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Original article

Efficacy of *Cinnamomum camphora* essential oil loaded chitosan nanoemulsion coating against fungal association, aflatoxin B_1 contamination and storage quality deterioration of *Citrus aurantifolia* fruits

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Summary The present study demonstrates the first time investigation on *Cinnamomum camphora* essential oil (CCEO) loaded chitosan nanoemulsion coating on *Citrus aurantifolia* fruits to ensure protection against *Aspergillus flavus* infestation and aflatoxin B_1 (AFB_1) contamination. Encapsulation of CCEO was confirmed through scanning electron microscopy (SEM), X-ray diffractometry (XRD) and Fourier transform infrared spectroscopy (FTIR). CCEO-loaded nanoemulsion displayed improved *in vitro* antifungal (1.0 μL mL^{-1}) and antiaflatoxigenic activity (0.8 μL mL^{-1}) as compared to unencapsulated CCEO. Additionally, CCEO nanoemulsion inhibited synthesis of ergosterol, enhanced the leakage of Ca^{2+}, K^+ and Mg^{2+} ions and retarded methylglyoxal biosynthesis in *A. flavus* cells. CCEO nanoemulsion coating on *C. aurantifolia* fruits remarkably reduced weight loss, total soluble solids and titrable acidity along with inhibition of *A. flavus* contamination and AFB_1 secretion during storage. Moreover, nanoemulsion coated fruits maintained the enzymatic antioxidant viz. superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities without affecting sensory attributes, thereby strengthening the practical applicability of CCEO nanoemulsion as nano-green smart fruit preservative.

Keywords Aflatoxin B_1, *Cinnamomum camphora* essential oil, coating, nanoemulsion.

Introduction

Citrus aurantifolia Swingle is extensively cultivated in tropical and subtropical regions of Asiatic countries. Fruits are rich in carotenoids, flavonoids, limonoids, ascorbic acid and phenols with beneficial activities against human diseases (Champa *et al*., 2020). However, during postharvest storage, fruit losses (20–90%) are recorded due to fungal infestation and mycotoxin contamination. Among different storage fungi, *Aspergillus flavus* is notoriously known for postharvest decay of *C. aurantifolia* fruits and attracted global attention because of aflatoxin B_1 (AFB_1) production. Recently, Das *et al*. (2021a) reported induced synthesis of aflatoxin in presence of methylglyoxal, thus deterioration in the quality and quantity of stored food commodities.

Different synthetic fungicides are used to control fungal infestation and AFB_1 contaminations, but their indiscriminate application can lead to health disorders, environmental pollution and induction of resistance in fungi (Singh *et al*., 2019). Recent decades have shown great contribution of essential oils in the inhibition of fungal and AFB_1 contamination due to their volatile nature, environmental friendliness and Generally Recognized As Safe (GRAS) status. However, direct application of essential oils on fruit surface may lead to changes in organoleptic properties and rapid oxidation of essential oil components, thereby causing loss in antifungal bioefficacy (Das *et al*., 2021b).

Recently, considerable attention has been paid to edible coatings of fruits as a suitable alternative of conventional preservation. Yang *et al*. (2021) investigated the antifungal effect of eugenol, cinnamaldehyde and carvacrol nanoemulsion-based edible coating on *Citrus reticulata* fruits against infestation of *Penicillium digitatum*. Antimicrobial efficacy and maintenance of *Citrus sinensis* fruit qualities using pectin-based edible coating containing orange peel essential oil has been recently reported by Radi *et al*. (2018). In this context, encapsulation of essential oils into biocompatible polymer

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matrix has been regarded as smart nanotechnology to improve essential oil efficacy and maintenance of fruit qualities under stored conditions. Among different biopolymers, chitosan has proven merit for encapsulation due to its biodegradability, and inclusion under GRAS category (Chaudhari *et al*., 2020). Additionally, coating with nanoencapsulated essential oils allows better protection of fruits due to nano-scale dimension, colloidal stability and controlled release of bioactive components (Manzoor *et al*., 2021).

Cinnamomum camphora (L.) J. Presl essential oil (CCEO), a valuable material for pharmaceutical, and food industries possesses strong antibacterial, antifungal and antioxidant activities (Chen *et al*., 2020). Till now, there is no study discussing the effect of CCEO and its nanoemulsion on maintenance of *C. aurantifolia* fruits quality by inhibiting fungal infestation and AFB_1 contamination.

Hence, the current study aimed to encapsulate CCEO into chitosan nanobiopolymer and investigated its efficacy as active coating on *C. aurantifolia* fruits to inhibit fungal association, AFB_1 contamination, and enhancement in fruit shelf-life. *In vitro* release behaviour was performed to determine controlled delivery of CCEO. The biochemical mechanisms of antifungal action *via* targeting the ergosterol, membrane permeability, and methylglyoxal biosynthesis have also been investigated. Furthermore, *in vivo* quality control of CCEO nanoemulsion coated *C. aurantifolia* fruits in terms of fruit weight, phenolic content, total soluble solid, titrable acidity, pH and sensory attributes have been performed for promising application of prepared nanoemulsion as nanogreen smart and eco-friendly delivery vehicle for fruit preservation.

