Research Article

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Eugenol Pickering emulsion stabilized by chitosan self-assembled nanoparticles: fabrication, emulsion stability, antioxidant and antimicrobial activity

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Abstract

Background: Eugenol, an important active ingredient in essential oils, effectively inhibits food-borne pathogens but is hindered by its high volatility. Pickering emulsion provides a suitable method to encapsulate, protect and enhance the absorption of these biologically active food components. This study investigated the encapsulation of different concentrations of eugenol Pickering emulsion stabilized with self-assembled chitosan nanoparticles by ultrasound-assisted emulsification. The effects of varying eugenol concentrations on Pickering emulsions' physical, stability, antioxidant and antimicrobial properties were analyzed.

Results: The integration of eugenol at different concentrations increased the droplet size of Pickering emulsion, and the value ranged from 20 to 142 nm during a 60-day storage. Eugenol (5%) significantly improved the antioxidant activity of the Pickering emulsion with a DPPH (2,2-diphenyl-1-picrylhydrazyl) value of 78%. In addition, eugenol effectively increased the antimicrobial activity of the Pickering emulsion against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) with inhibition zones of 14.1 and 17 mm, respectively. The stability of the Pickering emulsion increased with the increase in eugenol concentration throughout the storage period.

Conclusion: Pickering emulsions stabilized with self-assembled chitosan nanoparticles effectively enhanced the stability, antioxidant, and antimicrobial performance of eugenol. These results highlight the potential of such systems as natural and efficient delivery platforms for food and pharmaceutical applications.

Keywords: Pickering emulsion; eugenol; antioxidant; stability; antimicrobial

ABBREVIATIONS

ANOVA	One-way analysis of variance
CI	Creaming index
DPPH	2,2-diphenyl-1-picrylhydrazyl
EOs	Essential oils
FTIR	Fourier Transform Infrared spectroscopy
GRAS	Generally recognized as safe
MW	Molecular weight
NaOH	Sodium hydroxide
O/W	Oil-in-water
PDI	Polydispersity index
SD	Standard deviation
SE	Standard error
W/O	Water-in-oil

INTRODUCTION

Essential oils (EOs) are plant secondary metabolites, mostly consisting of terpenes and terpenoids, which have various pharmacological effects such as antimicrobial, antioxidant, antiviral, and anti-inflammatory properties.[1,2] Identified by their unique aroma and natural flavor compounds, EOs are extensively utilized in the food industry as food preservatives and flavor enhancers.[2,3] For instance, eugenol (4-allyl-2-methoxyphenol), a phenolic component derived from plant EOs such as clove, nutmeg, cinnamon, and bay leaves, is generally recognized as safe (GRAS) and non-mutagenic.[4] Eugenol can penetrate the bacterial cell wall and membrane, disrupting crucial cell mechanisms and ultimately

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leading to cell death.[5] Nevertheless, the antioxidant and antibacterial efficacy of eugenol are restricted in large-scale applications by their limited water solubility and high volatility.[6] Moreover, EOs are chemically unstable molecules, and their direct interactions with other components in food matrices may lower their biological effect.[7] Therefore, researchers have focused on the encapsulation of EOs by utilizing nano-scale emulsion or Pickering emulsion to increase their stability and prolong the release of active compounds.[8,9]

Emulsions are a form of colloidal dispersion consisting of two immiscible liquids that combine to form a homogeneous mixture, regardless of whether they are oil-in-water (O/W) or water-in-oil (W/O) dispersions.[2] Recent advances in nanotechnology have led to the successful transportation of lipophilic drugs and the development of food-derived bioactive compounds with large loading capacities using nanoemulsions.[10] However, the systems remain thermodynamically unstable, requiring a high-energy approach to achieve kinetic stability.[11] Eventually, they are susceptible to phase separation caused by degradation mechanisms including flocculation, creaming, coalescence, Ostwald ripening, and oiling off.[7] Traditionally, this instability has been addressed by integrating synthetic surfactants or emulsifiers (e.g., Tween 20, Tween 80, and sodium dodecyl sulfate), which usually form amphiphilic molecules to reduce the interfacial tension between oil and water and slow phase separation (Fig. 1).[6,12] However, awareness regarding the environmental impact, possible allergenicity, and health risks of synthetic surfactants has led to a sustainable approach by using natural products.[13] To address this problem, Pickering emulsions utilize biocompatible and non-toxic solid particles, including polysaccharides, flavonoids, and proteins, as interfacial stabilizers at the oil–water interface, reducing the need for chemical surfactants.[14]

The Pickering emulsion approach enhances emulsion stability while offering a more sustainable and eco-friendly alternative, making it particularly advantageous for applications in food, pharmaceuticals, and cosmetics.[15] For instance, in the food industry, Pickering emulsions are widely used for encapsulation and controlled release of bioactive compounds, such as essential oils, antioxidants, and probiotics, helping to enhance food preservation and shelf life.[16,17] In addition, these emulsions act as a controlled-release and biocompatible mechanism for hydrophobic drugs, improving bioavailability and targeted delivery, especially in the pharmaceutical field.[15] The concept of solid particles as stabilizers for Pickering emulsions goes back to Pickering, and their idea predates the contribution of Ramsden.[18,19] Unlike conventional emulsions stabilized by chemical surfactants, Pickering emulsions are generally formed with high resistance against coalescence, creating strong steric hindrance and forming rigid particles at the oil–water interface, resulting in greater stability and a longer shelf life.[2]

Chitosan is a biopolymer consisting of long chains composed of repeated units of D-glucosamine and N-acetyl-D-glucosamine.[20] These units are linked by $\beta(1 \rightarrow 4)$-glycosidic bonds. It is obtained by alkaline deacetylation of chitin, which occurs naturally in the exoskeletons of crustaceans and the cell walls of certain algae and fungi.[21] Chitosan is the second most abundant biopolymer among polysaccharides after cellulose. Due to its antibacterial activity, biocompatibility, and biodegradability, chitosan has recently received much attention for a variety of applications, including emulsion stabilization, drug delivery, and antimicrobial use.[22-24] A previous study suggested that self-assembled chitosan could be used to produce oil-in-water Pickering emulsions. However, no one has yet reported the effects of different concentrations of eugenol stabilized by a chitosan-Pickering emulsion on stability, rheology, antioxidant, and antimicrobial properties.

