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Impact of microfluidization or ultrasound processing on the antimicrobial activity against *Escherichia coli* of lemongrass oil-loaded nanoemulsions

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ABSTRACT

The effect of the ultrasonication and microfluidization processing conditions on the antimicrobial activity of lemongrass oil–alginate nanoemulsions was studied. Sonication led to less effective nanoemulsions against *Escherichia coli*, being the loss of antimicrobial potential dependent on the amplitude and treatment time applied. Namely, nanoemulsions sonicated at 100 μm for 180 s almost completely lost their bactericidal action, leading to 0.3 log-reductions of *E. coli* population after 30 min of contact time. On the contrary, nanoemulsions processed by microfluidization exhibited an enhanced antimicrobial activity, which was proportional to the number of cycles through the microfluidization chamber. In fact, whereas the coarse emulsion reduced the *E. coli* population up to 0.66, 2.25 and 5.85 log-units after 5, 15 and 30 min of contact time, the microfluidized nanoemulsions (10 cycles, 150 MPa) achieved 1.37, 5.29 and 7.07 log-reductions. Therefore, nanoemulsions with an improved functionally could be obtained by microfluidization, whereas ultrasounds seem to have a deleterious impact on the antimicrobial activity of lemongrass essential oil.

1. Introduction

Plant essential oils (EOs) have been widely used for many years due to their antimicrobial properties in foods and pharmaceutical products (Burt, 2004). In this sense, their well established bactericidal activity has made them object of numerous research studies dealing with food preservation (Holley & Patel, 2005; Tajkarimi, Ibrahim, & Cliver, 2010), as there is a strong consumer demand for safe and high-quality foods being free from synthetic additives. Antimicrobial action of EOs relies on their hydrophobic nature, which enables them to partition in the lipids of bacterial cell membrane and mitochondria, causing damages to these structures and rendering them more permeable (Bakkali, Averbeck, Averbeck, & Idaomar, 2008, Burt, 2004; Hammer, Carson, & Riley, 1999; Sikkema, De Bont, & Poolman, 1994). Leakage of ions followed by efflux of cytoplasmatic constituents cause the viability loss of microbial cells (Lambert, Skandamis, Coote, & Nychas, 2001; Walsh et al., 2003). However other mechanisms may be involved in microbial inactivation by EOs since they could interfere with membrane function, altering its electron transport, nutrient uptake, proteins, nucleic acid synthesis and enzyme activity (Bajpai, Baek, & Kang, 2011; Tiwari et al., 2009). Thus, the incorporation of EOs to food products is a potential alternative to chemical antimicrobial compounds regarding food preservation. However, their incorporation to foods still poses several drawbacks. On one hand, the type of essential oil to be incorporated to a food system should be accurately selected taking into account the organoleptical attributes of the final product (Tiwari et al., 2009). Moreover, the EO concentration needs to be reduced in order to minimize possible toxicological effects or consumer rejection (Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2011). On the other hand, due to their lipophilic nature, the formulation of foods containing EOs presents technological limitations considering their low solubility in aqueous media. Among EOs, lemongrass (*Cymbopogon citratus*) essential oil has been found to be effective against several foodborne pathogens when it was incorporated in minimally processed fruit (Raybaudi-Massilia, Mosqueda-Melgar, & Martín-Belloso, 2008; Raybaudi-Massilia, Rojas-Graü, Mosqueda-Melgar, & Martín-Belloso, 2008; Rojas-Graü et al., 2007), fruit juices (Duan & Zhao, 2009; Raybaudi-Massilia, Mosqueda-Melgar, & Martín-Belloso, 2006), minced meat (Barbosa et al., 2009), chocolate (Kotzekidou, Giannakidis, & Boulamatsis, 2008) or fish

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products (Mejlholm & Dalgaard, 2002). Therefore, it is a strong candidate to be used as natural antimicrobial in high perishable foods.

Nowadays, there is a growing interest in the use of antimicrobial delivery systems for food preservation. In this way, nanoemulsions are being regarded as an interesting approach to control the release of active ingredients in food formulations. Nano-sized emulsions with an increased active surface area may not only enhance the functionality of EOs (Flanagan & Singh, 2006; Imran et al., 2010), but also improve emulsion appearance and stability (Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005). However, the information about the feasibility to obtain nanoemulsions containing EOs is yet limited. In this sense, ultrasound and microfluidization treatments, as high-energy emulsification methods, have yielded emulsions with nano-sized droplets typically ranging between 30 and 600 nm (McClements & Rao, 2011). However, there is a lack of scientific evidence about the release of active compounds from nano-sized emulsions. In addition, the impact of particle size reduction treatments on the functionality of lipophilic compounds dispersed in oil-in-water nanoemulsions has not been previously studied. Thus, the purpose of the present work was to assess the influence of the lipid particle disruption method on the activity against *Escherichia coli* of lemongrass oil–alginate nanoemulsions.

2. Material and methods

2.1. Primary emulsion formation

Sodium alginate (1% w/v) (FMC Biopolymers, UK) was dissolved in hot water at 70 °C and continuous stirring until it was completely dissolved. A coarse or primary emulsion was made by mixing the sodium alginate solution as aqueous phase and lemongrass essential oil (1% v/v) (Laboratoris Dicana, Spain) as lipidic phase plus Tween 80 (1% v/v) (Scharlau, Spain) as surfactant, with a laboratory T25 digital Ultra-Turrax mixer (IKA, Staufen, Germany) working at 3400 rpm for 2 min. All samples were prepared using ultra pure water obtained from a Mili-Q filtration system.

