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Antimicrobial influence of nanoemulsified lemon essential oil and pure lemon essential oil on food-borne pathogens and fish spoilage bacteria

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ABSTRACT

The antimicrobial activities of lemon oil based nanoemulsion and two different concentrations of lemon essential oil (100% and 10%) on food-brne pathogens (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Salmonella* Paratyphi A) and fish spoilage bacteria (*Photobacterium damselae*, *Enterococcus faecalis*, *Vibrio vulnificus*, *Proteus mirabilis*, *Serratia liquefaciens*, and *Pseudomonas luteola*) were compared in terms of disc diffusion, minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). The constitutes of extracted lemon essential oil were identified by using GC–MS. Viscosity, the mean droplet size, thermodynamic stability and refractive index of nanoemulsions were determined. The main components detected in the lemon essential oil were D-limonene, p-cymene, β-pinene with percentages of 52.85%, 14.36%, and 13.69%, respectively. It was found that lemon nanoemulsion was more effective on food-borne pathogens except *K. pneumoniae* than 100% lemon essential oil. 10% lemon essential oil showed the highest inhibition effect on *S.* Paratyphi A. The conversion of the essential oil into nanoemulsion improved antimicrobial activity. According to value of MIC, both nanoemulsion and 100% essential oil inhibited bacterial growth of all of the pathogen bacteria tested whereas they were less effective on inhibition of fish spoilage bacteria. However, 10% essential oil was more effective on spoilage bacteria than pathogens. MBC showed that nanoemulsion and 100% lemon essential oil presented a noticeable bactericidal activity against *S.* paratyphi A whereas 10% lemon essential oil was found as ≥ 25 mg/mL against pathogens and spoilage bacteria. Therefore, the use of nanoemulsion based on lemon essential oil can have potential as a natural antimicrobial agent against food-borne pathogen and spoilage bacteria for fish processing industry.

1. Introduction

Nanotechnology is a newly emerging technique which has gained attention since it has a role in the development of functional foods and nano-sized food ingredients and additives, innovative food packaging and delivery systems for bioactive compounds in food and pharmaceutical industry (Handford et al., 2015; Huang et al., 2015). Especially, lipophilic active components such as bioactive lipids, flavours, antimicrobials can be encapsulated within colloidal-based delivery systems (e.g. nanoemulsions, emulsions), being suitable for oral consumption for health care (McClements et al., 2009).

Nanoemulsions can be described as oil-in-water (o/w) or water- in-oil (w/o) emulsions with mean droplet diameters ranging from 20 to 200 nm and also has optical transparency, high physical stability and bioavailability (Rao and McClements, 2012; Solans et al., 2005). Recently, consumer awareness in relation to the potential health risk of synthetic food additives and artificial antimicrobial preservatives in food industry has increased the interest in natural alternatives such as essential oil (Hamedo and Abdelmigid, 2009; Settanni et al., 2012).

Essential oils (EO) contain natural antimicrobial and antioxidant properties. Composition of EO includes a mixture of terpenoids, terpens, and other aromatic and aliphatic components (Bakkali et al., 2008). However, their compositions can be variable due to chemical and biological origin, and also different processing methods such as isolation, purification. The essential oil of lemon has previously been reported to possess antibacterial, antioxidant and fungicidal properties and is considered as generally recognized as safe (GRAS) by FDA 2018). Thus, it is applied in food industries as a preservative or flavoring agent (Sharma and Tripathi, 2008). The major flavour compound is citral, limonene, myrcene, octanal, and gamma-terpinene (Schieberle and Grosch, 1988). However, there are drawbacks for the use of essential oils as food preservatives, including the low water solubility, strong

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flavour, low stability (Prakash and Kiran, 2016). Nanotechnology has potential to overcome these issues, and also protecting food from some factors such as temperature, pH, water activity, thus enhanced antimicrobial activity.

