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Effect of carrier oil on the properties of sodium caseinate stabilized O/W nanoemulsions containing *Trans*-cinnamaldehyde

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ABSTRACT

In this study, the impact of different contents of medium-chain triacylglycerols (MCT) and long-chain triacylglycerols (LCT) as carrier oils mixed with *trans*-cinnamaldehyde (TCA) on the properties of sodium caseinate-stabilized nanoemulsions was investigated. The obtained results showed that the use of a carrier oil improves significantly the TCA emulsion stability, which can be attributed to a reduction of TCA solubility in the aqueous phase followed by a decrease of Ostwald ripening. This stability improvement was observed at different pHs (3, 7 and 11), salt concentrations (0–300 mmol/L), and temperature (4, 25, 60, and 80 °C). However, the carrier oil type (MCT or LCT) did not show any significant effect on the nanoemulsions properties whatever the environmental conditions. Antibacterial testing revealed that the addition of carrier oil increased the antibacterial activity of TCA against *Escherichia coli* and *Listeria innocua*. These results are useful for expanding the application of TCA as food preservative in food industry.

1. Introduction

It is well established that food preservation is a great challenge and a significant concern in the food industries and the most serious concern of food spoilage remains that of foodborne pathogens. To solve these problems, synthetic preservatives and chemical antimicrobial agents are extensively applied in preventing microbial deterioration of food products and increasing their shelf lives. However, given the growing public concern associated with the use of chemical preservatives, there is an urgent need to be substituted by natural antimicrobial agents. Several natural antimicrobial agents, such as essential oils (EOs), lysozyme, bacteriocins, chitosan and phenolic compounds were applied for food preservation in different ways (Saeed et al., 2019).

Trans-Cinnamaldehyde (3-phenyl-2-propenal, TCA) is the major constituent of the essential volatile oil extracted from cinnamon, a traditional flavoring agent. TCA is an aromatic aldehyde regarded as a “Generally Regarded As Safe” (GRAS) molecule (Adams et al., 2004). In addition to food applications as a flavoring agent in chewing gums, ice creams, candies, and beverages, it has been used to provide a cinnamon flavor to medical products, cosmetics, and perfumes (Shreaz et al., 2016). Over the years, numerous studies have indicated that TCA possessed brilliant antibacterial activity with broad-spectrum against

bacteria, yeasts, and molds, such as *Salmonella* spp., *Campylobacter jejuni*, *Cronobacter sakazakii*, *Listeria innocua* (Doyle & Stephens, 2019a), *Laetiporus sulphureus*, *Clostridium difficile* (Mooyottu et al., 2014) and *Saccharomyces cerevisiae* (Mochida et al., 1988). Thus, TCA may act as a good alternative to synthetic chemical preservatives due to its potential antibacterial activity and safety. Nonetheless, TCA, in its free form, has a low water-solubility and it is difficult to introduce this EO into aqueous-based foods. Moreover, TCA is a volatile compound easily denatured at room temperature, which limits its use in food processing (Jo et al., 2015).

Recently, encapsulation techniques, such as emulsion-based encapsulation process, are generally used as delivery systems for EOs. Particularly, oil-in-water emulsion systems are particularly suitable for encapsulating these lipophilic bioactive components and the average size can play an important role in the efficiency of these systems. Indeed, nanoemulsions may have better physical and chemical stability compared to conventional emulsions for encapsulating bioactive components because of their nanometric size (20–500 nm) and high surface area (Polychniatou & Tzia, 2018). Thus, nanoemulsion-based systems can provide many advantages, such as protecting hydrophobic bioactive agents from chemical degradation, limiting their volatility, increasing their biological activity, and minimizing the impact on the organoleptic

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characteristics of food products (Donsi et al., 2011). In addition, nanoemulsions are more kinetically stable than conventional emulsions but they are more susceptible to Ostwald ripening due to their small droplet size (McClements & Rao, 2011). As a result, they tend to undergo physical changes during storage, which may lead to undesirable alterations in appearance, texture, and shelf life of food products. Chen et al. (2018) showed that nanoemulsions containing pure cinnamaldehyde encapsulated by whey proteins is not physically stable due to a significant change in their size. This phenomenon might be attributed to the high water solubility of cinnamaldehyde, which causes droplet growth through Ostwald ripening (Riquelme et al., 2019). Accordingly, it is necessary to properly design nanoemulsion-based delivery systems to ensure that they have good physical stability in different conditions. Walker et al. (2017) indicated that carrier oil can be incorporated into the oily phase of nanoemulsions to improve its physical and oxidative stability. In another study reported by Donsi et al. (2012), the authors added sunflower oil to the lipid phase to overcome Ostwald ripening of carvacrol, α -limonene and *trans*-cinnamaldehyde nanoemulsions prepared by high pressure homogenization. In the same context, McClements (2012) developed beverage emulsions by addition of poorly water soluble oils to inhibit Ostwald Ripening. Walker et al. confirmed (2017) that the key to enhance the stability of nanoemulsions depends on the physical properties of the carrier oil, such as viscosity, density, and water-solubility. For instance, it has been shown that: (i) combining poor water-solubility carrier oils with hydrophobic bioactive phase, has been shown to enhance physical stability of nanoemulsions by inhibiting Ostwald ripening (Julian McClements et al., 2012); (ii) combining low-viscosity carrier oils with hydrophobic bioactive phase can be used to reduce the size of nanoemulsion droplets during high-pressure homogenization (Qian & McClements, 2011); and (iii) combining carrier oils containing natural antioxidant or antibacterial agents with hydrophobic bioactive phase may endow different bioactive properties (Meroni & Raikos, 2018).

In order to study, the effect of the chain length of oil carrier, two different triacylglycerols were used in this work to improve the properties of TCA oil-in-water nanoemulsions: a medium chain triacylglycerol (MCT) and a long chain triacylglycerol (LCT). The used MCT is typically rich in saturated medium chain fatty acids (C_{8:0} and C_{10:0}) and the LCT is derived from the linoleic acid and oleic acid (C_{18:0}). Both carrier oils show low water solubility compared with TCA, and they can be used to prepare nanoemulsions to inhibit Ostwald ripening. In addition, the consequences of these structural differences on the stability of emulsions, and consequently on their functional properties, deserve to be evaluated. Sodium caseinate was selected as emulsifier in this study because it is one of the most widely used proteins and has considerable emulsification, gelling and nutritional properties (Choi et al., 2009). The effects of parameters such as pH, ionic strength, and temperature on the stability as well as on the antibacterial activity of the nanoemulsions were also compared. The information generated by this study could be valuable for improving the functional performance and physical stability of food emulsion systems containing essential oil.

2. Materials and methods

2.1. Materials

Sodium caseinate powder was purchased from Fisher Scientific (United Kingdom). Protein content in sodium caseinate determined by the Kjeldahl method was 93.20% (nitrogen conversion factor N = 6.38). A medium chain triacylglycerol oil (MCT) was purchased from Gattefossé (Saint-Priest, France). A long chain triacylglycerol oil (sunflower oil, LCT) was purchased from a local Market. *Trans*-cinnamaldehyde (CA, 99%) was purchased from Sigma-Aldrich (St Quentin Fallavier, France). The summary of some physicochemical properties of three different oils are shown in Table 1. Analytical grade sodium chloride, imidazole, acetic acid, sodium hydroxide, and hydrochloric

Table 1
General physical properties of TCA, MCT and LCT.

