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Fabrication, stability and efficacy of dual-component antimicrobial nanoemulsions: Essential oil (thyme oil) and cationic surfactant (lauric arginate)

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ABSTRACT

The influence of a cationic surfactant (lauric arginate, LAE) on the physical properties and antimicrobial efficacy of thyme oil nanoemulsions was investigated. Nanoemulsions prepared from pure thyme oil were highly unstable due to Ostwald ripening, but they could be stabilized by adding a ripening inhibitor (corn oil) to the oil phase prior to homogenisation. The loading capacity and antimicrobial efficacy of thyme oil nanoemulsions were significantly increased by adding LAE. In the absence of LAE, at least 60 wt% corn oil had to be added to the lipid phase to inhibit Ostwald ripening; but in the presence of 0.1 wt% LAE, only 30 wt% corn oil was needed. LAE addition substantially increased the antimicrobial efficacy of the thyme oil nanoemulsions: 200 μg/ml thyme oil was needed to inhibit growth of a spoilage yeast (*Zygosaccharomyces bailii*) if LAE was added, whereas ⩾400 μg/ml was needed in the absence of LAE.

1. Introduction

Essential oils are natural compounds which can act both as flavouring agents and antimicrobial agents, and therefore they have been widely used as functional ingredients in food, cosmetic, and pharmaceutical applications (Bakkali, Averbeck, Averbeck, & Waomar, 2008). Essential oils contain a complex mixture of non-volatile and volatile compounds. Commercial essential oils are often a mixture of different constituents that vary in their molecular and physicochemical properties, for example, molecular weight, water solubility, polarity, and biological activity. The major molecular constituents within commercial essential oils can be classified into three broad classes: phenols, terpenes, and aldehydes (Bakkali et al., 2008; Burt, 2004; Ceylan & Fung, 2004). Many essential oils have been shown to exert strong antibacterial, antiviral, and antifungal activities (Burt, 2004; Ferreira et al., 2010; Giatrakou, Ntzimani, & Savvaidis, 2010), leading to their application as natural antimicrobial additives to extend the shelf life of food and beverage products. For example, thyme oil has been shown to have inhibitory activities against various bacteria and yeasts (Gaysinsky, Davidson, McClements, & Weiss, 2008). Thymol, the primary component of thyme oil (Gaysinsky, 2007), has also been reported to exhibit antimicrobial activity against many bacteria and fungi (Friedman, Henika, & Mandrell, 2002; Sivropoulou et al., 1996). The fact that essential oils are considered to be "natural" components makes them highly desirable for application in many commercial food and beverage products, since there is growing consumer demand for natural rather than synthetic additives.

However, essential oils are hydrophobic compounds and usually have quite low solubility in water, which limits their utilisation in aqueous-based foods and beverages. This problem could be simply resolved by encapsulating essential oil within emulsion-based delivery systems (Chang, McLandsborough, & McClements, 2012; Donsi, Annunziata, Sessa, & Ferrari, 2011; Donsi, Cuomo, Marchese, & Ferrari, 2014; Salvia-Trujillo, Rojas-Grau, Soliva-Fortuny, & Martin-Belloso, 2013, 2014; Wu, Lin, & Zhong, 2014; Ziani, Chang, McLandsborough, & McClements, 2011). After essential oils are encapsulated into suitable emulsion delivery systems, they can then be incorporated into aqueous-based foods (e.g., beverages) and other products by simple mixing. Based on their droplet size, emulsions may be divided into

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conventional emulsions (diameter > 200 nm) or nanoemulsions (diameter < 200 nm) (McClements, 2011, 2012; McClements & Rao, 2011). The diameter of the nanoemulsion droplets is often much smaller than the wavelength of light ($d \ll \lambda$), therefore the nanoemulsions are often transparent or only slightly turbid, since they do not scatter light strongly. This property is beneficial, making nanoemulsions suitable for transparent products, such as clear beverages, sauces, soups, and syrups. The small dimensions of the nanoemulsion droplets also mean that they typically have much better physical stability against gravitational separation, flocculation, and coalescence than conventional emulsions (Mason, Wilking, Meleson, Chang, & Graves, 2006; McClements, 2011; McClements & Rao, 2011; Tadros, Izquierdo, Esquena, & Solans, 2004). In addition, as droplets size decreases, the biological activity of the encapsulated active compounds within emulsions or nanoemulsions often increases (Acosta, 2009; Hatanaka et al., 2010; Huang, Yu, & Ru, 2010). Therefore, in many cases it is beneficial to encapsulate functional components into nanoemulsions, compared to conventional emulsions.