Although fish is an important animal protein source for the human diet, they are often associated with human disease. The presence of pathogenic bacteria in fish can results from their contaminated aquatic environment, diet, cultural practices, processing, and distribution of products. In addition, fish is one of the perishable food products and fish spoilage occurs mainly as a result of the microbial activity, causing undesirable or unacceptable product for consumption. Therefore, microbiological control of spoilage bacteria determines the quality and shelf life of fresh fish (Gram and Huss, 1996). How to control the types and quantities of microorganism is an essential problem for fish processing industry. Recently, researchers have focused on the use of natural antimicrobial additives such as essential oil or its nanoemulsion in fish and fish (Ozogul et al., 2017; Yazgan et al., 2017).

Some studies have demonstrated the antimicrobial activity of both pure essential oils and its nanoemulsification. Alfonzo et al. (2017) investigated the bio preservative effects of lemon essential oil microemulsions on salted sardines. The antagonistic activity of the essential oils which were extracted with hydro distillation method from pumelo, grapefruit, orange, kumquat, mandarin, and lemon was assessed against foodborne pathogen bacteria (43 strain of *Listeria monocytogenes*, 35 strains of *S. aureus* and 14 strains of *Salmonella enterica* (Settanni et al., 2012). Rao and McClements (2011) investigated conditions where stable microemulsions, nanoemulsions or emulsions could be fabricated using sucrose monopalmitate (SMP) as a surfactant and lemon oil as oil phase. They also studied the effect of lemon oil fold (1×, 3×, 5× and 10×) on the formation and properties of oil-in-water microemulsions and nanoemulsions (Rao and McClements, 2012). Ozogul et al. (2015) evaluated the antimicrobial activity of twelve essential oils (lemon, pine oil, eucalyptus, thyme, sage tea, lavender, orange, laurel, lemon, myrtle, rosemary and juniper) against nine food-borne pathogen bacteria by using disc diffusion method. The antimicrobial properties of various essential oils and their effects on wide range of microorganisms have already been studied (Al-Reza et al., 2010; Chikhoune et al., 2013; Govaris et al., 2010). However, antimicrobial effects of their conversion to nanoemulsion have not been studied extensively. Therefore, in this current study, antimicrobial activity of lemon oil based nanoemulsion on food-borne pathogens and fish spoilage bacteria was compared with pure lemon oil.

2. Materials and methods

2.1. Isolation and identification of bacterial strains

Spoilage bacteria, which were *Photobacterium damselae*, *Enterococcus faecalis*, *Vibrio vulnificus*, *Proteus mirabilis*, *Serratia liquefaciens*, and *Pseudomonas luteola*, were isolated from spoiled fish, including mackerel (*Scomber scombrus*), sardine (*Sardinella aurita*), anchovy (*Engraulis encrasicolus*) and rainbow trout (*Oncorhynchus mykiss*). They were obtained from Mediterranean Sea whereas rainbow trout was purchased from a local fish farm. The sample (10 g) was weighed from spoiled fish muscles into filtered stomacher bags (Fisher Scientific, Nepean, ON, Canada) in the aseptic condition and 90 ml of sterile saline was added, after which mixed well using a Stomacher (IUL, Barcelona, Spain) for 3 min. Further decimal dilution was made and then 0.1 mL of each dilution likely to be within counting range was pipetted onto the surface of plate count agar (PCA) in triplicate and spread over the surface. Plates were incubated for 2 days at 37 °C. Each of the individually selected bacterial colonies was streaked several times on the agar plate using a sterile loop to obtain pure colonies. Isolates were identified as described of the manufacturer's instructions for the API 20E and API 20NE strip system (BioMereux, France). The inoculated strip was incubated for 16–24 h at 37 °C and the colour reaction were noted ad either positive or negative. The result obtained was analyzed using the APILAB PLUS software (BioMereux). Polymerase chain reaction (PCR) targeted 16S rRNA gene region with 8F and 519R primers was used for detection and identification of *P. damselae*, *E. faecalis*, *V. vulnificus*, *P. mirabilis*, *S. liquefaciens*, and *P. luteola* isolated from spoiled fish (Lane, 1991; Turner et al., 1999). The nucleotide sequences of PCR generated fragments were confirmed with an Applied Biosystems DNA sequencer (Applied Biosystems, model 3130xl, Foster, USA). DNA sequencing reactions were conducted using the DNA sequencing kit (ABI BigDye®) supplied by Applied Biosystems (Foster, USA). Results were evaluated by BLAST search (www.ncbi.nlm.nih.gov/BLAST).

