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Physical Properties and Antimicrobial Efficacy of Thyme Oil Nanoemulsions: Influence of Ripening Inhibitors

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ABSTRACT: Thyme oil-in-water nanoemulsions (pH 3.5) were prepared as potential antimicrobial delivery systems. The nanoemulsions were highly unstable to droplet growth and phase separation, which was attributed to Ostwald ripening due to the relatively high water solubility of thyme oil. Ostwald ripening could be inhibited by mixing thyme oil with a water-insoluble ripening inhibitor (≥60 wt % corn oil or ≥50 wt % MCT in the lipid phase) before homogenization, yielding nanoemulsions with good physical stability. Physically stable thyme oil nanoemulsions were examined for their antimicrobial activities against an acid-resistant spoilage yeast, *Zygosaccharomyces bailii* (ZB). Oil phase composition (ripening inhibitor type and concentration) had an appreciable influence on the antimicrobial activity of the thyme oil nanoemulsions. In general, increasing the ripening inhibitor levels in the lipid phase reduced the antimicrobial efficacy of nanoemulsions. For example, for nanoemulsions containing 60 wt % corn oil in the lipid phase, the minimum inhibitory concentration (MIC) of thyme oil to inhibit ZB growth was 375 μg/mL, while for nanoemulsions containing 90 wt % corn oil in the lipid phase, even 6000 μg/mL thyme oil could not inhibit ZB growth. This effect is also dependent on ripening inhibitor types: at the same concentration in the lipid phase, MCT decreased the antimicrobial efficacy of thyme oil more than corn oil. For instance, when the level of ripening inhibitor in the lipid phase was 70 wt %, the MICs of thyme oil for nanoemulsions containing corn oil and MCT were 750 and 3000 μg/mL, respectively. The results of this study have important implications for the design and utilization of nanoemulsions as antimicrobial delivery systems in the food and other industries.

KEYWORDS: *emulsion, nanoemulsion, thyme oil, Ostwald ripening, ripening inhibitor, stability, antimicrobial, Zygosaccharomyces bailii*

INTRODUCTION

Essential oils contain a complex mixture of nonvolatile and volatile compounds produced by aromatic plants as secondary metabolites.[1,2] The major constituents in commercial essential oils can be classified into three classes: phenols, terpenes, and aldehydes.[1−3] Essential oils are natural compounds that have antioxidant, antiradical, and antimicrobial properties; therefore, they have been widely used as functional ingredients in food, cosmetic, and pharmaceutical applications.[2] Some essential oils have been shown to exert strong antibacterial, antiviral, and antifungal activities against food-borne pathogens,[1,4,5] leading to their broad applications as natural antimicrobial additives to extend the shelf life of food products. For instance, thyme oil has been shown to have inhibitory activities against various bacteria and yeasts.[6] Thymol, the major component of thyme oil,[7] has also been shown to exhibit antimicrobial activity against several bacteria and fungi.[8,9] The fact that essential oils are considered to be "natural" components makes them highly desirable for use in many food products, since there is growing consumer demand for natural rather than synthetic additives.[10−12]

Despite their potential applications as functional components in foods and beverages, the utilization of essential oils is often limited because of their relatively low water solubility. One feasible way to conquer this problem is to encapsulate essential oils into oil-in-water (o/w) emulsions or nanoemulsions. After encapsulation, the lipophilic antimicrobial components can be easily incorporated into foods and beverages due to their improved water dispersibility. Emulsion-based systems have been used as delivery systems for many years to encapsulate lipophilic bioactive components, such as antitumor agents,[5,13] anti-inflammatory agents,[13] vitamins,[14,15] and antimicrobials.[5,16−19]

Emulsion-based systems may be divided into either conventional emulsions (radius > 100 nm) or nanoemulsions (radius < 100 nm) depending on the dimensions of the droplets they contain.[20−23] Emulsions and nanoemulsions are both thermodynamically unstable systems that will eventually break down over time; therefore, they need to be carefully formulated to ensure that they will remain stable throughout their intended shelf life. Nanoemulsions have a number of potential advantages over emulsions for encapsulating functional lipophilic components.[24,25] Because of the small size of the droplets in nanoemulsions, destabilization mechanisms such as gravitational separation, flocculation, and coalescence often occur at a greatly reduced rate. Another potential advantage of using nanoemulsions is that the small droplet size means that they are transparent or only slightly turbid, which is an important characteristic for certain food and beverage applications. Finally, the small size of the droplets in nanoemulsions may alter the activity of any encapsulated

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components, for example, by increasing the fraction of the encapsulated component that reaches the intended site of action.