A major limitation to formulating antimicrobial nanoemulsions containing essential oils is that they have some water-solubility, which means that the oil droplets are prone to Ostwald ripening (Chang et al., 2012; Ziani et al., 2011). Ostwald ripening (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 (Kabalnov, 2001; Kabalnov & Shchukin, 1992; Wooster, Golding, & Sanguansri, 2008). The driving force for OR is the fact that the solubility of oil in the immediate vicinity of an oil droplet increases as the droplet diameter decreases. The Ostwald ripening rate usually increases with increasing solubility of the oil phase in the water phase (McClements, 2005, 2011; McClements & Rao, 2011; Wooster et al., 2008). OR can be prevented by incorporating sufficient amounts of highly water-insoluble oils in the droplets, since this generates an entropy of mixing effect that counteracts the imbalance of droplet size effect (Kabalnov & Shchukin, 1992; Wooster et al., 2008). These water-insoluble oils are typically referred to as "ripening inhibitors", and are usually highly non-polar substances with relatively high molecular weights, such as corn oil (Chang et al., 2012; McClements, Henson, Popplewell, Decker, & Choi, 2012; Ziani et al., 2011), sunflower oil (Donsi, Annunziata, Vincensi, & Ferrari, 2012), and medium chain triglycerides (MCT) (Liang et al., 2012; Terjung, Loffler, Gibis, Hinrichs, & Weiss, 2012). In our previous study, we showed that Ostwald ripening of thyme oil nanoemulsions could be inhibited by mixing thyme oil with sufficiently high amounts of water-insoluble oils (e.g., $\geqslant$60 wt% corn oil in the lipid phase) before homogenisation, (Chang et al., 2012; Ziani et al., 2011). This method can therefore be used to inhibit Ostwald ripening in nanoemulsions, provided that a sufficiently high quantity of water-insoluble oil is utilised.

Lauric arginate (LAE) is a food-grade cationic surfactant that is a highly potent antimicrobial active against a wide range of food pathogens and spoilage organisms (Brandt et al., 2010; Dai, Normand, Weiss, & Peleg, 2010; Theinsathid, Visessanguan, Kruenate, Kingcha, & Keeratipibul, 2012). In this study, we investigated the ability of LAE to improve both the physical stability and antimicrobial activity of thyme oil nanoemulsions. In particular, we hypothesized that utilising two different antimicrobial agents within a single nanoemulsion (LAE and thyme oil) may lead to synergistic antimicrobial activity. An acid-resistant spoilage yeast (*Zygosaccharomyces bailii*) was used as a model microorganism to assess the antimicrobial activity of the nanoemulsions. The results of this study have important implications for the design and utilisation of nanoemulsions as antimicrobial delivery systems in the food and other industries.

2. Materials and methods

2.1. Materials

Thyme oil was obtained from Optimal Health Solutions (La Pine, Oregon). Corn oil was purchased from a local grocery store. A non-ionic surfactant (Tween 80, T80) was purchased from Sigma–Aldrich Co. (St. Louis, MO), and the cationic surfactant lauric arginate (LAE) (MIRENAT-P/100) was provided by Grupo Lamirsa (Terrassa, Spain) which was reported to contain 85 wt% LAE.

2.2. Nanoemulsion preparation

To made thyme oil nanoemulsions, we first prepared aqueous phase and oil phase separately. The aqueous phase used to prepare the nanoemulsions consisted of 1.0 wt% Tween 80 or 0.9 wt% Tween 80 + 0.1 wt% LAE 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) prior to homogenisation. 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 homogenised by passing it five times through a high pressure homogeniser at 10 kPa (Microfluidics 110L, Microfluidics Corp., Newton, MA, USA) to further reduce the particle size. After preparation, the nanoemulsions formed were stored at 4 °C prior to analysis.

2.3. Particle size measurements

The mean particle diameters (*Z*-averages) of the nanoemulsions were measured using a dynamic light scattering instrument (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK), following the method described in our previous publication (Chang et al., 2012).

2.4. Particle charge measurements

The electrical charge (ζ-potential) of the droplets in the nanoemulsions was measured using a particle electrophoresis instrument (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK), following the method as described previously (Chang et al., 2012).