The aim of this study is to develop a eugenol Pickering emulsion stabilized by self-assembled chitosan nanoparticles and to investigate the effects of varying eugenol concentrations on its physical, stability, antioxidant, and antimicrobial properties. The preparation of Pickering emulsions stabilized with self-assembled chitosan particles has been described in some previous studies.[7,25,26] The effect of different eugenol concentrations on droplet size, polydispersity index (PDI), and zeta potential was analyzed. The rheological study of the Pickering emulsion was measured by apparent viscosity. The interaction of chitosan nanoparticles, eugenol and soybean oil was observed by Fourier Transform Infrared spectroscopy (FTIR) analysis. In this study, the effect of different concentrations of eugenol on the stability of the Pickering emulsion was investigated using various methods, including creaming index, storage stability and centrifugal stability. The antioxidant and antimicrobial properties of the Pickering emulsion were elucidated.

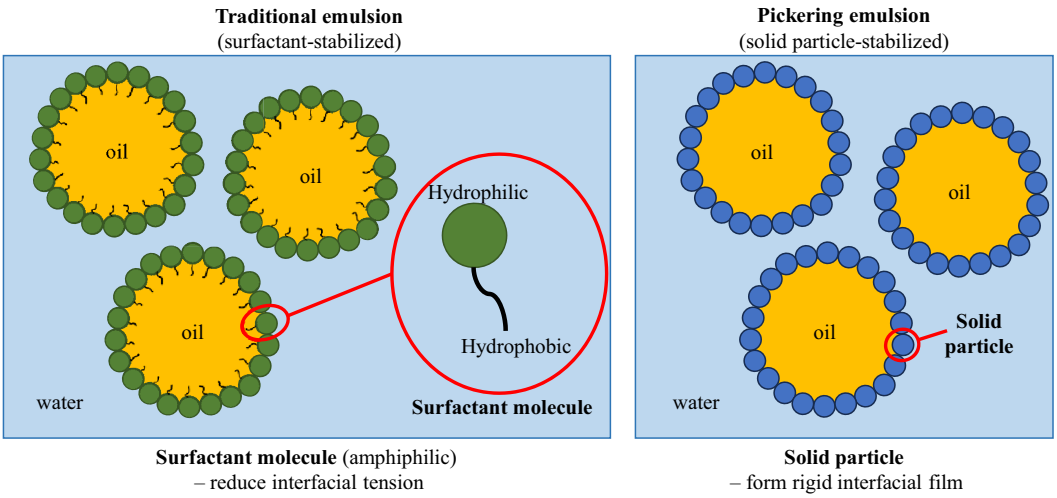

Figure 1. Comparison in mechanism stabilization between traditional and Pickering emulsion.

EXPERIMENTAL SECTION

Materials

Chitosan (medium MW, 190–310 kDa, deacetylation degree of 75–85%), eugenol (reagent plus, 99%), acetic acid, and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich (Selangor, Malaysia). Soya Lite Pure soybean oil made from 100% pure and non-GMO soybeans was bought from a supermarket in Pahang, Malaysia. All solutions were prepared using ultrapure water.

Preparation of chitosan self-assembled nanoparticles

The self-assembled chitosan nanoparticles were prepared according to the methods of Ahmed *et al*[25] Chitosan solution (2 mg/mL) was prepared by dissolving chitosan in 1 mL/100 mL (v/v) acetic acid solution. The mixture was stirred for 6 h at 1200 rpm and ambient temperature (25 ± 1 °C). The initial pH of the chitosan solution (pH 4.1) was adjusted to pH 6.0–6.5 with NaOH (5 M) solution.

Preparation of eugenol Pickering emulsion

The Pickering emulsion stabilized with chitosan nanoparticles was prepared according to a method previously described by Yue *et al*[26] In brief, a coarse emulsion was prepared by adding 50 mL of self-assembled chitosan particles (2 mg/mL) with different concentrations of eugenol and soybean oil in the ratios of 10:90, 30:70 and 50:50 mg/mL using a high-speed homogenizer-disperser (T25 digital Ultra-Turrax, IKA, Germany) at 12 000 rpm for 5 min. Subsequently, the coarse emulsion was further emulsified using an ultrasonic cell crusher (VCX 750, USA) with an ultrasonic cycle of 3 s and a pause of 5 s at an amplitude of 40 for the duration of 2 min. The Pickering emulsion developed is shown in Table 1 below.

Fourier transform infrared spectroscopy analysis

The Fourier Transform Infrared spectroscopy (FTIR) spectrum of the Pickering emulsion was recorded with an attenuated total reflection (ATR) part (Nicolet iS5 spectrometer, Thermo Fisher Scientific, United States). The FTIR spectra were determined in the wavelength region from 600 to 4000 cm^{-1} using OMNIC software.

Droplet size, polydispersity index and zeta potential measurement

The Pickering emulsions were diluted with ultrapure water before analysis.[8] The droplet size and the polydispersity index (PDI) of the Pickering emulsion were measured by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments, UK) at room temperature (25 ± 1 °C). The zeta potential was determined by phase-analysis light scattering.

Table 1. Formulation of eugenol Pickering emulsion

Sample	Eugenol (%v/v)	Soybean oil (%v/v)
eugPE0	–	10
eugPE1	1	9
eugPE3	3	7
eugPE5	5	5

Rheological measurement

The apparent viscosity properties of the Pickering emulsion were determined using a rotational rheometer (Anton Paar, MCR 301, Germany) with a parallel plate (diameter 50 mm, gap height 1 mm) at room temperature (25 ± 1 °C) according to the method of Huang *et al*.[8] The Pickering emulsion was placed between the plates and balanced for 2 min before measurement. The sample was continuously sheared from 0.1 to 1000 s^{-1} for the steady-state flow measurements.

Emulsion stability

Centrifugal stability

The centrifugation method was used to accelerate phase separation and to evaluate the stability of the Pickering emulsion. 1 mL of each sample was placed into centrifuge tubes and centrifuged at 10000 rpm for 10 min at room temperature using a centrifuge (Centrifuge 5810 R, Eppendorf). The samples were then visually examined for any signs of phase separation or creaming.

Storage stability

The Pickering emulsion was stored at room temperature (25 ± 2 °C) for a 60-day storage period. The samples were stored in airtight glass vials and the absence of direct light to prevent oxidation and photodegradation of active compounds. The storage stability of the Pickering emulsion was evaluated by measuring the creaming index (CI). The CI was calculated using the following Eqn. 1:

$$CI\,(\%) = \frac{H_s}{H_t} \times 100 \tag{1}$$

where H_s was the height of the serum layer and H_t was the total height of the emulsion.

