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RESEARCH

Encapsulation of Essential Oil-Cyclodextrin Inclusion Complexes in Electrospun Pullulan Nanofibers: Enhanced Storage Stability and Antibacterial Property for Geraniol and Linalool

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Abstract
In this study, the gamma-cyclodextrin (γCD) inclusion complexes with essential oil (EO) compounds linalool and geraniol were encapsulated in pullulan nanofibers using an electrospinning technique. The nanofibers were produced with the initial EO content of ~ 11% (w/w) and ~ 74 to 77% of these volatile compounds could be preserved in pullulan/EO-γCD nanofibers due to inclusion complexation. On the other hand, only ~ 15 to 23% of EO could be kept in the case of pullulan/EO nanofibers in the absence of γCD. The EO-γCD inclusion complexation also ensured improved thermal stability for EO compounds, and their volatilization shifted from ~ 119–139 to ~ 239–292 °C ranges for pullulan/EO-γCD nanofibers. Moreover, pullulan/EO-γCD nanofibers displayed substantial long-term storage stability and, ~ 48 to 64% of EO content was still preserved even after 4 weeks, while this ratio was in the range of ~ 0.3 to 4.5% in the case of pullulan/EO nanofibers. The pullulan/geraniol-γCD nanofibers displayed an effective antibacterial activity against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria, and overwhelmingly better performance compared to pullulan/geraniol nanofibers due to the inclusion complexation. Briefly, EO-incorporated nanofibers were generated using GRAS γCD molecules and an edible pullulan polymer, and this approach can be a promising alternative to be effectively used in the food industry for the encapsulation and delivery of volatile EO compounds.

Keywords Electrospinning · Cyclodextrin · Pullulan · Essential oil · Antibacterial · Linalool · Geraniol

Introduction

The development of functional encapsulation approaches from renewable resources has drawn the great attention of food and medicinal industries for decades to provide efficient packing, delivery, and protection for bioactive compounds. Here, biopolymers make ground as an encapsulation material against fossil fuel-based polymers thanks to their biocompatible, sustainable, and biodegradable natures (Gupta et al., 2022; Zhu et al., 2016). Polysaccharides derived from plants, animals, or microorganisms are commonly used in food and medicinal industries since they can be abundantly found in nature (Hassan et al., 2018; Yadav & Karthikeyan, 2019). Pullulan, a microorganism-based polysaccharide, can be efficiently obtained from the fermentation of starch syrup using the fungus-like yeast *Aureobasidium* pullulans (Rashid et al., 2024; Sugumaran & Ponnusami, 2017). Pullulan is comprised of maltotriose units (three α-(1, 4) linked glucose units) which connect each other by α-(1, 6) glycosidic bonds. Polysaccharides mostly show complex and branched chemical structures which can strain their processing; therefore, extra modification steps can be applied to resolve this complication (Jindal & Khattar, 2018). On the other hand, pullulan has a linear and stair-step macromolecular structure which eases its processing and releasing a product from this polymer. Moreover, pullulan is a tasteless, odorless, edible, and non-mutagenic polymer, making it an attractive polymer type for food, medicinal, and cosmetic applications (Rashid et al., 2024; Singh et al., 2017, 2019). Pullulan is commonly used as a gelling agent, adhesive, binder, thickener, or solubility enhancer, however, it can be also used to generate

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coating or packaging material due to its outstanding film/fiber-forming properties (Rashid et al., 2024; Sugumaran & Ponnusami, 2017). Besides all these advantages, the water solubility of pullulan also enables the production of uniform and free-standing nanofibers via the electrospinning technique without using a toxic solvent system (Angel et al., 2022).

As one of the most commonly known electrohydrodynamic atomization techniques, electrospinning presents feasibility and cost-effectiveness and enables the fabrication of encapsulation materials that can find the opportunity to be used for food and medicinal applications (Angel et al., 2022; Jain et al., 2020). Here, the main advantages of electrospun nanofibers are based on their large surface area, high porosity, lightweight, 3D continuous structure, and flexible feature. Additionally, these nanofibrous matrices can be modified and functionalized to gain superior properties by the inclusion of various functional components (Angel et al., 2022; Jain et al., 2020). Among other encapsulation techniques used for the preservation of essential oils (EOs) such as spray drying, freeze-drying, coacervation, emulsification, and liposomes, electrospinning can be considered a more feasible and proper technique for the encapsulation of volatile and thermally sensitive compounds since it is carried out at ambient conditions (Hossen et al., 2024; Mukurumbira et al., 2022; Saifullah et al., 2019; Sundar & Parikh, 2023). This is why electrospinning has also become an attractive way for the efficient encapsulation of EOs into nanofibrous matrices (Hossen et al., 2024; Saifullah et al., 2019; Sundar & Parikh, 2023). As is known, EOs are a complex blend of active compounds that are mostly volatile and have exclusive properties such as antioxidant, antibacterial, anti-inflammatory, and fragrance (Jugreet et al., 2020). Essential oils take a remarkable place in food, medicinal, and cosmetic industries owing to their attractive properties and relatively safe state (Devi et al., 2024; Falleh et al., 2020; Rout et al., 2022).

Linalool and geraniol are both monoterpenol types of EO compounds. Linalool, 3,7-dimethyl-1,6-octadien-3-ol, can be found in over 200 plant species, but it is particularly isolated from the EOs of lavender and coriander (Pereira et al., 2018). Geraniol, the *trans*-isomer of 3,7-dimethyl-2,6-octadien-1-ol, is also present in several aromatic plant species and mostly isolated from the rose oil (Chen & Viljoen, 2022; Lira et al., 2020). Linalool and geraniol, generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA), are well-known for their fragrance, flavor, and inclusive range of bioactive properties, therefore both of them are exploited as ingredients in cosmetics, pharmaceutical, personal care, and food products (Chen & Viljoen, 2022; Lira et al., 2020; Pereira et al., 2018). Like most EO compounds, linalool and geraniol also suffer from heat sensitivity, so they may need to be preserved by encapsulating in a proper carrier system during processing and storage.

As mentioned previously, electrospinning can be an attractive alternative route to encapsulate EO compounds into the nanofibers as a carrier platform (Hossen et al., 2024; Saifullah et al., 2019; Sundar & Parikh, 2023). Unfortunately, polymers can fail to preserve EOs per se because of their volatile nature, which can lead to evaporation during the electrospinning process. To address this issue, cyclodextrin molecules have been utilized to form inclusion complexes with EO compounds which were loaded in electrospun polymeric nanofibers (Celebioglu & Uyar, 2021; Celebioglu et al., 2023; Ertan et al., 2023; Kayaci et al., 2014; Yildiz et al., 2024). Cyclodextrins (CDs), cyclic oligosaccharides, form inclusion complexes by capturing molecules into their relatively hydrophobic cavity, and inclusion complexation can take the potential of these bioactive molecules a step further in several application areas including food, medicinal, cosmetic, etc. by enhancing their thermal/oxidation stability and water solubility and bioactivity (Cid-Samamed et al., 2022; Crini, 2014; Kali et al., 2023). As has been reported in previous studies, the thermal stability and aqueous solubility of both linalool (Camargo et al., 2018; Ceborska, 2016; He et al., 2023; Kou et al., 2023) and geraniol (Ceborska et al., 2015; Christoforides et al., 2020; He et al., 2023; Truzzi et al., 2021) have been also improved by forming inclusion complexes with various types of CDs. Moreover, the inclusion complexes of these EO compounds have been electrospun into nanofibers both in the existence (Gupta et al., 2024; Kayaci et al., 2014) or absence (Aytac et al., 2016, 2017) of a polymeric matrix. However, to the best of our knowledge, pullulan has not been used as the carrier polymer yet for the generation of electrospun nanofibers for the electrospinning of pullulan/linalool-γCD and pullulan/geraniol-γCD inclusion complex nanofibers with enhanced storage stability and antibacterial properties for these essential oils; geraniol and linalool.

As revealed by the previous reports, pullulan nanofibers can demonstrate remarkable potential in food application since food-grade nanofibrous materials can be practically generated by the modification of pullulan and/or its blend (whey, dextran, carboxymethyl cellulose, etc.) with fish oil (García-Moreno et al., 2017), folic acid (Aceituno-Medina et al., 2015), nisin (Soto et al., 2019), tea phenols (Shao et al., 2018), resveratrol (Seethu et al., 2020), and curcumin (Blanco-Padilla et al., 2015). Moreover, it has been revealed in the previous reports of our research group that, pullulan nanofibers can be functionalized with the CD inclusion complexes of different EO compounds including eugenol (Celebioglu & Uyar, 2021), carvacrol (Ertan et al., 2023) and cinnamaldehyde (Celebioglu et al., 2023). In this recent study, an effective encapsulation and carrying material for linalool and geraniol was developed in the form of free-standing nanofibers by using a sustainable and biocompatible pullulan polymer and a GRAS complexation agent of γCD.