2.5. Yeast strain

An acid resistant spoilage yeast, *Z. bailii* (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). The yeast strain was refreshed and cultured according to the method described by us previously (Chang et al., 2012), and then diluted to about 10^6 CFU/ml in MEB media (pH 3.5, 5 mM citrate buffer) to conduct the following antimicrobial assay.

2.6. Determination of antimicrobial activity

All nanoemulsions were filtered sterilized using 0.45 μm polyethersulfone membrane filters (F2500-14, Thermo Scientific, Germany) prior to carrying out the antimicrobial activity assays. The particle size distributions of the nanoemulsions did not change after filter sterilization (data not shown), which indicated that all the droplets passed through the filter.

Appropriate amounts of sterile thyme oil nanoemulsions were added to MEB media (pH 3.5, 5 mM citrate buffer), to make incubation media containing a serial of antimicrobial nanoemulsions. The

final concentrations of net thyme oil (not thyme oil emulsion) in each MEB media were 800, 400, 200, 100 μg/ml, respectively. The MEB media containing varying levels of antimicrobial emulsions were then inoculated with 1/100 ZB culture, to achieve initial cell levels around 10^4 CFU/ml. The surviving cell numbers were monitored after 0, 24, 60, 120 h incubation at 25 °C. Enumeration was carried out by using a spiral plater (Spiral Biotech, Norwood, Massachusetts). All experiments were conducted with duplicate samples of each treatment, and the entire study was carried out in triplicate.

2.7. 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.

3. Results and discussions

3.1. Impact of LAE on nanoemulsion formation, stability and loading capacity

If nanoemulsions are going to be used as delivery systems for antimicrobial agents, then it is important that they have good long-term stability. We therefore carried out a series of preliminary experiments to establish the physical stability of the nanoemulsions to droplet growth and phase separation.

Initially, we prepared a series of nanoemulsions stabilized by a non-ionic surfactant by homogenising 10 wt% oil phase and 90 wt% aqueous phase. The oil phase consisted of varying ratios of thyme oil (antimicrobial) and corn oil (Ostwald ripening inhibitor). The aqueous phase consisted of 1 wt% Tween 80 dissolved in buffer solution (5 mM citrate, pH = 3.5). After homogenisation, the samples were stored for 3 days at ambient temperature, mixed to ensure they were homogeneous, and then their mean particle sizes were measured. The size of the droplets after storage depended on the ratio of thyme oil to corn oil in the lipid phase (Fig. 1). At relatively high thyme oil levels (>40% in lipid phase) the emulsions were highly unstable to droplet growth and phase separation, but at lower levels they had relatively small diameters (d < 250 nm) and were stable to visible phase separation. As discussed previously, this effect can be attributed to the ability of the highly water-insoluble corn oil to act as a ripening inhibitor that retards droplet growth due to an entropy of mixing effect (Chang et al., 2012; McClements et al., 2012; Ziani et al., 2011).

We also examined the influence of cationic surfactant addition on the stability of the nanoemulsions. Nanoemulsions were prepared using the same approach, but using 0.9 wt% Tween 80 + 0.1 wt% LAE (instead of 1.0 wt% Tween 80). The physical stability of the thyme oil nanoemulsions was greatly improved in the presence of this cationic surfactant (Fig. 1). In particular, the mean droplet diameter was relatively small (d = 150 nm) at thyme oil levels from 30% to 70% in the lipid phase. At higher thyme oil levels (⩾80 wt%), the emulsions formed were highly unstable and rapid separated (data not shown). This experiment showed that the maximum amount of thyme oil that could be incorporated into the nanoemulsions, while maintaining their physical stability, was around 40 wt% in the lipid phase when only Tween 80 was used but around 70 wt% when a mixture of surfactants (Tween 80 and LAE) was used. Practically, one would like to maximise the amount of active ingredient (in this case thyme oil) present within a delivery system. Overall these results indicated that the incorporation of LAE into the nanoemulsions increased both their loading capacity and their stability. In addition, LAE is known to be an effective antimicrobial agent itself, and so we also examined the influence of its presence on the antimicrobial activity of the nanoemulsions (see later).

3.2. Impact of LAE on droplet charge

The electrical characteristics of the droplets in the thyme oil nanoemulsions were measured (Fig. 2), since droplet charge may have an important impact on nanoemulsion stability and antimicrobial efficacy. The ζ-potential of the thyme oil nanoemulsions (containing only Tween 80) were slightly negative (≈−1.5 mV), which can be attributed to the presence of some anionic impurities in the surfactant (such as free fatty acids) or preferential adsorption of anionic species from water (such as hydroxyl ions) to the droplet surfaces (McClements, 2005). When 0.1 wt% LAE was used as a co-surfactant, the ζ-potential of the thyme oil nanoemulsions became cationic (≈+18 mV), suggesting that at least some of the cationic surfactant molecules adsorbed to the oil droplet surfaces (Fig. 2).

