

Physical and Antimicrobial Properties of Peppermint Oil Nanoemulsions

Rong Liang,^{†,§} Shiqi Xu,[§] Charles F. Shoemaker,[#] Yue Li,[†] Fang Zhong,^{*,†} and Qingrong Huang^{*,§}

[†]State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi 214122, People's Republic of China

[§]Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, New Jersey 08901, United States

[#]Department of Food Science and Technology, University of California, Davis, California 95616, United States

S Supporting Information

ABSTRACT: The mixture of peppermint oil (PO) with medium-chain triacylglycerol was emulsified in water and stabilized with a food-grade biopolymer, modified starch, to form PO nanoemulsions. The effects of emulsifying conditions including homogenization pressure, the number of processing cycles, and oil loading on the mean diameters and viscosities of nanoemulsions were characterized by dynamic light scattering, optical microscopy, and rheological measurements. The formulated PO nanoemulsions with mean diameters normally <200 nm showed high stability over at least 30 days of storage time. Their antimicrobial properties related to those of PO have also been evaluated by two assays, the minimum inhibitory concentration (MIC) and time-kill dynamic processes, against two Gram-positive bacterial strains of *Listeria monocytogenes* Scott A and *Staphylococcus aureus* ATCC 25923. Compared with bulk PO, the PO nanoemulsions showed prolonged antibacterial activities. The results suggest that the nanoemulsion technology can provide novel applications of essential oils in extending the shelf life of aqueous food products.

KEYWORDS: peppermint oil, medium-chain triacylglycerol, nanoemulsions, antimicrobial activity, Gram-positive bacteria

■ INTRODUCTION

Essential oils (EOs) are natural concentrated aromatic hydrophobic liquid products obtained from plants by hydro-distillation or steam distillation, expression, or solvent extraction.^{1,2} Various EOs and their main components have been used for a wide variety of purposes including perfumes, cosmetics, foods and drinks, and medicines at different periods for thousands of years.³ In particular, some EOs exert an antimicrobial and antifungal activity that formed the basis of their applications as a natural antimicrobial additive in food manufacturing and preservation to extend the shelf life of food products.^{4,5} It is an effective method to reduce foodborne pathogens and simultaneously decrease the use of synthetic and semisynthetic antimicrobial compounds.

Unfortunately, some special properties, such as low water solubility, high volatility, and strong odor, of EOs limit their applications in foods and beverages. It is also a challenge to incorporate such oil-based compounds in aqueous food products and maintain the physical and chemical stability of foods.⁶ For decades, oil-in-water micro- and nanoemulsions have been considered to be efficient delivery systems for hydrophobic compounds by dispersing the lipid phase as a colloidal dispersion. After encapsulation, the lipophilic bioactive components can be easily incorporated into foods and beverages by increasing the water dispersibility, protecting them from degradation, oxidation, or interactions with the other food ingredients.^{7–11} The emulsion-based systems are widely used in food and drug formulations to entrap active phytochemicals/nutraceuticals,¹² pharmaceuticals,¹³ vitamins,^{14,15} enzymes,¹⁶ flavors,^{17,18} and EOs.^{19,20}

Numerous reports of the encapsulation of EOs to increase their stability and retain their flavor and functional properties have been recently published. However, the antimicrobial mechanism of EOs is not fully understood. It was proposed to involve membrane disruption by the lipophilic compounds.²¹ It has been demonstrated that the level of hydrophobicity of EOs affects the toxicity to bacteria.²² However, after being incorporated into oil-in-water (O/W) emulsions, the antimicrobial activity of EOs would be affected by the composition of the whole system. According to the literature, some contradictory results have been reported. For example, Gomes et al.²³ used poly(DL-lactide-co-glycolide) to prepare nanoparticles to entrap *trans*-cinnamaldehyde and eugenol and used poly(vinyl alcohol) as the stabilizing agent. Their results showed improved inhibiting effect against *Salmonella* spp. and *Listeria* spp. The same tendency was also reported by Donsì et al.²⁴ In contrast, Buranasuksombat et al.²⁵ prepared a series of lemon myrtle oil (LMO) emulsions with droplet sizes ranging from micro- to nanometers. The antimicrobial results showed the same level for all LMO emulsions, suggesting that the antimicrobial property of nanoemulsions may result from the active ingredients in the emulsions instead of the nanosized droplets. Therefore, it is important to design effective delivery systems to incorporate EOs and study how the different formulations may affect their antimicrobial activities.

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In this paper, peppermint (*Mentha canadensis* L.) oil (PO) was utilized as a model EO to formulate antimicrobial nanoemulsions. It is one of the most widely produced and consumed EOs in food flavors, cosmetic fragrances, and pharmaceutical products.^{26,27} Peppermint oil also exhibits antimicrobial, antiviral, and antifungal activities against various types of bacteria and yeasts. The antimicrobial activities of PO are mainly due to the combined effects of major compounds such as *L*-menthol, menthone, menthyl acetate, and limonene.^{2,26,28–30} Modified starch is a food-grade biopolymeric emulsifier commonly used to prepare emulsions in the food and beverage industry. It can provide stability against oil droplet coalescence caused by changes of pH, ionic strength, or temperature. The main objectives of this paper were to establish the parameters necessary to prepare stable peppermint oil-in-water nanoemulsions emulsified with modified starch and to monitor the change of particle sizes and rheological properties as a function of the storage time. Furthermore, the antimicrobial activities were tested for two common Gram-positive microorganism strains to gain more useful information for the EO nanoemulsions as antimicrobial delivery systems.