Here, pullulan nanofibers incorporated with linalool-γCD or geraniol-γCD inclusion complexes in a one-step process as shown in Fig. 1. The superiority of electrospun nanofibers from pullulan/EO-γCD inclusion complexes compared to pullulan/EO nanofibers without γCD was demonstrated using by diverse characterizations and testing methods.

Material and Methods

Materials

Linalool (98%, Alfa Aesar), geraniol (97%, Alfa Aesar), pullulan (Mw, 300 000 g/mol; TCI America), ethanol (Emsure®, Sigma-Aldrich), deuterated dimethylsulfoxide (*d6*-DMSO, 99.8%, Cambridge Isotope), and deuterium oxide (D_2O, 99.9%, Cambridge Isotope) were provided commercially. γCD (Cavamax W8 Food) was gifted from Wacker Chemie AG (USA). The chemicals were used without purification. The high-quality distilled water was supplied from the Millipore Milli-Q ultrapure water system (Millipore, USA).

Preparation of the Inclusion Complexes and Electrospinning Procedure

The inclusion complexes of EOs; linalool-γCD and geraniol-γCD were prepared using a 2:1 molar ratio (EO/CD). For this, γCD was dissolved in water at 23% (w/v, with respect to water) of solid content and then EOs were separately dropped into the aqueous solutions of γCD. The aqueous systems were stirred overnight at RT, and the solutions turned white along with the complex crystal formation. Afterward, pullulan (20%, w/v) was added into the linalool-γCD and geraniol-γCD inclusion complex systems to be stirred till it dissolved. Apart from these, pristine pullulan, pullulan/linalool, and pullulan/geraniol solutions were prepared for the generation of control samples. For all samples, pullulan concentration was kept at 20% (w/v) and the initial EO content was ~ 11% (w/w, with respect to total sample amount) for pullulan/EO and pullulan/EO-γCD systems. Prior to the electrospinning, the conductivity and viscosity of the solutions were also determined using a conductivity meter (FiveEasy, Mettler Toledo, USA) and rheometer (AR 2000 rheometer, TA Instrument, USA; 20-mm, 4° cone-plate

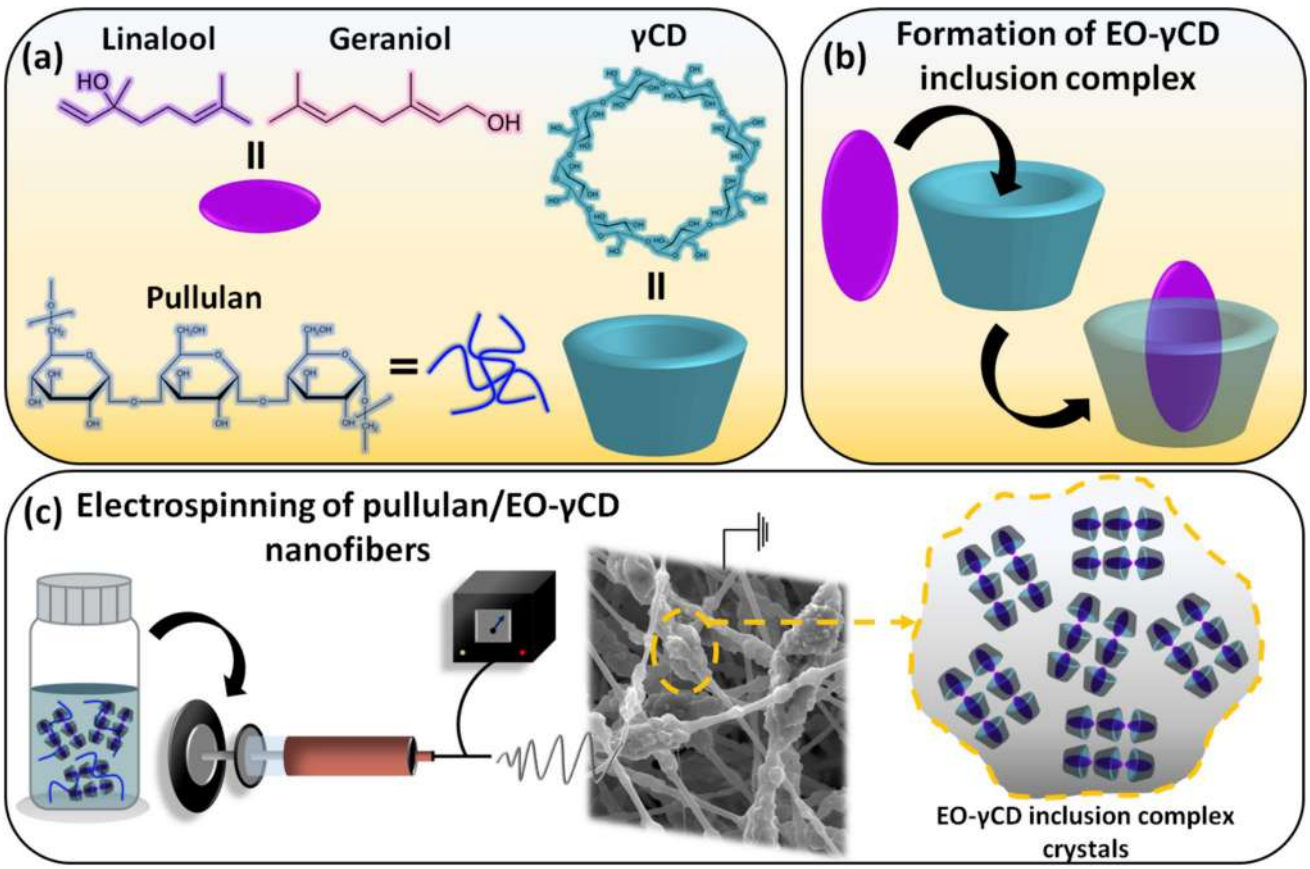

Fig. 1 **a** The chemical structure of essential oil (EO) compounds (linalool and geraniol), gamma-cyclodextrin (γCD), and pullulan. **b** The schematic representation of inclusion complex formation between γCD and EOs. **c** The schematic representation of the electrospinning of pullulan/EO-γCD nanofibers

spindle; shear rate of 0.01–1000 s^{-1}, 20 °C), respectively. Here, electrospinning equipment was used for the generation of nanofibers (Spingenix, model: SG100, Palo Alto, USA). Firstly, each solution was separately loaded into plastic syringes, and then these syringes were placed horizontally on the syringe pump. The stainless-steel needles (23–27 G) fixed to syringes were the spots where high voltage (15–18 kV) was applied while the electrospinning solutions were simultaneously being pumped with a continuous flow rate (0.5 mL/h). Consequently, nanofibers were randomly deposited on the metal collector covered with foil as a free-standing layer under the conditions of ~ 25% relative humidity and ~ 20 °C temperature.

2D-NMR (ROESY) Analysis

The Rotating frame Overhauser Effect Spectroscopy (ROESY) experiment was done for the inclusion complexes of linalool-γCD, and geraniol-γCD using a 600 MHz Varian INOVA nuclear magnetic resonance spectrometer. Here, D_2O:d_6-DMSO (3:2, v/v) mixture was used for the measurement (25 °C). First, linalool or geraniol was dissolved in d_6-DMSO in the existence of γCD to have a 1:4 molar ratio (EO/CD), and then D_2O was added to the mixture. For ROESY measurement, the molar ratio of the inclusion complex system was kept with a lower content of EO (1:4) compared to one used for the preparation of the sample (2:1) to eliminate the potential precipitation that can disturb the analysis.

Structural Characterization

Scanning electron microscopy (SEM, Tescan-MIRA3) was used for the morphological analysis of nanofibers, and samples were primarily coated with the thin layer of Au/Pd to eliminate their charging problem. The size of nanofibers (average fiber diameter (AFD)) was determined using ImageJ software (~ 100 nanofibers). The chemical characterization of samples was conducted by using a proton nuclear magnetic resonance (^{1}H-NMR) spectrometer having an autosampler (NMR, Bruker AV500). For this, EOs, γCD, pullulan nanofibers, pullulan/EO nanofibers, and pullulan/EO-γCD nanofibers were dissolved in *d6*-DMSO, and measurements were completed upon 16 scans. Mestranova software was used for both plotting of ^{1}H-NMR spectra and for the calculations of EO content in nanofibers. The attenuated total reflectance Fourier transform infrared spectrometer (ATR-FTIR, PerkinElmer, USA) was also run for further structural analysis and to record the FTIR spectra (4000–600 cm^{-1}; resolution of 4 cm^{-1}; 64 scans). The crystalline pattern of nanofibers was examined by X-ray diffractometer (XRD, Bruker D8 Advance ECO) using Cu Kα radiation ($2\theta = 5–30°$; 40 kV; 25 mA). Additionally, the thermal profiles of samples were evaluated to follow the differentiation at the thermal degradation profile of EOs that were encapsulated in nanofibers by using a thermogravimetric analyzer (TGA, Q500, TA Instruments, USA) (30–600 °C; heating rate of 20 °C/min; N_2).