The four food-borne pathogen bacteria were *Staphylococcus aureus* (ATCC29213), *Klebsiella pneumoniae* (ATCC700603) and *Enterococcus faecalis* ATCC29212, which were purchased from the American Type Culture Collection (Rockville, MD, USA), and also used *Salmonella* Paratyphi A (NCTC13) which were obtained from the National Collection of Type Cultures (London, UK). Nutrient broth (Merck 1.05443.0500, Darmstadt, Germany) was used for propagation of all bacterial cultures.

2.2. Essential oil

Lemon (*Citrus limonum*) plant was purchased from a national company (BIOMESI Bioagrotechnology R&D) in Adana, Turkey. Essential oils of lemon were removed by hydrodistillation using an industrial type of Clavenger device during period of 4 h. The Clevenger apparatus composed of a 1000-mL round–bottomed flask (Isolab, Wertheim Germany), a volatile oil determination tube and a reflux condenser (Norm Cam, Ankara, Turkey). All parts are connected via ground glass joints. The essential oil was kept in refrigerator at +4 °C until analysis of chemical composition.

2.3. Determination of volatile constitutes of lemon essential oil

Identification of the constitutes was carried out according to method of Ligor et al. (2013) with minor modification, using a Perkin Elmer Clarus 500 capillary gas chromatography (Waltham, MA, USA) directly coupled to the mass spectrometer system (Perkin Elmer Clarus). An SGE non-polar fused silica capillary column (60 m × 0.25 mm, ID; BPX5 0.25 um, Perkin Elmer, Shelton, CT, USA) was carried out by using of the following conditions: oven temperature held at 60 °C for 10 min after programmed to 250 °C min/1 and the final temperature kept for 10 min. Injector temperature was adjusted to 220 °C; helium was used as carrier gas, and flow rate was 1.5 mL/min. The volume of injected sample was 1 μL of diluted oil in hexane; a splitless injection technique was used; ionization energy was 70 eV in the electronic ionization (EI) mode; ion source temperature was 200 °C; the scan mass range was *m/z* 35–425 and the interface line temperature was 250 °C. The component of lemons was identified and calculated with regard to the retention time of a series of alkanes (C4-C28) used as the reference and the similarity of their mass spectra with those gathered in the NIST-MS and WILEY-MS libraries, or reported in the literature were used.

2.4. Preparation of nanoemulsions

Oil-in-water nanoemulsion was prepared using an oil phase constitutes 11% of the total nanoemulsion and water phase constitutes 89% of the total nanoemulsion according to (Moghimi et al., 2016) method with minor modification. Nanoemulsion was prepared from a mixture of lemon oil (10% w/w), Tween 80 (1% w/w) and water (89% w/w). Essential oil and Tween 80 were GRAS (generally recognized as safe). The mixture was homogenized by using an ultrasonic homogenizer (Optic Ivymen System CY-500, Barcelona, Spain) for 15 min at 72 amplitudes. The power of the ultrasonic homogenizer was 500 and frequency of the emitted ultrasound was 20 KHZ. The size of the sonotrode was 5.6 mm theta and 60 mm height. During this process, the