MATERIALS AND METHODS

Materials. Peppermint oil and medium-chain triacylglycerol (MCT) were selected as the oil phase. PO extracted by steam distillation from green leaves and flowers of *M. canadensis* L. was purchased from Nantong Menthol Factory Co., Ltd. (Nantong, China). According to the GC-MS result (Table S1, Supporting Information), menthol, which is a terpenoid compound showing slight water solubility, is the primary constituent of PO. MCT (Neobee 1053) with 44% C-10 and 56% C-8 was a gift from the Stepan Co. (Maywood, NJ, USA). Purity Gum 2000, which is a succinylated waxy maize starch, was donated by National Starch (Bridgewater, NJ, USA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. Deionized water obtained from a Milli-Q water purification system (Millipore Co., Bedford, MA, USA) was used in all experiments.

Preparation of Emulsions. An aqueous solution of Purity Gum 2000 at the concentration of 12% (w/w) was prepared by dispersing the dried powders in deionized water at room temperature and kept stirring overnight to enhance hydration of the starch prior to homogenization. Pure PO, MCT, and their mixture at ratios of 1:5, 1:1, and 5:1 (v/v) were used as core materials. The oil and aqueous phases were premixed with a high-speed homogenizer (Ultra-Turrax T25 IKA Works Inc., Wilmington, NC, USA) equipped with a S25 N18 G rotor operated at 24,000 rpm for 1 min at room temperature. These coarse emulsions were finely dispersed with a high pressure homogenizer (EmulsiFlex-C3, Avestin, Inc., Ottawa, Ontario, Canada) with pressures of 50, 100, and 150 MPa for 1, 3, 5, 7, 10, 15, or 20 cycles. During the process a heating exchanger was used to control the inlet, operational, and outlet temperatures at 15 °C.

Characterization of Physical Stability of Oil-in-Water Emulsions. Particle Size Distribution. The particle size distribution of the emulsions as a function of storage time was determined by a photon correlation spectroscopy (PCS)-based BIC 90 plus particle size analyzer equipped with a Brookhaven BI-9000AT digital correlator (Brookhaven Instrument Corp., New York, NY, USA). All measurements were made at a fixed scattering angle of 90° and a temperature of 25.0 ± 0.1 °C. The light source of the particle size analyzer is a solid-state laser operating at 658 nm with 30 mW power, and the signals were detected by a high-sensitivity avalanche photodiode detector. To avoid multiple scattering effects, emulsions were first diluted 100 times with deionized water and stirred continuously before the measurements to ensure the samples were homogeneous. The mean diameter of each emulsion was determined by Cumulant analysis of the intensity–intensity autocorrelation function, $G(q,t)$.

Rheological Measurements. Rheological measurements of PO nanoemulsions were performed at 25 °C using the Advanced Rheometric Expansion System (ARES, TA Instruments, New Castle, DE, USA) with a cone and plate geometry (cone diameter = 50 mm, angle = 4°, gap = 0.05 mm). For each measurement, 1.5 mL of the emulsion sample was loaded on the rheometer. The viscosity of nanoemulsions was measured by a steady state flow program with the shear rate ranging from 100 to 1000 s⁻¹ during 5 min. Experimental flow curves were fitted to a power law model

$$\eta = K\dot{\gamma}^{n-1} \quad (1)$$

where η was the viscosity (Pa·s), $\dot{\gamma}$ was the shear rate (s⁻¹), K was the consistency index (Pa·s^{*n*}), and n was the index that provided information about the flow behavior related to the effect of shear rate. There exist three value ranges for n : $n < 1$ for a shear-thinning fluid, $n = 1$ for a Newtonian fluid, and $n > 1$ for a shear-thickening fluid.³¹

Observation of Emulsion Droplets by Optical Microscopy. An optical microscope (Nikon TE-2000 inverted microscope, Nikon Corp., Japan) was used to observe the size of the emulsion droplet, which was diluted with deionized water at the ratio 1:100. Images of emulsions were taken at 40× magnification.

Microbial Cultures. The antimicrobial activity of the PO nanoemulsions was measured against two strains of foodborne Gram-positive pathogenic microorganisms, *Listeria monocytogenes* Scott A and *Staphylococcus aureus* ATCC 25923. The cultures were obtained from the Department of Food Science, Rutgers University, culture collection (New Brunswick, NJ, USA). Strains are stocked on trypticase soy agar plates (TSA, Becton Dickson and Co., Cockeysville, MD, USA) at 4 °C. Working cultures were prepared by transferring two colonies into trypticase soy broth (TSB), grown at 37 °C with agitation for 24 h to 10⁹ colony-forming units (CFU)/mL and diluted to 10⁴ CFU/mL before the test.