Antioxidant activity

The antioxidant activity of the emulsions was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, a widely used method for measuring free radical scavenging capacity.[27] The film samples (2 mL) were mixed with 2 mL of 0.2 mM DPPH solution. After incubating the emulsion for 4 h, absorbance (abs) was measured at 517 nm using a UV–visible spectrophotometer (U-1800, Japan). The results were expressed as % DPPH scavenging activity using the provided Eqn. 2.

$$\text{DPPH scavenging activity}(\%) = \left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\text{Abs}_{\text{control}}\right] \times 100 \tag{2}$$

Antimicrobial properties

The antimicrobial properties of the Pickering emulsions were evaluated using the inhibition zone method described by Fan *et al*.[28] The test was conducted using *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 6538), representing typical Gram-negative and Gram-positive bacteria, respectively. These strains are widely used in studies related to food and biomedical antibacterial applications. The bacterial cultures were maintained on nutrient agar slants at 4 °C and subcultured onto fresh media prior to use. For the studies, the bacteria were inoculated in nutrient broth and cultured at 37 °C for 18–24 h to achieve the logarithmic growth phase, hence ensuring uniformity in antimicrobial testing. The bacterial suspensions were evenly distributed on sterilized plates with Mueller-Hinton agar. Subsequently, 0.1 mL of each Pickering emulsions, the

positive control (Dettol) and the negative control (control Pickering emulsion) were added to the agar. The samples were incubated at 37 °C for 24 h, and the zone of inhibition formed by the emulsion was measured.

Statistical analysis

All experiments were conducted in triplicates, where the results were expressed as mean ± standard deviation (SD) or standard error (SE). The data were analysed using the IBM SPSS Statistics. Statistical analysis was performed using one-way analysis of variance (ANOVA) to measure the mean values between the groups. The multiple correction using Tukey HSD's test was used to determine significant differences at $P < 0.05$ level.

RESULTS AND DISCUSSION

FTIR analysis

Figure 2 shows Fourier transform infrared spectroscopy, which provides information about the functional groups and chemical bonds in chitosan nanoparticles, eugenol, and eugenol Pickering emulsions. The spectra provide information about the molecular structure and the interactions present in the sample based on adsorption peaks and bands. The chitosan nanoparticles shown by the purple line exhibit a broad band at 3277 cm^{-1} associated with the O—H stretching vibration. These absorption peaks correspond to the formation of inter- or intramolecular hydrogen bonds in the sample.[16] In addition, the band observed at 1636 cm^{-1} was assigned to the C═O stretching, known as the amide I band, which is commonly used to analyze protein secondary structure in chitosan nanoparticles.[22,29]

Conversely, the O—H stretching vibrations of the phenolic hydroxyl group appeared in the broad band from 3200 cm^{-1} to 3550 cm^{-1} in eugenol,[30] as indicated by the orange line in Fig. 2. The prominent peaks at 1430–1637 cm^{-1} are associated with the aromatic C═C stretching vibrations. The presence of the aromatic ring is emphasized by these peaks, which are noticeable in the FTIR spectra of aromatic compounds such as eugenol.[31] Another peak was the C—O stretching vibrations of the ether group in eugenol observed at 1230–1260 cm^{-1}.[30]

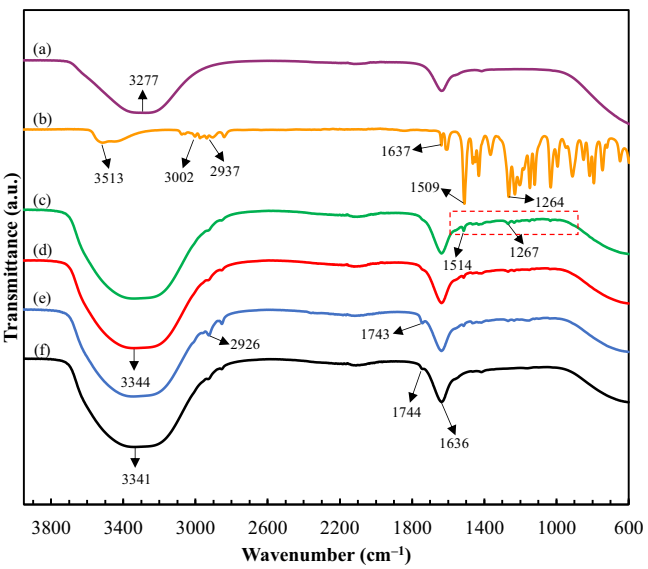

Figure 2. FTIR of (a) chitosan nanoparticles, (b) eugenol, (c) eugPE5, (d) eugPE3, (e) eugPE1, and (f) eugPE0.

The eugenol Pickering emulsion, represented by the blue, red, and green lines for different eugenol concentrations (1%, 3%, and 5%, v/v, respectively), shows important interactions between eugenol, chitosan nanoparticles, and soybean oil. Figure 2 shows that the O—H stretching peak becomes broader and more intense as the concentration of eugenol increases, indicating that the phenolic hydroxyl groups of eugenol are integrated into the emulsion. The results indicate that the hydroxyl groups of eugenol form hydrogen bonds with the chitosan nanoparticles, which leads to the stabilization of the emulsion. The integration of eugenol and soybean oil into a Pickering emulsion stabilized with chitosan resulted in a slight broadening of the —CH stretching peaks in the range of 2850 to 3000 cm^{-1}, indicating the addition of the alkyl group of eugenol to the emulsion.[32]

The visible peak around 1740–1750 cm^{-1} associated with C═O stretching of soybean oil, seen in the blue and black line (soybean oil Pickering emulsion), decreases in intensity with increasing concentration of eugenol.[32] The result indicates a possible interaction through hydrogen bonding or other intermolecular forces between eugenol and soybean oil. In addition, the enhancement of aromatic C═C stretching vibrations at 1637 cm^{-1} indicates an existing and increasing amount of aromatic structure of eugenol in the emulsion. Therefore, the change in peaks observed with increasing eugenol concentration indicates the importance of hydrogen bonding and other intermolecular interactions in the eugenol Pickering emulsion.

Droplet size, PDI, and zeta potential

Prior to ultrasound-assisted emulsification, the coarse emulsion prepared with chitosan nanoparticles exhibited an average droplet size of approximately 1980 nm and a polydispersity index (PDI) of 0.609, indicating poor dispersion and limited interfacial stability. After applying high-energy ultrasonic treatment, the emulsions formed more uniform and smaller droplets. Figure 3(a),(b) illustrate the changes in droplet size and PDI of the Pickering control emulsion (eugPE0) and different eugenol concentrations in the Pickering emulsion during the 60-day storage period. The control emulsion (eugPE0) displayed rapid growth in droplet size with values significantly larger than the eugenol-integrated Pickering emulsion ($P < 0.05$), indicating poor long-term stability. In contrast, emulsions with higher eugenol concentrations (eugPE3 and eugPE5) showed a slower increase in droplet size, ranging from 20 nm on the first day to about 68 nm after 60 days. This improved stability is attributed to the formation of a strong interfacial adsorption layer between oil droplets and chitosan nanoparticles in the oil–water interface, which prevents droplet coalescence in the system.[25,33] Therefore, increasing the concentration of eugenol helps to slow down droplet growth by strengthening the interfacial layer and enhancing the long-term stability of the emulsion over time.