Time-Dependent EO Preservation Performance of Nanofibers

To examine the storage stability, nanofibers were kept under 20–22 °C and 60–65% relative humidity for 1 day, 1 week, 2, and 4 weeks. Thereafter, samples were dissolved in the solvent system of ethanol/water (3/7, v/v) ($n = 3$), and the prepared solutions were examined using UV–vis spectroscopy (PerkinElmer, Lambda 35). The absorption intensity values at 190 nm for linalool and at 200 nm for geraniol were adapted to the calibration curves ($R^2 \geq 0.99$) to obtain % (w/w, with respect to total sample amount) preservation efficiency showing the remained EOs in the nanofibers.

Antibacterial Performance of Nanofibers

The growth kinetics of the bacterial strains in the presence of the samples were investigated by bacterial growth assay. *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 700926) were cultured overnight in LB broth with aeration at 37 °C. These were subcultured until the OD_{600} reached 1. 1 ml of each culture was aliquoted and centrifuged at 10,000 RPM for 5 min at RT. Pellets were washed in 1 ml of PBS and resuspended in LB media supplemented with 100 mM MOPS (3-(N-morpholino) propanesulfonic acid) (pH = 6.8). Nanofiber solutions prepared in DMSO were added to the media along with 10 µl of the bacterial suspension to make up the final volume of 1 ml. The final sample concentration was 5 mg/mL in the LB reaction mixture. Free geraniol and linalool were also tested for their ability to inhibit the growth of bacteria by using the EO concentration of 0.5 mg/mL which corresponds to the initial EO content within 5 mg/mL of nanofiber concentration. Reactions were added to dark 96-well flat bottom plates in replicates of three and inserted in the Biotek Synergy H1 microplate reader. Growth (OD_{600}) was read every 30 min for 24 h. Data were plotted on GraphPad Prism v9. The inhibition rate (%) was calculated by the equation below using sample-free DMSO as a control:

$$\text{Inhibition rate (\%)} = \frac{OD600_t^S - OD600_{t_o}^S}{OD600_t^C - OD600_{t_o}^C} \times 100$$

where $OD600^{S}_{t_0}$ is the initial OD600 value, and $OD600^{S}_{t}$ is the OD600 value of the samples at the 24th hour. $OD600^{C}_{t_0}$ and $OD600^{C}_{t}$ are the values of the DMSO measured at the beginning and after 24 h, respectively.

Statistical Analysis

The statistical analyses were carried out using the one-way/two-way analysis of variance followed by Tukey's test (ANOVA). OriginLab (Origin 2024, USA) was used for all these ANOVA analyses (0.05 level of probability).

Results and Discussion

2D-NMR Profile of EO-γCD Inclusion Complexes

In this study, the ROESY technique was employed to clarify the spatial characteristics of host-guest interactions within solutions of γCD with linalool and γCD with geraniol. The analysis of the ROESY spectra for these systems revealed simultaneous proton resonances involving the inner cavity protons (H_3 and H_5) of the γCDs and the protons of linalool and geraniol (Fig. 2a). Here, proton resonances were identified between the inner cavity protons of γCD (H_3 and H_5) and the protons of linalool (a, c, and d) and geraniol (c, g,

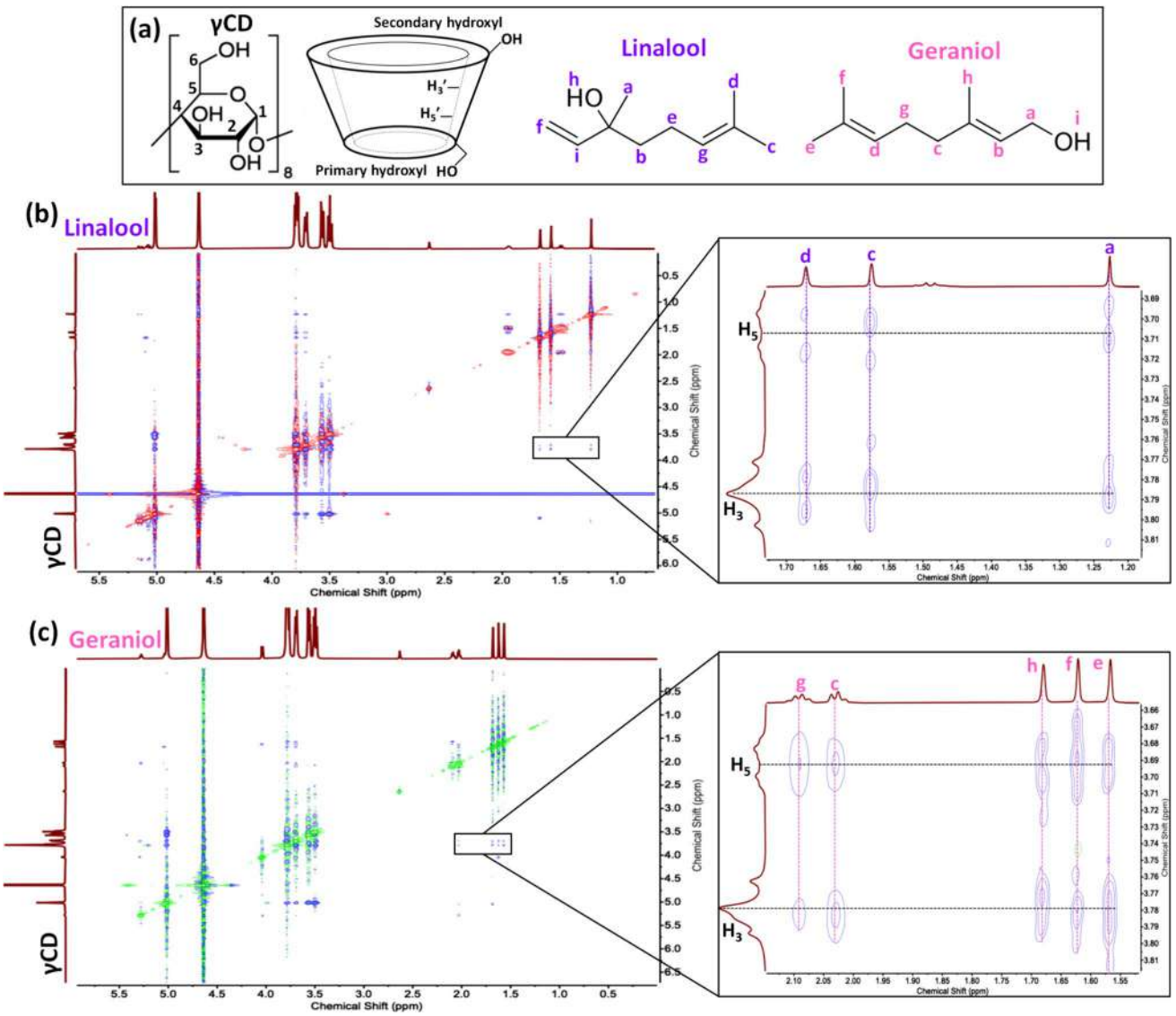

Fig. 2 **a** Chemical structure of γCD, linalool and geraniol. Full and expanded 2D-NMR (ROESY) spectra of **b** γCD-linalool and **c** γCD-geraniol inclusion complexes

e, f, and h) as pointed in Fig. 2b and c, respectively. These results confirmed the formation of inclusion complexes between γCD and both linalool and geraniol, suggesting that γCD effectively accommodates these molecules within its cavities.

