, reaching only 46.7% on day 7, while the control, β-CD, and carvacrol groups reached 100%, 100%, and 69.2%, respectively. The performance of β-CD-treated group indicates that it not only fails to inhibit the disease, but also as some study reported, serves as a carbon source for microorganisms and contributes to their growth [21]. The use of β-CD inclusion can significantly improve the thermal stability of such volatile agents, thereby preventing their rapid volatilization and achieving a long-term release antimicrobial effect. This inclusion property is utilized as a fumigant for disease control, which not only extends its effective duration, but also mitigates food damage caused by excessive instantaneous concentration [22]. Based on the design of cyclodextrins and plant-derived natural antimicrobials, many studies have produced similar results [43–45]. This demonstrates that this synthesis is a potentially effective antimicrobial approach for postharvest diseases control. Images of slices in storage (Fig. 5) also reflect these trends. The slices treated with inclusion complex exhibited the best appearance over the seven days. For the carvacrol-treated group, slices without fungal growth were selected to be photographed, and slight skin lesions or burns were observed on these slices. It has been reported that pure essential oils are highly volatile and reductive, and that high concentrations of essential oils can burn the skin of fruits and the taste can be unpleasant for consumers [5].

3.4. Quality evaluation

During fruit storage, weight loss is an important indicator for assessing fruit freshness. The primary cause of weight loss in fruit is the evaporation of water through the peel, skin, or rind, particularly via pores and wounds. For the intact slices, the main cause of weight loss is evaporation of water through the carpellary membrane of the slices. Fig. 6a illustrates a consistent increase in weight loss across all treatment groups during storage. On days 1–5, the control group exhibited a greater weight loss compared to the other three treatment groups (β-CD, carvacrol, and inclusion complexes) ($p < 0.05$). These findings suggest that the release of carvacrol from inclusion complexes can effectively mitigate the weight loss of *Shatangju* slices during storage. A similar study has also demonstrated that active cardboard trays coated with β-CD encapsulating EOs (carvacrol, oregano, and cinnamon) can effectively reduce weight loss and maintain the quality of cherry tomatoes [46].

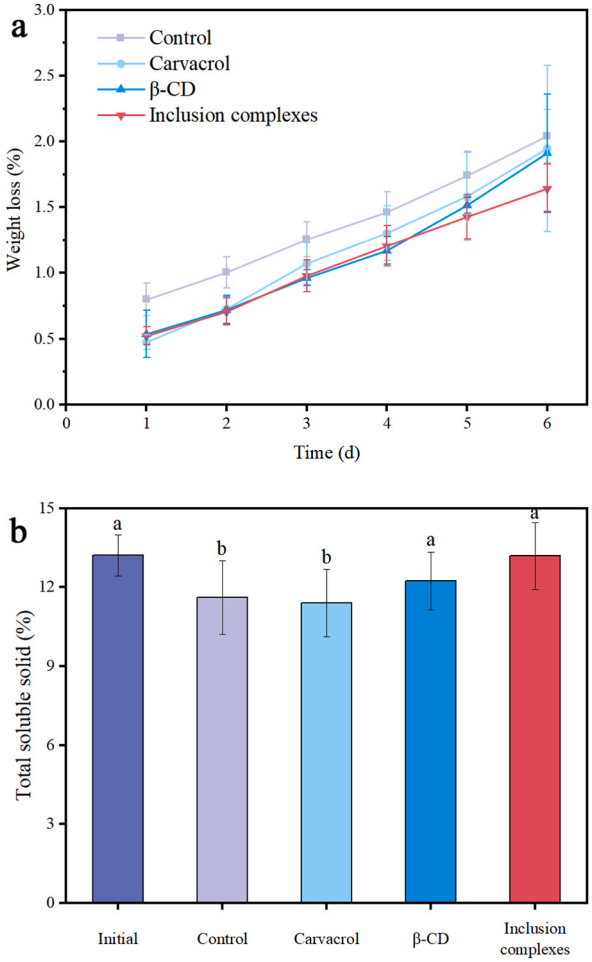

Fig. 6. Effects of different treatments on weight loss (a) and total soluble solid (b) of *Shatangju* after six days of storage at 25 °C. Different letters (a and b) above the columns indicate significant differences among the treatments based on Duncan's multiple range test ($p < 0.05$).

The sugar content of fresh fruit is crucial for maintaining its respiration and metabolism. However, it is inevitable that the TSS gradually decreases during postharvest storage [47]. As demonstrated in Fig. 6b, the TSS of the inclusion complex-treated sample did not exhibit a significant decrease compared to the initial value ($p \geq 0.05$), whereas that of both control and carvacrol groups showed a significant reduction ($p < 0.05$). In a study investigating the effects of *trans*-2-hexenal/β-CD inclusion complexes on citrus fruits, no significant differences in TSS were observed among various treatments during five-day storage [20]. Similarly, thymol/β-CD inclusion complexes did not significantly affect TSS levels in treated citrus fruit [48].