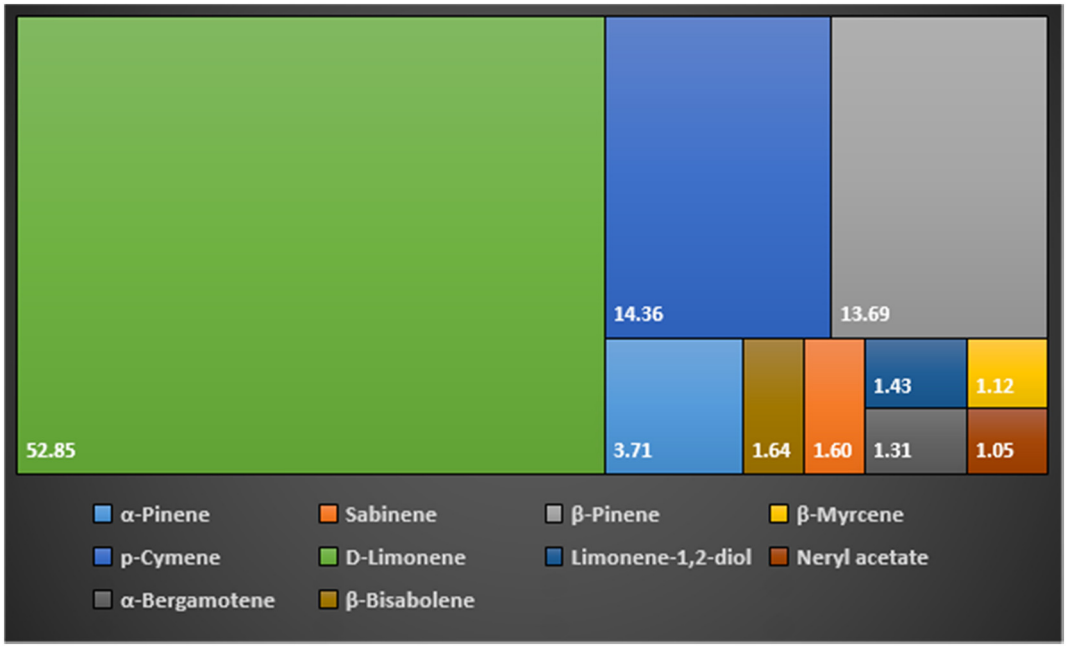

Fig. 1. Major volatile component of lemon essential oil identified by GC/MS analysis.
*Minor compounds of < 1% in the essential oil were not shown.

temperature of emulsion was controlled with the using of ice around the beaker.

2.5. Physical properties of nanoemulsion

The average particle size of droplets in the emulsion was measured using Mastersizer 2000 (Malvern, UK) based on laser diffraction. Viscosity of nanoemulsion was measured by a rheometer (TA Instruments ARES Rheometer). Thermodynamic stability of nanoemulsions was determined according to (Shafiq et al., 2007). The polydispersity index (PDI) of nanoemulsion was determined with using Dynamic light scattering (DLS) technique employing a Zetasizer Nano-ZS (Malvern instruments, Worcestershire, UK) according to the method of Hosseinnia et al. (2017). All measurement was carried out at 25 °C. Refractive index was measured using an Abbetype refractometer (Schimidt þ Haensch ATR W2, Berlin, Germany). Stability of nanoemulsion was observed for two weeks.

2.6. Screening of antimicrobial activity

Comparison of antimicrobial effects of oil-in-water nanoemulsion based on lemon essential oil and two different concentrations of lemon essential oil (100% and 10%) on six fish spoilage and four food-borne pathogen bacteria was carried out using paper disc diffusion method (Murray, 1995) with minor modifications. Antibiotics (tetracycline (30 µg), streptomycin (10 µg) and neomycin (5 µg) were used on fish spoilage and food-borne bacteria as positive control. Tween 80 was also used as negative control. Nutrient agar was carried out as the standard test medium for bacteria. Fifty microliters of nanoemulsions (10% lemon essential oil, 1% surfactant and 89% water) and lemon essential oil (100% and 10%) were pipetted on sterile filter paper disc (diameter 6 mm), which were permitted to dry in an open sterile petri dish in a biological cabinet vertical laminar flow. Bacteria at the concentration of 10^8 CFU/mL was spread onto the surface of agar media in petri dishes. Afterwards, four-paper disc with nanoemulsion, essential oil (100% and 10%), antibiotics and tween 80 were set on the inoculated agar surface separately. The diameter of an inhibition zone around the disc was measured after incubation of the bacterial plates at 37 ± 1 °C for 18–24 h. The values were recorded by average (mm) of four-disc diameter measurements.

2.7. Minimum inhibition (MIC) and bactericidal concentration (MBC)

Antimicrobial effects of nanoemulsion based on lemon essential oil and different concentrations of lemon essential oil (100% and 10%) on six fish spoilage and four food-borne pathogen bacteria were also determined using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) according to Clinical and Laboratory Standards Institute's methods (2008).

