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Technical Note

Effects of clove oil on physicochemical and functional properties of banana flour nanocomposite film

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Abstract: The effects were studied of clove essential oil (CEO) incorporation on the properties of banana-flour nanocomposite film made with a mixture of montmorillonite and banana starch nanoparticles. The CEO produced a significant increase in the yellow colour (*b**) with a slight decrease in the film opacity. Furthermore, the CEO reduced the film's tensile strength and melting temperature and enhanced water insolubility, water vapour barrier property and antioxidant capacity. However, the nanocomposite film incorporated with CEO had no antifungal activity.

Keywords: clove essential oil, banana flour, nanocomposite film, active food-packaging

INTRODUCTION

Clove (*Syzygium aromaticum)* is a herbal plant with a long history of use by humans. Bioactive compounds can be extracted from the dried flower buds of clove and used in traditional medicine as a bactericide, fungicide, anaesthetic and for other uses [1, 2]. The main constituents of clove are phenylpropanoids such as eugenol (more than 95%), carvacrol, thymol and cinnamaldehyde, with minor levels of β-caryophyllene and α-humulene [2]. Eugenol is the main volatile component which has antifungal properties against clinical isolates and collection strains of *Candida, Aspergillus* and dermatophytes [3]. Therefore, many researchers have tried to incorporate clove essential oil (CEO) in both synthetic and biopolymers [4-10]. The synthetic polymers can be used for food bio-preservative purposes, such as linear low-density polyethylene film for chicken meat packaging [8] and polylactide/poly(ε-caprolactone) for scrambled egg packaging [9]. Many

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researchers have tried to add CEO into biopolymers to form bioactive films for antimicrobial and antioxidant food-packaging purposes, such as sunflower protein film for fish patties [4], Type B gelatin composite film for shrimp packaging [5], citrus pectin film for antioxidant packaging [6], Job's tears (*Coix lachryma-jobi* L.) starch film for pork belly [7] and agar film for flounder (*Paralichthys orbignyanus*) fillets [10].

Banana flour, obtained from raw banana (*Musa* spp.), is abundant, cheap and a rich source of starch, and can be an alternative source of biopolymer for food-packaging material [11-16]. However, the hydrophilic property of banana flour film needs to be improved for its successful use as a packaging material for dried foods. From our previous studies, beeswax and two nanofillers—natural nanoclay (montmorillonite: MMT-Na^+) and banana starch nanoparticles (BSNs)—improved the water barrier and mechanical properties of banana flour film [14, 15]. In addition, garlic essential oil at 10 times the minimum fungicidal concentration incorporated in the banana flour film produced a very high level of antioxidant and antimicrobial properties in the preservation of roasted peanut [11].

The large and unacceptable loss of volatile compounds in CEO at room temperature needs to be addressed. Therefore, many nanocomposite films have been developed, including chitosan film [17], gelatin composite film [5], polylactide/poly(ε-caprolactone) film [9] and soy protein film [18], to retard the evaporation of CEO by using halloysite nanoclay, zinc oxide nanorods and nanoparticles and microfibrillated cellulose respectively. It is evident that appropriate nanoparticles are required to act as carriers and as a release-control system of CEO in active packaging. In our previous work [11], a mixture of MMT-Na^+ and BSNs was shown to act as a garlic essential oil carrier and release system in banana flour film. Consequently, the objective of the present study is to use a similar type of film for CEO and to study its effect on the properties of the film.

MATERIALS AND METHODS

Materials

Raw banana (*Musa sapientum* Linn at 112-116 days after petal fall) was obtained from an orchard on the Kasetsart University, Khamphaeng Saen campus, Nakhon Pathom, Thailand. Banana flour and banana starch were prepared using a modified method from our previous research [16]. Cyclohexane, Tween 80, glycerol, sorbitol, sucrose and $MgSO_4.7H_2O$ were obtained from Ajax Fine Chemicals (Australia). Sodium trimetaphosphate (STMP) was purchased from SIGMA (China). Sodium chloride was obtained from WVR International Ltd. (England). Sodium hydroxide and Span 80 were purchased from Merck (Germany). MMT-Na^+ was donated by Southern Clay Products (USA). CEO was obtained from Thai-China Flavours and Fragrances Industry (Thailand). 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (USA).