Materials and methods

Chemicals and solvents

Chemicals and solvents used for present study *viz*. chitosan (98% purity), ethylacetate (99% purity), sodium tripolyphosphate (S-TPP, 99% purity), phosphate buffer (99.5% purity), ethanol (98% purity), sucrose (≥97.5% purity), $MgSO_4.7H_2O$ (99% purity), KNO_3 (99% purity), yeast extract (97% purity), methanol (98.5% purity), chloroform (98% purity), KOH (99% purity), *n*-heptane (97% purity), KBr (99.5% purity), acetonitrile (≥99.5% purity) and Folin Ciocalteu (99% purity) were procured from Sisco Research laboratory (SRL), and Hi-Media Laboratory, Mumbai, India.

Fungal strain

Aspergillus flavus strain (AFLHPR14), previously isolated and identified in our laboratory was used for the present investigation (Das *et al*., 2020). Culture of the fungal strain was maintained on potato dextrose agar (PDA) medium in refrigerator at 4 °C (uncertainty value 0.1).

Extraction and chemical characterisation of *Cinnamomum camphora* essential oil (CCEO)

Fresh inflorescences of *Cinnamomum camphora* were subjected to Clevenger's hydro-distillation for 4 h at 70 °C (uncertainty value 0.1), followed by removal of water traces.

Chemical characterisation was performed by Thermo Scientific 1300-Gas Chromatograph coupled with DSQ II quadruple Mass Spectrophotometer. An aliquot of 1 μL CCEO was injected into DB-5MS capillary column (60 m length × 0.25 mm i.d., film thickness, 0.25 μm) and He was used as carrier gas (flow rate 1 mL min^{-1}). Temperature program for GC was set from 45 °C (uncertainty value 0.1) to 200 °C at a rate of 5 °C min^{-1} and extended up to 300 °C with the rate 40 °C min^{-1}. MS transfer line and ion source temperature were set as 250 and 230 °C respectively. Operation of mass spectrometer was performed at 70 ev. Components were identified and calibrated on the basis of retention time, elution order and relative retention indices by using *n*-alkanes series (C_9-C_{28} hydrocarbons) and compared with National Institute of Standard and Technologies (NIST) 2011 libraries.

Synthesis of CCEO loaded chitosan nanoemulsion (Ne-CCEO)

CCEO loaded chitosan nanoemulsion (Ne-CCEO) was prepared by the protocol of Das *et al*. (2021c). Different weight ratios (w/v) of chitosan to CCEO (1:0, 1:0.2, 1:0.4, 1:0.6, 1:0.8, and 1:1) were prepared accordingly.

Physico-chemical characterisation of Ne-CCEO

Morphology and surface characteristics of Ne-CCEO was observed through scanning electron microscopy (SEM, Hitachi S-530). X-ray diffraction (XRD) analysis of chitosan, chitosan nanoemulsion, and Ne-CCEO was done at 2θ value ranging from 5° to 50°. Fourier transform infrared spectroscopy (FTIR) analysis of chitosan, chitosan nanoemulsion, CCEO and Ne-CCEO was performed at wave number 500–5000 cm^{-1} with resolution of 4 cm^{-1}.

In vitro inhibitory activity of CCEO and Ne-CCEO against *A. flavus* growth and AFB_1 production

Antifungal and AFB_1 inhibitory activity of CCEO and Ne-CCEO was measured in SMKY medium by determining minimum inhibitory concentration (MIC) and minimum AFB_1 inhibitory concentration (MAIC) following culture incubation at 25 ± 2 °C (uncertainty value 0.1)

for 7 days. Concentration causing 100% growth inhibition of AFLHPR14 strain was considered as MIC.

To determine AFB_1 content, media was extracted with chloroform, evaporated, and dissolved into 1 mL methanol. AFB_1 separated on thin layer chromatography (TLC) plate was scrapped and transferred in methanol, centrifuged at 5000 ***g*** (4 °C; uncertainty value 0.1) and subjected to absorbance measurement at 360 nm (Chaudhari *et al*., 2020). Concentration representing 100% inhibition of AFB_1 was considered as MAIC.

Antifungal and antiaflatoxigenic mechanisms of action of CCEO and Ne-CCEO

Antifungal mechanism of action of CCEO and Ne-CCEO was determined through ergosterol assessment and ion leakage tests. For ergosterol determination, AFLHPR14 cells were treated with CCEO and Ne-CCEO, followed by extraction of ergosterol (Kedia *et al*., 2015). For ion's leakage determination, AFLHPR14 cells were fumigated with ½ MIC, MIC, and 2 MIC doses of CCEO and Ne-CCEO and concentration of Ca^{2+}, K^{+}, and Mg^{2+} ions was measured through atomic absorption spectroscopy.

Antiaflatoxigenic mechanism of action of AFLHPR14 cells treated with CCEO and Ne-CCEO was determined through methylglyoxal assessment (Das *et al*., 2021d).

Coating of *C. aurantifolia* fruits with Ne-CCEO and storage conditions: *In vivo* assay

Mature *C. aurantifolia* fruits were dipped into Ne-CCEO nanoemulsion for 1 min followed by 2–3 min air drying. Fruits immersed into distilled water served as uncoated control. Two different control and treatment sets were prepared on the basis of inoculation with 50 μL of AFLHPR14 spore suspension on the coated and uncoated surface of *C. aurantifolia* fruits. Finally, all the coated and uncoated fruits were stored at 4 °C (uncertainty value 0.1) for 30 days and assessed for fruit quality.