One milliliter of nanoemulsion or 100% and 10% essential oil (with stock solution of 50 mg/mL) was added to the first tube in each series and subsequently two fold serially diluted with Mueller Hinton Broth (MHB). The inoculum suspension (1 mL) of each bacterial strain (10^6 cfu/mL) was then added in each tube containing nanoemulsion or essential oil and MHB. The final concentrations of the extract were 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.19 mg/mL. Each tube was evaluated for bacterial growth and compared to the control. As a positive control, a tube containing MHB and bacterial suspension without emulsions was used. The tube containing nanoemulsion or essential oil or tween 80 with bacterial suspension was also used as negative control. The tubes were incubated at 35 °C for 18–24 h. The tube which shows no visible growth with the naked eye was recorded as MIC. MBC was determined by subculturing the contents of tubes of MIC into Mueller Hinton Agar (MHA) which show no growth.

2.8. Statistical analysis

To find the average value and standard deviation, the data obtained from the three samples for each treatment was used. The significance of differences ($P < 0.05$) was determined using Duncan's multiple comparison test with SPSS version 19.0 for Windows (SPSS Inc., Chicago, IL. USA).

3. Result and discussion

3.1. Volatile constitutes of lemon essential oil

Determination of qualitative and quantitative components of

essential oils are most important because of their antimicrobial potential. Variation in chemical composition of EOs would affect their biological activity. The constitutes of extracted lemon essential oil by GC/MS are shown in Fig. 1. The main components detected in the lemon essential oil were D-limonene, p-cymene, β-pinene with percentages of 52.85%, 14.36%, and 13.69%, respectively, whereas other compounds in low concentration follow as 3.73% α-pinene, 1.64% β bisabolene, 1.60% sabinene, 1.43% limonen-1.2-diol, 1.31% α-bergamotene, 1.12% β-mycene, 1.05% neryl acetate. Moosavy et al. (2017) reported that main compound ds of lemon peel essential oil were D-limonene (46.93%), γ-terpinene (16.89%), tri-cyclen (6.67%), 1-beta-pinene (4.69), and 2-beta-pinene (3.86%).

The high levels of D-limonene and β-pinene components were found in lemon essential oil in the current study. Similar results were obtained in previous study reported by Franceschi et al. (2004) who reported that lemon essential oil mainly include limonene (58,6%) and β-pinene (11.2%). Ozogul et al. (2015) showed that the main contents in the lemon essential oil were limonene (71.77%) and β-pinene (6.11%). Limonene (37.63–69.71%), beta-pinene (0.63–31.49%), gamma-terpinene (0.04–9.96%) and p-cymene (0.23–9.84%) were also reported as the main constitutes in lemon oil by Bourgou et al. (2012).

3.2. Physical properties of nanoemulsion

Mean droplet diameter (*Z*-average) and polydispersity index (PDI) of nanoemulsion based on lemon essential oil are given in Fig. 2. PDI value can be described as the particle size distribution of the droplets. A small PDI value represents a narrow particle size distribution (Liang et al., 2012). PDI value was found as 0.114 in this study. However, Walker et al. (2017) found much smaller value (< 0.22) for the PDI of the nanoemulsion containing lemon oil. The PDI values of the nanoemulsions prepared in four different formulations were reported as 0.18 for the first formulation (2%D-limonene in 8% sunflower oil and 3% soy lecithin), 0.14 for the second formulation (2%D-limonene in 8% sunflower oil and 3% pea proteins), 0.07 for the third formulation (2%D-limonene in 8% sunflower oil and 1% sugar ester), 0.09 for the fourth formulation (2%D-limonene in 8% sunflower oil and 0.5% glycerol monooleate 0.5% tween 20) (Donsi et al., 2012).