Properties	(TCA) <i>trans</i> -Cinnamaldehyde	MCT	LCT (Sunflower oil)
Density (g/cm ³)	1.05	0.945	0.920
Viscosity (mPa.s)	5.08	22.88	30.68
Interfacial tension (mN/m)	2.70	7.30	10.90
Solubility in water (mg/100 mL)	1.42	Insoluble	Insoluble

acid, were purchased from Sigma-Aldrich Chimie (St Quentin Fallavier, France). All the solutions were prepared in deionized water.

2.2. Preparation of nanoemulsions

A pre-emulsion was formulated by dispersing the lipid phase (10 g/100 g) in the continuous phase, which consisted of (1 g/100 g) sodium caseinate and (89 g/100 g) buffer solution (5 mmol/L acetic acid-imidazole, 50 mg/100 g sodium benzoate, pH 7). Firstly, the resulting suspensions were stirred at room temperature until the protein was completely dissolved. The oily phase (10 g/100 g) and the aqueous phase (90 g/100 g) were mixed using high-speed homogenization for 5 min under 13 500 rpm to form coarse emulsions. Finally, nanoemulsions were obtained by passing coarse emulsions through a microfluidizer (Microfluidics LM20, Microfluidics Corp., Newton, MA, USA) 5 times at 5×10^7 Pa. The pH of the nanoemulsion was then immediately adjusted to 7.0. Each emulsion was produced in duplicate. All samples were stored in 100 mL sealed glass bottles at room temperature in the dark for physical stability evaluation.

2.3. Effect of carrier oil concentration

The overall oil content of the nanoemulsions (TCA and carrier oil) was kept constant at 10 g/100 g. TCA was mixed with MCT or LCT in different ratios (100/0, 90/10, 70/30, 50/50, 30/70 and 0/100, m/m) under stirring (15 min, 750 rpm). In order to study colloidal stability under storage, the prepared nanoemulsions were stored for 28 days and characterized in terms of hydrodynamic size and zeta potential as a function of time ranging from 1 day to one month.

2.4. Droplet hydrodynamic size and size distribution

Droplet size and size distribution were determined by dynamic light scattering using a Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK) at fixed angle of 90°. To perform measurement, nanoemulsions were diluted to an oil droplet concentration around 0.1 wt% using imidazole acetate buffer to eliminate multiple scattering effects. After 90 s of equilibrium, each sample was analyzed three times. Then the average droplet size and polydispersity index (PDI) were obtained.

2.5. Zeta potential (ζ)

Nanoemulsions were diluted to a concentration around 0.05 wt% with imidazole acetate buffer to avoid multiple scattering effects. Diluted samples were transferred to the cell of disposable cuvettes for electrophoretic mobility measurement (Malvern Instruments, Worcestershire, UK). All measurements were performed three times and the ζ -potential values were reported as the average of three individual injections (\pm SD (standard deviation)).

2.6. Nanoemulsion stability to environmental stress

According to preliminary results, nanoemulsions containing mixed oily phase in the ratio TCA/MCT or LCT of 100/0 and 70/30 were investigated in the remainder of the study. The resultant nanoemulsions

were referred to as T100, T70M30 and T70L30 according to their oil compositions. To evaluate the effect of environmental stress on the properties of nanoemulsions, different pHs, salt concentrations, and temperatures were used to mimic scene which might be experienced during manufacture, cooking or utilization. For each dispersion, droplet size distribution and ζ -potential of samples were measured.

pH stability: Nanoemulsion samples were adjusted to pH values from 9.0 to 3.0 by adding 1.0 mol/L HCl or 1.0 mol/L NaOH and then stirred for 20 min to make sure the value of pH stable.

Salt stability: Nanoemulsion samples (pH 7.0) were mixed with different ratios of NaCl solution and distilled water while continuously stirring. The final NaCl concentration in the studied nanoemulsions were 0, 50, 100, and 300 mmol/L. Oil content and emulsifier contents after dilution were 5 g/100 g and 0.5 g/100 g, respectively.

Temperature stability: Selected nanoemulsion samples (pH 7.0) were heated in a water bath at different temperatures 60 °C and 80 °C for 1 h, and then cooled down to room temperature prior to analysis. Nanoemulsions stored in refrigerator (4 °C) and at room temperature (25 °C) were used as controls.

2.7. Viscosity

Viscosity of nanoemulsions was measured by Viscometers (LP-22, Brookfield, Canada) at 150 s⁻¹ at ambient temperature. Sample volume (0.5 mL) was kept constant and each measurement was repeated three times.

2.8. Interfacial tension

The oil-water interfacial tension was measured using an optical tensiometer equipped with an oscillating drop accessory (Tracker Teclis/IT Concept, France) at room temperature. In brief, the aqueous phase was placed in a glass cuvette, and the oil phase was injected into the aqueous phase using a syringe. The shape of the oil drop formed was recorded using a high-speed digital camera and the interfacial tension (mN/m) was calculated using the Tracker Software (Tracker Teclis/IT Concept, France). According to the density of the oil phase, the rising drop method or pendant drop method were used. Five different oil compositions (T100, T70M30, T70L30, M100 and L100) were studied and the initial volume of the oil drop was ~15 μ L. The aqueous phase contained 0.005 wt% caseinate in imidazole-acetate buffer (pH 7.0). The test was carried out at room temperature for 7200 s and each experiment was run in triplicate.

2.9. Antibacterial activity of nanoemulsions

The “zone of inhibition” assay on solid media was used for the determination of the antimicrobial effects of nanoemulsions against *Listeria innocua* (ATCC 33090) and *Escherichia coli* (ATCC 10536). Strains were stored at -20 °C in Tryptone Soy Broth (TSB) (Biokar diagnostics, Beauvais, France) with glycerol (15 mL/100 mL). One milliliter of the stock culture was transferred to 9 mL of TSB and incubated for 8 h at 37 °C. Then one milliliter of this pre-culture was transferred in 9 mL of TSB and incubated for 16 h at 37 °C. After that, pre-cultured bacteria were diluted in TSB to a final concentration of 10⁶ CFU/mL. One mL of bacterial suspension was incorporated at 5 mL/100 mL in melted Tryptone Soy Agar (TSA) and cooled in Petri dishes. A sterilized glass pipette (6 mm in diameter, Fisher Scientific) was used to make a well in the TSA Petri dishes inoculated. To maintain the same TCA concentration, 50 μ L of T70M30 and T70L30 and 35 μ L of T100 were placed in different wells. M100, L100, pure MCT and pure LCT were used as controls. The Petri dishes were then incubated for 2 h at 4 °C for making nanoemulsion diffusion and then incubated at 37 °C. Inhibition zone diameter was measured after 24 h and each experiment was repeated three times.

2.10. Statistical analysis

All assays were performed at least in triplicate. The data were presented as means \pm standard deviation of different samples. Statistical analyses were performed using one-way analysis of variance and multiple comparisons of means were done by Fishers Least Significant Difference (LSD) test. Statistical significance was defined as $p < 0.05$.