Antimicrobial Assays. Determination of Minimum Inhibitory Concentration (MIC). The agar dilution method was chosen to measure the antimicrobial activities of the bulk PO and PO nanoemulsions against the two microbial strains mentioned above. According to the procedure of Hammer et al.³² some modifications were made as follows: Briefly, a series of 2-fold dilutions of bulk PO and PO nanoemulsions, ranging from 4 to 0.0125% (v/v), was prepared in trypticase agar with 0.6% yeast extract supplement (TSAYE). Plates were solidified at room temperature for 30 min prior to inoculations with 5 μL spots containing approximately 10⁴ CFU/mL of each organism onto the agar surface using an autopipet. TSAYE with no oil and TSAYE with 4% (v/v) of emulsion without PO were used as controls. Inoculated plates were incubated at 37 ± 2 °C for 24 h. MICs against each strain were determined as the lowest concentration of PO and PO nanoemulsion inhibiting the visible growth of test microorganism on the agar plate. Experiments were repeated three times.

Time–Kill Studies. *L. monocytogenes* and *S. aureus* were tested to investigate the dynamic time–kill plots of bulk PO and PO nanoemulsions at MIC. Each assay included a growth control without test sample. Time–kill curves were constructed by monitoring the surviving cell numbers in samples after 0, 1, 3, 6, 9, 12, 24, and 36 h of incubation at 37 ± 2 °C. Enumeration was carried out by a standard plate count method. In brief, 0.1 mL of each sample was used to prepare decimal dilutions, which were spreaded in duplicate on TSAYE plates. The plates were incubated at 37 ± 2 °C for 24 h. Colonies were counted and calculated by dilution times. Experiments were carried out in triplicate.

Statistical Analysis. The whole experiment was conducted in duplicate, and all analyses were done at least in triplicate. A one-way analysis of variance (ANOVA) test was analyzed using the SPSS 17.0 package. Duncan's multiple-range test was used to determine the significant differences of mean values.

RESULTS AND DISCUSSION

Influence of the Composition of Oil Phase on Nanoemulsion Stability. Pure PO was dispersed into the aqueous solution of modified starch (MS) with the formulation of 12% PO and 12% MS (w/w) and then emulsified by high-pressure homogenization. The mean diameter of pure PO emulsions stabilized by MS could not be measured with a dynamic light scattering (DLS) instrument. Because the results fluctuated greatly from nano- to micro-sized particles during the measurement, the repeatability of three operations was found not to be consistent. Both the original and diluted emulsions were unstable during the measurement time.

With an increase of the homogenization pressure, the mean diameter of the emulsions also increased, as observed from the optical microscopy images. According to the scale bar in Figure 1, the size of the droplets ranged from 2 to 5 μm . Although the concentrations of the emulsions formed with different homogenization pressures were the same, the number of droplets observed in Figure 1a was less than that for the other two pressures in Figure 1b,c. This could be due to the lower detection limit of the optical microscope. When the droplet particle sizes decreased to <500 nm, they could not be observed with an optical microscope. This suggested that the average diameter of emulsion droplets under a pressure of 50 MPa appeared to be smaller than those formed with a pressure of 100 or 150 MPa. The visual observation of the emulsions showed greater instability for those formed with higher homogenization pressures.

This phenomenon was in good agreement with studies by Terjung et al., who showed that a critical concentration may exist for preparing emulsions containing carvacrol and eugenol.³³ When the loading of these flavor phytophenols was excessively high, that is, 50 and 30% (w/w in oil phase) for eugenol and carvacrol, respectively, a rapid growth of droplet size with increasing homogenization pressure would happen. The reason for the instability could be explained by the theory of Ostwald ripening, a process in which the mean droplet size of an emulsion increases over time caused by diffusion of oil molecules from small to large droplets through the intervening fluid.^{7,34} Due to the water solubility of the main flavor compounds, Ostwald ripening is a common problem for the instability of EO emulsions.³⁵

From previous studies, Ostwald ripening can be limited by adding a water-insoluble component in the oil phase, for example, a long-chain triacylglycerol. This type of molecule can retard Ostwald ripening by generating entropy of the mixing effect that counterbalances the curvature effects.^{36–38} In this study, a MCT with low polarity, high density, and high viscosity was used to compose the mixture of oil phase. MCT has been widely used as a solubilizer/carrier solvent for flavors, colors, essential oils, and vitamins. Some researchers have successfully used it to prepare nanoemulsions containing phytophenols with the goal of retarding Ostwald ripening.³³ Furthermore, from our previous research it can offer a more effective vehicle to deliver nutraceuticals by increasing the bioavailability in vitro than canola, coconut, and corn oils.³⁹

To test whether MCT was suitable for a modified starch-stabilized system, pure MCT was first added to MS solution and used to prepare a nanoemulsion with the high-pressure conditions of 150 MPa for 10 cycles. A nanoemulsion with a small mean diameter (146.0 ± 1.5 nm) was obtained. Furthermore, a series of different concentrations of MCT in