The polydispersity index (PDI) was used to assess the uniformity of droplet sizes within the emulsions, and all samples exhibited minimal variation over the storage period. As shown in Fig. 3(b), PDI values remained around 0.30, indicating that although droplet sizes increased over time, the size distribution remained relatively consistent. According to research by Danaei *et al.*[34] and Qiao *et al.*,[35] emulsion systems with a PDI lower than 0.3 were monodisperse (uniform droplet size), while systems with a PDI higher than 0.3 had a wider size distribution with some aggregation. This finding suggests that the developed emulsion effectively maintains droplet uniformity over the 60-day storage period.

The zeta potential is an essential indicator to determine the emulsion's stability, with a higher absolute value of more than

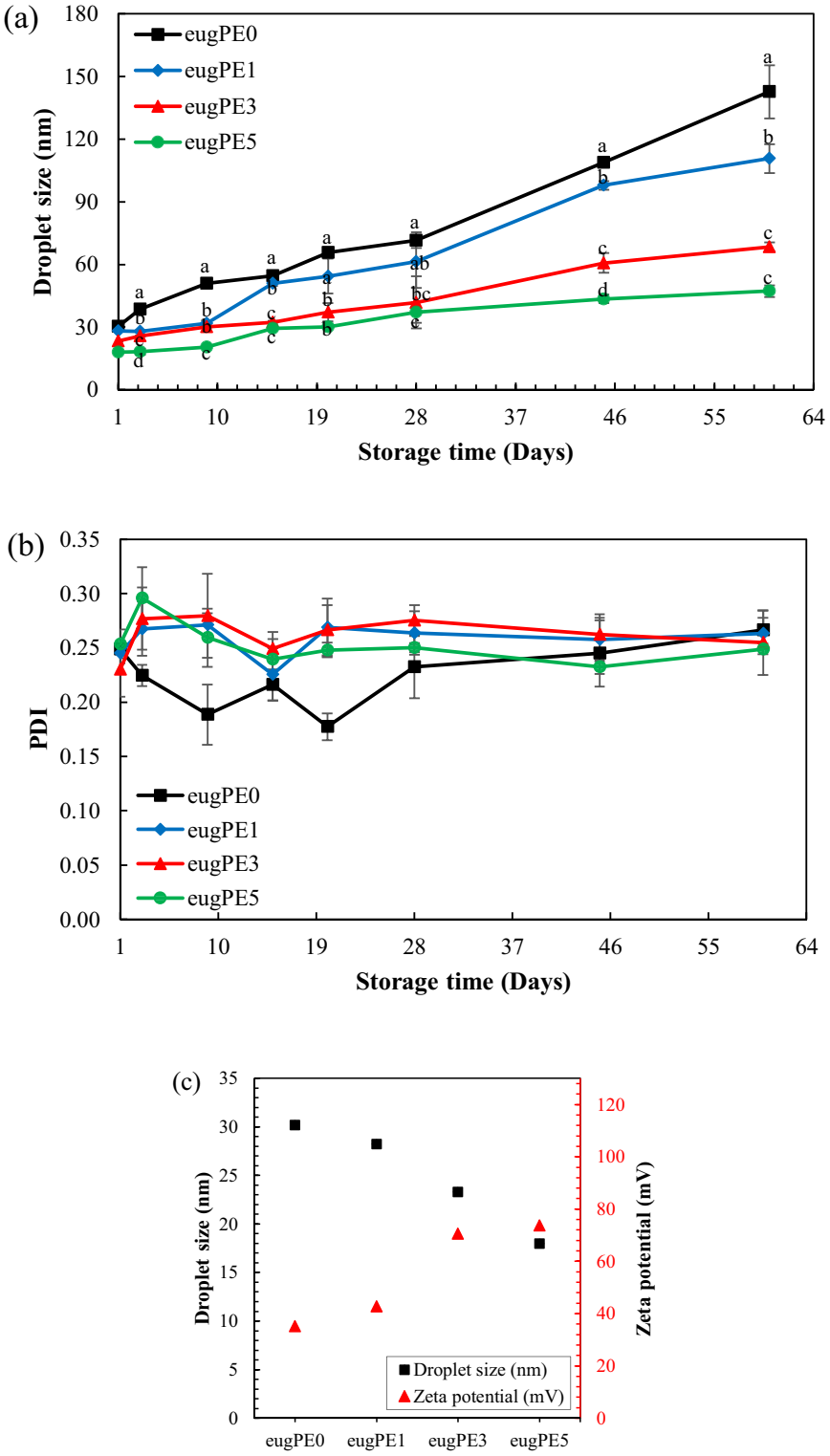

Figure 3. (a) Droplet size, (b) PDI, and (c) zeta potential of the Pickering emulsion during 28 days of storage at 25 °C.

30 mV indicating greater stability.[7,26] This is because of the strong electrostatic repulsion within the particles and the particles being less likely to aggregate in the emulsion. From Fig. 3(c), all the emulsions exhibit zeta potential values greater than 30 mV and have strongly positive charges. The presence of a residual protonated amino group (NH_3^+) in the chitosan polymer chain dominates the positive charge of zeta potential, as stated in the previous study.[26] The emulsion with 5%, v/v of eugenol exhibits a higher zeta potential with a value of 73.8 mV, and the value decreased to 70.7, 42.8 and 35.3 mV for the emulsion with 3%,

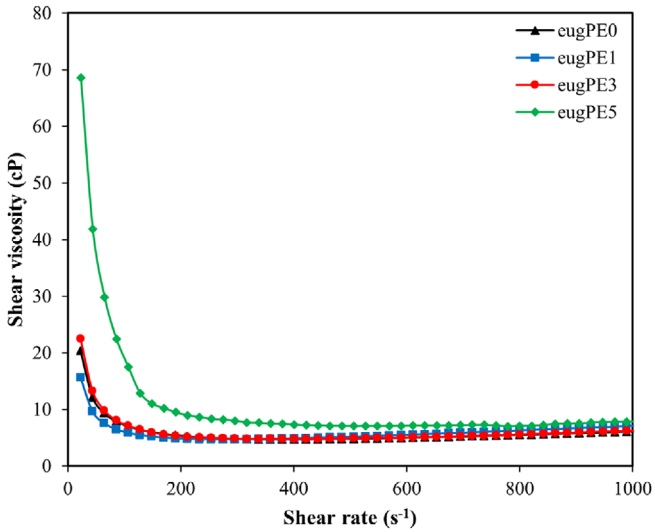

Figure 4. Apparent viscosity of the Pickering emulsion at different concentrations.