3. Results and discussion

3.1. Effect of carrier oil content on the properties of TCA nanoemulsions

The mean droplet size and polydispersity index of nanoemulsions containing different ratios of TCA/carrier oil (100/0, 90/10, 70/30, 50/50, 30/70 and 0/100) stored during 4 weeks were measured and the obtained results are reported in Fig. 1. Initially, all nanoemulsions had relatively small average hydrodynamic particle diameters ranging between 105.7–211.3 nm, and the lowest droplet size was obtained at the ratio TCA/LCT of 90/10. Except for nanoemulsions containing pure TCA (100/0), the other nanoemulsions have lower PDI value (<0.15) after one day storage. Low PDI value indicated that the droplet are narrowly size distributed. According to Stokes law, the smaller the droplet size was, the lower the sedimentation or creaming rate was (Paraskevopoulou et al., 2005). For nanoemulsions containing pure TCA, a transparent layer was detected in the top of the sample indicating the movement of the oil droplets to the bottom of the test tube during storage (data not shown). Results showed that, when the content of carrier oil was above 30% in the oil phase, caseinate-coated nanoemulsions containing the mixed oil phase can remain stable for a long time (>28 days), which suggests that they were stable to Ostwald ripening, coalescence, and flocculation. In fact, it is known that low size of droplets is more prone to Ostwald ripening during storage, i.e., diffusion of oil molecules from the smaller to the larger droplets (McClements et al., 2012, p.; Wooster et al., 2008). For this reason, it is possible for small droplets to be produced during homogenization process and then grow rapidly during storage. In our case, increased droplet size and relatively higher PDI value were present in the nanoemulsions containing oil phases with 100% and 90% TCA after two weeks. This is in agreement with results obtained for nanoemulsions containing pure thyme oil which was attributed to Ostwald ripening (Chang et al., 2012). In addition, it is worth noting that, by comparing the PDI values of nanoemulsions containing mixed oil phase of the 70/30, LCT as a carrier oil showed lower value of PDI than that of MCT, which may be attribute to the difference in properties between the two carriers. Similar results were reported by McClements (2012) who concluded that the properties of the droplets produced during homogenization depends on the viscosity and interfacial tension of the lipid phase.

Compared to nanoemulsions containing TCA, the improved stability observed after addition of a carrier oil could be attributed to a decrease in the solubility of the oily phase causing a drop in Ostwald ripening. In fact, TCA is slightly soluble in water (Seraj & Seyedarabi, 2019), while MCT and LCT have a relatively low water-solubility. Thus, the carrier oil can act as a ripening inhibitor through an entropy of mixing effect during homogenization (McClements et al., 2012). Density of oil is another factor that may affect the formation and stability of nanoemulsions. In fact, TCA has a higher density (1050 kg m⁻³) than water (997 kg m⁻³) at 25 °C, while MCT (945 kg m⁻³) and LCT (920 kg m⁻³) have a relatively lower density than water. The density of the mixed oil phase could be consequently close to that of the water phase which can reduce droplet creaming and sedimentation during long time storage. In addition, strong electrostatic repulsions between caseinate coated oil droplets is another important factor of nanoemulsion stability. On the other hand, the mean particle diameter of nanoemulsion increased with increasing carrier oil content in the organic phase, which could be probably attributed to the decreased diffusion of TCA from the oil droplets, resulting in an increase of particle diameter after

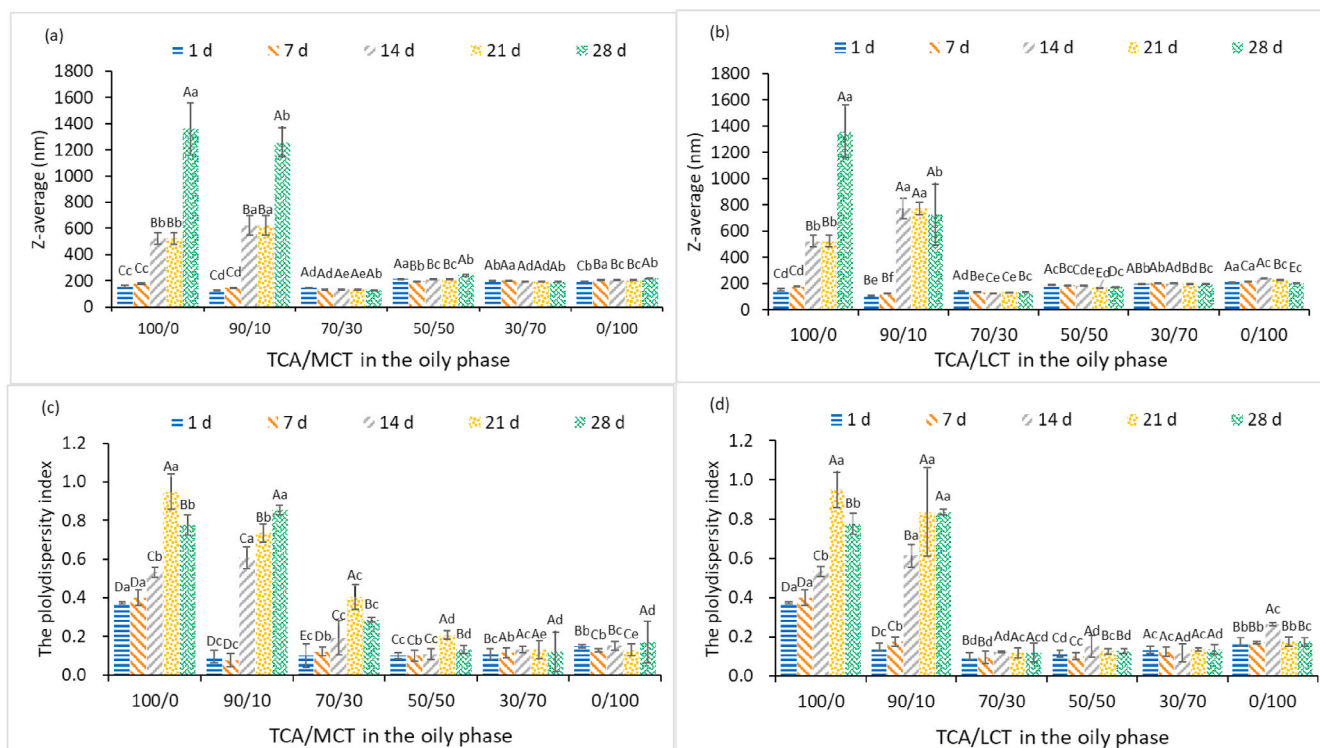


Fig. 1. Effect of MCT or LCT content on particle size (a and b) and polydispersity index (c and d) of sodium caseinate stabilized TCA nanoemulsions as a function of storage time. Samples with different uppercase superscripts (A–E) differ significantly ($p < 0.05$) when different nanoemulsions are compared at the same storage time; Samples with different lowercase superscripts (a–f) differ significantly ($p < 0.05$) when the same nanoemulsions are compared at different storage times.

homogenization.