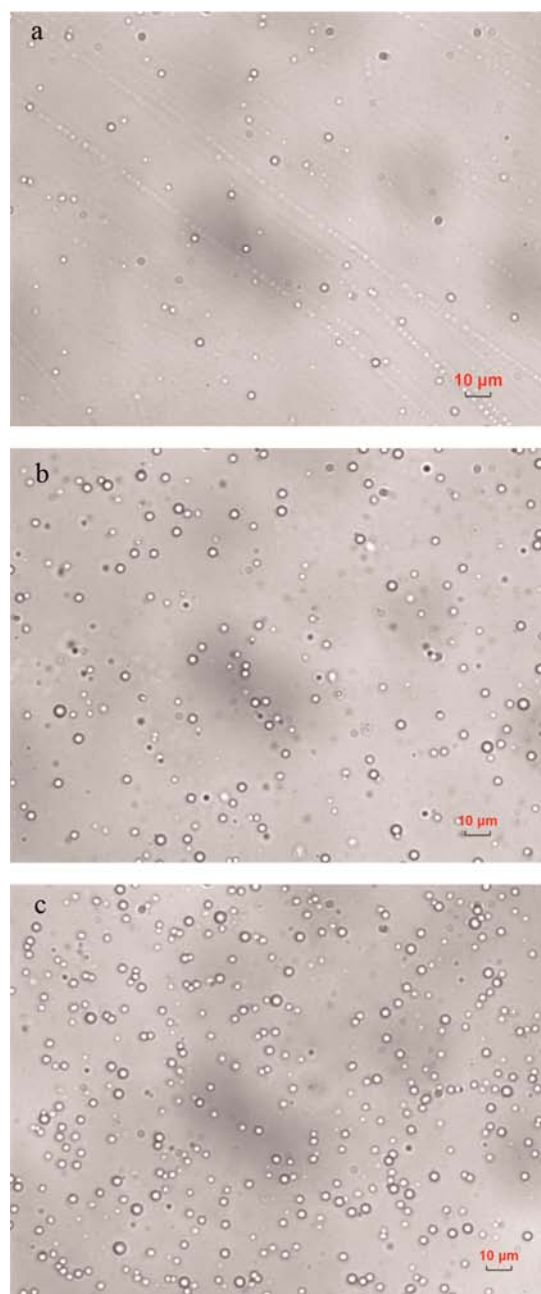


Figure 1. Optical microscopic images of pure peppermint oil (PO) nanoemulsions stabilized by modified starch (MS) processed at different homogenization pressures: (a) 50 MPa; (b) 100 MPa; (c) 150 MPa. The formulation of nanoemulsion contains 12% (w/w) MS and 12% (w/w) pure PO, and the number of homogenization cycles is 10.

the oil phase were prepared and encapsulated with the MS aqueous dispersion. The trend of decreasing mean diameter was found with increasing concentrations of MCT, and the results are shown in Figure 2. With the lowest concentration (16.7%, v/v) of MCT in the oil phase, the mean diameter dropped dramatically from a microscale (4 μm) of pure PO emulsion to a nanoscale (222.0 ± 2.6 nm). The apparent viscosities of nanoemulsions measured at same shear rate (120 s^{-1}) increased from 24.3 to 38.7 mPa·s with increasing concentrations of MCT (Figure 2). This trend of droplet diameters and viscosities of PO emulsions was consistent with the research from Wang et al.¹² According to the theoretical

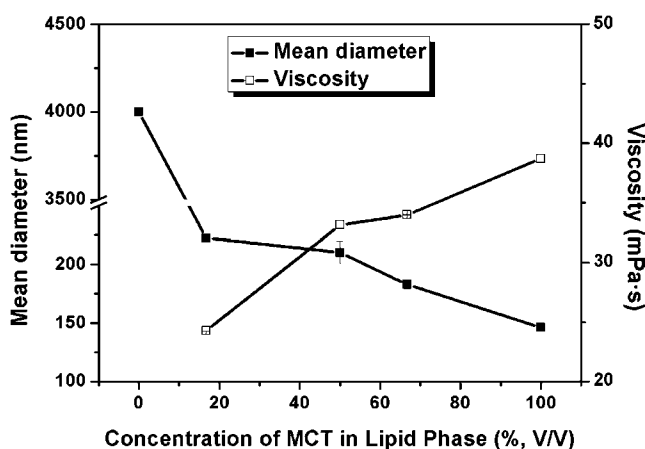


Figure 2. Effects of the addition of medium-chain triacylglycerol (MCT) to peppermint oil (PO) on the mean diameters and viscosities of nanoemulsions containing 12% (w/w) modified starch (MS) and 12% (w/w) oil phase (PO + MCT). The nanoemulsions were prepared with a homogenization pressure of 150 MPa and 10 processing cycles.

analysis from Pal, the droplet diameter strongly affected the rheological behavior of emulsions, and a decrease in the mean diameter is expected to cause an increase in viscosity at any given shear rate.⁴⁰ Overall, the decrease of particle size and an increase in apparent viscosity would improve the stability of emulsions, and mixing MCT with PO before high-pressure homogenization was a useful method to inhibit the Ostwald ripening of PO emulsions.