In this study, we investigated the potential of using nanoemulsions as antimicrobial delivery systems suitable for utilization within the food and other industries. To be a successful antimicrobial delivery system, the nanoemulsions must meet at least two requirements: (i) they should remain physically stable throughout long-term storage, and (ii) they should maintain their good antimicrobial efficacy. Although nanoemulsions are relatively stable to gravitational separation, flocculation, and coalescence because of their small sizes, they are often more prone to Ostwald ripening (OR), which occurs when the oil phase has an appreciable solubility within the aqueous phase.[26−28] OR is the growth of large oil droplets at the expense of smaller oil droplets due to diffusion of oil molecules through the intervening aqueous phase. The driving force for OR is the fact that the solubility of oil in the immediate vicinity of an oil droplet surface increases as the droplet size decreases.[26−28] The OR rate generally increases with increasing solubility of the oil phase in the water phase.[20−22,26] Antimicrobial essential oils have an appreciable solubility in aqueous solutions; therefore, nanoemulsions prepared from them are particularly prone to OR. Previous studies have shown that OR can be inhibited by incorporating sufficient levels of highly water-insoluble oils in the droplets, since this generates a compositional ripening effect that opposes the OR effect.[26,28,29] This second oil has been referred to as a "ripening inhibitor" and is usually a highly nonpolar substance with a relatively high molecular weight, such as corn oil,[30,31] sunflower oil,[32] and medium chain triglycerides (MCT).[33,34]

Although it is well-known that ripening inhibitors can stabilize essential oil nanoemulsions by retarding OR, little is known about how they affect the antimicrobial activities of essential oils loaded in nanoemulsions. In this study, we therefore used thyme oil as a model essential oil to form nanoemulsions and used corn oil and MCT oil as ripening inhibitors. We then examined the influence of ripening inhibitor type and concentration on the physical stability and antimicrobial activity of thyme oil nanoemulsions against a model yeast strain (*Zygosaccharomyces bailii*, ZB). The results of this study have important implications for the design and utilization of nanoemulsions as antimicrobial delivery systems in the food and other industries.

MATERIALS AND METHODS

Materials. Thyme oil was purchased from Optimal Health Solutions (La Pine, Oregon). Corn oil was obtained from a local supermarket. MCT (Miglyol 812) was purchased from Sassol Germany GmbH (Witten, Germany). The manufacturer reported that the MCT used was composed of 50−65% of caprylic acid (C8:0) and 30−45% of capric acid (C10:0) in terms of its fatty acid composition. A nonionic surfactant (Tween 80, T80) was purchased from Sigma-Aldrich Co. (St. Louis, MO). Double-distilled water was used for the preparation of all aqueous solutions.

Nanoemulsion Preparation. The aqueous phase used to prepare the nanoemulsions consisted of 1.0% (w/w) Tween 80 dispersed in an aqueous buffer solution (5 mM citrate buffer, pH 3.5). Lipid phases were prepared by mixing different mass ratios of thyme oil and ripening inhibitor (corn oil or MCT oil, from 0 to 100% w/w) prior to homogenization. The lipid phase (10% w/w) was mixed with the aqueous phase (90% w/w) using a high-speed blender for 2 min. The resulting crude emulsion was then homogenized by passing it five times through a high pressure homogenizer at 10 kPa (Microfluidics 110L, Microfluidics Corp., Newton, MA) to further reduce the particle size. After preparation, the nanoemulsions formed were stored at 20 °C prior to analysis.

Particle Size Measurements. The mean particle diameters (*Z*-averages) of the nanoemulsions were measured using a dynamic light scattering instrument (Zetasizer Nano ZS, model ZEN 3600, Malvern Instruments, Malvern, United Kingdom). This instrument determines the particle size from intensity-time fluctuations of a laser beam (633 nm) scattered from a sample at an angle of 173°. Each individual measurement was an average of 13 runs. The nanoemulsions were diluted using buffer solution (5 mM citrate buffer, pH 3.5) prior to analysis to avoid the effects of multiple scattering.

Yeast Strain. An acid-resistant spoilage yeast, ZB was used as a target microorganism to examine the antimicrobial effects of different nanoemulsions. The strain was obtained from the Pepsico R&D Culture Collection (Valhalla, NY). Yeast stock culture was kept frozen at −70 °C in 25% glycerol. The yeast strain was refreshed on malt extract agar plates (Becton Dickinson, Sparks, MD), and a single yeast colony from the plate was then inoculated into 10 mL of malt extract broth (MEB) media (Becton Dickinson), which was previously adjusted to pH 3.5 by citrate buffer with a final strength of 5 mM. The culture was incubated at 32 °C under mild agitation (150 rpm in a rotary shaker) for 2−3 days until the optical density (turbidity) at 600 nm (OD_{600}) was around 1.0 (1 cm path length). As a guideline, an OD_{600} of 1.0 corresponds to approximately 5×10^6 CFU/mL for cultures of yeast strains. The culture was then diluted to about 10^6 CFU/mL using fresh MEB (pH 3.5) to conduct the following antimicrobial assay.

Determination of Antimicrobial Activity. All of the nanoemulsions subject to antimicrobial assay were filtered sterilized using 0.45 μm polyethersulfone membrane filters (F2500-14, Thermo Scientific, Germany). The mean particle sizes and distributions of the nanoemulsions did not change after the filter sterilization (data not shown). A certain amount of the sterile nanoemulsions was then mixed with appropriate amounts of double strength MEB and single strength MEB media to achieve 10 mL of single strength MEB media (pH 3.5, 5 mM citrate buffer) containing a final concentration of 6000 μg/mL thyme oil. Five milliliters of the above media was then diluted with the same amount of single strength MEB media, and this procedure was continued so as to make successive 2-fold dilutions until the thyme oil concentration dropped to 188 μg/mL. The final concentrations of thyme oil in each MEB media were therefore 6000, 3000, 1500, 750, 375, and 188 μg/mL, respectively. Prior to exposure to antimicrobial treatments, the target strain ZB was freshly subcultured and diluted to about 10^6 CFU/mL as stated previously and was then 1/100 inoculated into MEB media containing varying levels of thyme oil to achieve initial cell levels around 10^4 CFU/mL. The surviving cell numbers were monitored after 0, 6, 12, 24, 48, 96, and 120 h of incubation at 25 °C. Enumeration was carried out by using a spiral plater (Spiral Biotech, Norwood, MA). Controls consisted of MEB media only (without nanoemulsion), corn oil nanoemulsions without thyme oil, and MCT oil nanoemulsions without thyme oil. All experiments were conducted with duplicate samples of each treatment, and the entire study was carried out in triplicate.