Preparation of Banana Starch Nanoparticles

The banana starch nanoparticles were prepared using a water-in-oil mini-emulsion cross-linking technique modified from our previous study [16]. First, the aqueous phase was prepared by mixing the hydrophilic natural materials (1% w/v of banana starch in 50 mL of 0.5N NaOH solution) with the cross-linker substances (0.5 g STMP and 0.375 g NaCl) using a controlled-temperature magnetic stirrer at 90°C for 30 min. Then the oil phase was obtained from blending 1% w/v emulsifier (Span-80:Tween-80 = 84:16) into 300 mL cyclohexane using a high-speed homogeniser at 6,000 rpm for 2 min. The prepared aqueous phase was added dropwise (1.5

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mL/min.) into the oil phase with vigorous stirring. The starch granules were washed with acetone to separate them from the emulsion. Then they were precipitated using acetic acid. The precipitated starch granules were washed with purified water until neutral and kept frozen (-40°C) for 24 hr prior to lyophilisation with a freeze dryer (Model CoolSafe 110-4 Pro, Labogene, Denmark).

Preparation of Fungi

A strain of *Aspergillus flavus* was provided by the Central Laboratory and Greenhouse Complex of Kasetsart University, Nakhon Pathom, Thailand. *A. flavus* was cultivated for 7 days at 27±2°C on yeast extract sucrose agar; then it was used to recover fungal spores according to the method of Orsuwan and Sothornvit [11]. Suspensions of 1.0×10^4 CFU/mL of *A. flavus* were prepared.

Antimicrobial Activity of CEO

The minimum inhibitory concentration (MIC) of CEO was obtained using macrodilution in yeast extract broth test tubes [11]. To determine the minimal fungicidal concentration (MFC), 100 µL of the non-growth suspensions (MIC and higher concentrations) were pipetted and seeded with a sterile L-rod onto Petri dishes containing yeast extract sucrose agar. Each sample was placed in an incubation cabinet at 27±2°C for 5 days. The lowest CEO concentration showing zero colony growth was defined as the MFC.

Banana Flour Nanocomposite Film Preparation

The banana flour nanocomposite (BFN) film was prepared using our solution casting method [11, 13]. A sample of banana flour powder (2 g) was dispersed in 75 mL of distilled water with a mixture of glycerol and sorbitol as plasticiser at a ratio of 1:1 (40% w/w of banana flour). The suspension was heated to 90 °C and held for 20 min. with vigorous stirring. A mixture (0.5 g) of an equal amount of MMT-Na^+ and BSNs was dispersed overnight in 10 mL water using a magnetic stirrer at room temperature (27±2ºC). This was added dropwise into the above suspension and the film solution was kept at 90°C for 10 min. CEO (0.2 and 1.0 mg/mL of the film solution) was mixed with Tween 80 (25% w/w of CEO) in 15 mL of purified water. After the film solution had cooled to 60°C, the CEO emulsion was slowly added (1.5 mL/min.) with continuous stirring. Then the resulting mixture was cast into Petri dishes (diameter 9 cm) and dried in a hot-air oven at 40±5°C for 16 hr. The dried film was peeled off and preconditioned in a chamber under constant humidity (RH 50±5%) and temperature (27±2°C) with magnesium nitrate for at least 48 hr prior to further analysis. The film thickness was measured using a manual micrometre (No. 7326, Mitutoyo Manufacturing, Japan) to the nearest 0.00254 mm. The mean thickness of each film was determined from an average of five random measurements.

Characterisation of Nanocomposite Film

Infrared spectroscopy

The BFN film was scanned using an attenuated total-reflectance Fourier-transform infrared (ATR-FT-IR) spectrometer (Spectrum Two, Perkin Elmer, USA). Spectra in the range 4000–600 cm^{-1} with automatic signal gain were collected in 32 scans at a resolution of 4 cm^{-1}. The interference of water and CO_2 from air was deducted during scanning.

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Surface colour and opacity

The surface colour of the BFN film was measured using a colour spectrophotometer (BYK-Gardner GmbH, Germany). CIELAB colour parameters (L^*, a^* and b^*) were averaged from five readings for each sample. A white standard colour plate (L^* = 95.83, a^* = -0.78 and b^* = -0.02) was used to calibrate colour parameters and as a background. The total colour difference (ΔE^*) was calculated based on equation (1):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

where ΔL^*, Δa^* and Δb^* are the differences between each colour value of the white standard colour plate and the film specimen.