Effects of Homogenization Pressure and Processing Cycles. Because Ostwald ripening could be limited by using a mixed PO oil phase with MCT, the next step was to study the conditions to reduce the sizes of the nanoemulsions by varying the pressure of homogenization. The pressure value and process cycles of the operation are two main parameters to determine the properties of emulsions.⁷ According to Stokes' law, the smaller radius of droplets can decrease the velocity of gravitational separation further to increase the stability of emulsion, so the mean diameter of emulsions was used to determine the proper homogenization pressure and processing cycles.

Increasing the homogenization pressure and processing passes resulted in a significant decrease in mean diameters (Figure 3), which was consistent with the results of several studies.^{41,42} In the case of current emulsion formulation [12% MS, 12% mix oil (16.7% PO + 83.3% MCT)], the mean diameter progressively dropped from the cycle 1 to the cycle 10 at three levels of pressure, respectively. However, after 10 cycles, no further significant decrease in mean diameter was observed. These results are in agreement with the findings of Tan et al.⁴³ The small particle size and uniform droplet distribution also rely on the pressure level. When the pressure increased from 50 to 100 MPa, the mean diameter decreased dramatically. Further increase of homogenizer pressure from 100 to 150 MPa resulted in negligible change in droplet sizes. Therefore, a homogenization pressure of 100 MPa and 10 processing cycles were used to prepare PO nanoemulsions.

Effect of Oil Loading on the Stability of Nanoemulsions. It is often desirable for O/W emulsions to contain high contents of oil phase to increase the amount of hydrophobic active ingredients. It is known that oil content can also influence the emulsion droplet sizes through

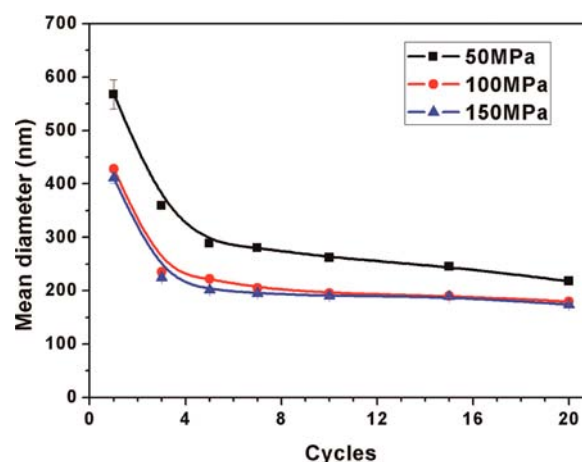


Figure 3. Effect of homogenization pressure and number of processing cycles on mean diameters of peppermint oil (PO) nanoemulsions containing 12% (w/w) modified starch (MS) and 12% (w/w) mixed oil (PO:MCT = 1:5, v/v): 50 MPa (squares); 100 MPa (circles); 150 MPa (triangles).

coalescence phenomenon.⁶ Thus, the oil loading is an important parameter to evaluate the stability of O/W emulsions produced. The oil phase in this paper is the mixture of PO and MCT.

The mean diameter and size distributions of nanoemulsions prepared by different oil phase to modified starch ratios are presented in Table 1. Here the concentration of MS was fixed

Table 1. Particle Mean Diameter and Size Distribution of Peppermint Oil (PO)/Medium-Chain Triacylglycerol (MCT) Nanoemulsions Prepared with Different Oil Phases: Modified Starch Ratios (w/w) (Mean \pm SD, $n = 3$)^a

oil concentration (mass ratio of oil:MS, w/w)	mean diameter (nm)	PDI ^b
1:3	184.0 \pm 10.6 A	0.254 \pm 0.003 c
2:3	196.0 \pm 1.0 A	0.302 \pm 0.013 de
3:3	189.0 \pm 5.3 A	0.320 \pm 0.013 ef
4:3	228.0 \pm 15.1 B	0.332 \pm 0.020 f

^aThe emulsions were produced using MS at a concentration of 12% (w/w) and mix oil phase at PO:MCT = 1:5 (v/v) with high-pressure homogenization pressure at 100 MPa and 10 cycles, respectively. For the particle size values, data followed by different capital letters (A, B) are significantly different ($P < 0.05$). For the PDI values, data followed by different lower case letters are significantly different ($P < 0.05$).