Statistical Analysis. Microsoft Excel software was used to determine *P* values using a Student's *t* test. Significant differences were concluded when P was <0.05 for pairwise comparisons with Students' two-tailed *t* test.

RESULTS AND DISCUSSIONS

Influence of OR on Thyme Oil Emulsion Stability. If nanoemulsions are going to be used as delivery systems for antimicrobial agents, then it is important that they have good physical stabilities during long-term storage. Initially, we attempted to prepare a nanoemulsion by homogenizing pure thyme oil and an aqueous nonionic surfactant solution: 10% thyme oil and 1.0% Tween 80, pH 3.5. However, the resulting emulsion was highly unstable to droplet growth and phase

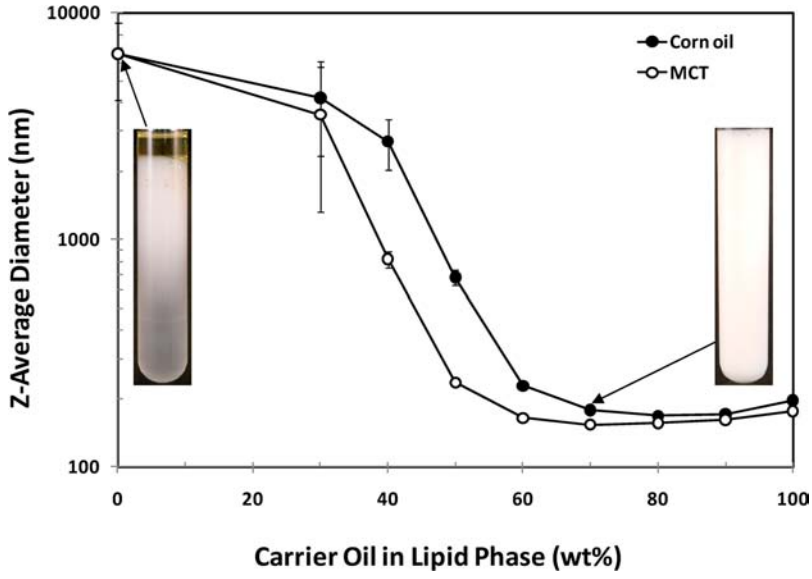

Figure 1. Dependence of mean droplet diameter after 3 days of storage at ambient temperature on the oil phase composition for 10% oil-in-water emulsions containing different amounts of thyme oil and ripening inhibitor (corn oil or MCT) in the lipid phase.

separation—after 3 days of storage, the mean particle diameter was >7000 nm, and there was visible evidence of creaming and oiling off (Figure 1). Indeed, within the first hour after homogenization, light scattering measurements showed that some of the smaller oil droplets had started to grow into larger ones, resulting in emulsions containing two main size classes with peaks around 120 and 1300 nm in diameter (Figure 2a). The oil droplets continued growing during storage, and after 3 days, the population of smaller-sized droplets had disappeared, and only a population of larger droplets with diameters around 7000 nm was observed (Figure 2a).

The high instability can be attributed to the fact that thyme oil has an appreciable water solubility (≈1 g L^{-1} for thymol at 25 °C, MSDS sheet), which means that it is highly susceptible to OR.[26−28,35] OR is a common problem for the instability of essential oil emulsions, and the instability phenomena caused by OR have been reported by many researchers. For example, emulsions made by pure lemon oil,[30] thyme oil,[31] carvacrol or eugenol,[34] peppermint oil,[33] and D-limonene[36] all exhibited rapid growth of droplet sizes after homogenization.

Influence of Ripening Inhibitors on Nanoemulsion Formation and Stability. As mentioned previously, OR can be inhibited in emulsions and nanoemulsions by incorporating a ripening inhibitor into the oil phase prior to homogenization.[26,28,29] In this study, we examined the possibility of improving the OR stability of thyme oil nanoemulsions by including either corn oil or MCT into the oil phase, because both of these oils are food-grade materials that can be used as part of edible delivery systems. Corn oil is primarily comprised of high molecular weight triacylglycerols with very low water solubilities and is therefore a highly effective ripening inhibitor.[29−31] MCT is primarily comprised of medium molecular weight triacylglycerols with relatively low water solubilities and has also been shown to be effective at inhibiting OR.[33,34] However, the water solubility of MCT is somewhat higher than that of corn oil; therefore, one might expect that it would be less effective at inhibiting OR.