To further explore the effect of the properties of carrier oil on the stability of nanoemulsions, the viscosity of nanoemulsions containing mixed oil phases in the ratio 100/0 and 70/30 were investigated (Fig. 2a). According to the results reported in this figure and measured at 22 °C, pure LCT showed highest viscosity (30.68 mPa s), while pure TCA showed the lowest one (5.08 mPa s) and the mixtures TCA/MCT and TCA/LCT showed intermediate viscosity values (7.56 mPa s and 9.87 mPa s, respectively). Therefore, the addition of carrier oil significantly increased the viscosity of the oily phase. As stated by Qian et al. (2011), the increased viscosity could improve stability of nanoemulsions, but their droplet size will be slight increased, which was consistent with our previous results (Fig. 1). Fig. 2b showed the interfacial tension reduction kinetics between different oily phases and sodium caseinate aqueous solution. Whatever the oily phase composition, the interfacial tension decreased over time, which can be attributed to the adsorption of caseinate molecules to the oil-water interface. Pure LCT and pure MCT showed that the equilibrium interfacial tensions were relatively high (around 10.9 mN/m and 7.3 mN/m, respectively), while pure TCA showed an equilibrium interfacial tension around 2.7 mN/m. This result can be attributed to the difference in polarity between TCA, LCT and MCT. Interestingly, the equilibrium interfacial tensions of the two mixed oil systems showed almost the same equilibrium interfacial tensions than that of pure TCA, suggesting that the interfacial tension between oil and water was mainly dependent on the presence of TCA. Similar results were found by Chen et al. (2018) as well as Felix et al. (2019). Indeed, Chen et al. (2018) found that, in the presence of TCA, the surface of the oil droplets stabilized by whey protein became crinkly, while the shape of the oil droplets without TCA did not change. This result indicates that TCA has the ability to improve the adsorption of caseinate to the oil-water interface, resulting in smaller size oil droplets after homogenization (Fig. 1).

As shown in Fig. 3, the ζ -potential of the nanoemulsions containing the two different carrier oils were measured within 4 weeks. At the first

day, all nanoemulsions had a relatively high negative surface potential (−50 to −30 mV), which can be attributed to the fact that the aqueous phase pH (7.0) during the preparing process was above the isoelectric point of the caseinate molecules (Wang et al., 2019). As described by Lieberman and Rieger (2008), when the absolute value of ζ -potential is greater than 25 mV, the electrostatic repulsions are high enough to maintain good colloidal stability of the prepared emulsion. Thus, it could be deduced that the presence of caseinate could provide high repulsive forces between the droplets to have good physical stability whatever the oil composition. However, the surface potential of oil droplets changed significantly after one week, especially for nanoemulsions containing pure TCA or a low amount of carrier oil (T90M10 and T90L10), which could indicate a change in the interface composition and/or structure.

Except for the system containing pure TCA (TCA100), the ζ -potential of the nanoemulsions became more negative with increasing carrier oil content, which means that the electrostatic repulsions between oil droplets are more intense when the carrier oil content was increased. Several different physicochemical mechanisms might account for the observed changes in surface charge of nanoemulsions. Firstly, the composition and structure of the adsorbed caseinate layer may depend on the properties of the oil phase (such as size, polarity and viscosity), which could indirectly change their surface potential (li Zhai et al., 2013). Secondly, there is a hypothesis that TCA may have chemically reacted with the adsorbed caseinate due to the presence of an aldehyde group in TCA molecules generally used as a cross-linking agent (Marin et al., 2014), and then changed their structure and electrical characteristics. Thirdly, there may be charged surface-active impurities in TCA, MCT and LCT that may affect the electrical properties of the droplets. Finally, as stated by Zhu (2017), cinnamaldehyde can rapidly be oxidized to cinnamic acid (Fig. 1. Supplementary data) during storage. Thus, the presence of cinnamic acid can affect the environmental properties, like pH, and then affect the ζ -potential of caseinate coated droplets.

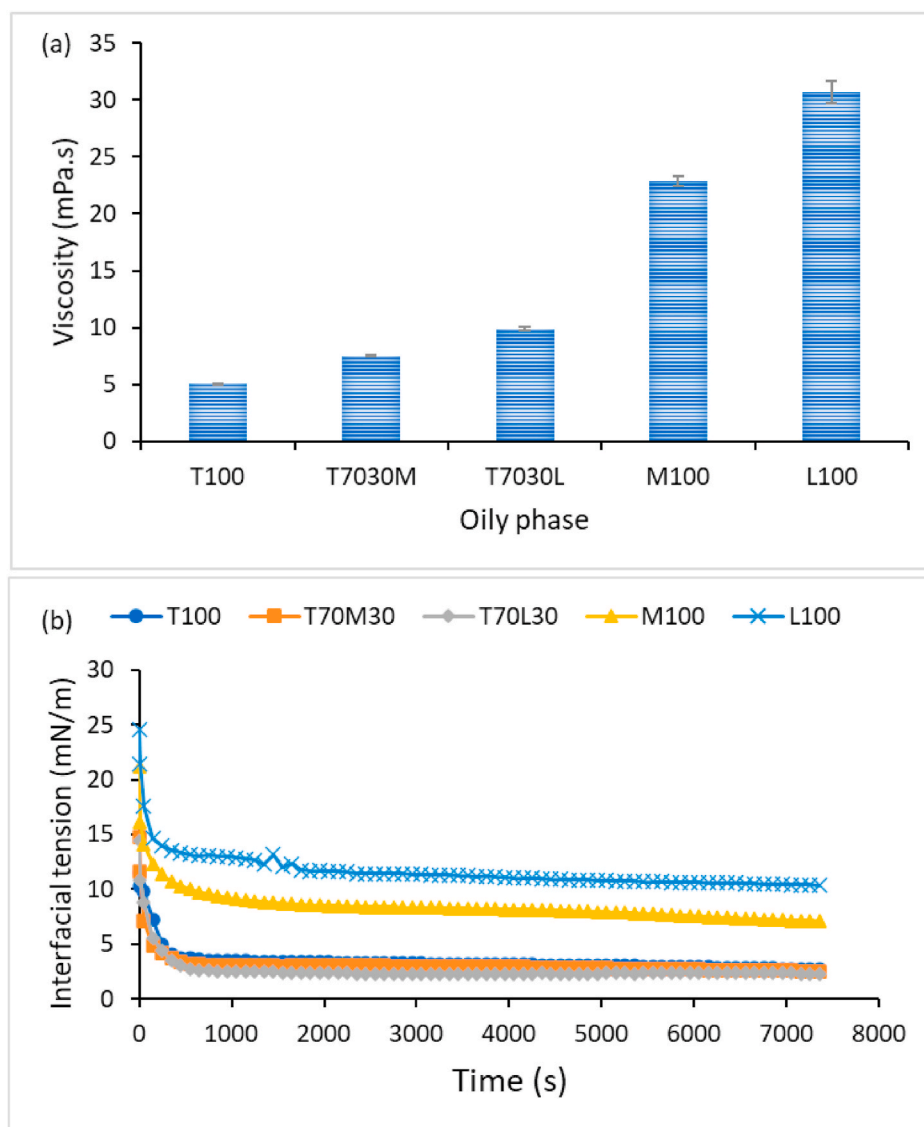


Fig. 2. Viscosity of five different oil compositions (a) and interfacial tension over time of five different oil compositions in the presence of 0.005 wt% sodium caseinate in the aqueous phase (b).

To explore this perspective, five nanoemulsions containing different oil mixtures (T100, T70M30, T70L30, TOM100 and TOL100) were selected and the pH of each sample was measured within 4 weeks (Fig. 4). The pH of nanoemulsions containing pure TCA or mixtures of TCA and carrier oils decreased with storage time, while the pH of nanoemulsions containing pure carrier oils (MCT and LCT) remained unchanged. This result can also explain the fact that the zeta potential of nanoemulsions containing high contents of carrier oil was relatively stable (Fig. 3). Interestingly, compared to pure TCA nanoemulsions, the nanoemulsions containing mixed oil phase (T70M30 and T70L30) appeared to be able to slow down the pH to the same extent after three weeks. In addition, LCT as carrier oil in nanoemulsions exhibited more ability ($p < 0.05$) to change the pH than MCT as carrier oil. This result can be explained by comparing the changes in PDI values of T70M30 and T70L30 as a function of time (Fig. 1c and d). In fact, a significant increase of PDI value was found when MCT was used as carrier oil after one week, while the PDI of T70L30 nanoemulsions remained stable. These effects may therefore depend on the physical properties of carrier oils. Similar tendency was also reported by Walker (2017) who found that the oxidation rate of fish oil was dependent on the carrier oil type.