^bPDI, polydispersity index.

at 12% (w/w), and the ratio of PO:MCT in the oil phase was fixed at 1:5 (v/v). When the oil/starch ratio increased from 1:3 to 3:3 (w/w), the change of mean diameter was not significant ($P > 0.05$). Further increase of the oil phase resulted in a clear ($P < 0.05$) increase in the mean diameter from 189 nm (3:3) to 228 nm (4:3). In this case the excessive oil volume fraction could not be covered by the emulsifier, and part of the free oil droplets would affect the droplet diameter of the emulsion.⁴²

The polydispersity index (PDI) value represents the particle size distribution of the droplets. A small PDI value indicates a narrow particle size distribution. According to the results shown in Table 1, the PDI values were normally < 0.35 , indicating that all of the emulsions had a relatively narrow range of size distribution. It increased significantly ($P < 0.05$) from 0.254 to 0.302 as the mass ratio increased from 1:3 to 2:3, but the

further increase of oil phase did not change the PDI value significantly until the ratio was higher than 4:3. This trend confirmed the results of mean diameter. When the oil to MS mass ratio is 3:3, the nanoemulsion showed a small mean diameter and a narrow size distribution.

Storage Stability of Nanoemulsions. One objective of this study is to prepare O/W nanoemulsions with a high concentration of PO. To get more information about the stability of these nanoemulsions, extended storage tests were carried out, and the mean diameter and rheological properties were measured during the storage time.

The effect of storage time on mean diameter of nanoemulsions with different ratios of PO:MCT (12% oil phase) was measured at days 1, 15, and 30 (Figure 4). The storage test

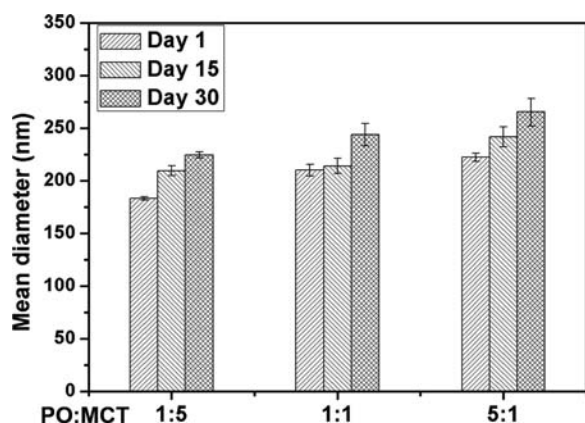


Figure 4. Evolution of mean diameters over time upon storage at room temperature for modified starch-based nanoemulsions containing 12% oil phase prepared with different PO to MCT ratios with homogenization pressure and processing cycles fixed at 100 MPa and 10 cycles, respectively.

was carried out at room temperature. The initial nanoemulsions (day 1) exhibited an increased mean diameter from 180 to 220 nm as the ratio of PO:MCT increased from 1:5 to 5:1. During the 30 day storage test, an increase of 20–30 nm in mean diameter was observed in the emulsions with PO:MCT of 1:5, 1:1, and 5:1(v/v), respectively. Over the storage times there was no obvious phase separation or creaming observed for any of the samples. The consistent mean diameter of these emulsions over storage times indicated a better stability of PO/MCT oil-in-water nanoemulsions as compared to emulsions with PO only.

The rheological properties of the nanoemulsions with different concentrations of PO were also investigated as a measure of their stability. Most of the emulsion-based delivery systems used in foods and beverages show shear-thinning behavior, which is important for lowering the viscosity under flow during consumption.⁴⁴ The shear rate dependence of apparent viscosities of PO nanoemulsions has been investigated (Figure 5). The nanoemulsions showed different degrees of shear-thinning behavior. The viscosities of nanoemulsions increased when the ratios of MCT in the oil phase increased, as indicated from the data listed in Table 2, which showed the rheological parameters fitted with a power law function for PO nanoemulsions prepared with different PO concentrations. The square of the correlation index (R^2) was >0.98 , suggesting that the model is suitable for the emulsions studied in this work. During storage the K values decreased for three nanoemulsion