We prepared a series of emulsions with different initial lipid phase compositions, that is, thyme oil mixed with different types and amounts of ripening inhibitor (corn oil or MCT:thyme oil = 0:100, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, and 100:0), to examine the effect of ripening inhibitor on the stability of thyme oil emulsions. After homogenization, the emulsion samples were stored for 3 days at ambient temperature and mixed to ensure they were homogeneous, and then, their mean particle sizes were measured (Figure 1). As mentioned earlier, nanoemulsions prepared using a lipid phase consisting of only thyme oil (0% ripening inhibitor) were highly unstable and susceptible to droplet growth, creaming, and oiling off as a result of OR. In contrast, when mixed with certain amounts of ripening inhibitor (corn oil or MCT), the thyme oil emulsions gained higher stability. For both of the ripening inhibitors, as their concentration in the lipid phase was increased, the extent of droplet growth decreased (Figure 1). From 0 to 60 wt % MCT or 0 to 70 wt % corn oil in the lipid phase, there was a steep decrease in mean particle diameter ($P < 0.05$) with increasing ripening inhibitor concentration, which can be attributed to the ability of these triacylglycerol oils to inhibit OR.[29,37] A further increase in ripening inhibitor concentration did not change the mean droplet diameters dramatically. At MCT concentrations from 60 to 90 wt %, the droplet diameter was about 160 nm, while at corn oil concentrations from 70 to 90%, the droplet diameter was about 170 nm. There was a significant increase in the mean droplet diameter of the nanoemulsions when the ripening inhibitor concentration was further increased so that the droplets contained either 100% MCT (d = 176 nm) or 100% corn oil (d = 196 nm) (Figure 1). The droplet diameters in the systems produced using relatively high levels of ripening inhibitors were in the range where they could be considered to be nanoemulsions, that is, $d < 200$ nm.[23] For systems containing the same amount of ripening inhibitor, the diameter of the ones containing MCT was significantly smaller than the ones containing corn oil ($P < 0.05$).

The decrease in droplet diameter with increasing ripening inhibitor in the oil phase can be attributed to two different effects: (i) the ripening inhibitor affected the size of the droplets initially generated by the homogenizer, and (ii) the ripening inhibitor affected the stability of these droplets to growth after homogenization. Measurements of the droplet size

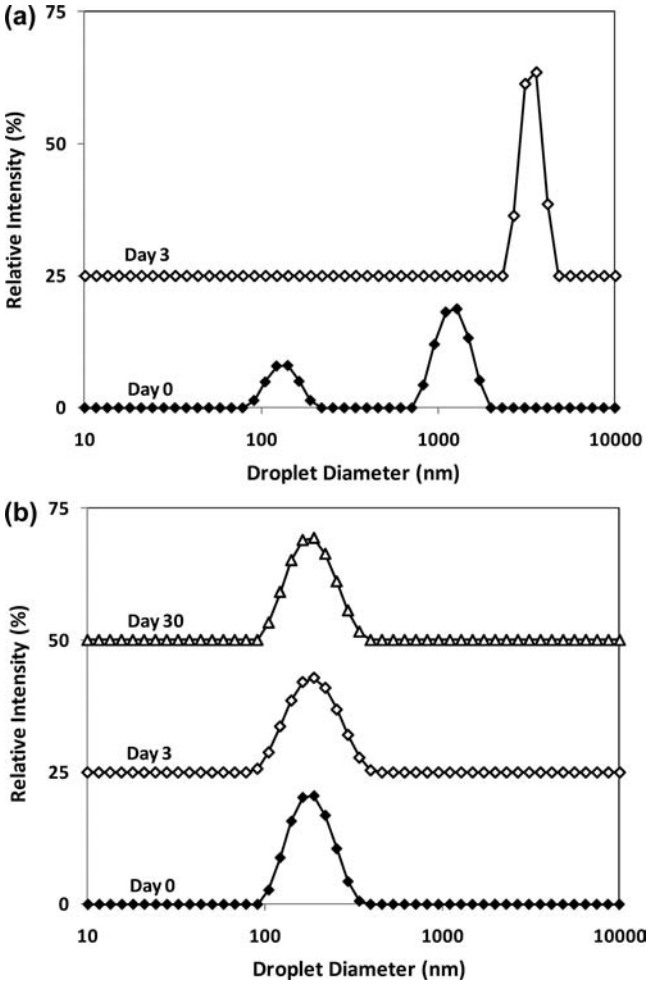

Figure 2. Dependence of particle size distributions of two representative nanoemulsions before and after a certain time storage at ambient temperature. (a) Particle size distributions with time for nanoemulsion containing pure thyme oil only (no ripening inhibitors in lipid phase). The pure thyme oil nanoemulsion was highly unstable, with small emulsion droplets starting to grow into larger ones instantly, and formed into droplets with very large sizes. (b) Particle size distributions with time for nanoemulsion containing 30 wt % thyme oil and 70 wt % corn oil in the lipid phase. The nanoemulsion containing ripening inhibitor was stable, without apparent changes in particle size distributions even after 30 days of storage.