3.2. Physical stability of nanoemulsions against environmental stress

Caseinate stabilized nanoemulsions are especially sensitive to environmental conditions which could affect their physical stability. The main three influencing parameters are pH, ionic strength, and temperature whose effects have been studied in the following sections.

pH stability: Three different pHs (3.0, 7.0 and 11.0) were used to study the effect of pH on the stability of nanoemulsions (Fig. 5). As shown in Fig. 5a, no significant difference was found in particle size of the three samples prepared at different pH values. For droplet size distribution, nanoemulsions containing mixed oil phases showed very low PDI values indicating that they were highly concentrated, while nanoemulsions containing pure TCA showed very high PDI values in each pH condition. In fact, as the pH increased from 3.0 to 11.0, the particle size of the three nanoemulsions slightly increased. This may be attributed to the electrostatic repulsion forces among amino and carboxyl groups of caseinate (Lovett et al., 2015). On the other hand, aqueous pH is related to the solubility of caseinate which will affect the particle size distribution of nanoemulsions after homogenization. As stated by Mezdour et al. (2008), the increase of the caseinate solubility allows to form thicker adsorption oil-in-water layer. The ζ -potential of the droplets in

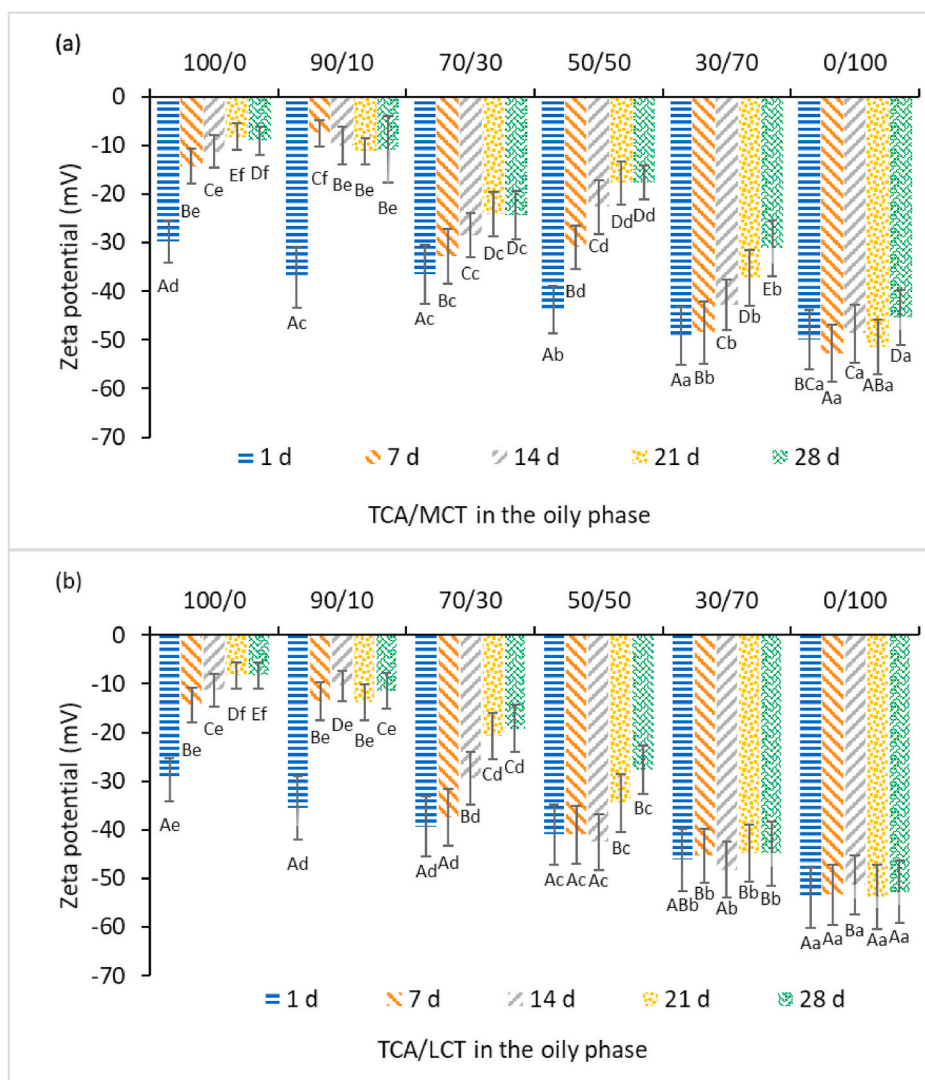


Fig. 3. Effect of MCT(a) or LCT (b) content on the ζ -potential of caseinate stabilized TCA nanoemulsions as a function of storage time. Samples with different uppercase superscripts (A–E) differ significantly ($p < 0.05$) when different nanoemulsions are compared at the same storage time; Samples with different lowercase superscripts (a–f) differ significantly ($p < 0.05$) when the same nanoemulsions are compared at different storage times.

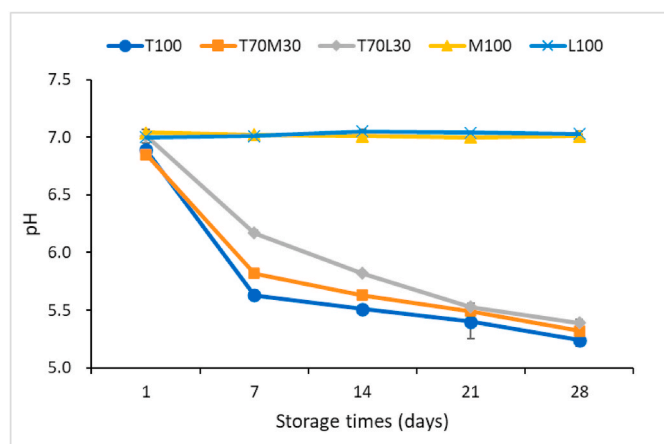


Fig. 4. pH values of caseinate stabilized nanoemulsions containing different oily phases during storage.

these emulsions was also evaluated (Fig. 5b). For all samples, the ζ -potential showed the similar trend and changed from positive to negative as the pH changed from 3 to 9, which can be attributed to the fact that the isoelectric point (I_p) of caseinate is around 4.5. The results are consistent with our previous studies that have shown that caseinate-coated oil droplets are highly unstable and aggregate in the vicinity of this pH (Wang et al., 2019). This result also showed that ζ -potential of emulsions mainly depends on the caseinate whatever the composition of the oily phase.