The absorbance of the BFN film was determined using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan) at a wavelength of 600 nm (A_{600}) according to Park et al. [19]**.** Then the film's opacity was calculated based on equation (2):

$$\text{Film opacity} = \frac{A_{600}}{\text{Film thickness}} \quad (2)$$

Mechanical properties

Tensile strength, elastic modulus and elongation at break of the film were determined according to the ASTM standard method D882 [20] using an Instron Universal Testing Machine (Model 5569, Instron Engineering Corporation, USA) with a 0.5 kN load cell. Film specimens were cut into a rectangular shape from the centre (0.8 cm wide and 5 cm long). The initial gauge gap and speed of the crosshead were set at 5 cm and 5 cm/min. respectively.

Thermal properties

A differential scanning calorimeter (Diamond DSC, Perkin Elmer, USA) was used to determine the thermal properties of the film. The instrument was calibrated using pure indium. Approximately 5 mg of film was weighed, placed in an aluminium pan and hermetically sealed using an encapsulating press. Samples were heated from 50 to 220ºC at a rate of 10ºC/min. An empty pan was used as a reference. During data collection, the differential scanning calorimeter cell was flushed with nitrogen at 20 mL/min. to maintain an inert environment. The melting temperature (onset and peak temperatures) was determined from the thermogram according to the ASTM standard method D-3418 [21]. The onset temperature is the initial melting temperature and the peak temperature is the temperature at which all substances melt. Each sample mean was calculated from three individual samples.

Moisture content

The moisture content of the film was determined according to the method of Orsuwan and Sothornvit [15]. Each film sample was cut into a square of 3 cm × 3 cm and subsequently dried at 105°C for 24 hr using a hot-air oven. The moisture content was calculated from the weight difference of the sample before and after drying divided by the weight before drying and expressed as a percentage.

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Water vapour permeability

Water vapour permeability of the BFN film was determined using the gravimetric modified-cup method modified from the ASTM standard method E-96 [22]. Briefly, the selected film without pinholes and defects was cut and sealed to a cup (diameter 50.8 mm, area of circular opening 2.03×10^{-3} m^2) previously containing 7 mL of distilled water. The sealed cup was then placed into a pre-equilibrated cabinet with controlled air flow at 27±2°C and 50±5% RH, where relative humidity was maintained using a desiccant (silica gel) in the chamber. The water vapour permeability of the film was calculated based on equation (3):

$$\text{Water vapour permeability} = \frac{\text{WVTR} \times d}{P_{A1} - P_{A2}} \quad (3)$$

where WVTR is water vapour transmission rate (g/day.m^2), d is the film thickness (mm) and P_{A1} and P_{A2} are water vapour partial pressures (kPa) inside and outside the cup respectively.

Water solubility

The water solubility of the BFN film was obtained according to Sothornvit et al. [23] and calculated based on equation (4):

$$\text{Water solubility} = \frac{W_1 - W_2}{W_1} \times 100\% \quad (4)$$

where w_1 and w_2 are the initial and final dried weights of film respectively after immersing in 30 mL of distilled water at 27±2°C for 24 hr and drying in a hot-air oven at 105°C for 24 hr.

Antioxidant activity

A sample extract solution was prepared by mixing 1 g of the film into a test tube containing 10 mL of 95% ethanol using a high-speed homogeniser (Polytron PT3100, Kinematica AG, Switzerland) at 6,000 rpm for 5 min. Then the precipitate was separated from the solution by passing through Whatman No.1 filter paper. The supernatant was used to determine the antioxidant activity of the BFN film that was evaluated using a DPPH free radical scavenging assay according to Orsuwan and Sothornvit [11]. The percentage of DPPH free radical scavenging activity was determined based on equation (5):

$$\text{DPPH free radical scavenging activity} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\% \quad (5)$$

where A_{blank} and A_{sample} are the absorbance values at 517 nm for the methanolic solution of blank (methanol : 0.1 mM DPPH = 3:1) and the sample extract (sample extract : 0.1 mM DPPH = 3:1) respectively.

The DPPH radical scavenging activity was calculated against a calibration curve established with trolox and expressed as mg trolox equivalent/g of film.