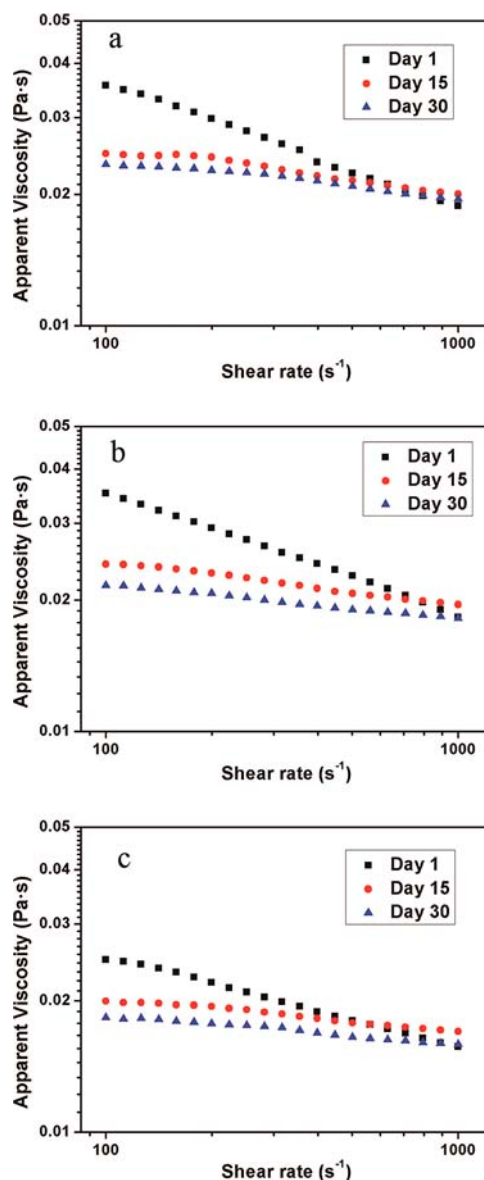


Figure 5. Effects of storage time on the rheological properties of nanoemulsions containing 12% oil phases at different PO:MCT ratios: (a) 1:5; (b) 1:1; (c) 5:1. The storage temperature is ambient temperature (25 ± 2 °C).

formulations, in agreement with the decrease of viscosity according to the flow curves in Figures 5. All flow behavior indices (n) obtained were <1.0 (Table 2), further indicating the shear-thinning nature of the nanoemulsions. For each nanoemulsion, the value of n increased; that is, for nanoemulsion with PO:MCT = 1:5, n changed from 0.709 (day 1) to 0.86 (day 30), which demonstrated a more apparent Newtonian nature for emulsions during the storage period. This change of emulsion viscosities was in good agreement with previously published results from Pal, suggesting that emulsions of smaller sizes had much higher viscosities than the coarse emulsions and that the shear-thinning effect is much stronger in the case of smaller-sized emulsions.⁴⁵ The change of rheological properties was mainly due to the change in droplet mean diameter. Fine emulsions with smaller droplet size showed higher viscosity in the beginning, and the loss of shearing thinning and viscosity during storage would be due to the loss of nanoemulsion

Table 2. Rheological Parameters Obtained from Fitting Using Power Law Model for Peppermint Oil (PO) Nanoemulsions Prepared with Different PO Concentrations ($n = 3$)^a

ratio of PO:MCT (v/v)	storage time (days)	K (Pa·s ⁿ)	N	R ²
1:5	1	0.1381	0.709	0.9973
	15	0.0525	0.844	0.9865
	30	0.046	0.86	0.9827
1:1	1	0.1292	0.719	0.999
	15	0.0489	0.85	0.996
	30	0.039	0.874	0.9924
5:1	1	0.0666	0.79	0.9982
	15	0.0352	0.881	0.9896
	30	0.0304	0.893	0.9866

^aThe emulsions were produced using 12% (w/w) modified starch (MS) and the oil/MS ratio of 3:3 (w/w) with high-pressure homogenization pressure of 100 MPa and 10 processing cycles.

structure by an increase of particle diameter during storage. Overall, all of the emulsions exhibit good stability with no visual instability such as creaming during storage. The results also proved that the use of MCT can effectively produce a stable PO O/W nanoemulsion by inhibiting Ostwald ripening.

Antimicrobial Activity of Peppermint Oil Nanoemulsions. According to the GC-MS data, the compositions of pure and emulsified PO were quite similar (Table S1, Supporting Information), so the PO nanoemulsions were considered to be a potential antimicrobial delivery system. According to the results reported in Table 3, MIC values

Table 3. Antimicrobial Properties of Pure Peppermint Oil (PO) and PO Nanoemulsions against *L. monocytogenes* and *S. aureus* ($n = 3$)^a

microbial strain	antimicrobial component	final amount of PO added to TSAYE ^b (% v/v)				
		4	2	1	0.5	0.25
<i>L. monocytogenes</i>	pure PO	–	–	–	–	+
	PO emulsion	–	–	–	–	+
<i>S. aureus</i> ATCC 25923	pure PO	–	–	–	–	+
	PO emulsion	–	–	–	–	+

^aThe nanoemulsions were produced using MS at concentration of 12% (w/w) and mix oil phase at PO:MCT = 5:1 (v/v) with 100 MPa homogenization pressure and 10 cycles. ^bTSAYE, trypticase agar with 0.6% yeast extract supplement.

against two Gram-positive bacteria of *L. monocytogenes* and *S. aureus* of nanoencapsulated PO and bulk PO were determined using an agar dilution method. Compared to bulk PO, the MIC values for PO nanoemulsions showed the same level (0.5%, v/v) for these two strains. The MIC value for PO was consistent with the results from Gupta and van Vuuren et al., who found moderate inhibition of the growth of microorganisms.^{46,47} For the PO emulsions, it was concluded that this emulsion system stabilized by modified starch did not compromise the antimicrobial activity. Similar results have also been reported for lemon myrtle oil emulsions stabilized by Tween 80.²⁵

A time–kill dynamic experiment was conducted to compare the addition of either bulk PO or PO nanoemulsions toward the growth of *L. monocytogenes* and *S. aureus*. The final concentration of PO in either pure form or nanoemulsion was 0.5%. According to the results in Figure 6, for both strains the