Ionic force: Nanoemulsions can be used as delivery systems in some food matrices containing different salt concentrations, and therefore it is important to verify the effect of ionic strength on droplet stability. Thus, the effect of different NaCl concentrations on the mean particle diameter and ζ -potential of nanoemulsions was examined (Fig. 6). All nanoemulsions showed an increase of the droplet size and PDI when NaCl concentration was increased for 0–300 mmol/L, especially for nanoemulsions containing pure TCA ($p < 0.05$, significantly). For nanoemulsions containing mixed oily phases, addition of low content of salt (<200 mmol/L) to the nanoemulsions caused little changes in the mean particle diameter after homogenization, but addition of higher salt content promoted a certain degree of droplet aggregation ($p < 0.05$). This droplet aggregation observed at higher salt concentrations is due to

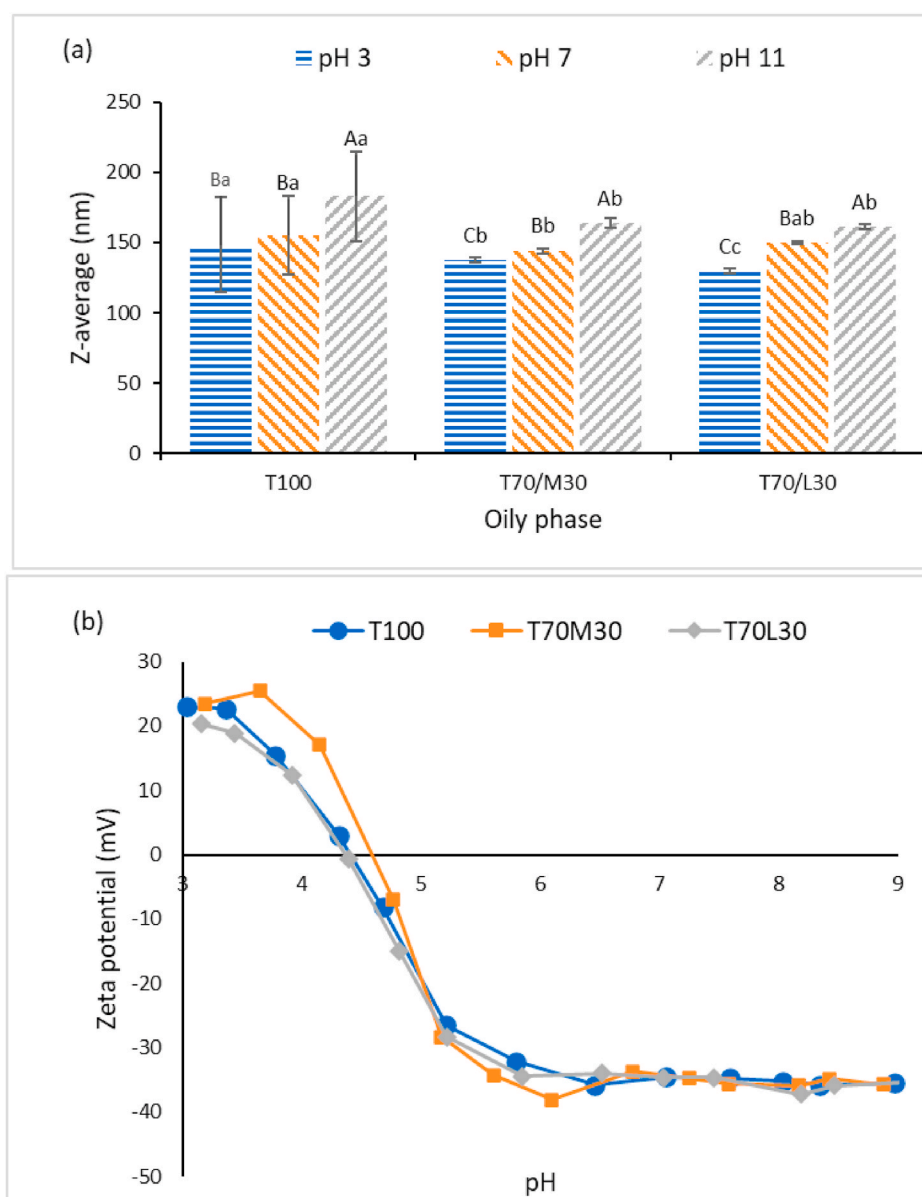


Fig. 5. Effect of pH on particle size (a) and ζ -potential (b) of caseinate-stabilized nanoemulsions containing different oily phases. The italic number above each bar refers to the PDI value. Samples with different uppercase superscripts (A–C) differ significantly ($p < 0.05$) when the same nanoemulsions are compared at different pHs; Samples with different lowercase superscripts (a–c) differ significantly ($p < 0.05$) when the different nanoemulsions are compared at the same pH.

a reduction in the electrostatic repulsion between the protein-coated droplets caused by electrostatic screening and ion binding effects.

Similar phenomenon was found upon addition of NaCl to β -lactoglobulin-stabilized emulsions (Qian et al., 2011). Moreover, our results showed that, in the presence of NaCl, the droplet size of nanoemulsions containing pure TCA turned to bimodal distribution (two peaks in one curve, data not shown), while the particle size distributions of nanoemulsions containing mixtures of TCA and carrier oil remained monomodal, though their peak shapes and locations were changed slightly. Thus, addition of carrier oil can prevent the emulsions to form bimodal distributions, which was consistent with the results obtained by Zhao et al. (2015). This phenomenon can be attributed to the fact that an increase in particle size leads to an increase in the rate of creaming of droplets in nanoemulsions (Paraskevopoulou et al., 2005). Fig. 6b showed that the ζ -potential of nanoemulsions was significantly affected by ionic strength. All samples showed a decrease trend when the NaCl concentration was increased suggesting an accumulation of counter-ions around oil droplets surfaces screening caseinate charged groups

preventing repulsions between oil droplets (Öztürk, 2017).

Temperature stability: In many commercial applications, it seems important that emulsions can withstand high temperatures during their production, storage or use. For this reason, the effect of heating treatments (60 °C and 80 °C) on the stability of nanoemulsions was examined, and 4 °C as well as 25 °C were used as control (Fig. 7). With the exception of pure TCA-containing nanoemulsions treated at 80 °C, all other nanoemulsions appeared to be relatively stable to heat treatment and showed a unimodal particle size distribution (Fig. 7a). This could be attributed to the good thermal stability of caseinate-coated droplets owing to their relatively high negative charge at pH 7, which led to stronger electrostatic repulsions between them. Meanwhile, caseinate stabilized emulsions generally have better stability during heating, because of the protein structure which does not affected by the heat-induced conformation changes as for globular proteins (Srinivasan et al., 2002). For nanoemulsions containing pure TCA, a slight increase in size was observed at 60 °C, but when the heating temperature was increased to 80 °C, the size increased significantly ($p < 0.05$). After