of the systems immediately after homogenization and after 3 days of storage indicated that those samples with relatively low ripening inhibitor contents in the lipid phase (<50% for MCT or <60% for corn oil) were unstable to droplet growth and creaming (Figures 1 and 2a), whereas those with higher ripening inhibitor contents were stable (Figures 1 and 2b). This suggests that the dependence of droplet diameter on ripening inhibitor concentration at relatively high MCT or corn oil levels was due to the influence of the ripening inhibitors on droplet disruption within the homogenizer, whereas the dependence at relatively low levels was mainly due to OR. The size of the droplets produced during high pressure homogenization usually decreases as the oil phase viscosity and interfacial tension decrease because this facilitates droplet disruption.[38] Essential oils have lower viscosities and interfacial tensions than medium or long chain triacylglycerol oils. One would therefore expect smaller droplets to be formed within the homogenizer as the ripening inhibitor concentration decreased. In practice, we observed that the droplet size first decreased and then increased as the ripening inhibitor concentration in the oil phase was increased from 0 to 100% (Figure 1). We postulate that very small droplets were formed within the homogenizer at low ripening inhibitor concentrations but that these quickly grew due to OR. The increase in droplet size observed at high ripening inhibitor concentrations (from 80 to 100%) can be attributed to the influence of oil type on droplet disruption within the homogenizer. In addition, the reason that the droplets in the MCT nanoemulsions were smaller than those in the corn oil nanoemulsions (Figure 1) can be attributed to the lower viscosity and interfacial tension of MCT as compared to corn oil.[39]

In general, the amount of ripening inhibitor required to prevent OR depends on the concentrations, molecular weights, and water solubilities of the different components in the oil phase.[28] Practically, one would like to maximize the amount of an active ingredient (in this case, thyme oil) present in an antimicrobial delivery system. In this respect, our results suggest that MCT is a more effective ripening inhibitor than corn oil since only 50 wt % MCT was required to inhibit droplet growth, whereas 60% corn oil was required. This was surprising since MCT has a higher water solubility than corn oil and would therefore be expected to undergo faster OR. However, MCT also has a lower molecular weight than corn oil, which means that the entropy of mixing effect that opposes droplet growth through OR is greater. Presumably, the latter effect therefore dominates in the systems used in this study, since nanoemulsions containing pure MCT or corn oil were both stable to droplet growth during storage, suggesting that their water solubilities were low enough to retard OR.

We also measured the long-term stability of selected systems by measuring the change in their particle size during 1 month of storage at room temperature. Only those systems that were found to be stable to droplet growth during the first 3 days of storage in our preliminary experiments were selected for the long-term storage study, that is, ≥50% MCT or ≥60% corn oil in the oil phase. We found that there was no appreciable change (less than 5% of the initial mean diameter) in any of these nanoemulsions during storage (Figure 3), which suggests that once a sufficient amount of ripening inhibitor was included in the oil phase prior to homogenization, the nanoemulsions were highly stable to droplet growth.

Influence of Oil Phase Composition on Antimicrobial Activity of Thyme oil Nanoemulsions. The antimicrobial activities of a series of thyme oil nanoemulsions that exhibited good physical stability (MCT or corn oil in the oil phase ≥60%) were then determined against a model yeast strain ZB. Initially, the nanoemulsions were filter sterilized to avoid the influence of any endogenous microorganisms, and then, different amounts of these sterile nanoemulsions were added to a broth containing ZB cell levels of $\sim 10^4$ CFU/mL. Growth curves were obtained by monitoring the cell numbers against storage time throughout a total incubation period of 5 days at room temperature. Nanoemulsions prepared using 100% corn oil or 100% MCT as the oil phase were also tested as controls, and neither of them exhibited any antimicrobial effects (data not shown), which indicate that it was the thyme oil (rather than the ripening inhibitors or surfactant) that had antimicrobial activity. We did not examine the antimicrobial efficacy of nanoemulsion prepared using pure thyme oil (i. e., without ripening inhibitors), because it is extremely physical unstable.

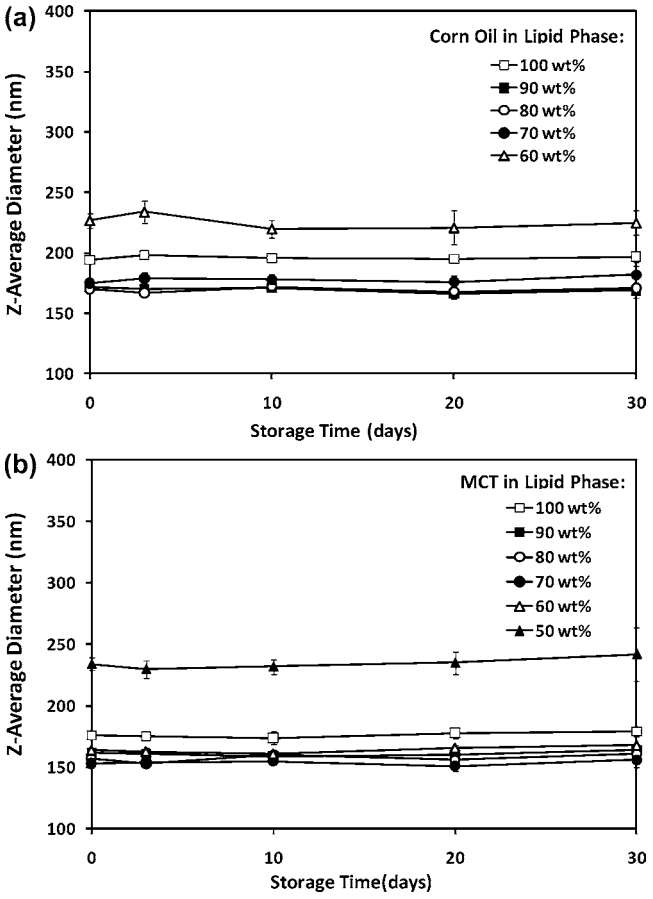

Figure 3. Evolution of mean droplet diameter with time at ambient temperature for oil-in-water emulsions containing different ratios of thyme and ripening inhibitor in the lipid phase. (a) Corn oil served as a ripening inhibitor; (b) MCT served as a ripening inhibitor.