2.2. Nanoemulsion formation

2.2.1. Ultrasonication

A UP400S Hielscher sonifier (Hielscher Ultrasound Technology, Teltow, Germany), of 400 W nominal power and a frequency of 24 kHz equipped with a 22-mm sonotrode, was used to perform the ultrasonic treatment. The coarse emulsion was pumped into a stainless steel ultrasonic flow cell by means of a peristaltic pump (model Selecta- PR 2003) set at 100 mL/min giving a residence time of 16.5 s. The ultrasonic flow cell was equipped with a water-cooled jacket to avoid excessive heating of nanoemulsions. Several treatments were applied to produce nanoemulsions, varying the ultrasound amplitude (30, 60 and 100 μm) and treatment time (0, 30, 60, 120, 180 s). The maximum temperature of the sample registered at the outlet of the system was 47 °C.

2.2.2. Microfluidization

A microfluidizer (M110P, Microfluidics, Massachusetts, USA) was used to treat the coarse emulsion to obtain nanoemulsions. This device pumped the emulsion towards an interaction chamber where the product was accelerated at high velocity, and the high-shear forces created inside reduced the droplet size of the emulsion. Afterwards, the product passed immediately through a coiling coil immersed in a water bath with ice, keeping the outlet temperature below 20 °C. The coarse emulsion was passed through the system several times (1, 2, 3, 4, 5 and 10) at different pressures (50, 100 and 150 MPa).

Table 1
Droplet diameter (nm) and interfacial ζ-potential (mV) of lemongrass oil–alginate emulsions or nanoemulsions produced by sonication at different processing amplitude (μm) and sonication times (s).

Amplitude (μm)	Sonication time (s)	Droplet diameter (nm)	ζ-Potential (mV)
0	0	1410 ± 365	−17.6 ± 2.9
30	30	34.9 ± 8.5	−38.0 ± 10.4
	60	15.3 ± 2.9	−37.7 ± 3.2
	120	6.9 ± 1.3	−42.0 ± 3.6
	180	5.1 ± 0.2	−55.8 ± 6.4
60	30	18.6 ± 5.1	−30.9 ± 3.4
	60	8.8 ± 1.5	−36.4 ± 2.7
	120	6.1 ± 1.5	−46.0 ± 5.6
	180	4.3 ± 0.1	−47.1 ± 8.9
100	30	10.6 ± 1.8	−32.8 ± 0.1
	60	6.0 ± 0.1	−44.7 ± 0.1
	120	5.6 ± 0.9	−41.5 ± 3.2
	180	4.3 ± 0.2	−46.0 ± 3.7

Values are expressed as mean ± standard deviation.

2.3. Droplet size characterization

The emulsion and nanoemulsion droplet diameter was measured by dynamic light scattering (DLS) with a Zetasizer NanoZS laser diffractometer (Malvern Instruments Ltd, Worcestershire, UK). The ζ-potential of oil droplets, to determine the interfacial electrical charge of lipid nanodroplets, was measured by phase-analysis light scattering (PALS) with a Zetasizer NanoZS laser diffractometer (Malvern Instruments Ltd, Worcestershire, UK). The physicochemical properties of nanoemulsions produced by ultrasounds or by microfluidization were published previously in two different journal papers (Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2012; Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2013). Nevertheless, the effect of ultrasound or microfluidization processing parameters on the average droplet diameter and ζ-potential nanoemulsions are shown in Tables 1 and 2, respectively.

2.4. Antimicrobial activity assay

The antimicrobial activity of lemongrass oil–alginate nanoemulsions was assessed by evaluating the *in vitro* inhibition of *E. coli*. The method used was a modification of that previously described by

Table 2
Droplet diameter (nm) and interfacial ζ-potential (mV) of lemongrass oil–alginate emulsion or nanoemulsions produced by microfluidization at different processing pressure (MPa) and number of cycles.

Pressure (MPa)	Cycles	Droplet diameter (nm)	ζ-Potential (mV)
0	0	1410 ± 365	−17.6 ± 2.9
50	1	53.1 ± 5.9	−45.8 ± 4.6
	2	37.6 ± 3.1	−44.4 ± 4.7
	3	35.1 ± 6.2	−50.2 ± 5.1
	4	23.0 ± 2.4	−46.9 ± 6.7
	5	16.0 ± 0.4	−51.9 ± 5.3
	10	10.8 ± 2.3	−49.2 ± 9.1
100	1	45.8 ± 7.5	−42.3 ± 3.1
	2	18.5 ± 2.6	−40.5 ± 7.7
	3	13.6 ± 2.3	−43.0 ± 4.3
	4	7.4 ± 0.1	−49.8 ± 5.9
	5	11.1 ± 2.3	−47.4 ± 4.7
	10	6.1 ± 1.0	−49.0 ± 3.3
150	1	23.5 ± 2.5	−42.3 ± 4.9
	2	14.1 ± 2.4	−44.3 ± 4.5
	3	7.3 ± 1.7	−36.6 ± 5.1
	4	7.8 ± 2.0	−43.7 ± 4.5
	5	7.1 ± 0.6	−38.6 ± 7.7
	10	5.8 ± 0.1	−41.1 ± 3.9

Values are expressed as mean ± standard deviation.