Morphological Analysis of Electrospun Nanofibers

In this study, the incorporation of pullulan nanofibers with the inclusion complexes of linalool-γCD and geraniol-γCD was performed using a one-step approach. Here, the inclusion complexes of EOs and γCD were formed in the aqueous medium as suspension, followed by the dissolution of polymer in the same medium which was ultimately electrospun into nanofibers. Figure 3 displays the photos of electrospinning solutions and the nanofibers that were generated from these solutions. The clear solution of pullulan (Fig. 3a-i) turned into a white color for EO-γCD incorporated systems (Fig. 3c and e-i) as a result of inclusion complex formation. On the other hand, the control samples of pullulan/EO were turbid due to their emulsion characteristic (Fig. 3b and d-i). Eventually, free-standing and easily foldable nanofibers were successfully generated from all these systems using the electrospinning technique (Fig. 3). The SEM imaging showed homogenous and defect-free morphology of pullulan nanofibers (Fig. 3a-ii). At the same time, few beads were noticed for pullulan/EO nanofibers of which electrospinning solutions contained oil drops that could result in beaded parts throughout the fibers (Fig. 3b and d-ii) (Angeles et al., 2008; García-Moreno et al., 2016). For pullulan/linalool-γCD (Fig. 3c-ii) and pullulan/geraniol-γCD (Fig. 3e-ii) nanofibers, the heterogenous segregates detected

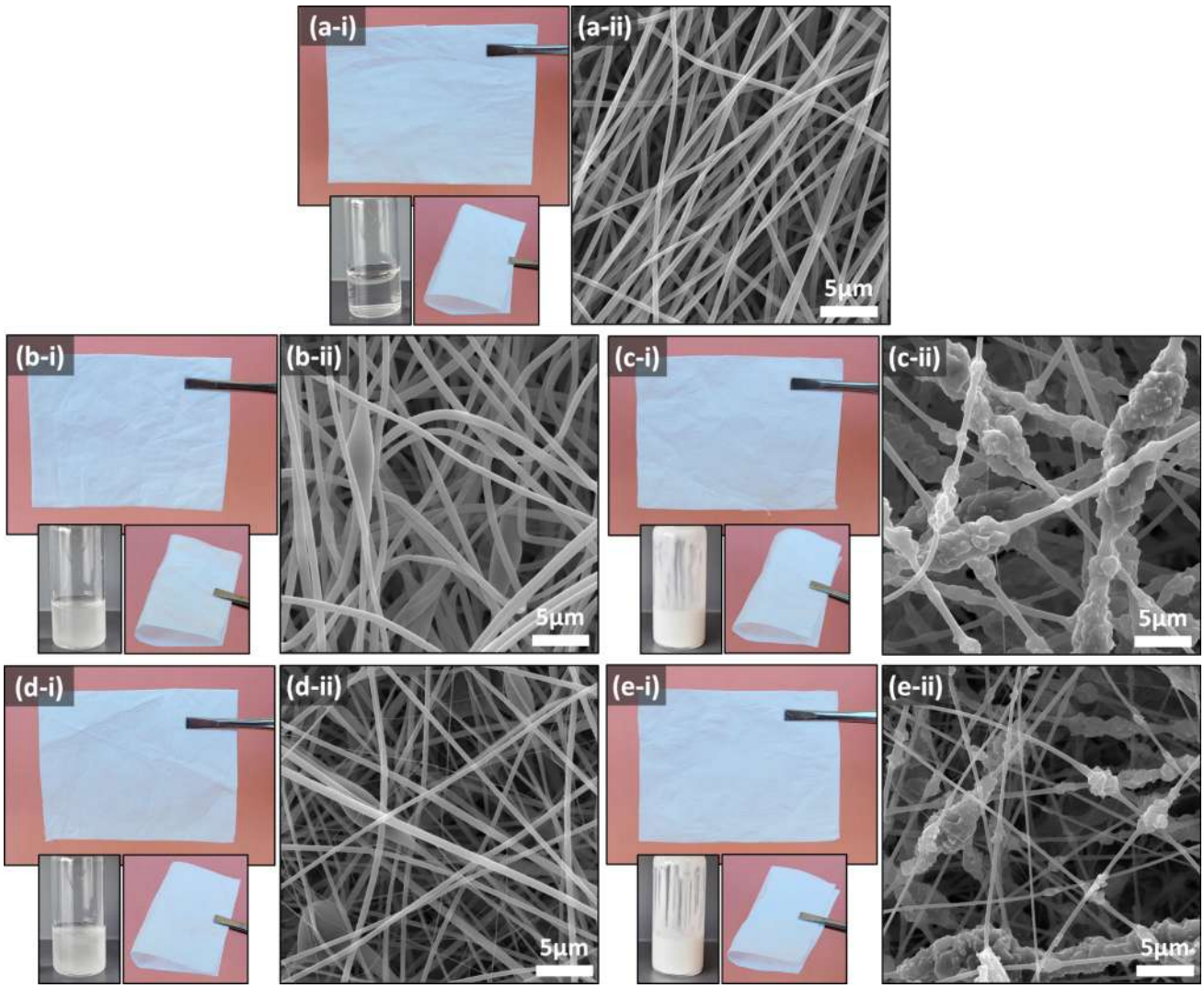

Fig. 3 (i) The photos of electrospinning solutions/electrospun nanofibers and (ii) SEM images of the nanofibers of **a** pullulan, **b** pullulan/linalool, **c** pullulan/linalool-γCD, **d** pullulan/geraniol, and **e** pullulan/geraniol-γCD

in SEM images were due to the dispersion of linalool-γCD and geraniol-γCD inclusion complex crystals within the pullulan nanofiber matrix (Fig. 1c).

Table 1 indicates the properties of electrospinning solutions and the AFD of nanofibers electrospun from these systems. Here, EO-γCD included pullulan solutions showed higher viscosity and conductivity values compared to pristine pullulan and pullulan/EO systems. There is a more significant increase in the case of viscosity compared to the conductivity of solutions by the incorporation of EO-γCD. However, this did not result in a coherent effect on the AFD of nanofibers. For pullulan/linalool-γCD nanofibers, thicker fibers were observed (635 ± 135 nm) than pullulan nanofibers (485 ± 70 nm) while thinner fibers were detected in the case of pullulan/geraniol-γCD nanofibers (355 ± 80 nm). Principally, higher viscosity or lower conductivity of solutions causes less stretching of the electrospinning jet and this promotes thicker fiber formation (Uyar & Besenbacher, 2008). Here, the heterogenous distribution of EO-γCD crystals around polymer chains might have inhibited the monolith response within the electrospinning jet during the stretching process so this might have induced different AFD trends for linalool-γCD and geraniol-γCD based systems. It was also detected that linalool-included nanofibers have significantly higher AFD values compared to others ($p < 0.05$). This is most probably due to the higher additive effect of linalool on the surface tension of aqueous systems compared to geraniol as reported by Lewandowski and Szymczyk (2019). This resulted in thicker fiber formation for linalool-based systems since beyond the critical surface tension value that needs to be overcome for the bead-free fiber formation, it is known that lower surface tension values can favor thinner fiber production (Guo et al., 2022).

Structural Analysis of Nanofibers

FTIR spectroscopy was used for the structural examination of samples and Fig. 4 indicates the FTIR graphs of EOs, γCD powder, and nanofibers of pullulan, pullulan/EO, and pullulan/EO-γCD. FTIR spectrum of γCD exhibited absorption bands at 3266 cm^{-1}, 2925 cm^{-1}, 1641 cm^{-1}, 1153 cm^{-1}, and 1077 cm^{-1}/1020 cm^{-1} which corresponds to symmetrical and asymmetrical stretching of ν(O – H), stretching of methyl/methylene ν(C – H), H – O – H bending, asymmetric ν(C – O – C) link stretching, and the ν(C – O)/ ν(C – C) stretching, respectively (Celebioglu & Uyar, 2021). γCD is composed of glucopyranose sub-units linked by α-(1,4) glycosidic bonds. On the other hand, pullulan is constructed by maltotriose (three glucose units connected by α-(1,4) glycosidic bonds) units linked by α-(1,6) glycosidic bonds (Yang et al., 2020). Due to the similarity of their chemical structure, analogous absorption bands at the parallel region were detected in the FTIR graphs of γCD and pullulan. Accordingly, pullulan-based samples also displayed absorption peaks of ν(O – H) stretching, ν(C – H) stretching, and H – O – H bending at 3313 cm^{-1}, 2925 cm^{-1}, and 1641 cm^{-1}, respectively (Shao et al., 2018; Yang et al., 2020).