The growth curves for the nanoemulsions containing corn oil are shown in Figure 4, and similar trends were also observed for the nanoemulsions containing MCT (Figure 5). To compare the antimicrobial effects of different nanoemulsions at varying concentrations, the number of yeast cells remaining after 5 days of incubation were determined from the growth curves for nanoemulsions containing either corn oil (Figure 6a) or MCT (Figure 6b). These results clearly show that increasing the level of ripening inhibitor in the lipid phase reduced the antimicrobial efficacy of the thyme oil in the nanoemulsions. For instance, the minimum amount of thyme oil required to inhibit ZB growth during 5 days of storage was around 375, 750, 1500, and >6000 μg/mL for nanoemulsions containing 60, 70, 80, and 90 wt % corn oil in the lipid phase, respectively, demonstrating the negative effects associated with increased levels of ripening inhibitor. Similarly, nanoemulsions with elevated MCT levels (60, 70, 80, and 90 wt % in lipid phase) also displayed increased minimum inhibitory concentrations against ZB (750, 3000, >6000, and >6000 μg/mL). Interestingly, no growth inhibition was observed at the highest tested thyme oil levels (6000 μg/mL) for the nanoemulsions containing 90 wt % of either ripening inhibitor in the lipid phase.

The ability of the ripening inhibitors to decrease the antimicrobial efficacy of the thyme oil depended on their nature, with MCT reducing the antimicrobial efficacy more than corn oil (Figure 6a,b). For instance, when the level of

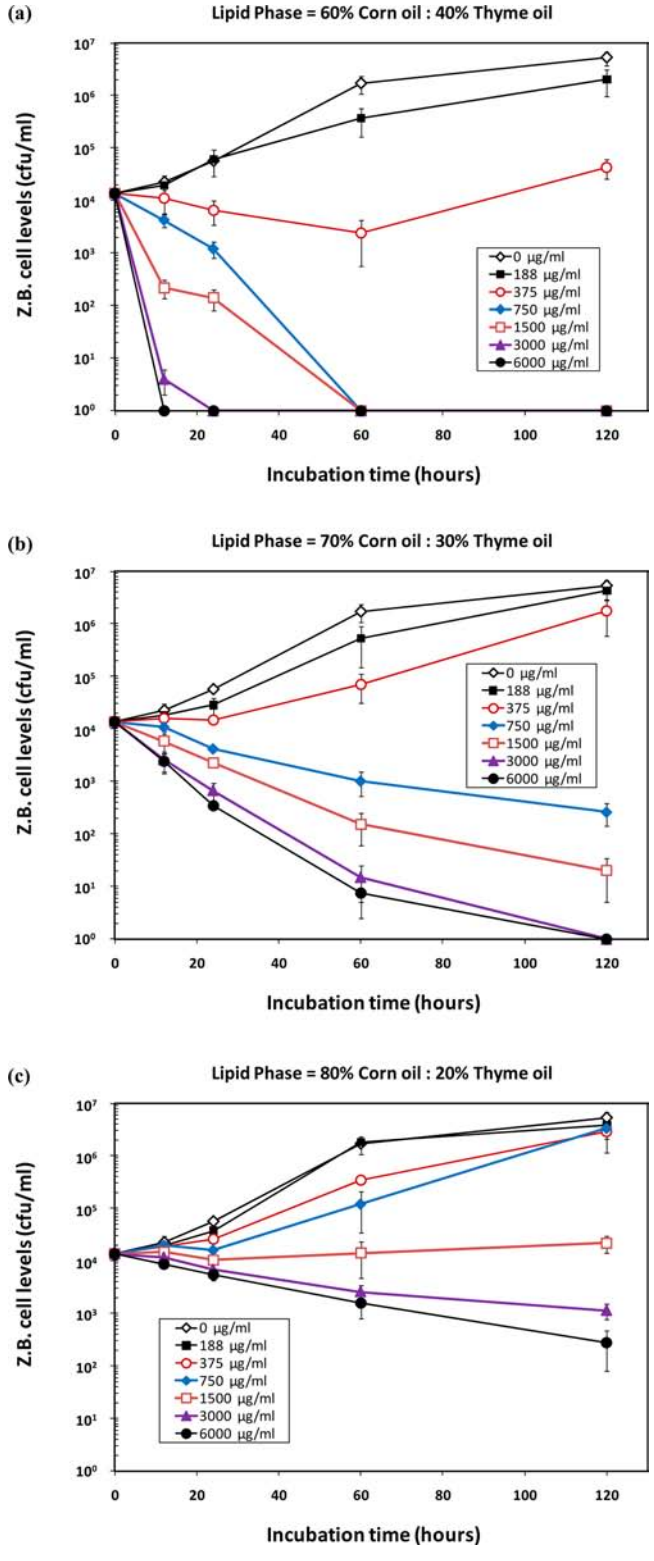

Figure 4. Growth behavior of ZB incubated for 120 h at ambient temperature (22−25 °C) in the presence of different droplet concentrations of thyme oil loading emulsions with corn oil as the ripening inhibitor. The thyme oil concentration as indicated in the legend was based on the pure thyme oil levels in the growth media. (a) Emulsions with a lipid phase composed of 60 wt % corn oil and 40 wt % thyme oil. (b) Emulsions with a lipid phase composed of 70 wt % corn oil and 30 wt % thyme oil. (c) Emulsions with a lipid phase composed of 80 wt % corn oil and 20 wt % thyme oil.