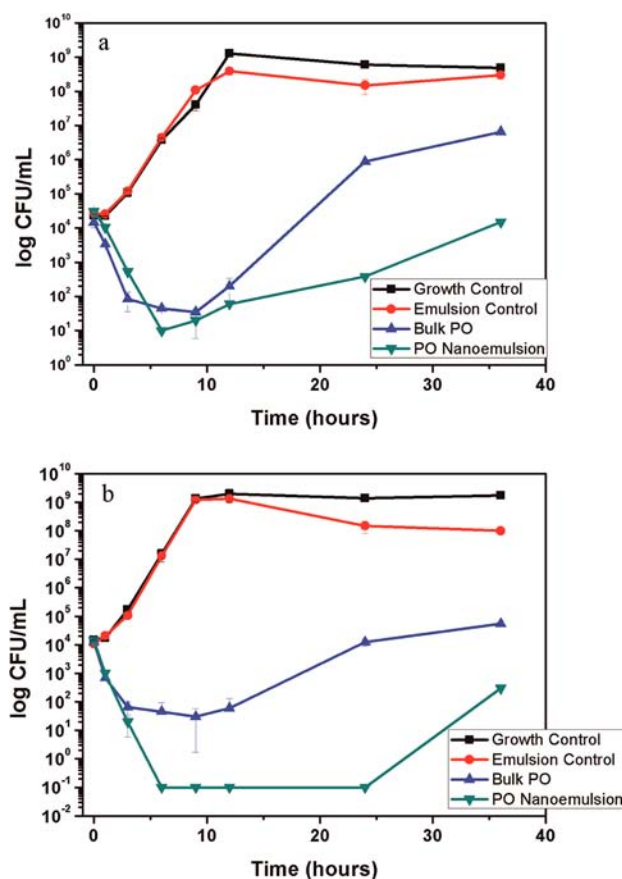


Figure 6. Time–kill plots for bulk peppermint oil (PO) and PO nanoemulsions against *L. monocytogenes* (a) and *S. aureus* (b). The PO concentration used in the test was fixed at 0.5% (v/v), which is the minimum inhibitory concentration (MIC) of PO. The oil phase of PO nanoemulsion is a mixture of PO and MCT with a PO to MCT ratio of 5:1 (v/v).

nanoemulsions without PO showed no inhibitory effect on bacterial growth, and the test organisms grew at similar levels as growth controls. With the addition of PO in either pure form or emulsion, a strong initial inhibitory effect for both bacteria was observed. For *L. monocytogenes* the bacterial numbers went down to almost 10¹ CFU/mL during the first 8 h; afterward, the bacterial cells rapidly increased to higher levels. As observed in Figure 6a, the growth of the bacteria was slower with PO nanoemulsions and the bacterial number reached about 10⁴ CFU/mL after 36 h, whereas with pure PO it went up to around 10⁷ CFU/mL. *S. aureus* was more sensitive to the action of PO when compared with *L. monocytogenes*. Figure 6b shows that the bacterial numbers were inhibited at a lower level (near 10¹ CFU/mL) in the first 3 h, and this inhibitory effect was continued until 24 h for PO nanoemulsions. Finally, the bacterial numbers went up to 10² and 10⁵ CFU/mL for PO nanoemulsion and bulk PO, respectively.

On the basis of the results described above, PO nanoemulsions showed the same value of MIC as the bulk PO but had a long-term inhibition of the bacteria growth of *L.*

monocytogenes and *S. aureus*. The possible reason for this phenomenon was that the emulsion system would be useful to increase the stability and solubility of PO in the culture medium to further control the release and extend the antimicrobial activity. However, the emulsion system may also limit the contact of PO with the membrane of bacteria. A compromised result was chosen for PO nanoemulsions. The same results were also found by Wang et al.⁴⁸ At the same time some researchers reported a reduction of antimicrobial activity for compounds after encapsulation.^{38,49} Therefore, more and more researchers tried to analyze the parameters affecting the antimicrobial activities of EOs emulsions. It was shown that the antimicrobial activities were determined not only by the particle size of emulsions but also by the structure of emulsions, for example, the location of active compounds in the system and the concentration of active compounds in aqueous phase.^{33,50} Further work is still being done to reveal the antimicrobial mechanisms for PO entrapped in nanoemulsion.

In summary, stable PO nanoemulsions have been produced by incorporating PO with MCT. For a long storage time, the particle size and viscosity of the nanoemulsions containing different concentrations of PO remained stable, and no obvious creaming and phase separation were observed. The MIC values for two strains of microorganisms, *L. monocytogenes* and *S. aureus*, were similar for both bulk PO and PO nanoemulsions. However, our time–kill kinetic study indicated that the formulated PO nanoemulsions showed an improved long-term antimicrobial activity compared to the pure PO. The knowledge obtained from this study will be helpful in the design and development of new intervention strategies for foodborne pathogens using food-grade essential oils.

■ ASSOCIATED CONTENT

📄 Supporting Information

Additional experimental details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (F.Z.) +86 13812536912; (Q.H.) +1 (732) 932-7193.
E-mail: (F.Z.) fzhong@jiangnan.edu.cn; (Q.H.) qhuang@aesop.rutgers.edu.

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Notes

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