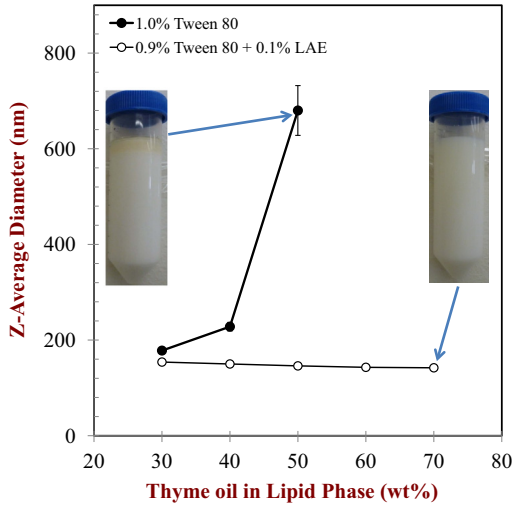

Fig. 1. Dependence of mean droplet diameter after 3 days of storage at ambient temperature on oil phase composition for 10% oil-in-water emulsions containing different amounts of thyme oil and corn oil in the lipid phase (5 mM citrate buffer, pH 3.5). Surfactant was either 1.0% Tween 80 or 0.9% Tween 80 + 0.1% LAE.

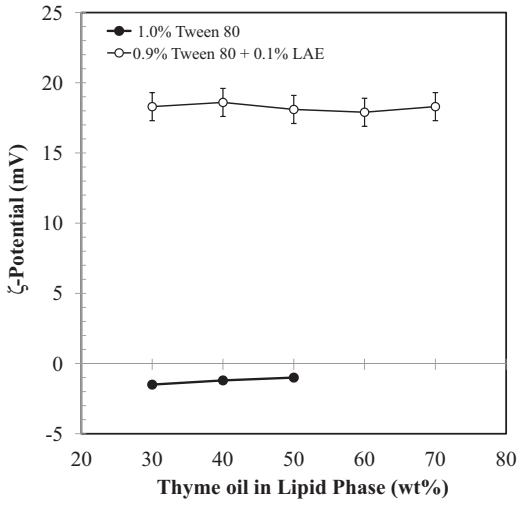

Fig. 2. Dependence of ζ-potential of emulsion droplets on oil phase composition for 10% oil-in-water emulsions containing different amounts of thyme oil and corn oil in the lipid phase (5 mM citrate buffer, pH 3.5).

3.3. Storage stability of nanoemulsions

For most commercial applications it is important that nanoemulsion-based delivery systems maintain their physically stability throughout their intended shelf-life, i.e., there is little change in particle size or evidence of phase separation. We therefore examined the stability of the antimicrobial nanoemulsions over 1 month storage at ambient temperature. A series of nanoemulsions were prepared that consisted of 10 wt% oil (30–70% thyme oil in the lipid phase), 0.9 wt% Tween 80, 0.1 wt% LAE, and 80 wt% aqueous phase (pH 3.5 buffer). All of these systems remained stable throughout the entire storage period, with the percentage change in mean particle diameter being less than 3% for all samples (data not shown). Indeed, the particle size distributions were very similar before and after storage (Fig. 3), again highlighting their high stability to droplet growth. In a previous study (Chang et al., 2012) we showed that systems containing 10 wt% oil (30% or 40% thyme oil in the lipid phase) and 1 wt% Tween 80 (no LAE) were also stable to droplet growth over 30 days storage.

When applied to food systems, the thyme oil nanoemulsions are usually highly diluted, we therefore also examined the stability of the diluted nanoemulsions (10 and 100 times diluted in pH 3.5 citrate buffer) over 30 days storage at room temperature. The results indicated that the diluted nanoemulsions all had very good stability to droplet growth, i.e., no appreciable increase in mean particle diameter after storage (data not shown).

