Food Research International 101 (2017) 114–128

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Do oil-in-water (O/W) nano-emulsions have an effect on survival and growth of bacteria?

Hani El Kadri[a], Putu Virgina Partha Devanthi[a], Tim W. Overton[a,b], Konstantinos Gkatzionis[a,b,*]

[a] School of Chemical Engineering, University of Birmingham, Birmingham B152TT, United Kingdom
[b] Institute of Microbiology & Infection, University of Birmingham, B152TT Birmingham, United Kingdom

ARTICLE INFO

Keywords:
Nano-emulsion
Antimicrobial activity
Flow cytometry
Bacterial membrane integrity
Caprylic acid
Emulsion stability

ABSTRACT

Nano-emulsions (typically droplet diameter < 1 μm) are common in foods, and have been extensively reported to present antimicrobial activity, however, the mechanism is not well defined, and some studies reported no effect. A review of the literature was conducted and revealed strongly contradictory reports regarding the antimicrobial effect of nano-emulsions even in reference to similar microbial species and formulations. Following up, this study aimed to investigate the effect of nano-emulsions on four bacterial species (*Staphylococcus epidermidis*, *Bacillus cereus*, *Lactobacillus acidophilus* and five *Escherichia coli* strains) possessing different surface charge and hydrophobicity. Model oil-in-water (O/W) emulsions with different size of oil droplets were prepared with sunflower oil stabilised by polysorbate 80 (Tween80) emulsifier (hydrophilic), using high shear mixing followed by ultrasonication. The viability of bacteria was monitored by culture, membrane integrity was assessed with flow cytometric analysis with propidium iodide (PI) staining and fluorescence microscopy monitored the spatial distribution of cells within the O/W emulsions. The stability of the nano-O/W emulsions in the presence of bacteria was assessed by monitoring the droplet size [D (4, 3)] and creaming height. In contrast to other reports the survival and growth of bacteria was not affected by the size of the oil droplets, no damage to the bacterial membrane was evident with flow cytometry and emulsion stability was not affected by the presence of bacteria during 7 days of storage. Furthermore, the antimicrobial activity of caprylic acid (CA) was compared between O/W coarse and nano-emulsions while varying the concentration of the hydrophilic surfactant Tween80. The activity of CA was similar in nano-emulsion and coarse emulsion; however, it was higher than in bulk oil and was reduced with increasing Tween80 concentration, suggesting that its efficacy is dictated by formulation rather than oil droplet size. The results demonstrated no enhanced antimicrobial activity due to nano-sized oil droplets and that conclusions on nano-emulsions should be taken with caution.

1. Introduction

Nano-emulsions (typically with droplet diameter < 1 μm) gained popularity in food production due to improving food properties and formulations, for example, use of less fat and emulsifiers, increased emulsion stability and improved optical appearance, enhancement of taste and sensory perception of ingredients or masking of certain ingredients (Chaudhry & Castle, 2011). Nano-emulsion manufacturing requires more energy than emulsions with smaller droplet sizes (Gupta, Eral, Hatton, & Doyle, 2016) and they possess different physicochemical properties to coarse emulsions (McClements, 2010) due to their nano-sized droplets (Baglioni & Cherazzi, 2013) and increased interface. Nano-emulsions have shown antimicrobial activity against a variety of Gram-positive and Gram-negative bacteria including *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus megaterium*, *Bacillus subtilis* and *Bacillus circulans* (Baker, Wright, Hayes, Hamouda, & Brisker, 2000; Bhargava, Conti, da Rocha, & Zhang, 2015; Hamouda et al., 1999; Jo et al., 2015; Lu et al., 2017; Majeed et al., 2016; Teixeira et al., 2010). Furthermore, nano-emulsions were found to selectively disrupt the membrane of prokaryotic cells but not eukaryotic cells (Baker et al., 2000), which could expand their applications in managing safety and microbial growth in food through formulation. The antimicrobial effect of nano-emulsions has been attributed to their structure itself and the nano-sized droplets. When nano-emulsions are formed under high shearing forces (e.g. ultrasonication, high-pressure homogenisation or high-shear mixing) they acquire significant amount of energy as they are formed (Lee, Karthikeyan, Rawls, & Amaechi, 2010). The nano-droplets are thermodynamically driven to fuse with lipid-containing micro-organisms and

* Corresponding author at: School of Chemical Engineering, University of Birmingham, Birmingham B152TT, United Kingdom.
E-mail address: k.gkatzionis@bham.ac.uk (K. Gkatzionis).

http://dx.doi.org/10.1016/j.foodres.2017.08.064
Received 28 June 2017; Received in revised form 21 August 2017; Accepted 28 August 2017
Available online 06 September 2017

Table 1
The antimicrobial activity of nano-emulsions correlated with their mean droplet size, ingredients (oil phase, stabilisers, antimicrobials) and the micro-organism it is tested against. The impact of the nano-emulsion is classified as positive (+) when the nano-sized droplets improves the antimicrobial activity with respect to the control, as negative (−) when it decreases the antimicrobial activity, and as neutral (±) when no significant change is observed or a significant change was observed only due to incorporation of antimicrobials and not because of nano-size droplets. The method of emulsification is mentioned as: HPH—High Pressure Homogenisation, HSH—High Shear Homogenisation, US—Ultrasonication, MFZ—Microfluidizer, CPI—Catastrophic Phase Inversion.