1%, and 0%, v/v of eugenol, respectively. In addition, as can be seen from the Fig. 3(a),(c), the trend shows that the zeta potential value increased and was reversely related to the droplet size of the emulsion. This study indicates that the magnitude of the zeta potential was affected by the concentration of eugenol in the emulsion.

Apparent viscosity

The rheology plays an important role in understanding the physical properties of the emulsion, such as its appearance, stability, and intricate structures, through its flow characteristics.[26] Fig. 4 illustrates the apparent viscosity of the control Pickering emulsion (eugPE0) and different concentrations of eugenol in the Pickering emulsion for varied shear rates ranging from 1 to 1000 s^{-1}. Initially, all emulsions exhibited high viscosity at low shear rates, and the values gradually dropped at certain rates and reached a constant value as the shear rate increased. The emulsion with 5% of eugenol displayed a higher apparent viscosity, starting at 68.6 cP, and the value was reduced to a constant value of 8 cP at a higher shear rate. Meanwhile, the apparent viscosity of the

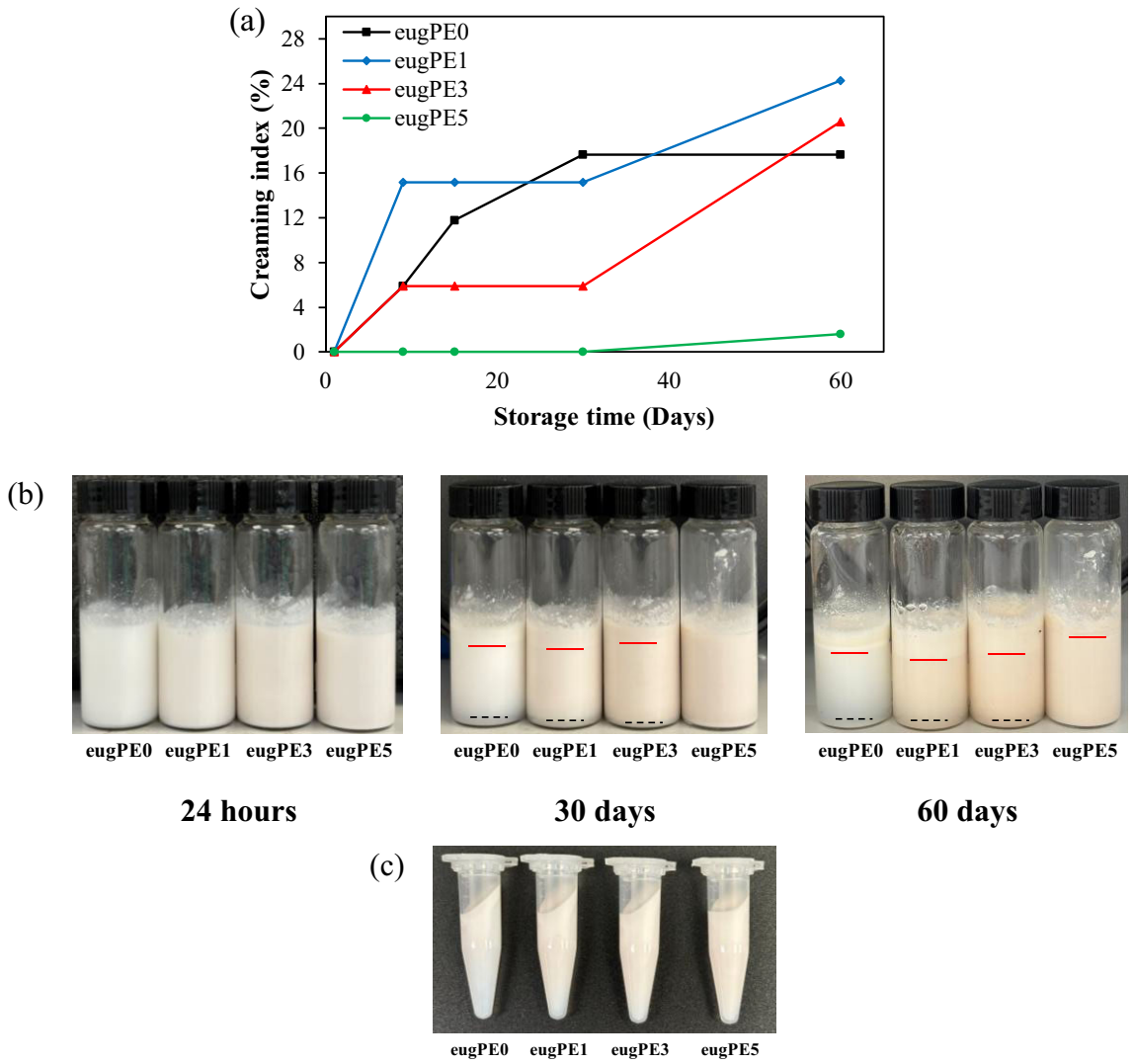

Figure 5. (a) Creaming index, (b) storage stability, and (c) centrifugal stability of the Pickering emulsion.

control emulsion and eugenol-integrated emulsion with eugenol less than 5% decreased from approximately 22 cP to a stable value that reached around 7 cP. The result indicates that all the Pickering emulsions demonstrated shear thinning behavior, where the viscosity and flow resistance dropped as the shear rate increased, surpassing Brownian motion.[36] Besides that, at a high shear rate, the apparent viscosity of the emulsion with 5% of eugenol is slightly higher as compared to the other emulsion. The observed trend shows that as the eugenol concentration increased, the apparent viscosity was inversely proportional to the droplet size of the emulsion. This phenomenon is most likely caused by the size of the droplets in smaller diameters being close to each other, which enhances the interaction between them, resulting in stronger structures that are less susceptible to shearing disruption.[7]

Appearance, creaming index and storage stability

The stability of all the emulsions was evaluated by measuring changes in their characteristics during storage at room temperature (25 ± 2 °C) for 60 days. The samples were stored in airtight glass vials in the absence of direct light to prevent oxidation and photodegradation throughout the storage period. From Fig. 5(a), eugPE1 exhibited the highest CI (24.24%) at day 60, followed by eugPE3 (20.59%) and euPE0 (17.65%), while eugPE5 maintained the lowest value (1.61%), indicating superior stability. This pattern may be attributed to the influence of droplet size, interfacial film strength, and particle distribution. According to Stokes' Law, the rate of creaming in an emulsion is influenced by the size of the dispersed droplets, meaning that smaller droplets remain suspended longer and improve emulsion stability.[37] Besides, an adequate number of particles can facilitate the development of a network structure and create additional steric hindrance to prevent the particles from agglomerating and enhance emulsion stability.[25] Therefore, smaller droplets and ensuring adequate particle coverage are essential for reducing coalescence and creaming, ultimately enhancing the long-term stability of Pickering emulsions.