Antifungal characterisation

A suspension of 1.0×10^4 CFU/mL of *A. flavus* was obtained based on the preparation mentioned earlier [24]. A 100 µL of the suspension was pipetted and seeded using a sterile L-rod onto yeast extract sucrose agar Petri dishes. The BFN film (diameter 20 mm) samples were placed onto the agar that had already been inoculated. The result was recorded by measuring the diameter of the clear or inhibition zone after 48 hr of incubation at 27±2ºC.

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Statistical analysis

A completely randomised experiment was used to determine the effects of CEO concentration on BFN film properties. At least three replicates were used to determine each property. One-way analysis of variance and Duncan's multiple range tests were used to determine the treatment effects at 95% confidence interval. The statistical analysis was conducted using SPSS 23.0 for Windows software package (SPSS Inc., USA).

RESULTS AND DISCUSSION

Results of the preliminary experiment showed that the MIC of pure CEO against *A. flavus* was 0.2 mg/mL. Therefore, 0, 1 and 5 times the MIC of CEO were incorporated in the BFN film and the film properties were characterised.

IR Spectra

The ATR-FT-IR spectra of the neat BFN and the CEO-BFN blend films are shown in Figure 1. Both the BFN and CEO-BFN blend films have similar basic absorption peaks. There is no shift in the position of the peaks after the CEO was incorporated into the film matrix. The incorporation of CEO into the film matrix may produce some small peaks in the BFN film spectra such as at 1560 cm^{-1} (C=C stretching of an aromatic ring), 1430 cm^{-1} (CH stretching of an aromatic ring) and 1350 cm^{-1} (C–O stretching of an aromatic ester) [25, 26]. This indicates that the addition of the CEO does not affect the BFN film structure and there is only a weak interaction between the aromatic ring in the structure of the phenylpropanoid (eugenol) from the CEO and the polymer via Tween-80 surfactant [25, 26]. These findings are similar to the studies on the incorporation of CEO into Job's tears (*Coix lachrymal-jobi* L.) starch film [7].

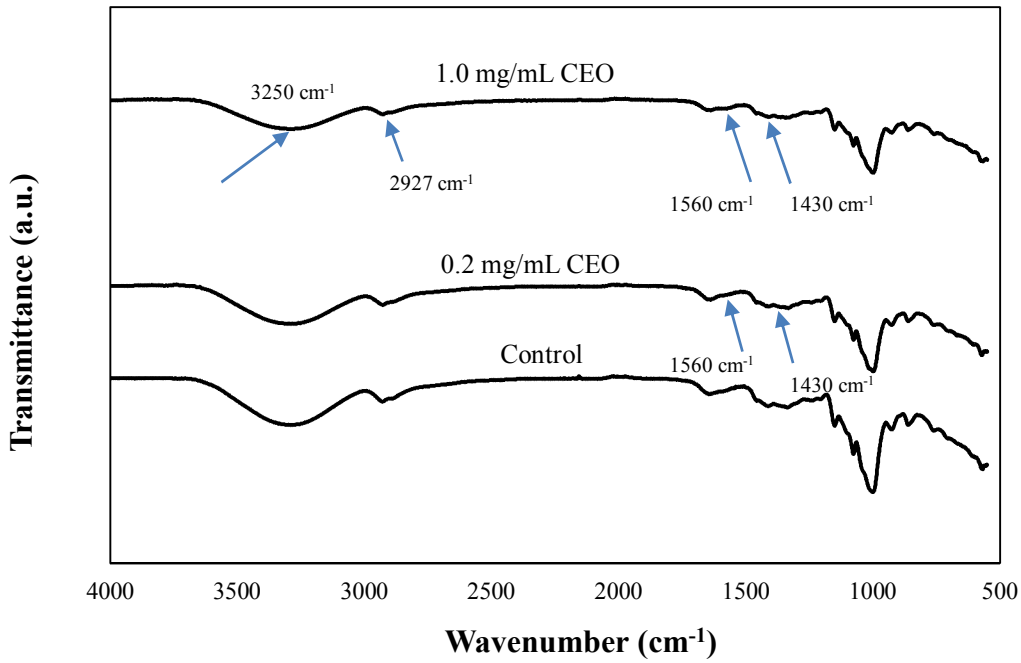

Figure 1. ATR-FT-IR spectra of BFN film (control) incorporated with CEO (0.2 and 1 mg/mL)

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Moisture Content, Water Solubility and Water Vapour Permeability

The addition of CEO into the BFN film seems to change the hydrophilic/hydrophobic ratio of the film network and tends to reduce the water solubility and water vapour permeability of the film (Table 1). However, the increase in moisture content might have been due to the breaking up of the film network by the evaporation of CEO, allowing water molecules to replace and interact with the polymer chains [27]. Nevertheless, the addition of CEO in Job's tear starch film produced opposite results when the CEO content increased, namely a decrease in the moisture content and an increase in the water vapour permeability [7]. However, there was a similar trend in the reduction of water solubility of the Job's tear starch film. These different trends might be attributed to the change in the film structure, specifically the presence of a porous structure in the starch film, which was believed to have occurred during the evaporation of CEO that retained a vacuum during film forming and drying [5].