For pullulan/linalool-γCD nanofiber, the characteristic peaks of linalool at 1641 cm^{-1}, 1450/1411 cm^{-1}, and 1375 cm^{-1} corresponding to ν(C = C) stretching, ν(C – H) bending of methylene, and ν(C – H) bending of methyl group were detected as shown in the expanded FTIR graph of Fig. 4a (Das et al., 2021; Kanekar et al., 2022). In the case of pullulan/geraniol-γCD nanofiber, the characteristic peaks of geraniol were also detected in a similar region with linalool due to their similar chemical structures (Fig. 4b). Here, the absorption bands of geraniol located at 1667 cm^{-1}, 1440 cm^{-1}, and 1376 cm^{-1} also rises from the ν(C = C) stretching, ν(C – H) bending of methylene, and ν(C – H) bending of methyl group (Ding et al., 2024; P. Gupta et al., 2024). For both pullulan/EO-γCD nanofibers, the characteristic peaks of EOs highlighted in the FTIR spectra verified the encapsulation of linalool and geraniol in the nanofibers. Moreover, the shifts of peaks observed from 1450/1411 to 1413 cm^{-1} for pullulan/linalool-γCD nanofiber and from 1440 to 1415 cm^{-1} for pullulan/geraniol-γCD nanofiber confirmed the inclusion complex formation between EOs and γCD loaded in electrospun nanofibers. In spite of that, the given peaks of EOs were not almost detected for pullulan/EO nanofibers, just ν(C = C) stretching peak at around 1640–1670 cm^{-1} boosted the absorption intensity at the same region compared to the pristine pullulan nanofibers. This can be an indication of the higher amount of EOs

Table 1 The properties of electrospinning solutions and the electrospun nanofibers

Sample	Pullulan conc. (%, w/v)	γCD conc. (%, w/v)	Molar ratio (EO/γCD)	Viscosity (Pa·s)	Conductivity (μS/cm)	Average fiber diameter (nm)
Pullulan	20			0.5882	43.97	485 ± 70
Pullulan/linalool	20			0.5851	48.90	625 ± 120
Pullulan/linalool-γCD	20	23	2:1	1.2775	51.03	635 ± 135
Pullulan/geraniol	20			0.5635	52.74	435 ± 155
Pullulan/geraniol-γCD	20	23	2:1	1.1223	59.72	355 ± 80

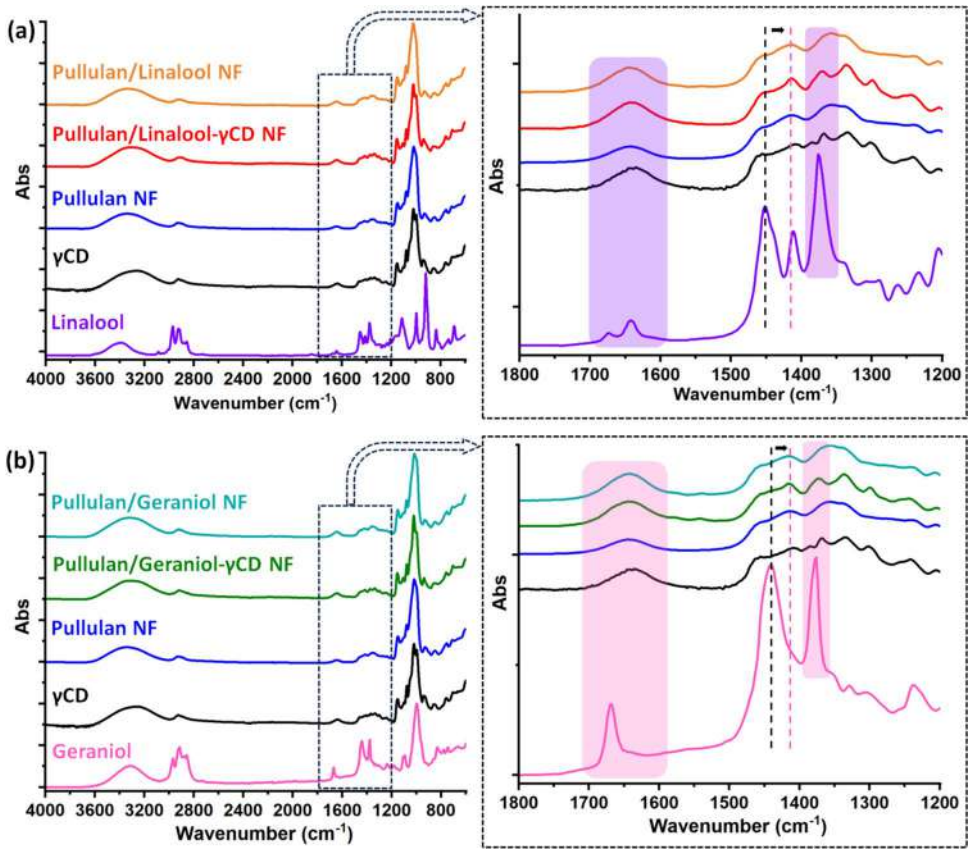

Fig. 4 The full and expanded range FTIR spectra of **a** linalool, γCD, pullulan NF, pullulan/linalool NF, and pullulan/linalool-γCD NF and **b** geraniol, γCD, pullulan NF, pullulan/geraniol NF, and pullulan/geraniol-γCD NF (NF: nanofibers)

encapsulated in the pullulan/EO-γCD nanofibers than pullulan/EO nanofibers owing to inclusion complexation.

The inclusion complex formation between EOs and γCD was further analyzed using XRD. Figure 5 indicates the XRD pattern of γCD powder and nanofibers of pullulan, pullulan/linalool, pullulan/linalool-γCD, pullulan/geraniol, and pullulan/geraniol-γCD. The pristine γCD has "cage-like" crystalline packing which represents the blocked CD cavities by neighboring CDs and generates XRD pattern with peaks at $2\theta = 5.2°$, 12.4°, 14.0°, 16.5°, 18.8°, and 21.8° (Fig. 5). On the other hand, γCDs reorganize into the "channel-type" lattice upon inclusion complexation and γCD molecules form cylindrical channel structure by stacking on top of each other. This leads to a XRD pattern having peaks at $2\theta = 7.0°$, 14.0°, 15.0°, 16.0°, and 17.0° (Celebioglu et al., 2017). As expected, pristine pullulan nanofibers exhibited

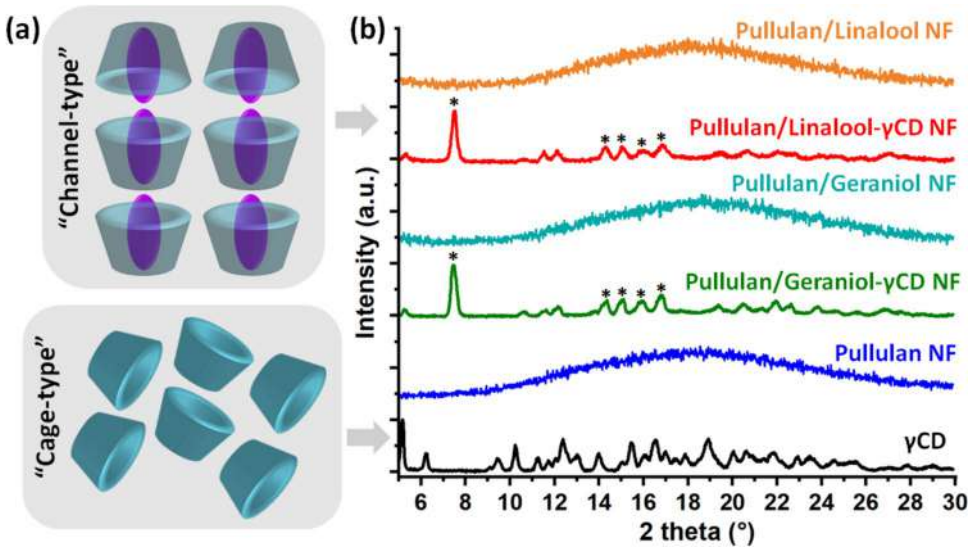

Fig. 5 **a** Schematic representation of channel and cage type of crystal packing. **b** XRD pattern of γCD, pullulan NF, pullulan/geraniol-γCD NF, pullulan/geraniol NF, pullulan/linalool-γCD NF and pullulan/linalool NF (NF: nanofibers)

a broad pattern at $2\theta = 18.5°$ due to the *d*-spacing of 4.52 Å (Seethu et al., 2020). Pullulan/EO nanofibers showed a similar amorphous XRD pattern to pristine pullulan nanofibers since they are just the physical mixture of pullulan and EOs (Fig. 5). In contrast, pullulan/EO-γCD nanofibers indicated the characteristic peaks of inclusion complex crystals of EO-γCD by dominating the amorphous pattern of pullulan nanofiber. Here, the distinct XRD peaks detected for pullulan/EO-γCD nanofibers originated from the "channel-type" of packaging of CDs, and this verified the modification of pullulan nanofibers with EO-γCD inclusion complexes (Celebioglu & Uyar, 2021; Celebioglu et al., 2017). XRD findings are coherent with the SEM imaging results where the EO-γCD crystals were apparently observable in the matrix of nanofibers (Fig. 3).