Impact of Ne-CCEO coating on fungal and AFB_1 contamination of *C. aurantifolia* fruits

For determination of *in vivo* antifungal activity, fruit juice of both uncoated and coated fruits was extracted, inoculated in PDA medium and incubated for 10 days. Percent protection was determined on the basis of inhibition of fungal occurrence.

For AFB_1 content determination, 2 mL of fruit juice was mixed with methanol and centrifuged at 10 000 ***g*** for 10 min (4 °C) (uncertainty value 0.1). Separated upper layer was diluted in chloroform and KBr mixed distilled water followed by AFB_1 determination. A solvent system comprising of methanol, acetonitrile and water (17:19:64 v/v/v) at a flow rate of 1 mL min^{-1} was used for high pressure liquid chromatography based AFB_1 detection.

Effect of Ne-CCEO coating on weight, total phenolic content (TPC), total soluble solid (TSS), titrable acidity (TA) and juice pH of *C. aurantifolia* fruits

Weight loss was determined on the basis of differences in final and initial fruit weight after 30 days. Fruit juice was used for the determination of TPC, TSS and TA. For TPC, 20 mL of fruit juice was mixed with 1.16 mL of distilled water followed by addition of 100 mL of Folin Ciocalteu solution. TPC was expressed as μg mL^{-1} gallic acid. TSS was determined by hand refractometer. Acid content in coated and uncoated fruits was determined by titration and expressed as % citric acid (Yao *et al*., 2018). pH of fruit juice was measured using digital pH metre.

Enzymatic antioxidant activity of Ne-CCEO coated *C. aurantifolia* fruits

Juice isolated from uncoated and coated fruits was used for the determination of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activity and expressed as U g^{-1} according to the protocol of Yang *et al*. (2021).

Sensory evaluation

Sensory evaluation of uncoated and coated *C. aurantifolia* fruits for texture, flavour, odour, and colour was conducted by 10 panellists from Banaras Hindu University Varanasi, India, aged between 25 and 50 years. The panellists were informed about the methodology and asked to sign a consent pertaining to reagents used for the preparation of nanoemulsion. Two minute was provided to each panellist for scoring sensory parameters of fruits. Intensity of each sensory property was evaluated by five point hedonic scale (Das *et al*., 2021c).

Statistical analyses

Analyses of samples were performed in triplicate and results were expressed as mean ± standard error. Statistical comparison was done by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test at P values < 0.05.

Results and discussion

Extraction and chemical characterisation of CCEO

Yield of CCEO was 4 mL kg^{-1} of fresh inflorescences. GC-MS analysis of CCEO revealed 20 components

Table 1 GC-MS of CCEO

Retention time	% Composition	Components	Retention index
4.28	0.62	Tricyclene	926
4.74	**3.98**	**Camphene**	954
5.79	0.03	Mesitylene	995
4.84	0.28	Carane	1013
6.54	4.76	o-Cymene	1026
6.59	**10.05**	**D-Limonene**	1029
8.69	**8.23**	**Fenchone**	1086
9.91	0.11	à-Campholenal	1126
10.76	**60.27**	**(-)-Camphor**	1146
10.91	0.07	Terpineol <cis-β->	1144
11.65	0.07	Methyl isoborneol <2->	1181
12.27	1.17	p-Cymen-8-ol	1182
15.28	0.2	Carvenone	1258
16.87	0.12	Carvacrol	1299
18.71	**5.02**	**β-caryophyllene**	1422
24.8	0.02	Kessane	1530
25.23	0.02	Limonen-10-ol	1289
29.29	0.04	Apiol	1678
30.13	0.02	Cadinol	1654
30.61	0.03	aR-Turmerone	1669
Total	95.11		

Note: Components in bold are major components.

with camphor, D-limonene, fenchone, β-caryophyllene and camphene as major components (Table 1). Chen *et al*. (2020) reported linalool and eucalyptol as major components of CCEO. Borneol was identified as major ingredient of CCEO (Xiao *et al*., 2021). In both cases, leaves were used for CCEO extraction. However, our result contradicted previous investigation which might be due to utilisation of inflorescence for essential oil extraction. Such component differences depend on chemotypes, geographical variation and altitudinal effects (Das *et al*., 2021b).

Encapsulation of CCEO into chitosan nanoemulsion (Ne-CCEO)

Ne-CNEO was synthesised *via* electrostatic interaction between positively charged NH_3^+ groups of chitosan with negatively charged PO_4^{3-} group of S-TPP. Different chitosan to CCEO ratio (w/v) was prepared to optimise maximum encompassment of CCEO.

Physico-chemical characterisation of Ne-CCEO

SEM analysis

Particles size of chitosan nanoemulsion was 20.36–51.21 nm. Incorporation of CCEO enhanced the size from 60.34 to 89.55 nm (Fig. 1a, b) and is in agreement with the observations of Rajkumar *et al.* (2020). Particles of chitosan and Ne-CCEO nanoemulsion were spherical in shape with agglomeration at certain locations.