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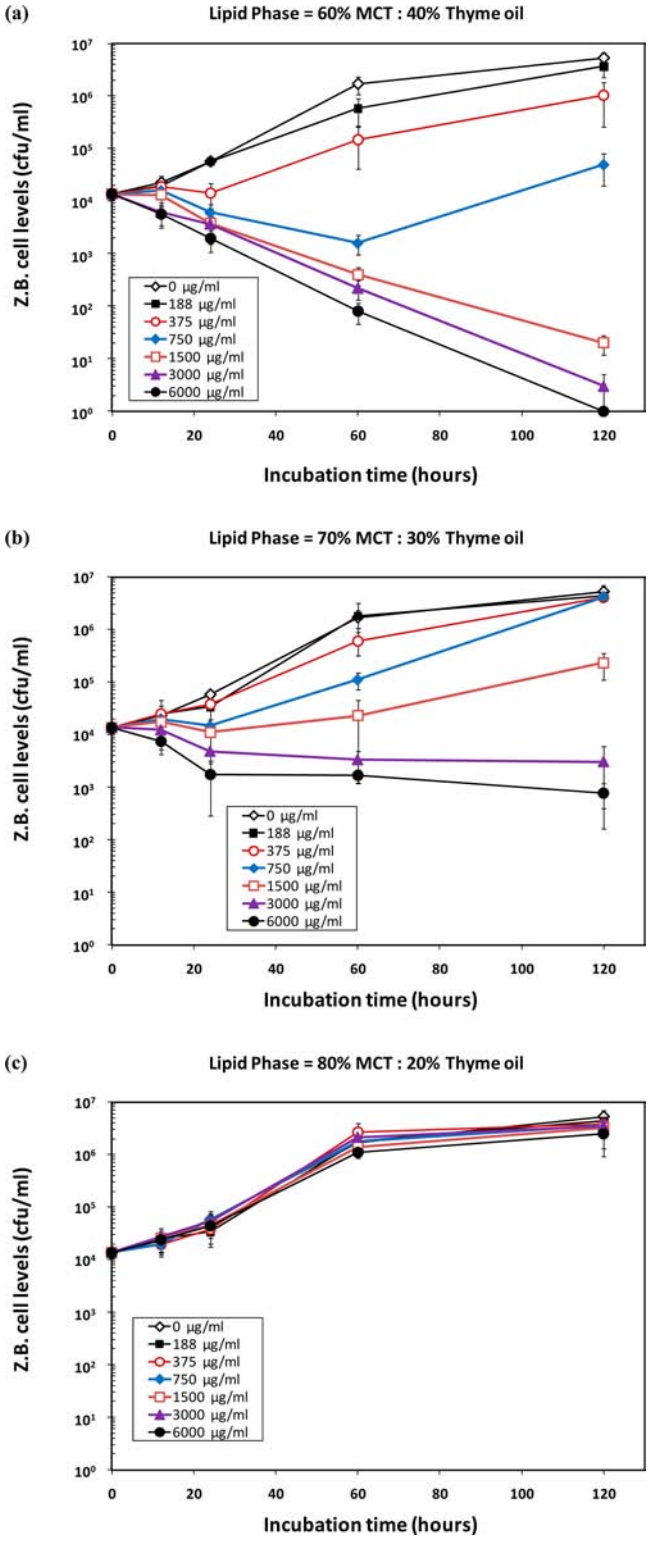

Figure 5. Growth behavior of ZB incubated for 120 h at ambient temperature (22−25 °C) in the presence of different droplet concentrations of thyme oil loading emulsions with MCT as the ripening inhibitor. The thyme oil concentration as indicated in the legend was based on the pure thyme oil levels in the growth media. (a) Emulsions with a lipid phase composed of 60 wt % MCT and 40 wt % thyme oil. (b) Emulsions with a lipid phase composed of 70 wt % MCT and 30 wt % thyme oil. (c) Emulsions with a lipid phase composed of 80 wt % MCT and 20 wt % thyme oil.