TGA was used to examine the thermal decomposition of samples, and Fig. 6 shows the thermograms and the derivatives (DTG) of γCD, EOs, nanofibers of pullulan, pullulan/EO, and pullulan/EO-γCD. TGA findings revealed the volatile nature of linalool and geraniol by the maximum evaporation that occurs at ~ 119 °C and ~ 139 °C, respectively. The thermograms of pullulan nanofibers and pullulan/EO nanofibers are composed of two main weight losses; water dehydration (till ~ 110 °C) and the main degradation of pullulan (~ 325 to 327 °C). Here, the volatilization of EOs was not detected as a separated weight loss step in the thermogram of pullulan/EO nanofibers (Fig. 6a and b-ii). The evaporation of EOs that happens in a similar temperature range as water dehydration and/or the massive loss of these EOs that could not be preserved during the process can be the reasons for this result. While the physical mixture of EOs and pullulan is present for pullulan/EOs nanofibers, inclusion complexation that can lead to a shift in the evaporation of volatile compound to the higher temperature ranges is the case of pullulan/EO-γCD nanofibers (Mura, 2015; Wadhwa et al., 2017). For pullulan/EO-γCD nanofibers, another weight loss step was detected in addition to water evaporation (~ 110 °C) and main degradation of pullulan (~ 325 °C). This additional step occurred respectively at ~ 239 °C and ~ 292 °C for pullulan/linalool-γCD and pullulan/geraniol-γCD nanofibers and corresponds to the volatilization of EOs that formed inclusion complexes within γCD (Fig. 6a and b-ii). These obvious shifts from ~ 119 to ~ 239 °C for linalool and from ~ 139 to ~ 292 °C for geraniol verified the enhanced thermal stability of these EOs owing to inclusion complexation in the case of pullulan/EO-γCD nanofibers.

Figure 7 displays ^{1}H-NMR spectra of EOs, γCD, and nanofibers of pullulan, pullulan/EO, and pullulan/EO-γCD which were recorded just after the electrospinning process. Here, the characteristic peaks of EOs highlighted with letters were observed clearly in the ^{1}H-NMR spectra of pullulan/EO-γCD nanofibers while it is quite difficult to detect these peaks in the ^{1}H-NMR spectra of pullulan/EO nanofibers (Fig. 7). This finding refers to the higher EO content of pullulan/EO-γCD nanofibers compared to pullulan/EO nanofibers. The preserved chemical structure of EOs during the

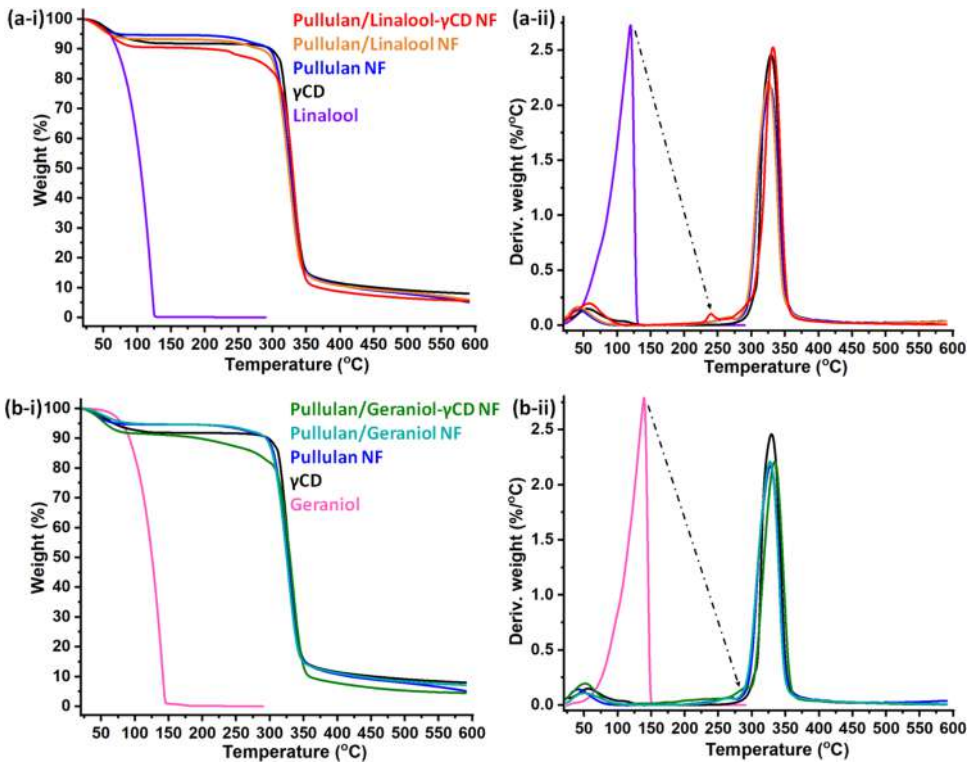

Fig. 6 (i) TGA thermograms and (ii) DTG of **a** linalool, γCD, pullulan NF, pullulan/linalool NF, and pullulan/linalool-γCD NF and **b** geraniol, γCD, pullulan NF, pullulan/geraniol NF, and pullulan/geraniol-γCD NF (NF: nanofibers)

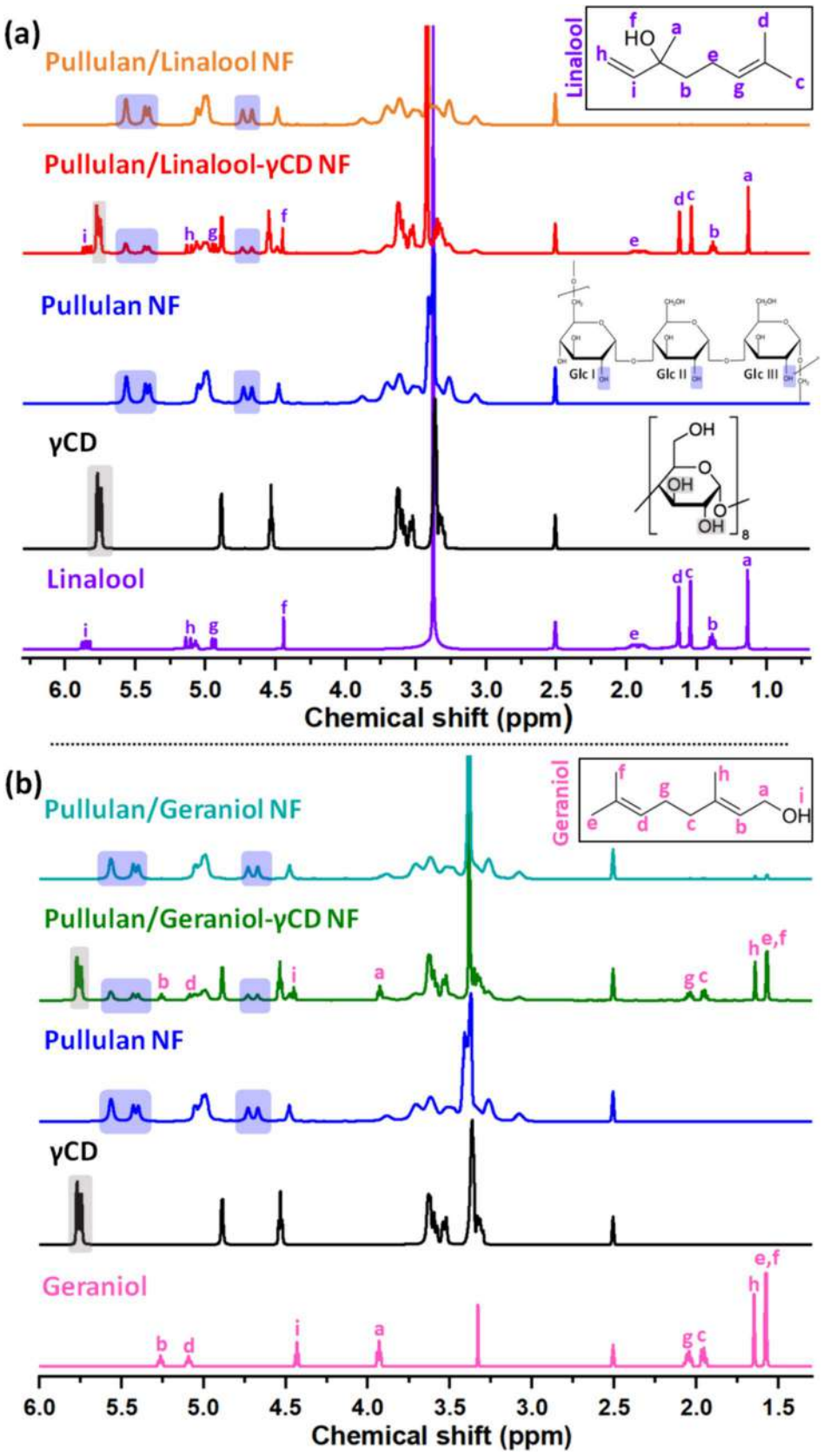

Fig. 7 ^{1}H-NMR spectra of **a** linalool, γCD, pullulan NF, pullulan/linalool NF, pullulan/linalool-γCD NF, and **b** geraniol, γCD, pullulan NF, pullulan/geraniol NF, pullulan/geraniol-γCD NF (NF: nanofibers)