Mean droplet diameter of lemon essential oil based nanoemulsion was 181.5 nm. The mean droplet size of nanoemulsions prepared with carvacrol and bergamot, mandarin and lemon essential oils using tween 20 were reported in the range of 133.4–176.4 nm (Severino et al., 2015). Walker et al. (2017) reported that droplet diameter of lemon oil based nanoemulsion produced with Tween 80 and a high-pressure homogenizer method was 91 nm. Zhang et al. (2014) reported that droplet size of nanoemulsions which contain D-lemon oil, various concentrations of nisin (0%, 0.5%, 1.5%, 3%) and tween 80 (6%w/w) were 16.34 nm, 16.34 nm, 18.92 nm and 18.92 nm respectively. In another research by Donsi et al. (2012) who reported the droplet size for the first formulation (2% D-limonene in 8% sunflower oil as organic phase and 3% soy lecithin as emulsifier) as 239 nm, 184 for the second formulation (2% D-limonene in 8% sunflower oil as organic phase and 3% pea proteins as emulsifier), 169 for the third formulation (2% D-limonene in 8% sunflower oil as organic phase and 1% sugar esters as emulsifier), 228 for the fourth formulation (2% D-limonene in 8% sunflower oil as organic phase and 0.5% glycerol monooleate 0.5% tween 20 as emulsifier).

Table 1
Physicochemical properties of nanoemulsion based on lemon essential oil.

Temperature (°C)	25
Viscosity (cP)	0.88
Surface tension	32.59
Thermodynamic stability	+ +

+ + explains good thermodynamic stability. Nano emulsification, + phase separation within 1 week, + + stable up to 2 weeks.

Viscosity is an important parameter for physicochemical characterization of nanoemulsion. Determination of viscosities confirms whether the system is O/W or W/O emulsion. Low viscosity of systems shows that it is O/W type whereas high viscosity indicates that it is water in oil type system (Baboota et al., 2007). Viscosity of nanoemulsion was found as 0.88 cP (Table 1). However, viscosity of lemon essential oil was determined as 5.3 (cP) in the research by Rao and McClements (2012).

Lemon nanoemulsion was stored at ambient temperature and stability of the nanoemulsion was observed for up to 15 days. Stability of nanoemulsion was determined by centrifuging samples at 2000 x g for 30 min at 4 °C and 45 °C every two days. After that, testing tubes were left at room temperature (23-24 °C). No layer of oil was separated out even after two weeks. Nanoemulsion exhibited good stability during this period (Table 1).

3.3. Antimicrobial activity of pathogen bacteria

Essential oils by means of their phenolic compounds, interact with the lipids of the cell membrane, thus improving the membrane permeability, disturbing structures of cells and causing homeostasis,

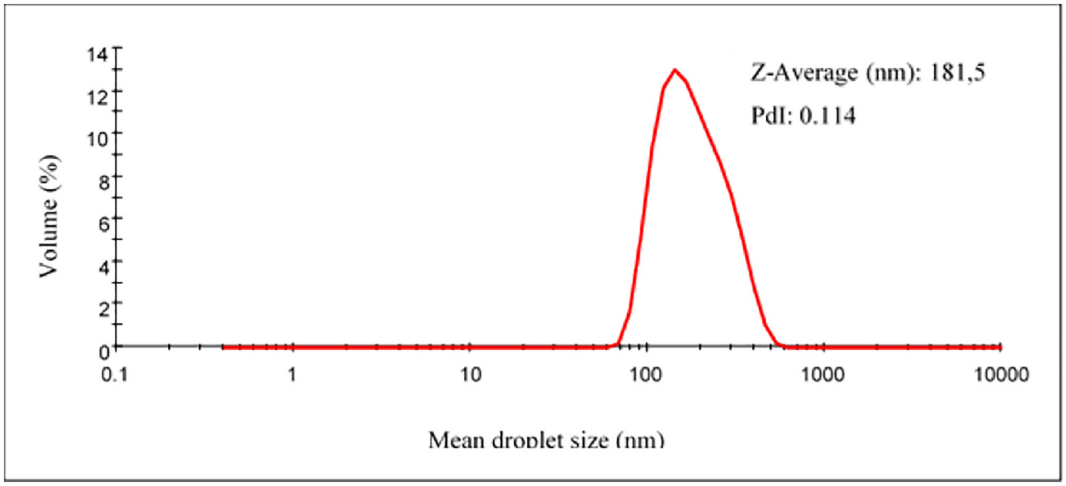

Fig. 2. The particle size distribution of nanoemulsion based on lemon essential oil.
PDI: polydispersity index.