FTIR analysis

FTIR spectra of chitosan, chitosan nanoemulsion, CCEO and Ne-CCEO nanoemulsion are presented in Fig. 1c. Characteristic peaks of chitosan were observed at wave number 1080 (C–N), 1088 (C–O–C), 1379 (H–C–H, C–O–H), 1647 (amide I), 2925 (–CH stretching) and 3430 (–NH_2/OH bending) cm^{-1}. Peaks of chitosan nanoemulsion were similar to chitosan except some peak shifting. CCEO displayed specific peaks at wave number 810-860 (aromatic ring), 1450 (C–OH bending), 1573 (C=C vibration), 1626, 1679 (C=O stretching vibration), 2960 (asymmetric –CH_3 stretching) and 3331 (–OH stretching) cm^{-1}. However, increasing intensity of different absorption bands, especially P=O groups and amide I peaks in Ne-CCEO suggested encapsulation of CCEO into chitosan nanobiopolymer (Amiri *et al*., 2021).

XRD analysis

Figure 1d presents X-ray diffractogram of chitosan, chitosan nanoemulsion, and Ne-CCEO. A specific peak at 2θ diffraction angle of 20° indicated crystalline nature of chitosan powder. Chitosan nanoemulsion, due to ionic cross-linking of chitosan amino group with S-TPP, showed loss of crystallinity in chitosan. Incorporation of CCEO into chitosan nanoemulsion showed broadening associated with the loss of crystalline structure and formation of modified chitosan with amorphous phase (Chaudhari *et al*., 2020).

Loading capacity (LC) and encapsulation efficiency (EE) of Ne-CCEO

LC and EE of Ne-CCEO ranged between 0.41–3.69% and 54.18–94.12% respectively (Table S1). LC and EE increased with rise in concentrations of CCEO and maximum value was recorded at 1:1 ratio (w/v) of chitosan to CCEO. Similar increasing trend has been demonstrated by Singh *et al*. (2019) during encapsulation of *Ocimum sanctum* essential oil. High LC and EE value is an important and preferred attribute for enhancement in shelf-life of essential oil (Hasani *et al*., 2018), which could maximise their application in long-term preservation of stored fruits.

In vitro release of Ne-CCEO

The amount of CCEO released under *in vitro* condition was determined at absorption maxima 289 nm. Biphasic release pattern *viz*. initial fast release (23.69%) within 0–8 h, followed by slow release (3.39%) between 48 and 96 h was observed. After 96 h, the release rate reached in the form of plateau (Fig. 2a). Rapid release was probably due to chitosan surface adsorbed CCEO. Our result is consistent with