3.4. Influence of electrostatic repulsion on nanoemulsion stability

We hypothesized that the good stability of the nanoemulsions containing the cationic surfactant were at least partly due to the presence of positively charged groups at the droplet surfaces (Fig. 2), as these would generate an electrostatic repulsion between droplets. To test this hypothesis, we carried out the following experiment: a high-loading thyme oil nanoemulsion was prepared (7% thyme oil, 3% corn oil, 0.9% Tween 80, 0.1% LAE) at pH 3.5, and then alkali (NaOH) solution was added to adjust the pH to 7.0 or salt (NaCl) solution was added to increase the ionic strength (to 200 mM NaCl). After these adjustments, the nanoemulsions rapidly became unstable to droplet aggregation, with evidence of oiling-off and phase separation within a few minutes (Fig. 4). When the pH was changed from acidic to neutral, the LAE molecules ($pK_a = 6.5$) lose their positive charge, thereby reducing the electrostatic repulsion between droplets. When the salt concentration was increased, the counter ions in the aqueous solution will electrostatically screen the droplet charge, thereby also reducing the electrostatic repulsion. On the other hand, nanoemulsions stabilized only by Tween 80 remained stable after adjustment to neutral pH or high ionic strength (data not shown). This effect can be attributed to the ability of the hydrophilic head groups of the non-ionic surfactant molecules to generate strong short range steric repulsions.

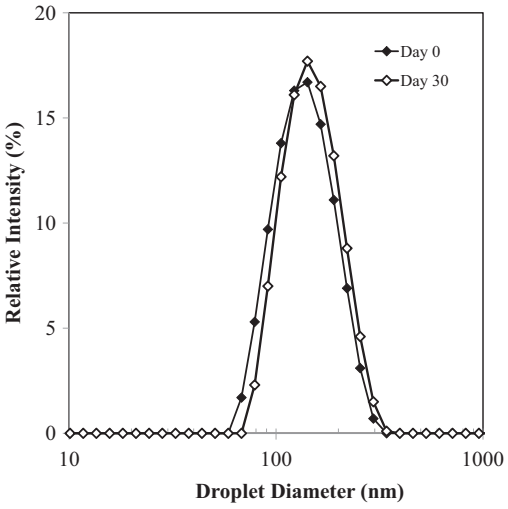

Fig. 3. Dependence of particle size distributions before and after storage of 10% oil-in-water emulsions containing 70% thyme oil and 30% corn oil in the lipid phase (0.9% Tween 80 + 0.1% LAE, 5 mM citrate buffer, pH 3.5).

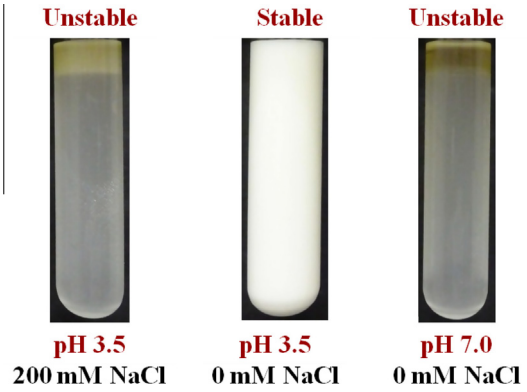

Fig. 4. Sensitivity of nanoemulsions at neutral pH or high ionic strength (200 mM NaCl). The nanoemulsions contained 7% thyme, 3% corn oil, 0.9% Tween 80, and 0.1% LAE.

3.5. Influence of oil phase composition and LAE on nanoemulsion antimicrobial activity

The antimicrobial activities of a series of thyme oil nanoemulsions that were shown to have good physical stability were determined against a model spoilage yeast strain (*Z. bailii*, ZB). Initially, nanoemulsions were filter sterilized to remove endogenous microorganisms, and then different amounts of the resulting sterile nanoemulsions were added to a broth containing ZB cells ($\sim 10^4$ CFU/ml). Growth curves were obtained by measuring the cell numbers present during storage throughout a total incubation period of 5 days at ambient temperature (Fig. 5). A nanoemulsion prepared using 100% corn oil (no thyme oil) as the oil phase and Tween 80 as the surfactant was tested as a control. This sample did not exhibit any antimicrobial effects (data not shown), which suggests that it was the thyme oil (rather than the corn oil or Tween 80) that exhibited antimicrobial activity. We also examined the antimicrobial effect of the cationic surfactant (LAE) dispersed in aqueous solutions, and found that it had a minimal inhibitory concentration (MIC) against ZB of around 25 μg/ml (μg/ml).