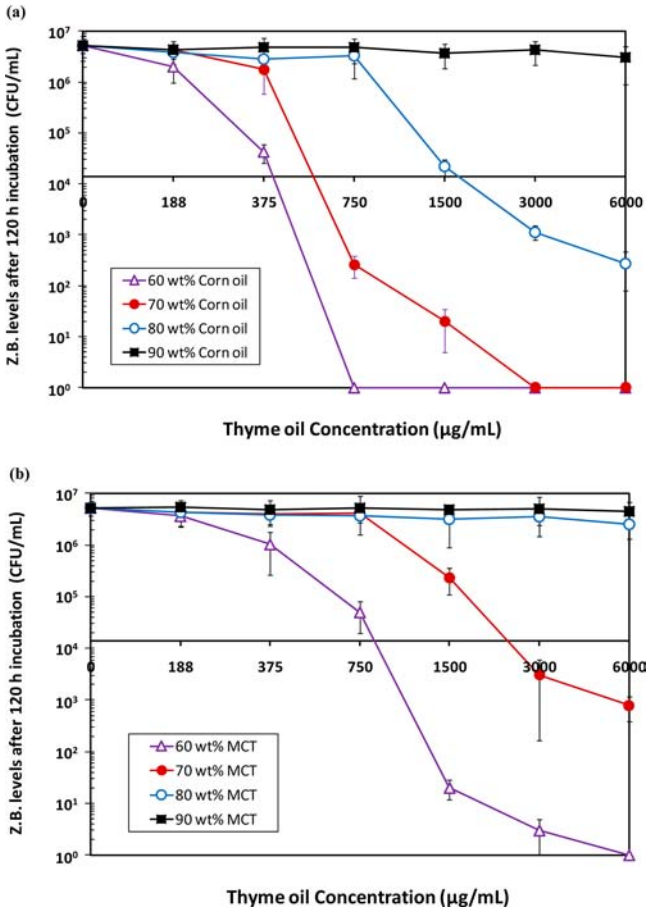

Figure 6. ZB levels after 120 h of incubation in the presence of different concentrations of thyme oil loading nanoemulsions with varying levels of ripening inhibitors in the lipid phase. The *X*-axis crosses the *Y*-axis at ZB levels of 1.4×10^4 CFU/mL, which equals the initial ZB levels upon inoculation. (a) Corn oil served as a ripening inhibitor; (b) MCT served as a ripening inhibitor.

ripening inhibitor in the lipid phase was 70 wt %, the minimal concentration of thyme oil required to inhibit ZB growth for nanoemulsions containing corn oil and MCT were 750 and 3000 μg/mL, respectively, indicating that different ripening inhibitors had varying effects on reducing the antimicrobial efficacy of thyme oil.

Overall, this study demonstrates that the oil phase composition (ripening inhibitor type and concentration) had a significant influence on the antimicrobial activity of thyme oil nanoemulsions. We postulate that the physicochemical origin of this effect is the reduction in effective antimicrobial concentration within the microorganisms due to partitioning into the oil phase of the nanoemulsions. A lipophilic antimicrobial agent will partition between oil phases, aqueous phases, and microorganism cell walls depending on their relative concentrations and oil−water partition coefficients. This process will occur very quickly in nanoemulsions due to the very small dimensions of the droplets and cells.[20] As the total amount of the oil phase in a nanoemulsion increases, more antimicrobial agent will partition into it; therefore, there will be less available to partition into the aqueous phase where the microorganisms exist. In addition, the oil−water partition coefficient depends on the nature of the oil phase in a system. We postulate that the oil−water partition coefficient for thyme oil is higher in MCT than in corn oil; that is, at similar

concentrations, there will be a higher amount of thyme oil dissolved in MCT than in corn oil. Indeed, other experiments in our laboratory with flavor oil nanoemulsions found that citral partitioned more into MCT than corn oil (data not published). This would then account for the fact that MCT nanoemulsions cause a bigger reduction in the antimicrobial efficacy of thyme oil than corn oil nanoemulsions (Figures 4 and 5). Recent studies have also suggested that the antimicrobial activities of essential oils are influenced by their physical location within nanoemulsions and emulsions due to this partitioning effect.[32,34] It would be useful to know the oil−water partition coefficients of thyme oil in corn oil and MCT to confirm our hypothesis. However, this is difficult to establish in practice since thyme oil is a compositionally complex mixture of many different constituents, such as thymol, carvacol, γ-yerpinene, and p-cymene.[1,40,41] In the future, it may be desirable to utilize individual thyme oil components to fabricate nanoemulsions so as to provide a better fundamental understanding of their impact on antimicrobial mechanisms. In particular, a better understanding of how essential oil components incorporated within nanoemulsion droplets interact with microbial cells may lead to the design of more effective antimicrobial delivery systems.

In summary, we have shown that physically stable antimicrobial nanoemulsions could be fabricated by mixing thyme oil with an appropriate amount of ripening inhibitor, such as MCT or corn oil. However, incorporating the ripening inhibitors reduced the antimicrobial activity of the thyme oil in the nanoemulsions. The ripening inhibitor type and concentration had an appreciable influence on the antimicrobial activity of the thyme oil nanoemulsions. In general, increasing the ripening inhibitor levels in the lipid phase reduced the antimicrobial efficacy of the nanoemulsions, with the effect depending on ripening inhibitor type. At the same concentration in the lipid phase, MCT decreased the antimicrobial efficacy of thyme oil more than corn oil. It is thus important to optimize the ripening inhibitor type and level to maximize both physical stability and antimicrobial activity.

The results of this study have important implications for the design and utilization of nanoemulsions as antimicrobial delivery systems in the food and other industries. The physical phenomenon reported in this study may also have important implications for the application of essentials oils as antimicrobials in foods in general. The antimicrobial efficacy of an essential oil may be greatly reduced in foods containing high levels of fats due to partitioning of the active components into the fat phase rather than aqueous phase where microbial cells exist.

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Notes

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