Moreover, from Fig. 5(b), none of the emulsions showed any signs of phase separation during the first 24 h. This result was aligned with the creaming index values (Fig. 5(a)), indicating that the oil particles were resistant to coalescence, which prevents the formation of a distinct oil layer on the surface. However, at day 30 of storage, noticeable phase separation had appeared for all of the emulsions except for the emulsion with 5% of eugenol. In addition, the formation of a cream layer (Fig. 5(b): red line) was observed in all emulsions, while eugPE5 did not show any stratification phenomenon during storage. This observation indicates that higher eugenol concentrations contribute to greater emulsion stability by promoting the formation of a strong adsorption layer between oil droplets and chitosan nanoparticles at the oil–water interface, thereby preventing droplet coalescence and oiling off.[33]

Besides, Fig. 5(c) presents the effects of centrifugation on the stability of Pickering emulsions with different eugenol concentrations. All emulsions exhibited good centrifugal stability, demonstrating their ability to withstand the pressure exerted by centrifugation without phase separation.

In addition, the observed color changes during storage are likely attributed to oxidative reactions involving the oil phase, eugenol, and chitosan nanoparticles, which can lead to the formation of colored oxidation byproducts over time.[38] The breakdown of the interfacial film due to droplet collisions and coalescence may have facilitated oxygen penetration, accelerating oxidation processes, and intensifying color changes.[39] Phase separation can also contribute to uneven color distribution as the oil and aqueous phases separate. Therefore, storage conditions, including temperature, light exposure, and airtight packaging, are crucial in minimizing oxidative degradation and maintaining emulsion stability.

DPPH scavenging activity

The antioxidant activity of the eugenol Pickering emulsion was evaluated using the DPPH scavenging assay. Figure 6 shows the inhibition efficiency of the Pickering emulsion against DPPH free radicals. The control Pickering emulsion exhibited a DPPH scavenging activity of 51%, primarily due to the residual free amino and hydroxyl groups in chitosan, which can interact with free radicals and form stable macromolecular radicals and ammonium groups.[40] A study by Subramani and Manian *et al.*[41] reported a similar DPPH scavenging activity of 55.6% in chitosan-based films, supporting the antioxidant potential of chitosan. In addition, the eugenol-integrated Pickering emulsion (eugPE1, eugPE3, eugPE5) showed a significantly higher DPPH scavenging activity ($P < 0.05$) compared to the control emulsion (eugPE0) with values of 71%, 76%, and 78%, respectively. Furthermore, the highest antioxidant activity was observed in the 5 wt% eugenol emulsion, indicating that this concentration is the most effective in neutralizing DPPH radicals. This enhanced activity is attributed to the hydroxyl group in eugenol, which donates hydrogen atoms to free radicals, thereby inhibiting the radical chain reaction.[42] Previous studies have shown that Pickering emulsions stabilized by essential oils, tannins, and chitosan particles exhibit strong antioxidant properties, typically ranging from 80% to 90%, due to their ability to scavenge free radicals, suppress singlet oxygen, and chelate transition metals.[7]

Antimicrobial properties

Figure 7 displays the inhibition diameters of eugenol Pickering emulsion against *Escherichia coli* (*E. coli*) and *Staphylococcus*

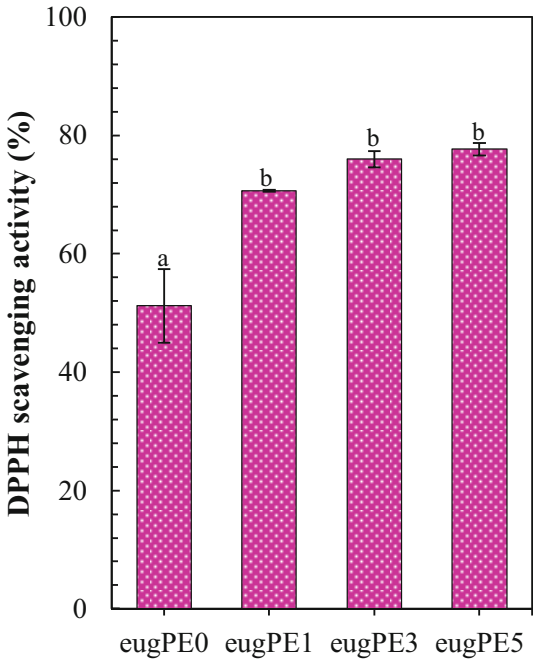

Figure 6. DPPH scavenging activity of the Pickering emulsion at different concentrations.

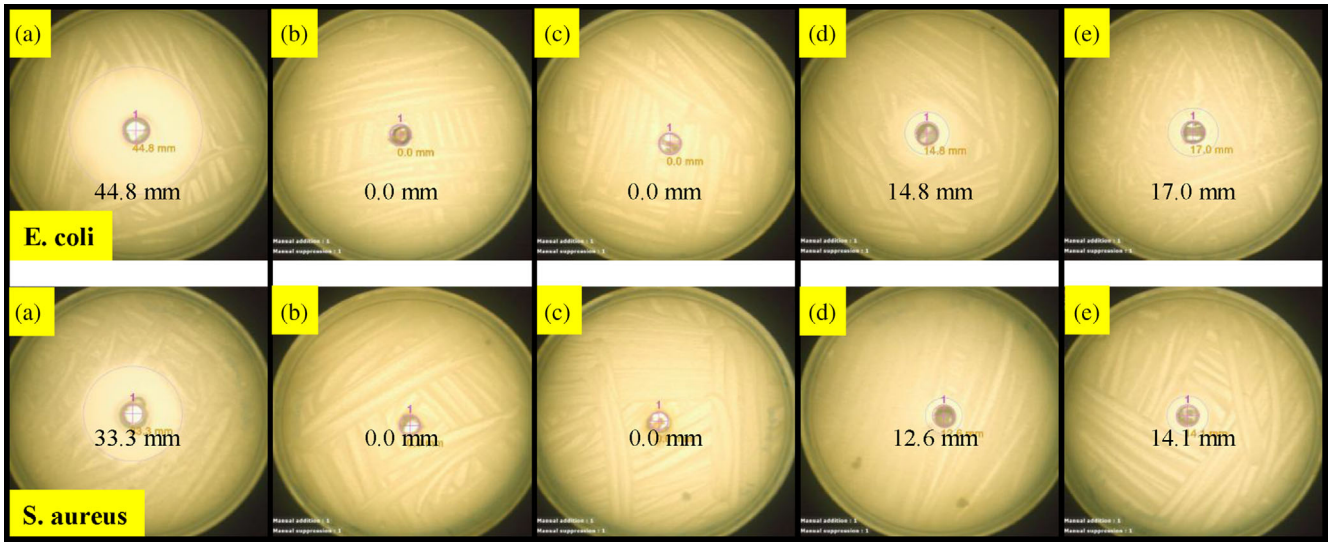

Figure 7. Antimicrobial activity of (a) positive control (Dettol), (b) negative control (eugPE0), (c) eugPE1, (d) eugPE3, and (e) eugPE5 against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*).