The efficacy of the antimicrobial nanoemulsions clearly depended on the amount of thyme oil and LAE they contained (Fig. 5). All the nanoemulsions were diluted so that they contained a specific amount of thyme oil in the final system e.g., 0, 100, 200, 400, or 800 μg/ml. In the presence of lowest levels of thyme oil (100 μg/ml), microbial growth was observed in all of the systems (data not shown). In the presence of low levels of thyme oil (200 μg/ml), the microbial growth was inhibited for some nanoemulsions. At the highest level of thyme oil (800 μg/ml), a reduction in microbial numbers was observed for all of the nanoemulsions.

In the absence of LAE, the antimicrobial efficacy increased as the concentration of thyme oil in the original nanoemulsion increased from 3% to 4% (Fig. 5a and c). In the presence of LAE, the antimicrobial effectiveness also increased as the thyme oil concentration in the original nanoemulsions increased (Fig. 5b, d, e and g). It should be noted that this effect is not because of differences in the final

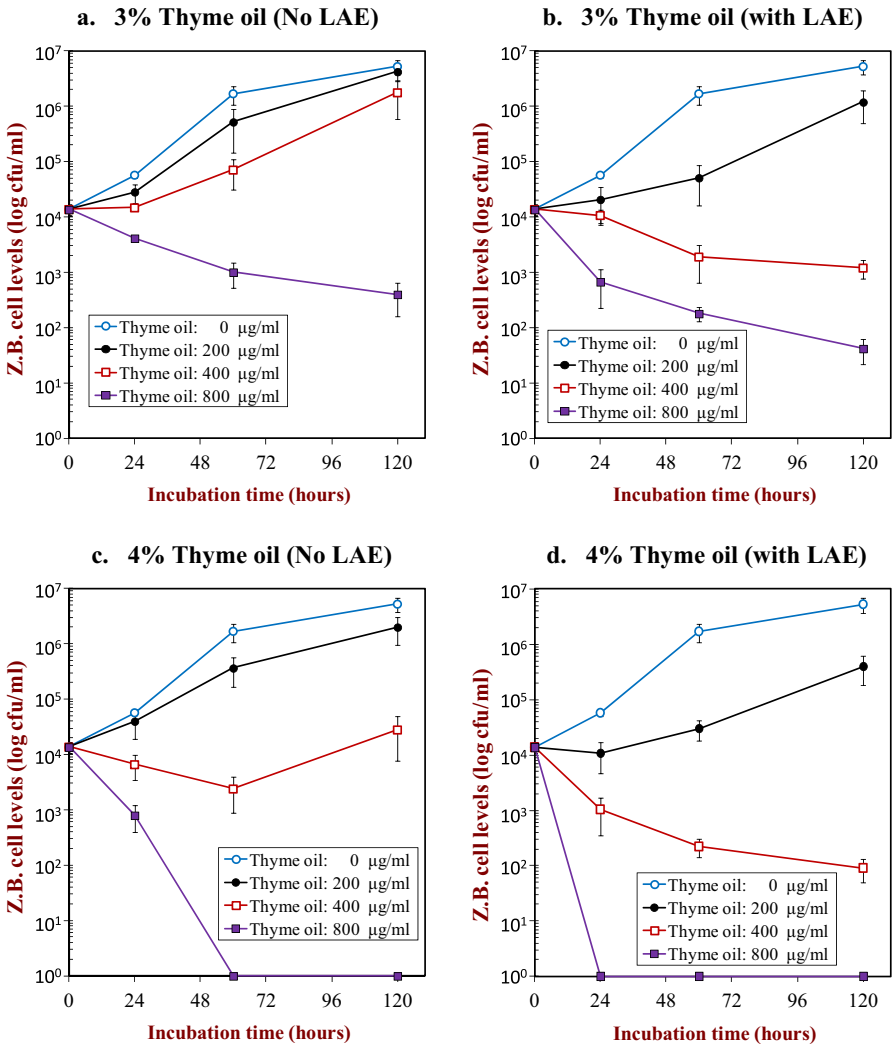

Fig. 5. Antimicrobial efficacy of nanoemulsions with different initial compositions against an acid-resistant spoilage yeast (*Z. bailii*). The original nanoemulsions contained 10% total oil (thyme oil + corn oil) and 1% total surfactant. The ratio of thyme oil to corn oil within the oil phase was varied. The amount of LAE in the surfactant phase was also varied: "No LAE" (1% Tween 80) or "with LAE" (0.1% LAE, 0.9% Tween 80). Appropriate amounts of nanoemulsions were added to MEB media so that the final concentrations of net thyme oil (not thyme oil emulsion) were 0, 200, 400 or 800 μg/ml. The media were then inoculated with *Z. bailii* and the cell levels were enumerated at intervals during five days incubation at 25 °C. The detection limit of *Z. bailii* was 10^0 CFU/ml.