Table 1. Moisture content, water solubility and water vapour permeability of BFN film incorporated with CEO

CEO (mg/mL)	Moisture content (%)	Water solubility (%)	Water vapour permeability (g.mm/kPa.day.m^2)
Control	15.99^{a}±0.52	1.70^{b}±0.06	57.25^{b}±1.29
0.2	19.98^{b}±0.23	1.76^{b}±0.06	50.43^{ab}±7.55
1.0	23.09^{c}±0.71	1.58^{a}±0.07	49.31^{a}±0.21

[a, b] Different superscript letters in the same column indicate significant differences between treatments at 95% confidence interval.
Note: Values are given as mean ± standard deviation.

Mechanical Properties

Table 2 shows the tensile properties of the BFN and CEO-BFN blend film. As expected, the incorporation of CEO into the BFN film significantly decreases the tensile strength of the polymer, but it does not affect the elongation at break and elastic modulus compared to the neat BFN film. The decrease in tensile strength of the polymer is clearly associated with the existence of structural discontinuities in the film network or the heterogeneous film structure. Nevertheless, the decrease in the tensile strength in the current study is opposite to the result from the addition of garlic oil into the BFN film in our previous study [11]. This might be attributed to the interactions between the different chemical compositions of the natural active compounds and the polymer matrix. In addition, the organosulphur compounds in garlic oil, such as diallyl trisulphide, diallyl disulphide and diallyl sulphide, play an important role in the plasticising effect in the BFN film and they do not evaporate at room temperature [11] while the CEO contains many volatile compounds. Chitosan-gum arabic complex film containing cinnamon and CEO also had reductions in the tensile strength [28], which was primarily attributed to the partial replacement of the stronger intermolecular polymer interactions and polymer-polymer interaction by the weaker polymer-oil interactions in the film matrix. The addition of CEO into Type B gelatin film and chicken feather protein/gelatin film also produced similar results, which was possibly due to the CEO acting as a plasticiser in the

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gelatin film matrix or the replacement of protein molecules by clove oil in the film matrix [5, 29], thus lowering the strength of the intermolecular forces along the protein matrix [5]. Nisar et al. [6] found that the addition of CEO as a plasticiser extended the elongation at break of pectin film.

Table 2. Tensile strength, elastic modulus, elongation at break, onset and peak melting temperatures of BFN film incorporated with CEO

CEO (mg/mL)	Tensile strength (MPa)	Elastic modulus (MPa)	Elongation at break (%)	Onset temperature (°C)	Peak temperature (°C)
Control	4.29^{b}±0.02	33.94^{a}±0.21	26.55^{a}±1.83	175.79^{b}±4.71	178.35^{b}±6.01
0.2	3.80^{a}±0.10	35.57^{a}±2.69	23.11^{a}±2.01	171.48^{ab}±2.16	172.74^{ab}±1.63
1.0	3.87^{a}±0.05	31.80^{a}±2.84	24.44^{a}±2.04	164.27^{a}±6.85	165.16^{a}±7.05

[a, b] Different superscript letters in the same column indicate significant differences between treatments at 95% confidence interval.
Note: Values are given as mean ± standard deviation.

Thermal Properties

The onset and peak melting temperatures of the polymer are displayed in Table 2. In the blend film with 1.0 mg/mL CEO these temperatures are significantly lower compared to the control film. This might be attributed to the change in the film structure after the CEO has evaporated during film drying [5], resulting in an increase in water absorption in the amorphous region of the starch molecules. This indicates a decreased thermal stability of the BFN film with increased CEO content.