aureus (*S. aureus*) at different eugenol concentrations. The results demonstrated that the Pickering emulsion with a higher concentration of eugenol (eugPE5) exhibits larger zones of inhibition of 17.0 mm and 14.1 mm against *E. coli* and *S. aureus*, respectively. In addition, no inhibition zones were observed at lower eugenol concentrations (eugPE0 and eugPE1), indicating that the emulsion was concentration-dependent and had no bacteriostatic effect to inhibit the microorganisms. Furthermore, the study suggests that eugenol is more susceptible in Gram-negative bacteria (*E. coli*) than in Gram-positive bacteria (*S. aureus*). This may be due to the structural properties of bacterial cell walls, with the less dense peptidoglycan layer of the Gram-negative bacillus of *E. coli* being more efficiently affected by phenolic compounds like eugenol.[43] In conclusion, the essential oil in the emulsion plays an important part in inhibiting the growth of bacteria by several mechanisms, thereby providing valuable insights for the development of eugenol-based antimicrobial formulations.

CONCLUSION

This study demonstrates that Pickering emulsions stabilized with self-assembled chitosan nanoparticles serve as an effective delivery system for eugenol, an essential oil component with strong antioxidant and antimicrobial properties. The encapsulation of eugenol within the emulsion improves its dispersion and bioactivity, while also contributing to the overall stability of the system. The results indicate that increasing the eugenol concentration in the emulsion enhances both antioxidant and antimicrobial activities, with the 5% (v/v) eugenol formulation exhibiting the highest DPPH scavenging activity (78%) and the most significant antibacterial effects against *S. aureus* and *E. coli*. In addition, the study found that higher eugenol concentrations contributed to improved emulsion stability, as evidenced by minimal phase separation and controlled droplet growth over 60 days of storage. These findings suggest that eugenol-loaded chitosan-stabilized Pickering emulsions could offer a promising strategy for the food and pharmaceutical industries, enhancing the stability, antioxidant activity, and antimicrobial properties of bioactive compounds.

ACKNOWLEDGEMENT

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DATA AVAILABILITY STATEMENT

The data is accessible upon reasonable request from the corresponding author.

REFERENCES

1 Sharma S, Barkauskaite S, Jaiswal AK and Jaiswal S, Essential oils as additives in active food packaging. *Food Chem* **343**:128403 (2021).
2 Santos PH, Camani PH, Surek M, de Souza WM, Setz LFG and dos Santos Rosa D, Clove leaf essential oil Pickering emulsion stabilized by octenyl succinic anhydride modified waxy maize starch: an eco-friendly alternative. *Colloids Surf A Physicochem Eng Asp* **698**:134540 (2024).
3 Feng T, Wang X, Fan C, Wang X, Wang X, Cui H *et al.*, The selective encapsulation and stabilization of cinnamaldehyde and eugenol in high internal phase Pickering emulsions: regulating the interfacial properties. *Food Chem* **401**:134139 (2023).
4 Sharma S, Perera KY, Jaiswal AK and Jaiswal S, Natural antimicrobials from fruits and plant extract for food packaging and preservation, in *Food Packaging and Preservation*. Elsevier, pp. 133–152 (2024). https://doi.org/10.1016/B978-0-323-90044-7.00008-2.
5 Nisar T, Wang ZC, Yang X, Tian Y, Iqbal M and Guo Y, Characterization of citrus pectin films integrated with clove bud essential oil: physical, thermal, barrier, antioxidant and antibacterial properties. *Int J Biol Macromol* **106**:670–680 (2018).
6 Fan L, Su W, Zhang X, Yang S, Zhu Y and Liu X, Self-assembly of sophorolipid and eugenol into stable nanoemulsions for synergetic antibacterial properties through alerting membrane integrity. *Colloids Surf B Biointerfaces* **234**:113749 (2024).
7 Niu J, Lin Q, Li X, McClements DJ, Ji H, Jin Z *et al.*, Pickering emulsions stabilized by essential oil-tannin-chitosan particles: microstructure, stability, antibacterial activity, and antioxidant activity. *Food Hydrocoll* **154**:110145 (2024).
8 Huang M, Wang H, Xu X, Lu X, Song X and Zhou G, Effects of nanoemulsion-based edible coatings with composite mixture of rosemary extract and ε-poly-l-lysine on the shelf life of ready-to-eat carbonado chicken. *Food Hydrocoll* **102**:105576 (2020).
9 Sultan M, Elsayed H, Abdelhakim AEF and Taha G, Active packaging gelatin films based on chitosan/Arabic gum/coconut oil Pickering nano emulsions. *J Appl Polym Sci* **139**:51442 (2022).
10 Jena GK, Panigrahi KC and Nayak CK, Applications of nanotechnology in nutrigenomics, in *Role of Nutrigenomics in Modern-Day Healthcare*