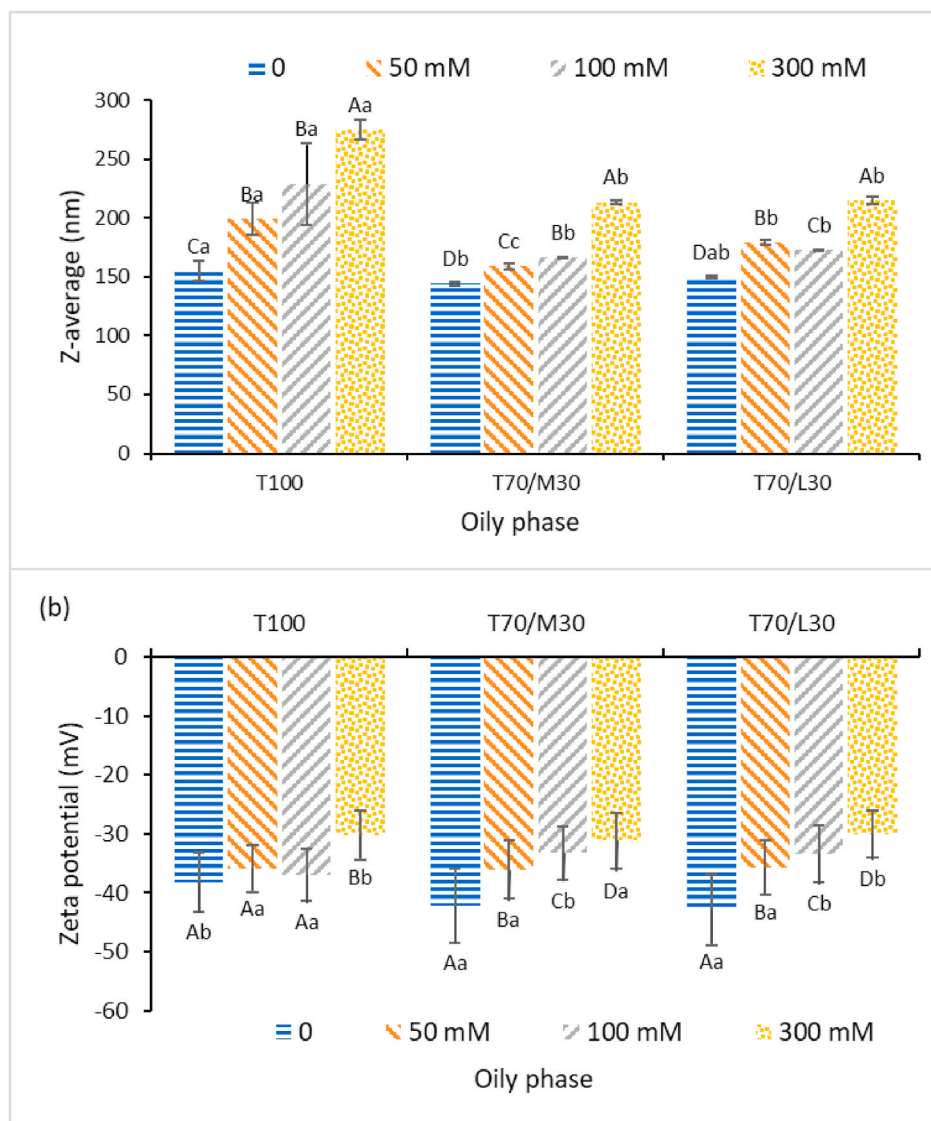


Fig. 6. The effect of salt concentration on particle size (a) and ζ -potential (b) of caseinate-stabilized nanoemulsions containing different oily phases. The italic number above each bar refers to the PDI value. Samples with different uppercase superscripts (A–D) differ significantly ($p < 0.05$) when the same nanoemulsions are compared at different salt concentrations; Samples with different lowercase superscripts (a–c) differ significantly ($p < 0.05$) when the different nanoemulsions are compared at the same salt concentration.

heating, the ζ -potential of nanoemulsions were also measured. According to Fig. 7b, there was no significant change in the ζ -potential for all nanoemulsions upon thermal treatments, suggesting that heating did not strongly influence the overall composition or structure of the caseinate coatings. The extreme condition may affect the physical stability of the nanoemulsion, however, MCT oil and LCT oil as carrier oils into the oil phase showed better tolerance than pure TCA.

3.3. Antibacterial activity of nanoemulsions

Nanoemulsions were studied for their antibacterial activity against a Gram-positive (*Listeria innocua*) and a Gram-negative (*Escherichia coli*) bacteria. Based on the same TCA content, three nanoemulsions (T100, T70M30 and T70L30) were added to each well of the bacterial-containing medium, and the same formulation without TCA as a control. Inhibition zones of nanoemulsions on medium containing bacteria after 24 h is shown in Fig. 8a. The three nanoemulsions showed clear inhibition zones against the two bacteria, whereas the control without TCA showed no inhibition zones. The size of the inhibition zones was measured by the software Image J (National Institutes of Health, USA) and the obtained values are shown in Fig. 8b. The antimicrobial activity of TCA is attributed to its aldehyde functional group and the antibacterial mechanism of TCA is probably due to the increase in permeability

of bacteria cell wall (Doyle & Stephens, 2019b). As stated by Weiss et al. (2009, pp. 425–479), encapsulation of TCA at the nanoscale could increase the physical stability of bioactive compounds and increases their bioactivity through activation of cell absorption mechanism. In addition, there was no significant disparity between antimicrobial activity of nanoemulsions containing MCT and LCT as carrier oils, but these two nanoemulsions exhibited larger inhibition zones than nanoemulsions containing pure TCA ($p < 0.05$). This phenomenon can be attributed to the fact that the addition of a carrier oil increases emulsion stability during storage, which can further increase its biological activity. Another reason may be considered to explain this result, is that nanoemulsions containing pure TCA may lose a small amount of TCA, because TCA is volatile and subject to oxidation. Meanwhile, the size of inhibition zones can be affected by several parameters, such as the amount of nanoemulsion, the spread area of nanoemulsions, the mechanism of TCA release, the number of bacteria in the medium, and the time of measuring inhibition zone. As far as possible, we have tried in this work to keep all these parameters constant and to vary only the composition of the emulsion in order to detect the effect of the carrier oil.