electrospinning process was also validated through ^{1}H-NMR measurements since the characteristic peaks of EOs were attained in the same way for the pullulan/EO-γCD nanofibers (Fig. 7). Here, ^{1}H-NMR measurement confirmed the loading of EO within electrospun nanofibers, additionally, it enabled the calculation of the approximate encapsulated amount of EO in pullulan/EO-γCD nanofibers for the early period of the process. The labeled discrete peaks of EOs, γCD, and pullulan which did not overlap with each other were used for the calculations (Fig. 7). On the other hand, the peaks of EO did not show a meaningful intensity to get an accurate integration value for the determination of EO amount in pullulan/EO nanofibers. Here, the linalool and geraniol contents were roughly found to be ~ 9.0% (w/w) and ~ 9.2% (w/w) for pullulan/linalool-γCD and pullulan/geraniol-γCD nanofibers which revealed that ~ 80% and ~ 81% of the initial EO content (~ 11% (w/w)) was preserved for the given nanofibers, respectively.

Long-Term Storage Stability Profile

Due to the higher aqueous solubility of γCD (23%, w/v) compared to βCD (1.85%, w/v), electrospun nanofibers can be incorporated with a higher concentration of CD inclusion complexes and so active compounds (Celebioglu & Uyar, 2021; Ertan et al., 2023). Additionally, as was shown in one of the related studies, γCD is the most effective type of CD among others (αCD and βCD) in order to encapsulate geraniol within polyvinyl alcohol (PVA) nanofibers thanks to its better size match with geraniol (Kayaci et al., 2014). Correspondingly, the electrospinning solutions of both EO compounds (linalool and geraniol) were prepared using γCD and 2:1 (EO/CD) molar ratio that corresponded to ~ 11% (w/w) EO content within nanofibers. It is important to state that the given loading quantity (~ 11% (w/w)) did not negatively influence the nanofibers' electrospinning process. In this study, pullulan/EO and pullulan/EO-γCD nanofibers were kept under room conditions (20–22 °C and 60–65% relative humidity) for 1 day, 1 week, 2 weeks, and 4 weeks to examine the storage stability. Then the preserved EO content within these samples was determined by dissolving them in an ethanol/water (3/7, v/v) blend. For the 1st day, it was found that the preservation efficiency of pullulan/linalool-γCD and pullulan/geraniol-γCD nanofibers were respectively 77.2 ± 1.2% and 74.2 ± 2.4% which corresponds to the EO concentrations of ~ 8.6% (w/w) and ~ 8.3% (w/w) (Fig. 8). The results of 1st day were approximately coherent with the ^{1}H-NMR measurements which were performed right after the electrospinning since the preserved % of EOs within samples had been calculated as ~ 80% and ~ 81% for pullulan/linalool-γCD and pullulan/geraniol-γCD nanofibers, respectively. On the other hand, preservation efficiency of pullulan/linalool and pullulan/geraniol nanofibers were found to be

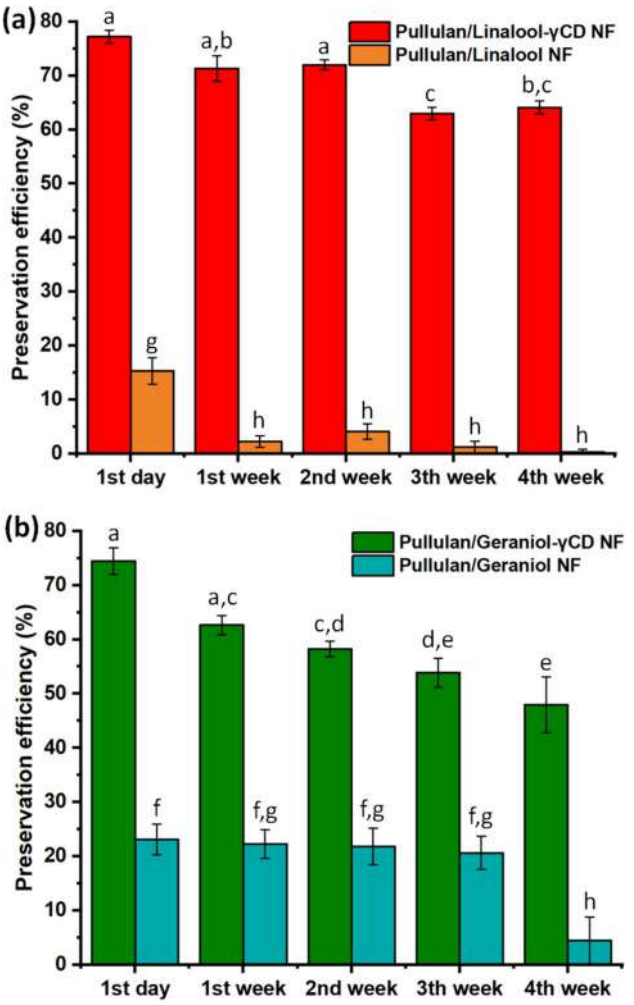

Fig. 8 Time-dependent stability graph of **a** linalool loaded (%) in pullulan/linalool NF, pullulan/linalool-γCD NF, and **b** geraniol loaded (%) in pullulan/geraniol NF, pullulan/geraniol-γCD NF (means that do not share a letter are significantly different; $p < 0.05$) (NF: nanofibers)

15.5 ± 2.5% and 23.1 ± 2.8% which corresponds to ~ 1.7% (w/w) and ~ 2.6% (w/w) of EO content, respectively (Fig. 8). Statistically, the inclusion complexation within CD cavities ensured significantly better preservation for both linalool and geraniol within pullulan/EO-γCD nanofibers compared to pullulan/EO nanofibers without γCD ($p < 0.05$). Here, the uncomplexed EO molecules within pullulan/EO nanofibers were lost during the preparation and electrospinning steps by vaporizing from the polymer matrix.

The preservation efficiency (%) results belonging to the next 1-week, 2-week, and 4-week periods were also given in Fig. 8. It was detected that 64.1 ± 1.2% and 48.0 ± 5.1% of linalool and geraniol were still preserved within pullulan/linalool-γCD and pullulan/geraniol-γCD nanofibers, respectively, even after 4 weeks. However, the 4-week value was just 0.3 ± 0.5% and 4.5 ± 4.3% for pullulan/linalool and

pullulan/geraniol nanofibers, respectively. Consequently, pullulan/EO-γCD nanofibers provided a significantly higher preservation efficiency for linalool and geraniol than pullulan/EO nanofibers during this prolonged storage time thanks to their superior feature rising from EO-γCD inclusion complexation ($p < 0.05$). On the other part, it was noticed that a significantly higher amount of EO (%) was kept in nanofibers of pullulan/geraniol compared to pullulan/linalool ($p < 0.05$). Even though both linalool and geraniol were just physically mixed with pullulan in the case of pullulan/EO nanofibers, the lower vapor pressure and so lower volatility of geraniol than linalool can be the reason for the higher preservation efficiency of this EO compound in pullulan/geraniol nanofibers. However, a similar trend was not observed for the results of the 2–4 weeks of pullulan/EO-γCD nanofibers, and pullulan/linalool-γCD nanofibers demonstrated better preservation effect than pullulan/geraniol-γCD nanofibers for the given storage period ($p < 0.05$). This might be due to the more lipophilic nature of linalool compared to geraniol molecules which was also demonstrated by the comprehensive study reported by He et al. (2023). In this related study, it was demonstrated that linalool can form more efficient and stable inclusion complexes with γCD compared to geraniol due to its relatively higher lipophilic nature (He et al., 2023). Therefore, this phenomenon can also explain our recent findings in which relatively better complexation and preservation efficiency were provided in the case of pullulan/linalool-γCD nanofibers compared to pullulan/geraniol-γCD nanofibers.