thyme oil concentrations in the systems, since they were diluted by different amounts to attain the same final range of essential oil levels (0, 200, 400, or 800 μg/ml). For example, a nanoemulsion initially containing 2% thyme oil and 8% corn oil was diluted 1000 times to get to a final thyme oil concentration of 200 μg/ml (so the final corn oil concentration was 800 μg/ml). On the other hand, a nanoemulsion initially containing 4% thyme oil and 6% corn oil was only diluted 500 times to get to a final thyme oil concentration of 200 μg/ml (so the final corn oil concentration was then 300 μg/ml). The observed effects are therefore attributed to the fact that the Ripening inhibitor (corn oil) decreased the antimicrobial efficacy of the thyme oil, by an amount that was concentration dependent, i.e., increasing the level of ripening inhibitor in the lipid phase reduced the antimicrobial efficacy of the thyme oil in the nanoemulsions. Thyme oil partitions between the non-polar regions within the cell membranes of the microorganisms and the non-polar regions within the oil droplet interiors. If there is a higher amount of corn oil present within a nanoemulsion, then less of the thyme oil will partition into the microbial cell membranes, and therefore it will have less antimicrobial activity.

At the same initial thyme oil level, the antimicrobial activity was higher for the nanoemulsions containing LAE than in those containing no LAE (Fig. 5), which indicates that the cationic surfactant also contributed to their antimicrobial efficacy. The MIC of the thyme oil in the different nanoemulsions was calculated from the growth curves and is reported in Table 1. The concentration of LAE in these nanoemulsions at the MIC of the thymol oil is also reported. These results show that relatively low levels of thyme oil (200 μg/ml) and lauric arginate (2.9 μg/ml) (for the nanoemulsion whose original composition was: 7% thyme oil + 3% corn oil, 0.9% Tween 80, 0.1% LAE) are required to effectively reduce the numbers of this microorganism. The level of LAE in this nanoemulsion is well below the MIC of LAE on its own (25 μg/ml), which suggests that there is a synergistic antimicrobial effect of the essential oil and cationic surfactant.

There are a number of potential mechanisms that might account for the observed improvement in antimicrobial efficacy of the nanoemulsions upon addition of the cationic surfactant. First, LAE is a potent antimicrobial itself, and therefore it may have interacted directly with the yeast cells. Second, the positively-charged nanoemulsion droplets would be attracted to the negatively-charged yeast cells, which would increase the concentration of essential oil at the site of action (i.e., microbial cell membranes). Third, a lower amount of corn oil was needed to form