and Drug Discovery. Elsevier, pp. 547–555 (2023). https://doi.org/10.1016/B978-0-12-824412-8.00009-6.
11 Rai VK, Mishra N, Yadav KS and Yadav NP, Nanoemulsion as pharmaceutical carrier for dermal and transdermal drug delivery: formulation development, stability issues, basic considerations and applications. *J Control Release* **270**:203–225 (2018).
12 Mushtaq A, Mohd Wani S, Malik AR, Gull A, Ramniwas S, Ahmad Nayik G *et al*., Recent insights into Nanoemulsions: their preparation, properties and applications. *Food Chem X* **18**:100684 (2023).
13 Mwangi WW, Lim HP, Low LE, Tey BT and Chan ES, Food-grade Pickering emulsions for encapsulation and delivery of bioactives. *Trends Food Sci Technol* **100**:320–332 (2020).
14 Chevalier Y and Bolzinger MA, Emulsions stabilized with solid nanoparticles: Pickering emulsions. *Colloids Surf A Physicochem Eng Asp* **439**: 23–34 (2013).
15 Albert C, Beladjine M, Tsapis N, Fattal E, Agnely F and Huang N, Pickering emulsions: preparation processes, key parameters governing their properties and potential for pharmaceutical applications. *J Control Release* **309**:302–332 (2019).
16 Wang Z, Zhang M, Liang S and Li Y, Enhanced antioxidant and antibacterial activities of chitosan/zein nanoparticle Pickering emulsion-incorporated chitosan coatings in the presence of cinnamaldehyde and tea polyphenol. *Int J Biol Macromol* **266**:131181 (2024).
17 Liu Z, Lin D, Shen R and Yang X, Characterizations of novel konjac glucomannan emulsion films incorporated with high internal phase Pickering emulsions. *Food Hydrocoll* **109**:106088 (2020).
18 Pickering SU, CXCVI.—emulsions. *J Chem Soc* **91**:2001–2021 (1907).
19 Ramsden W, Separation of solids in the surface-layers of solutions and 'suspensions' (observations on surface-membranes, bubbles, emulsions, and mechanical coagulation).—preliminary account. *Proc R Soc Lond* **72**:156–164 (1903).
20 Mwangi WW, Ho KW, Tey BT and Chan ES, Effects of environmental factors on the physical stability of pickering-emulsions stabilized by chitosan particles. *Food Hydrocoll* **60**:543–550 (2016).
21 Sharkawy A, Barreiro MF and Rodrigues AE, Chitosan-based Pickering emulsions and their applications: a review. *Carbohydr Polym* **250**: 116885 (2020).
22 Xiao J, Gu C, Zhu D, Huang Y, Luo Y and Zhou Q, Development and characterization of an edible chitosan/zein-cinnamaldehyde nanocellulose composite film and its effects on mango quality during storage. *LWT* **140**:110809 (2021).
23 Mesgari M, Aalami AH and Sahebkar A, Antimicrobial activities of chitosan/titanium dioxide composites as a biological nanolayer for food preservation: a review. *Int J Biol Macromol* **176**:530–539 (2021).
24 Altunkaynak F, Okur M and Saracoglu N, Controlled release of paroxetine from chitosan/montmorillonite composite films. *J Drug Deliv Sci Technol* **68**:103099 (2022).
25 Ahmed R, Wang M, Qi Z, Hira N, Jiang J, Zhang H *et al*., Pickering emulsions based on the pH-responsive assembly of food-grade chitosan. *ACS Omega* **6**:17915–17922 (2021).
26 Yue M, Huang M, Zhu Z, Huang T and Huang M, Effect of ultrasound assisted emulsification in the production of Pickering emulsion formulated with chitosan self-assembled particles: stability, macro, and micro rheological properties. *LWT* **154**:112595 (2022).
27 Ramakrishnan R, Kulandhaivelu SV and Roy S, Alginate/carboxymethyl cellulose/starch-based active coating with grapefruit seed extract to extend the shelf life of green chilli. *Ind Crops Prod* **199**:116752 (2023).
28 Fan S, Wang D, Wen X, Li X, Fang F, Richel A *et al*., Incorporation of cinnamon essential oil-loaded Pickering emulsion for improving antimicrobial properties and control release of chitosan/gelatin films. *Food Hydrocoll* **138**:108438 (2023).
29 Surewicz WK and Mantsch HH, New insight into protein secondary structure from resolution-enhanced infrared spectra. *Biochimica et Biophysica Acta (BBA): Protein Structure and Molecular Enzymology* **952**:115–130 (1988).
30 Balyan S, Dadwal V and Patil BS, Lactobacillus-isolated exopolysaccharide as emulsifier ensure extended stability of eugenol encapsulation, potent anti-microbial activity, and application on fresh produce. *Food Biosci* **61**:104632 (2024).
31 Mondéjar-López M, López-Jimenez AJ, García Martínez JC, Ahrazem O, Gómez-Gómez L and Niza E, Comparative evaluation of carvacrol and eugenol chitosan nanoparticles as eco-friendly preservative agents in cosmetics. *Int J Biol Macromol* **206**:288–297 (2022).
32 Sienkiewicz AM and Czub P, The unique activity of catalyst in the epoxidation of soybean oil and following reaction of epoxidized product with bisphenol a. *Ind Crops Prod* **83**:755–773 (2016).
33 Ebrahimi R, Fathi M and Ghoddusi HB, Pickering emulsions stabilized by cellulose nanocrystals extracted from hazelnut shells: production and stability under different harsh conditions. *Int J Biol Macromol* **258**:128982 (2024).
34 Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A *et al*., Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics* **10**:57 (2018).
35 Qiao H, Chen L, Rui T, Wang J, Chen T, Fu T *et al*., Fabrication and in vitro/in vivo evaluation of amorphous andrographolide nanosuspensions stabilized by D-α-tocopheryl polyethylene glycol 1000 succinate/sodium lauryl sulfate. *Int J Nanomedicine* **12**:1033–1046 (2017).
36 Hosseini RS and Rajaei A, Potential Pickering emulsion stabilized with chitosan-stearic acid nanogels incorporating clove essential oil to produce fish-oil-enriched mayonnaise. *Carbohydr Polym* **241**:116340 (2020).
37 Wang XY and Heuzey MC, Chitosan-based conventional and Pickering emulsions with long-term stability. *Langmuir* **32**:929–936 (2016).
38 Yang L, Fan C, Gao Q, Xie Q, Jiang C, Wang S *et al*., Physicochemical characterization, sensory quality analysis, and shelf-life prediction during the storage of a total nutrient formula emulsion. *LWT* **204**: 116427 (2024).
39 Bist Y, Kumar Y and Saxena DC, Enhancing the storage stability of Pickering emulsion using esterified buckwheat starch with improved structure and morphology. *LWT* **161**:113329 (2022).
40 Wang Z, Yan Y, Zhang Z, Li C, Mei L, Hou R *et al*., Effect of chitosan and its water-soluble derivatives on antioxidant activity. *Polymers (Basel)* **16**:867 (2024).
41 Subramani G and Manian R, Bioactive chitosan films: integrating antibacterial, antioxidant, and antifungal properties in food packaging. *Int J Biol Macromol* **278**:134596 (2024).
42 Bhowmik S, Agyei D and Ali A, Bioactive chitosan and essential oils in sustainable active food packaging: recent trends, mechanisms, and applications. *Food Packag Shelf Life* **34**:100962 (2022).
43 Pasquina-Lemonche L, Burns J, Turner RD, Kumar S, Tank R, Mullin N *et al*., The architecture of the gram-positive bacterial cell wall. *Nature* **582**:294–297 (2020).