Emulsion type	Oil phase	Stabilisers	Continuous phase	Antimicrobials	Mean droplet size (nm)	Method of emulsification	Impact of nano-emulsion	Micro-organism	Controls	Author
O/W nano-emulsion	Euganol 5–12.5% (w/w)	Tween20	Water	None	50–110 nm	US	+	*Fusarium oxysporum f.* sp. *vasinfectum*	Untreated sample	Abd-Elsalam and Khokhlov (2015)
O/W emulsion	Oregano oil 0.05 or 0.1% (w/w)	Tween80	Water	None	148 nm	US	+	*L. monocytogenes* *S. Typhimurium* *E. coli* O157:H7	Water	Bhargava et al. (2015)
O/W nano-emulsion	Grindsted Acetem 90–50 K 10–15% (w/w)	Tween60	Water	Cinnamaldehyde 3–10% (w/w)	79 ± 2 nm	HPH	−	*L. monocytogenes* *E. coli* O157:H7	Nano-emulsion without cinnamaldehyde	Bilbao-Sainz et al. (2013)
O/W nano-emulsion	**LMO** Lemon myrtle 5% (w/w) **SBO** Soybean oil 16% (w/w) **BCTP** Soybean oil 16% (w/w)	**LMO** Tween 80 **SBO** Tween80 **BCTP** Triton X-100, tributyl-*n*-phosphate	Water	None	97 ± 2 nm	MFZ	±	*E. coli* *L. monocytogenes* *S. Typhimurium* *P. aeruginosa* *B. cereus*	Coarse emulsion	Buranasuksombat et al. (2011)
O/W nano-emulsion	Thyme and corn oil 10% (w/w)	Tween 80 or Tween and LAE	Water	None	< 200 nm	HPH	+	*Z. bailii*	Thyme or corn oil nano-emulsion with no LAE	Chang, McLandsborough, and McClements (2015)
O/W nano-emulsion	Thyme oil and corn oil or MCT medium chain triglyceride (MCT) (from 0 to 100% (w/w)) (10% (w/w))	Tween 80	Aqueous buffer solution (5 mM citrate buffer, pH 3.5)	None	160–196 nm	HPH	+	*Zygosaccharomyces bailii*	Nano-emulsion with corn or MCT but no thyme oil	Chang et al. (2012)
O/W nano-emulsion	D-Limonene 5% (w/w) or a mixture of terpenes 5% (w/w)	Soy lecithin Solec Ip, Tween 20 and glycerol monooleate and CLEARGUM CO 01	Water	D-Limonene and a mixture of terpenes extracted from *Melaleuca alternifolia* (0.1–10% (w/w)	74.4–156.6 nm	HPH	+	*E. coli* *L. delbrueckii* *S. cerevisiae*	Sunflower oil with D-limonene (50:50) 10% (w/w) or palm oil with a mixture of terpenes (50:50) 10% (w/w)	Donsì et al. (2011)
O/W nano-emulsion	Sunflower oil 8% (w/w)	Lecithin, pea proteins, sugar ester, and a combination of Tween20 and glycerol monooleate	Water	Carvacrol, D-limonene and cinnamaldehyde 2% (w/w)	170–240 nm	HPH	+	*E. coli* *L. delbrueckii* *S. cerevisiae*	Water	Donsì et al. (2012)
O/W nano-emulsion	**BCTP** Soybean oil 16% (v/v) **BCTP-CPC** Soybean oil 16% (v/v) **TEOP** Ethyl oleate 3% (v/v)	**BCTP** Tri-*n*-butyl phosphate, and Triton X-100 **BCTP-CPC** Tri-*n*-butyl phosphate, and Triton X-100 **TEOP** *n*-Pentanol and Tween80	Water	**BCTP** Water **BCTP-CPC** CPC 0.25% (w/v) **TEOP** None	N.A.	US	±	*S. aureus* *E. coli* *L. monocytogenes*	Water, CPC solution 0.25% (w/v), tributyl phosphate solution, bulk soybean oil, Triton X-100, Tween80 or *n*-pentanol solution	Ferreira et al. (2010)
O/W nano-	Basil oil 6% (v/v)	Tween80	Water	None	29.3 nm	US	+	*E. coli*	*PBS*	Ghosh et al. (2013)

(continued on next page)