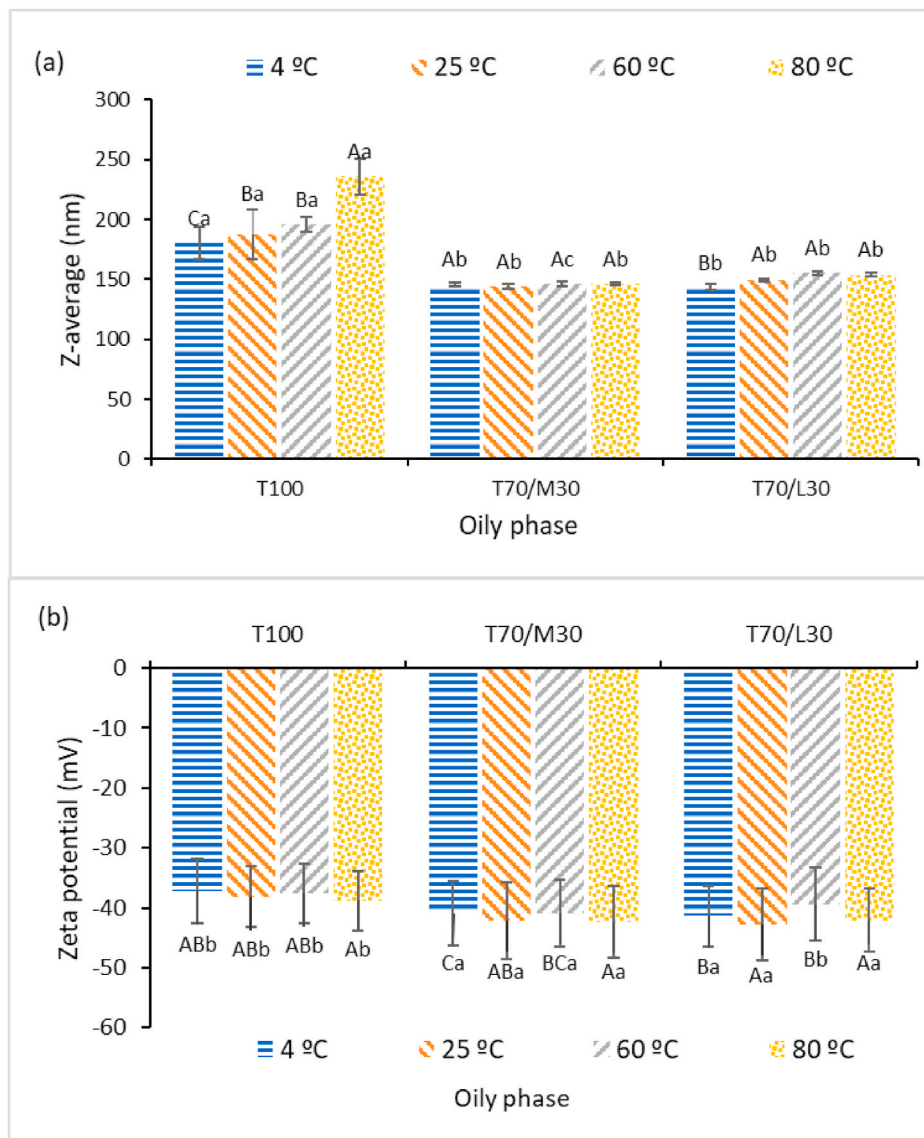


Fig. 7. The effect of temperature on particle size (a) and ζ -potential (b) of caseinate-stabilized nanoemulsions containing different oily phases. The italic number above each bar refers to the PDI value. Samples with different uppercase superscripts (A–D) differ significantly ($p < 0.05$) when the same nanoemulsions are compared at different temperatures; Samples with different lowercase superscripts (a–c) differ significantly ($p < 0.05$) when the different nanoemulsions are compared at the same temperature.

4. Conclusions

This study compared the ability of two different carrier oils to improve the properties of emulsions containing *trans*-cinnamaldehyde and stabilized by sodium caseinate. The influence of the oil type and content on the physical stability of nanoemulsions was investigated under different pHs, salt concentrations and temperature as well as their antibacterial activity against two common gram-positive and gram-negative bacteria. The analyses revealed that stable caseinate-coated nanoemulsions are difficult to formulate using pure TCA. However, nanoemulsions containing TCA mixed with carrier oil could keep stable for a relatively long period, especially when LCT is used as carrier oil. This stability improvement can be attributed to a reduction of TCA diffusion in the aqueous phase and an inhibition of Ostwald ripening. Antibacterial effect revealed that, compared to nanoemulsions containing pure TCA, the activity of nanoemulsions containing mixtures of TCA and carrier oils exhibited better antibacterial activity against *Escherichia coli* and *Listeria innocua*. The development of these nanoemulsions is in line with the growing interest of the food industry and consumers in using products based on natural substances and the incorporation of carrier oils into nanoemulsion-based systems containing TCA is a promising technique that can extend the shelf life of

perishable foods.

CRediT authorship contribution statement

Wei Liao: Conceptualization, Formal analysis, Writing – original draft, conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version. **Adem Gharsallaoui:** Conceptualization, Formal analysis, Writing – original draft, conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version. **Emilie Dumas:** Conceptualization, Formal analysis, Writing – original draft, conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version. **Sami Ghnimi:** Conceptualization, Formal analysis, Writing – original draft, and conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version. **Abdelhamid Elaissari:** Conceptualization, Formal analysis, Writing – original draft, conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual

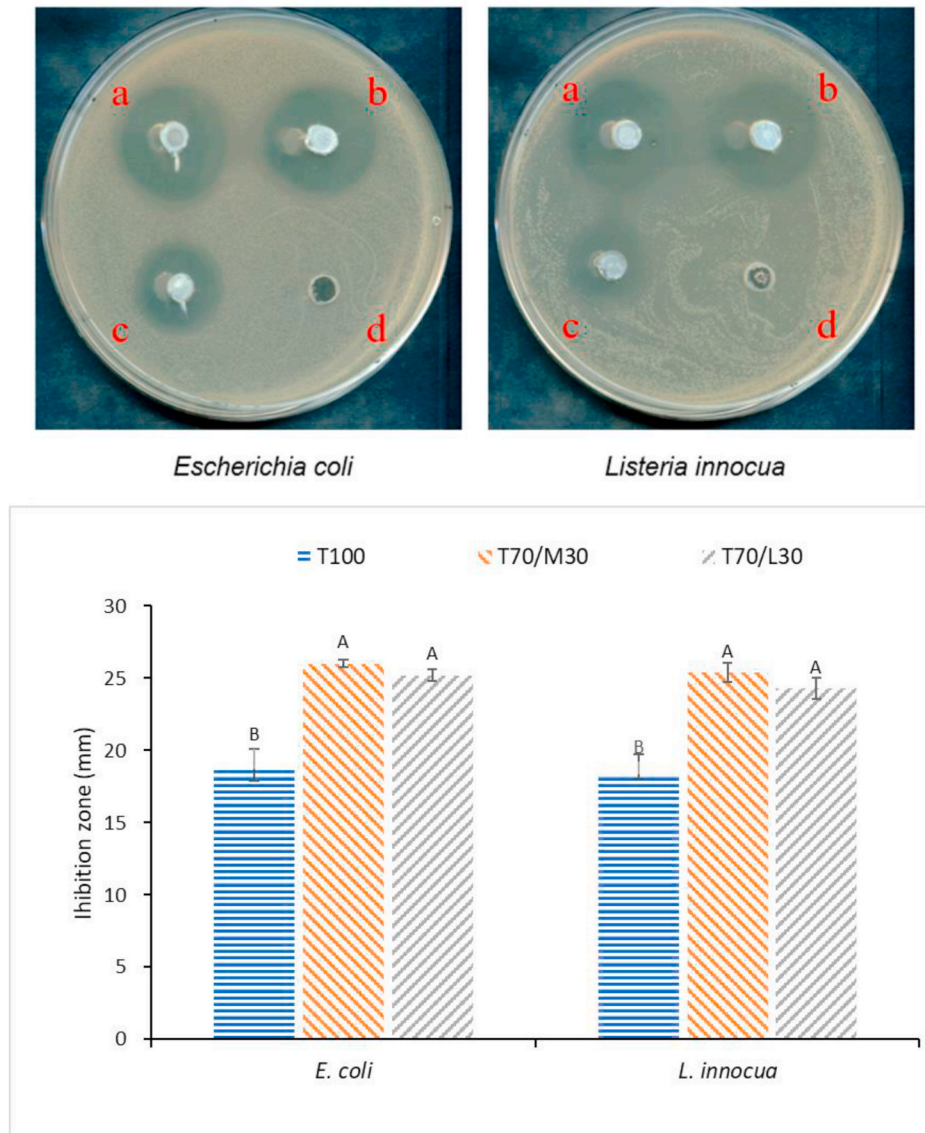


Fig. 8. Top: Example of inhibition zones on TSA containing *Escherichia coli* and *Listeria innocua* with four nanoemulsions: T100 (a), T70M30 (b), T70L30 (c) and M100 (d). Bottom: Measured diameter of inhibition zone of three nanoemulsions against *Escherichia coli* and *Listeria innocua*: T100, T70M30 and T70L30. Samples with different uppercase superscripts (A–B) differ significantly ($p < 0.05$).

content; and (c) approval of the final version.

Declaration of competing interest

The authors declare no conflicts of interest that pertain to this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2021.111655>.

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