Antibacterial Properties of Nanofibers

The growth curve assay was carried out to evaluate the antibacterial performance of nanofibers, and graphs plotted against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria were depicted in Fig. 9. Here, streptomycin, a common antibiotic having a broad spectrum of antibacterial activity was used as a positive control. It was observed that pure geraniol (0.5 mg/mL) demonstrated a similar antibacterial activity against both *S. aureus* and *E. coli* as potently as streptomycin (Fig. 9a and b). On the other hand, pure linalool (0.5 mg/mL) showed significantly less growth inhibition capacity compared to geraniol as given in Fig. 9. As reported previously, the antibacterial activity of monoterpene compounds might be due to the destruction of the lipidic part of the microorganism plasmic membrane which leads to changes in the membrane feature, and thus penetration of these compounds into the cell to interact with the intracellular sites (Cristani et al., 2007; Hou et al., 2022). Here, it was also mentioned that the physiochemical properties of compounds such as water solubility or lipophilicity and the surface charge of bacterial membranes can vary the antibacterial activity of these EO compounds (Cristani

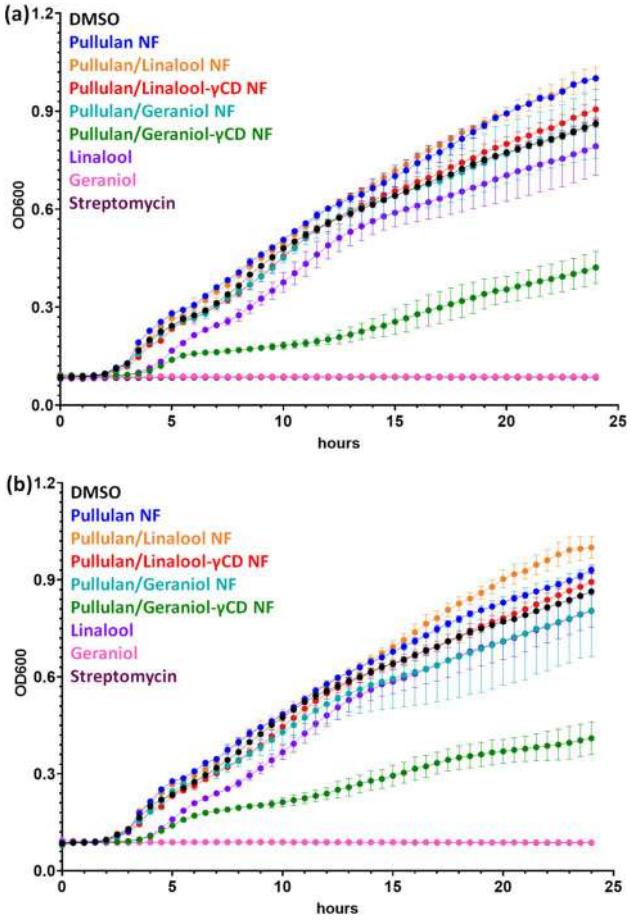

Fig. 9 Growth curves of samples against **a** *E. coli*, and **b** *S. aureus*

et al., 2007; Hou et al., 2022). In one of the related studies, Togashi et al. (2010) reported that linalool and geraniol had close inhibition performance against *S. aureus* compared to other terpene alcohols. However, there was still less amount of geraniol (28.0 µg/mL) needed than linalool (42.0 µg/mL) to inhibit the bacterial strain by 50% (ID_{50}) (Togashi et al., 2010). In another study reported by Friedman et al. (2004), similar results were demonstrated against *E. coli*, and bactericidal activity values (BA_{50}) of geraniol were found to be less compared to linalool for different temperature ranges, and this confirmed the better inhibition performance of geraniol. Briefly, our findings where geraniol showed better antibacterial activity compared to linalool are correlated with the previous studies, and these observations can be due to the relatively less lipophilic nature of geraniol compared to linalool (He et al., 2023). As reported in a related study where the antibacterial performance of four different monoterpenes was examined, the less lipophilic molecule among others demonstrated the fastest kinetic to move through the aqueous medium and hence interacted quicker with the lipid bilayers simulating the lipidic fraction of cell membranes (Cristani et al., 2007). In other words, geraniol

might have also shown faster kinetic compared to linalool in the medium to interact with and inhibit the bacterial strains.

As expected, the bacterial inhibition performance of pullulan nanofibers was similar to that of sample-free DMSO. In line with the difference between linalool and geraniol antibacterial activity, pullulan/linalool and pullulan/linalool-γCD nanofibers could not restrict the bacterial growth independently of their linalool contents (Fig. 9). On the other hand, pullulan/geraniol-γCD nanofibers extensively restricted the bacterial growth of both *E. coli* and *S. aureus* (Fig. 9). The treatment with pullulan/geraniol-γCD nanofibers substantially prevented the growth of bacterial strains up to 10 h, and a very limited growth rate was observed for both *E. coli* and *S. aureus*. Here, growth rates increased after 10 h and continued until the end of the assay. The error bars showed that the bacteria are struggling to grow and may spout mutations to escape the effect of the samples. As shown in Fig. 9, pullulan/geraniol nanofibers demonstrated a very limited antibacterial activity when compared to pullulan/geraniol-γCD nanofibers. This finding is coherent with the geraniol content of samples which were found to be ~ 8.3% (w/w) and ~ 2.6% (w/w) for pullulan/geraniol-γCD and pullulan/geraniol nanofibers, respectively. Here, a higher amount of geraniol was preserved in the case of pullulan/geraniol-γCD nanofibers due to inclusion complexation, and accordingly, a higher amount of active compound took place in the bacterial inhibition process compared to pullulan/geraniol nanofibers. Briefly, these results verified that pullulan/geraniol-γCD nanofibers can powerfully inhibit the growth of both Gram-positive and Gram-negative strains of bacteria proving its potential as an antibacterial material.

Conclusion

To conclude, electrospinning enabled the successful development of nanofibers from the combination of biocompatible pullulan polymer and the inclusion complexes of γCD and well-known EO compounds, linalool and geraniol. The ROESY (2D-NMR) findings confirmed the effective inclusion complexation between γCD and EO compounds. Here, pullulan/EO-γCD nanofibers were generated in a one-step process using water as a solvent system, and the ultimate nanofibers of pullulan/linalool-γCD and pullulan/geraniol-γCD were obtained with ~ 635 nm and ~ 355 nm of average fiber diameters, respectively. The inclusion complexation ensured enhanced thermal stability for both linalool and geraniol, and this was depicted by the shift of evaporation from ~ 119 to ~ 239 °C for linalool and from ~ 139 to ~ 292 °C for geraniol. Moreover, due to the inclusion complex formation, a higher preservation effect was provided for both linalool and geraniol in the case of pullulan/EO-γCD nanofibers. Here, pullulan/linalool-γCD and pullulan/geraniol-γCD nanofibers preserved respectively ~ 77% and ~ 74% of their initial EO content while this value was ~ 15% and ~ 23% for pullulan/linalool and pullulan/geraniol nanofibers. The preservation trend of nanofibers was also followed during a prolonged storage period, and ~ 64% and ~ 48% of EO were still kept within pullulan/linalool-γCD and pullulan/geraniol-γCD nanofibers, respectively, even after 4 weeks. On the other hand, the 4-week value was just ~ 0.3% and ~ 4.5% for pullulan/linalool and pullulan/geraniol nanofibers, respectively. Due to better preservation effect, an enhanced antibacterial performance was also obtained in the case of pullulan/EO-γCD nanofibers compared to pullulan/EO nanofibers against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria. There is still a great demand for natural and safe alternatives against synthetic products, and here, we have developed an effective encapsulation and delivery systems for highly volatile EO compounds of linalool and geraniol by using an edible pullulan polymer and GRAS γCD molecules. Here, the electrospun nanofibers having inclusive properties such as high surface area, lightweight, and lithe features with acceptable mechanical integrity provided an integrable platform along with the incorporation of EO compounds known for their attractive bioactive properties. The electrospun nanofibers functionalized with the inclusion complexes of EO compounds can be directly coated on the food by using a properly modified electrospinning setup or can be integrated into the appropriate and desired part of food packaging as a layer. Here, CD inclusion complexes will guarantee preserving EO's bioactive feature during processing and storage. Briefly, these functional materials can be easily adapted into various application forms in the food industry and this approach can be the source of inspiration for developing edible food supplements or novel packaging and covering materials.

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Data Availability Data is provided within the manuscript or supplementary information files.

Code Availability Not applicable.

Declarations

Competing Interests The authors declare no competing interests.

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