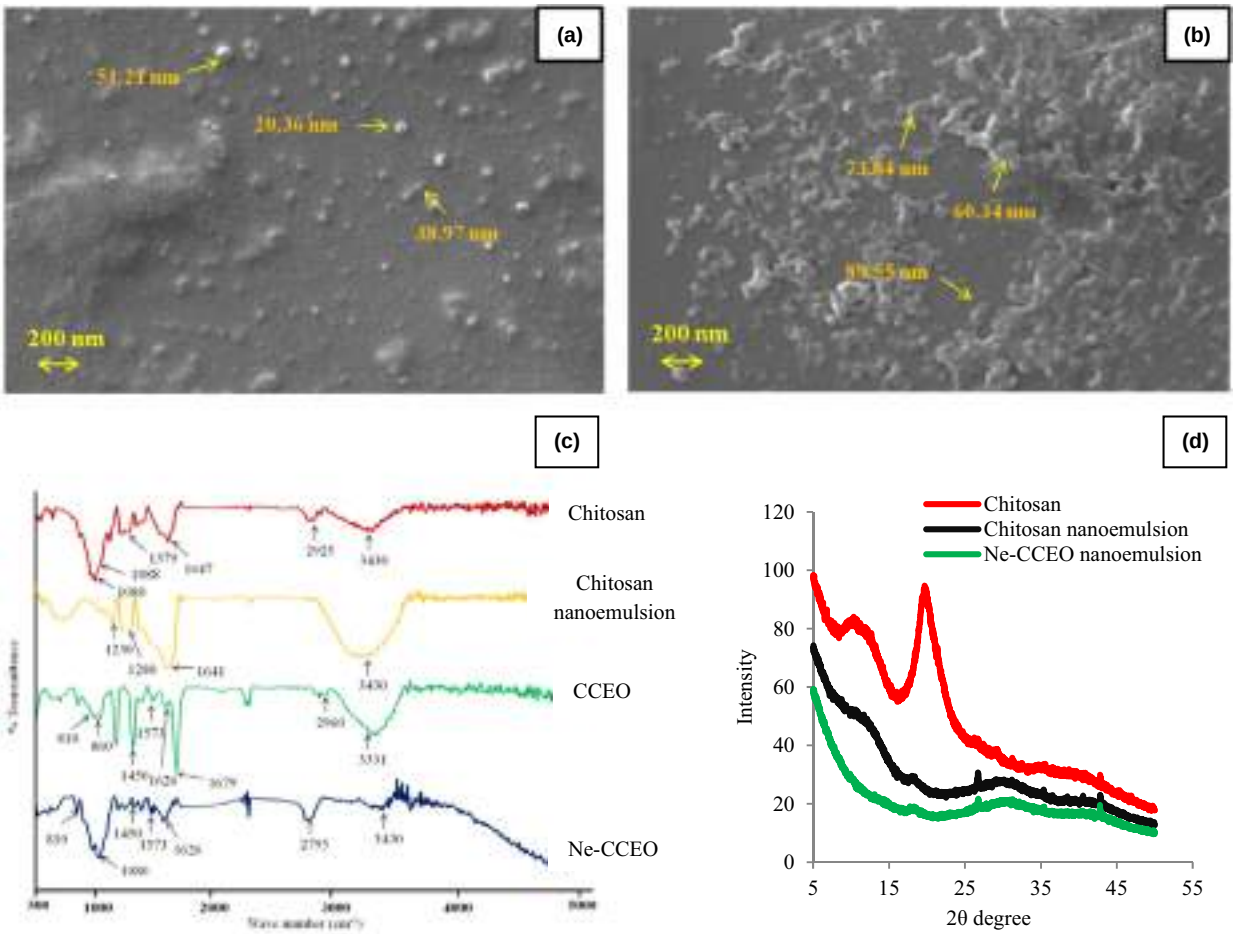

Figure 1 Scanning electron microscopy of chitosan nanoemulsion (a), Ne-CCEO nanoemulsion (b), FTIR of chitosan, chitosan nanoemulsion, CCEO and Ne-CCEO nanoemulsion (c), XRD analysis of chitosan, chitosan nanoemulsion and Ne-CCEO nanoemulsion (d).

Hosseini *et al.* (2013) suggesting controlled delivery of oregano essential oil.

In vitro inhibitory activity of CCEO and Ne-CCEO against *A. flavus* growth and AFB_1 production

MIC and MAIC of CCEO against AFLHPR14 cells were recorded at 2.5 and 2.0 μL mL^{-1} respectively. MIC and MAIC of Ne-CCEO was found as 1.0 and 0.8 μL mL^{-1} respectively (Fig. 2b, c). Better inhibitory action of Ne-CCEO against fungal growth and AFB_1 production has been associated with nanometric size and controlled delivery (Chaudhari *et al.*, 2020).

Antifungal and antiaflatoxigenic mechanisms of action of CCEO and Ne-CCEO

Ergosterol is required for fungal membrane fluidity, and cellular metabolism (Kedia *et al.*, 2015). Decrement in ergosterol content of AFLHPR14 cells (Fig. 2d, e) was observed after treatment with CCEO and Ne-CCEO likely due to inhibition of lanosterol-14α-demethylase (Das *et al.*, 2021d). Fumigation of AFLHPR14 cells with CCEO and Ne-CCEO led to efflux of Ca^{2+}, K^{+}, and Mg^{2+} ions (Fig. 2f) causing destabilisation of cellular homoeostasis. CCEO and Ne-CCEO treatment showed considerable inhibition of cellular methylglyoxal (an AFB_1 inducer) biosynthesis (Fig. 2g).

In vivo antifungal and antiaflatoxigenic efficacy of Ne-CCEO coated *C. aurantifolia* fruit

Coating of *C. aurantifolia* fruits by Ne-CCEO nanoemulsion showed 79.26 and 100% protection against fungal infestation at MIC and 2 MIC doses. Incomplete inhibition of *A. flavus* infestation observed at MIC was expected to be due to absorption of volatile components by fruit itself. However, at both MIC and 2 MIC doses of Ne-CCEO, 100% inhibition of