3.5. Sensory analysis

Prior to the application of a new preservative, it is imperative to ascertain whether its implementation will have an impact on consumers' affective responses and purchase intentions. A sensory panel was used to assess the impact of inclusion complexes on the appearance, smell, flavor, mouthfeel, and texture of the fruit. The star map of sensory indicators in the first three days of the experiment is shown in Fig. 7. Most of the sensory indicators and the overall acceptability score of the inclusion complex-treated samples were comparable to those of control samples. However, the fruit treated with carvacrol exhibited the lowest overall acceptability during the 3-day period. Due to its high volatility, the carvacrol-treated samples received the lowest score for odor on days 1 and 2. This indicates that a too-high concentration of carvacrol has a negative effect on fresh fruits, while volatilized carvacrol has a slight impact on fresh citrus. However, the application of inclusion complexes to *Shatangju* slices resulted in a higher score compared to pure carvacrol, indicating that β-CD encapsulation can effectively mask the unpleasant odor of carvacrol.

4. Conclusion and prospect

This study describes the production of inclusion complex particles composed of β-CD and carvacrol. The successful formation of

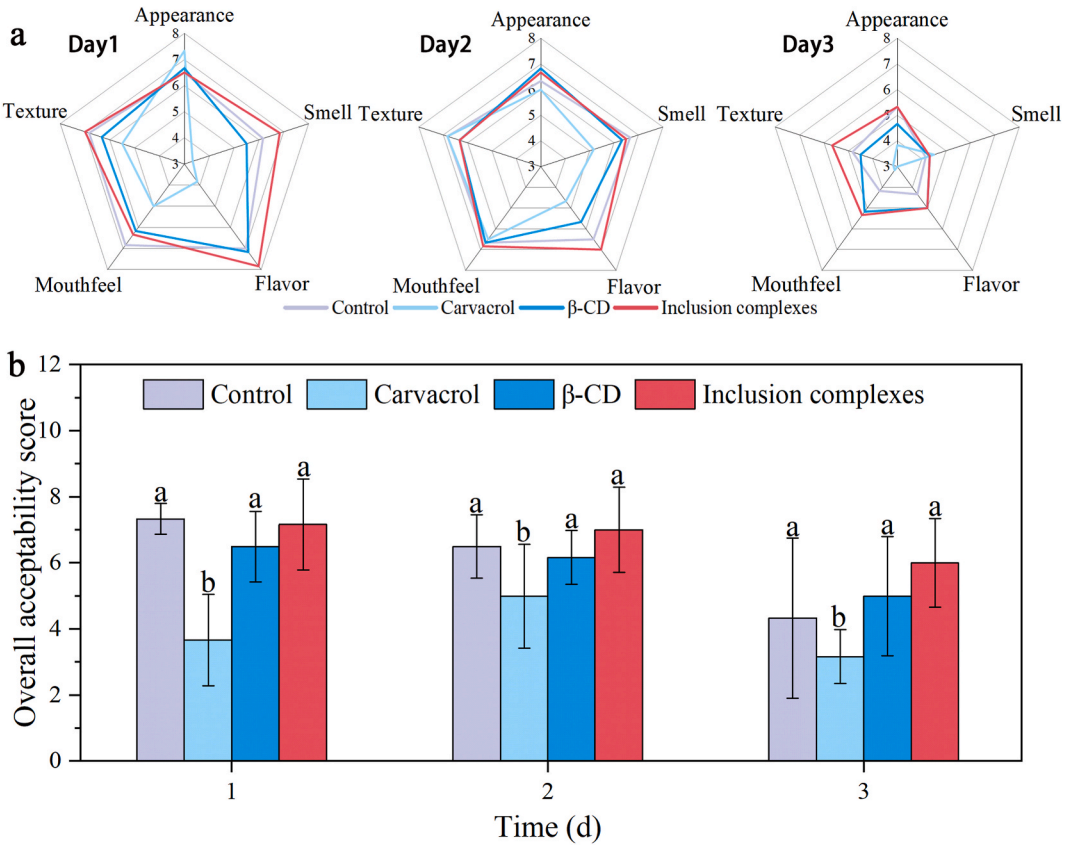

Fig. 7. Sensory characteristics scales (a) and general acceptability scores (b) of the *Shatangju* from different groups on days one to three post-treatment. Different letters (a and b) above the columns indicate significant differences among the treatments based on Duncan's multiple range test ($p < 0.05$).

inclusion complexes was demonstrated by a series of material characterization techniques, including electron microscopy, particle size distribution analysis, FTIR, XRD, TGA, and DTA. The inclusion complexes exhibited *in vitro* activity against *P. digitatum* and *in vivo* capacity to inhibit green mold decay caused by *P. digitatum* in *Shatangju* slices during storage. Moreover, the inclusion complexes had no significant impact on weight loss, TSS, or sensory acceptability. In conclusion, these findings suggest that the carvacrol/β-CD inclusion complexes have the potential to be used as non-contact preservatives for fresh-cut fruits. However, as a fumigation preservative, its effective duration and dose are closely related to its stability or volatility under various conditions. Therefore, further research should focus on—which is also an aspect that was not addressed in the current study—determining whether its release duration is sufficient to cover the growth cycle of microorganisms, and whether the cumulative concentration produced by it is just adequate for inhibiting microorganisms without harming food quality.

Declarations

Ethics statement

This study complies with all regulations and has obtained informed consent from all participants.

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Author contribution statement

Yumeng Liu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Luo Weng, Ying Lin: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Daijie Lin, Linsheng Xie: Performed the experiments; Analyzed and interpreted the data.
Tian Zhong: Conceived and designed the experiments; Wrote the paper.

Data availability statement

Data will be made available on request.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18804.

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