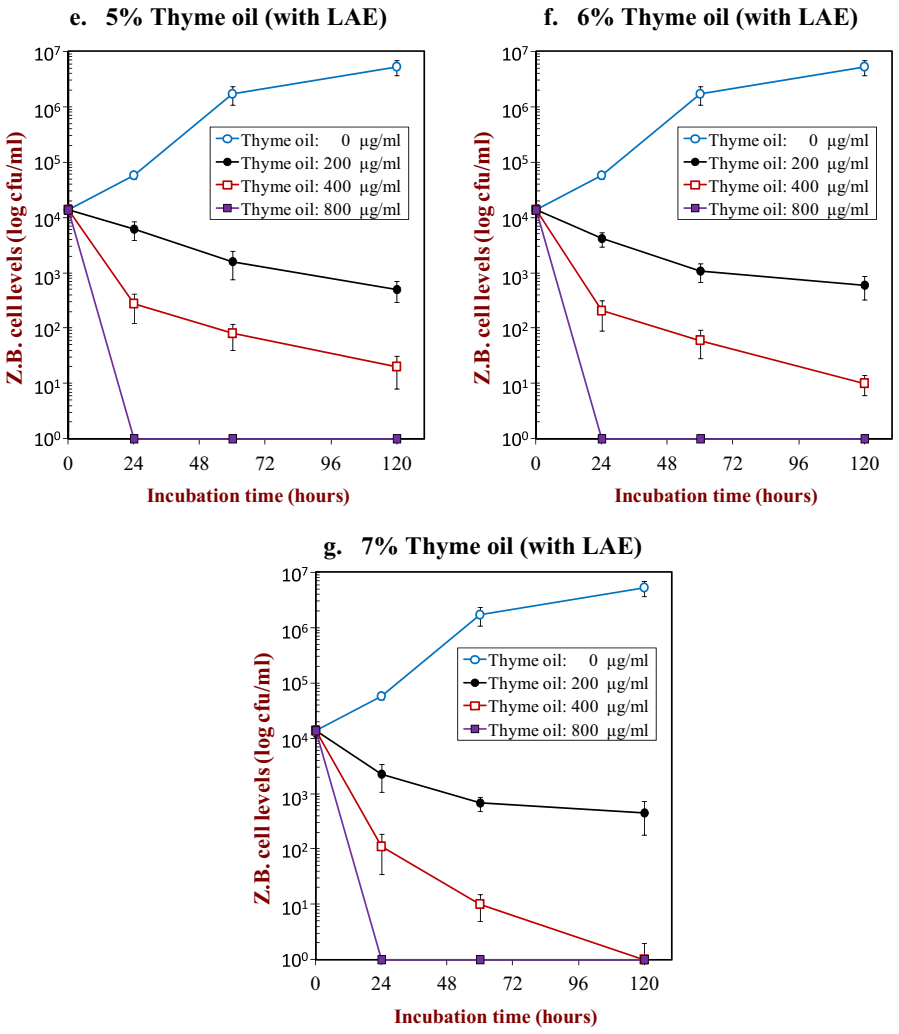

Fig. 5 (*continued*)

Table 1
Initial composition and antimicrobial efficacy of thyme oil nanoemulsions in the absence and presence of lauric arginate (LAE). The antimicrobial efficacy is expressed as the minimal inhibitory concentration (MIC) of thyme oil against *Zygosaccharomyces bailii*, a spoilage yeast.

Initial composition		No LAE	With LAE	
Thyme oil (%)	Corn oil (%)	Thyme oil MIC[a] (μg/ml)	Thyme oil MIC[a] (μg/ml)	[LAE][b] (μg/ml)
3	7	800	400	13.3
4	6	400	400	10
5	5	(Unstable)	200	4
6	4	(Unstable)	200	3.3
7	3	(Unstable)	200	2.9

[a] The MIC shown here is the minimum inhibitory concentration of thyme oil against the acid resistant yeast *Zygosaccharomyces bailii*, in the MEB media (5 mM citrate buffer, pH 3.5).
[b] The concentration of lauric arginate [LAE] reported is the final LAE level present in the MEB media at the MIC of thyme oil (not the MIC of LAE!).

stable nanoemulsions when LAE was present, and so there would have been a greater partitioning of thyme oil into the microbial cell membranes (rather than into the corn oil droplets).

4. Conclusions

Overall, our study has shown that the introduction of a cationic surfactant (LAE) into thyme oil nanoemulsions is beneficial in a number of ways. The loading capacity of the essential oil in the nanoemulsions could be significantly increased. Without LAE, at least 60 wt% corn oil was required in the oil phase to inhibit Ostwald ripening and form stable nanoemulsions, but with LAE (0.1 wt%), the amount of corn oil needed was reduced to 30 wt%. On the other hand, emulsions stabilized by LAE were highly sensitive to changes in pH and salt addition, becoming unstable at neutral pH or at high ionic strength, which was attributed to a reduction in electrostatic repulsion.

The addition of LAE was also found to substantially increase the antimicrobial efficacy of the nanoemulsions: 200 μg/ml thyme oil was needed to inhibit growth of an acid-resistant spoilage yeast (*Z. bailii*) if LAE was added, whereas $\geqslant$400 μg/ml was needed in

the absence of LAE. This effect may be attributed to: (i) cationic LAE-coated thyme oil nanoparticles were attracted to anionic microbial surfaces; (ii) LAE molecules disrupted microbial cell membranes; (iii) a reduction in the amount of corn oil needed to form stable nanoemulsions in the presence of LAE increased the amount of thyme oil available to interact with the microbial cells.

Our results indicated that utilising two different antimicrobial agents within a single nanoemulsion (LAE and thyme oil) may lead to synergistic antimicrobial activity. These results have important implications for the design and utilisation of nanoemulsions as effective antimicrobial delivery systems in the food and other industries. Nevertheless, it should be noted that any antimicrobial delivery system should not adversely affect the stability or desirable sensory attributes of the product it is incorporated into. For example, essential oils often have a strong flavour profile that is incompatible with certain types of food and beverage products.

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