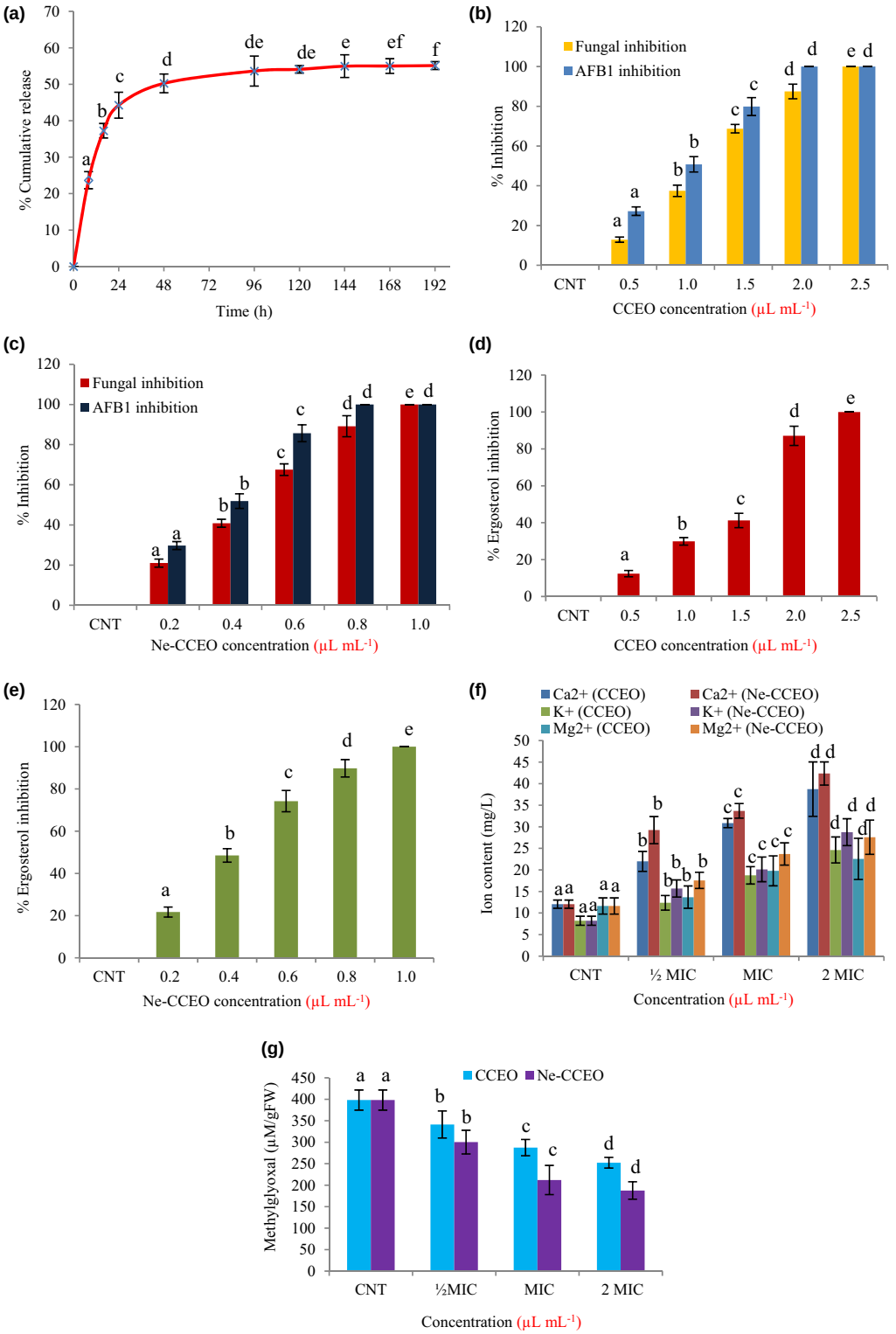

Figure 2 *In vitro* release of Ne-CCEO (a), Inhibition of fungal growth and AFB_1 production by CCEO (b), inhibition of fungal growth and AFB_1 production by Ne-CCEO (c), inhibition of ergosterol by CCEO (d), inhibition of ergosterol by Ne-CCEO (e), effect of CCEO and Ne-CCEO on ions leakage (f), effect of CCEO and Ne-CCEO on cellular methylglyoxal (g).

Table 2 Effect of Ne-CCEO coating on fruit weight, total phenolic content, juice pH, titrable acidity, total soluble solid, fungal and AFB_1 protection of *C. aurantifolia* fruits

Treatment	Weight loss (%)	Phenolic content (μg mL^{-1} gallic acid)	Juice pH	Titratable acidity (%)	Total soluble solid (%)	Fungal protection (%)	AFB_1 protection (%)
Uninoculated control (uncoated fruits)	9.03 ± 0.014^{b}	9.22 ± 0.015^{a}	4.10 ± 0.023^{a}	0.54 ± 0.024^{ab}	30.41 ± 1.16^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Inoculated control (uncoated fruits)	9.25 ± 0.026^{a}	9.11 ± 0.020^{a}	4.03 ± 0.012^{a}	0.57 ± 0.023^{a}	32.45 ± 0.69^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Uninoculated Ne-CCEO nanoemulsion treatment (MIC dose)	6.17 ± 0.029^{d}	12.84 ± 0.034^{b}	5.02 ± 0.027^{bc}	0.46 ± 0.020^{b}	20.95 ± 0.73^{c}	79.26 ± 2.69^{b}	100 ± 0.00^{b}
Inoculated Ne-CCEO nanoemulsion treatment (MIC dose)	6.51 ± 0.027^{c}	13.01 ± 0.032^{c}	4.92 ± 0.042^{b}	0.47 ± 0.017^{b}	24.34 ± 0.49^{b}	74.12 ± 4.21^{c}	100 ± 0.00^{b}
Uninoculated Ne-CCEO nanoemulsion treatment (2 MIC dose)	4.94 ± 0.045^{f}	13.47 ± 0.040^{d}	5.11 ± 0.021^{c}	0.35 ± 0.023^{c}	19.37 ± 0.49^{c}	100 ± 0.00^{d}	100 ± 0.00^{b}
Inoculated Ne-CCEO nanoemulsion treatment (2 MIC dose)	5.10 ± 0.011^{e}	13.88 ± 0.032^{e}	5.10 ± 0.011^{c}	0.29 ± 0.005^{c}	20.82 ± 0.75^{c}	100 ± 0.00^{d}	100 ± 0.00^{b}

Note: Values are mean ($n = 3$) ± SE, the means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

AFB_1 production was recorded (Table 2). Better anti-aflatoxigenic potentiality of Ne-CCEO coating as compared to antifungal activity may be due to inhibition of some key enzymes involved in carbohydrate catabolism and aflatoxin transportation. Interference in some prime metabolic steps of *A. flavus* cells, on the other hand, may also hinder AFB_1 biosynthesis (Tian *et al.*, 2012).

Effect of Ne-CCEO coating on fruit weight, TPC, juice pH, TA, and TSS of *C. aurantifolia* fruits

Ne-CCEO coating on *C. aurantifolia* fruits reduced weight loss ($P < 0.05$) (Table 2). Ne-CCEO coating on fruits maintained TPC after 30 days of storage at 4 °C (uncertainty value 0.1; Table 2). Loss in TPC of uncoated fruits may be due to high respiration rate, alteration in microbial metabolism and degradation of phenolic constituents by enzymes (Arabpoor *et al.*, 2021). In this context, better antifungal activity of CCEO may suppress the oxidation of phenolic content of coated fruits. In addition, Ne-CCEO coating increased juice pH as compared to uncoated fruits (Table 2). TA values of Ne-CCEO coated fruits were significantly reduced (Table 2), suggesting delay in ripening process caused by modified internal atmosphere and reduced fruit respiration (Yang *et al.*, 2021). Significant reduction in TSS was observed for Ne-CCEO coated *C. aurantifolia* fruits (Table 2). Maximum TSS in uncoated fruits has been associated with increased fungal infestation leading to raised respiration, polysaccharide degradation, and subsequent fruits decay, while improved oxygen barrier potentiality of Ne-CCEO coating was responsible for considerable reduction in TSS (Manzoor *et al.*, 2021). Most importantly, fungal infestation modulated tricarboxylic acid cycle and pentose-phosphate-pathway causing changes in organic acids and carbohydrates (Tang *et al.*, 2012). Hence, inhibition of fungal infestation and AFB_1 contamination by Ne-CCEO *via* targeting ergosterol and methylglyoxal could facilitate the maintenance of fruit qualities.