Colour and Opacity

The surface colour values, especially the yellowness (b^*) and the total colour difference (ΔE^*) of the CEO-BFN blend film are significantly influenced by the incorporation of 1% CEO in the film (Table 3). The b^* value of the film significantly increases, most probably due to the colouring components contained in both the CEO and Tween-80, which affect the change in the b^* value of the film [1-6]. Consequently, the total colour difference (ΔE^*) of the CEO-BFN blend film also changes significantly with the same trend. The visual appearance is similar to that reported in our previous study where garlic oil was incorporated in banana nanocomposite film [11] even though garlic oil and clove oil have different colouring constituents.

Antioxidant Activity

The radical scavenging activity of the film is shown in Table 3. The antioxidant activity of the neat banana flour film is based on DPPH assay [11, 30]. In general, phenolic compounds and other phytochemical agents contained in the banana flour have been proved to be free radical scavengers and a UV barrier [31]. The incorporation of the CEO significantly increases the antioxidant capacity of the BFN film due to eugenol and various other compounds present in the CEO. Furthermore, the antioxidant activity of the CEO-BFN blend film (Y) linearly increases with

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Table 3. Colour (*L**, *a** and *b**), total colour difference (Δ*E**), opacity and DPPH radical scavenging activity of BFN film incorporated with CEO

CEO (mg/mL)	*L**	*a**	*b**	*ΔE**	Opacity (AU/mm)	DPPH radical scavenging activity (mg trolox eq./g of film)
Control	76.52^{a}±0.27	7.24^{a}±0.23	23.39^{a}±0.52	31.40^{a}±0.59	3.01^{a}±0.10	16.27^{a}±1.52
0.2	76.84^{a}±0.74	7.40^{a}±0.48	22.19^{a}±0.80	30.37^{a}±1.18	3.07^{a}±0.25	19.55^{b}±0.96
1.0	76.48^{a}±0.06	7.33^{a}±0.11	26.73^{b}±0.37	34.01^{b}±0.28	2.61^{a}±0.29	38.16^{c}±0.73

$^{a, b, c}$ Different superscript letters in the same column indicate significant differences between treatments at 95% confidence interval.

Note: Values are given as mean ± standard deviation.

increased CEO concentration X in the equation Y = 22.282X + 15.747 (R^2 = 0.9974), with approximate increases of 20% and 130% for 0.2 mg/mL and 1.0 mg/mL respectively of CEO. However, the CEO-BFN blend film with 1.0 mg/mL of CEO has 30% lower antioxidant activity compared to film containing the same content of garlic essential oil in our previous research [11]. This may be attributed to the different chemical compositions of the two essential oils and the fact that there are more volatile components in the CEO, which might have evaporated during the film drying step, resulting in a reduction in the antioxidant activity efficiency of the CEO in the BFN film.

Antifungal Activity

As expected, the control BFN film showed hardly any antifungal activity. Similarly, the antifungal activity from the addition of CEO showed that the CEO-BFN blend film did not exert a definite inhibitory effect on the growth of *A. flavus* mycelia (no clear or inhibition zone was observed). As mentioned earlier, this might be attributed to the high volatility of CEO and the weak interaction between CEO and the polymer matrix that resulted in the loss of volatile compounds during the film-forming process [26]. The remaining active hydrophobic substances in the CEO-BFN blend film were insufficient to induce fungal cell lysis, resulting in no inhibition of fungal growth. However, the effective antimicrobial concentrations of CEO against pathogenic bacteria have been reported to be relatively high compared to the current study. Ejaz et al. [5] found that Type B gelatin film containing 2% zinc oxide nanorods loaded with 50% CEO (15 mg/mL) had a maximum antimicrobial activity (4 log reduction) against *Listeria monocytogenes* and *Salmonella typhimurium*. Sahlan et al. [32] investigated the microencapsulation of CEO with a casein encapsulator using spray drying and found that the CEO encapsulated in the microcapsule had an MIC of 250 mg/mL against *Streptococcus mutans*.

CONCLUSIONS

The inclusion of CEO in the BFN film leads to a significant increase in the yellow colour (*b**) of the active compounds in the CEO, with a slight decrease in the opacity. Furthermore, the incorporation of the CEO into the BFN blend film results in lower tensile strength and melting temperature but better water insolubility, water vapour barrier property and antioxidant capacity. However, the highest concentration of CEO applied in this study (1.0 mg/mL) did not produce any

noticeable antifungal activity against *A. flavus*. Further studies are planned using higher concentrations of CEO with a proper encapsulated material and process.

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