Effect of Ne-CCEO coating on antioxidant enzymatic activities of *C. aurantifolia* fruits

C. aurantifolia fruits coated with Ne-CCEO at MIC and two MIC doses induced the production of SOD, CAT, and APX, which was significantly higher (14.20%, 27.83% and 11.34% respectively) as compared to uncoated fruit during 30 days of storage periods (Fig. 3a–c). Most notably, during postharvest storage, fruits were senesced and ripened by accumulation of reactive oxygen species (ROS) causing deterioration of nutritional qualities (Yang *et al.*, 2021). Low level of antioxidant enzymes in uncoated fruits has been linked with scavenging of ROS during storage period. However, Ne-CCEO coating inhibited fungal infestation, AFB_1 contamination and methylglyoxal

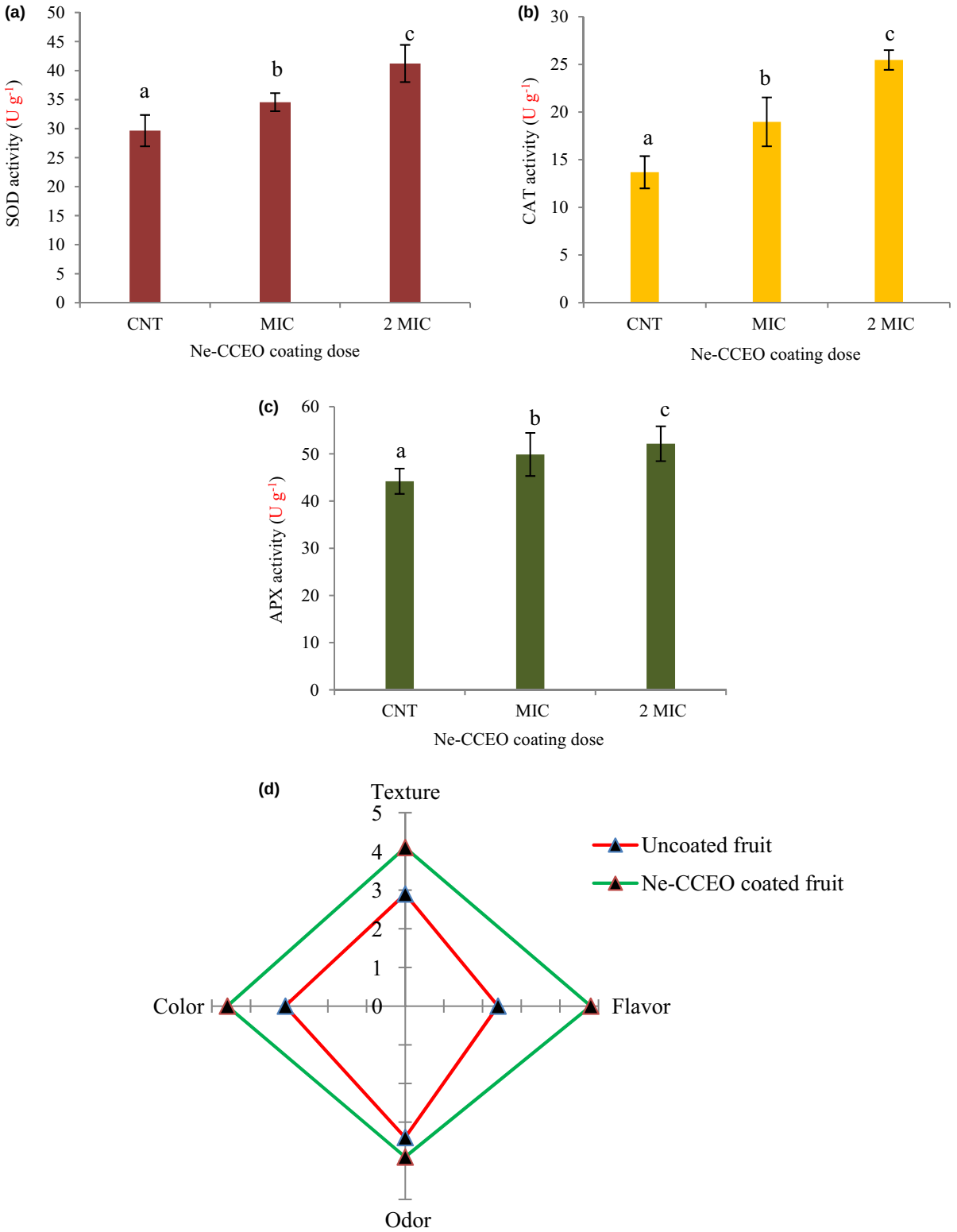

Figure 3 Effect of Ne-CCEO coating on SOD (a), CAT (b) and APX (c) activity in *C. aurantifolia* fruits, effect of Ne-CCEO coating on texture, flavour, odour and colour of *C. aurantifolia* fruits (d).

biosynthesis, due to raised fruit antioxidant enzymatic activities (Song *et al*., 2016).

Sensory evaluation of Ne-CCEO coated *C. aurantifolia* fruits

Lower scores (<3) were recorded for all sensory parameters in case of uncoated fruits after 30 days of storage. Fungal infestation led to variation in colour, flavour and odour (Grande Tovar *et al*., 2019). However, Ne-CCEO coated fruits maintained acceptable scores recorded for texture, colour, flavour and odour ($P < 0.05$; Fig. 3d).

Conclusion

CCEO-loaded chitosan nanoemulsion (Ne-CCEO) showed strong antifungal and antiaflatoxigenic activity at 1.0 and 0.8 μL mL^{-1} respectively as compared to unencapsulated CCEO. At the MIC (1.0 μL mL^{-1}) dose, Ne-CCEO completely inhibited ergosterol synthesis, enhanced cellular cations loss and inhibited methylglyoxal synthesis. Nanoemulsion coating displayed *in vivo* preservation potentiality for *C. aurantifolia* fruits with 79.26 and 100% protection against fungal infestation and AFB_1 contamination. Besides, the Ne-CCEO nanoemulsion coating facilitated reduction in fruit weight loss, increase in phenolic content and alleviation in degradation of titrable acidity and total soluble solids. Most importantly, Ne-CCEO coating maintained SOD, CAT and APX activities of *C. aurantifolia* fruits between 11.34% and 27.83% without altering sensory attributes, thus strengthening its practical potentiality as smart fruit preservative.

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Conflict of interest

The authors reported no conflict of interest.

Author contribution

Somenath Das: Conceptualization (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Validation (equal); Visualization (equal); Writing – original draft (equal); Writing – review & editing (equal). **Vipin K Singh:** Visualization (equal); Writing – review & editing (equal). **Anand K Chaudhari:** Data curation (equal); Formal analysis (equal); Validation (equal). **Abhishek Kumar Dwivedy:** Visualization (equal). **Nawal Dubey:** Conceptualization (equal); Supervision (equal); Writing – review & editing (equal).

Ethical approval

Ethics approval was not required for this research.

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.15618.

Data availability statement

Research data are not shared.

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Hosseini, S.F., Zandi, M., Rezaei, M. & Farahmandghavi, F. (2013). Two-step method for encapsulation of oregano essential oil in chitosan nanoparticles: preparation, characterization and *in vitro* release study. *Carbohydrate Polymers*, **95**, 50–56.

The standardization of *in vitro* release of *Cinnamomum camphora* essential oil from chitosan nanoemulsion used in our study was based on above cited reference. Our results also corroborated with the findings of Hosseini et al. (2013) suggesting the controlled delivery of oregano essential oil from chitosan matrix system during a period of 144 h. The above reference is pioneer study in the area of essential oil encapsulation.

Kedia, A., Prakash, B., Mishra, P.K. *et al.* (2015). *Trachyspermum ammi* L. essential oil as plant based preservative in food system. *Industrial Crops and Products*, **69**, 104–109.

Chaudhari, A.K., Singh, A., Kumar Singh, V. *et al.* (2020). Assessment of chitosan biopolymer encapsulated α-Terpineol against fungal, aflatoxin B_1 (AFB_1) and free radicals mediated deterioration of stored maize and possible mode of action. *Food Chemistry*, **311**, 126010.

The above cited reference has dealt with the determination of antimicrobial activity of essential oil component α-Terpineol to protect the stored food commodities. Our study has also focused on the management of food commodity losses caused by fungal infestation and aflatoxin B_1 contamination. The determination of loading capacity and encapsulation efficiency for *Cinnamomum camphora* essential oil entrapped into chitosan nanoemulsion was calculated based on the protocol of above reference.

Manzoor, S., Gull, A., Wani, S.M. & *et al.* (2021). Improving the shelf life of fresh cut kiwi using nanoemulsion coatings with antioxidant and antimicrobial agents. *Food Bioscience*, **41**, 101015.

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Tian, J., Huang, B., Luo*et al.* (2012). The control of *Aspergillus flavus* with *Cinnamomum jensenianum* Hand.-Mazz essential oil and its potential use as a food preservative. *Food Chemistry*, **130**, 520–527.

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Yang, R., Miao, J., Shen, Y. *et al.* (2021). Antifungal effect of cinnamaldehyde, eugenol and carvacrol nanoemulsion against *Penicillium digitatum* and application in postharvest preservation of citrus fruit. *LWT*, **141**, 110924.

The results of above cited reference are similar to our study reporting delayed degradation of titrable acidity linked with the utilization of organic acid as respiratory substrate. Moreover, the above referenced work has presented reactive oxygen species mediated deterioration of storage quality and is in agreement with our findings.

Yao, S., Cao, Q.I., Xie, J., Deng, L. & Zeng, K. (2018). Alteration of sugar and organic acid metabolism in postharvest granulation of Ponkan fruit revealed by transcriptome profiling. *Postharvest Biology and Technology*, **139**, 2–11.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supinfo S1 Materials and methods

Table S1 Loading capacity and encapsulation efficiency of Ne-CCEO