Table 2
Antimicrobial activity of lemon essential oil and its nanoemulsion against food borne pathogen bacteria.

Food borne pathogens	Inhibition zone (mm)						
	Lemon essential oil (100%)	Lemon essential oil (10%)	Lemon nanoemulsion	Tween 80	Tetracycline	Streptomycin	Neomycin
Staphylococcus aureus	11.63 ± 0.48^{C}	16.25 ± 1.50^{b}	16.63 ± 0.48^{b}	0.00 ± 0.00^{d}	20.38 ± 0.63^{a}	15.13 ± 0.63^{c}	19.75 ± 0.50^{a}
Enterococcus faecalis	19.00 ± 0.41^{d}	10.50 ± 0.58^{e}	24.25 ± 0.5^{c}	0.00 ± 0.00^{f}	30.50 ± 0.41^{a}	10.02 ± 0.00^{e}	25.25 ± 0.96^{b}
Klebsiella pneumoniae	19.75 ± 0.50^{c}	11.50 ± 0.58^{e}	14.38 ± 0.63^{d}	0.00 ± 0.00^{f}	27.00 ± 0.91^{a}	10.75 ± 0.50^{e}	24.00 ± 0.82^{b}
Salmonella Paratyphi A	13.38 ± 0.75^{e}	24.25 ± 0.50^{a}	17.88 ± 0.48^{c}	0.00 ± 0.00^{f}	23.38 ± 0.75^{a}	16.50 ± 0.91^{d}	21.00 ± 0.71^{b}

Values represents mean ± SD. The same superscript (a–f) in the same row were not significantly different ($P > 0.05$).

known as the state of steady internal physical and chemical conditions maintained by living systems, and resulting the leakage of ions and cytoplasmic content (Seow et al., 2014). Since nanoemulsions both enhance the dispersibility in food matrices and improve physicochemical stability of EO, they have a significant influence on their interaction with microbial cells and their biological activity (Donsi and Ferrari, 2016).

Most essential oils contain terpenes, terpenoids, and other aromatic and aliphatic constituents with low molecular weights. The major component (90%) of bioactive essential oils consists of monoterpenes hydrocarbons such as *p*-cymene, limonene, *α*-pinene, and *α*-terpinene (Bakkali et al., 2008). Our results also support the previous findings, essential lemon oil showing good antimicrobial effects on both food-borne and spoilage bacteria probably due to D-limonene (52,85%), p-cymene (14.36%) and β-pinenes (13.69%) as found in this study.

The antimicrobial activities of two different concentrations of lemon essential oil and its nanoemulsion against food-borne pathogen bacteria were presented in Table 2. Lemon nanoemulsion and 10% essential oil showed higher antimicrobial activity ($P < 0.05$) against *S. aureus* with 16.63 and 16.25 mm inhibition zone diameters than that of 100% lemon essential oil (11.63 mm). However, Hsouna et al. (2017) obtained higher inhibition zone diameter (22 mm) with lemon (*Citrus limon*) essential oil for *Staphylococcus aureus* ATCC 923. The reason for this difference may be associated with chemical contents of essential oils. The chemical content of essential oils, even within the same species, varies depending on the environmental conditions of the plant, extraction process, purification methods, and also influenced from genotypes, chemotypes of plant (Djenane, 2015; Martinez et al., 1996). In the current study, the higher inhibition activities of lemon nanoemulsion (16.63 mm) and 10% lemon essential oil (16.25) against *S. aureus* was observed compared to that of streptomycin (15.13 mm) used as a positive control. However, tetracycline and neomycin antibiotics exhibited stronger antimicrobial activities with 20.38 and 19.75 mm zone diameter against this bacteria, respectively than the other treatment groups. Bozkurt et al. (2017) reported that the zone diameter of tetracycline against *S. aureus* was 22 mm which is higher that the result of the present study. The tween 80 did not have any antimicrobial properties which are used only for negative control purposes.

E. faecalis was more sensitive to nanoemulsion with 24.25 mm inhibition zone diameter, whereas *E. faecalis* showed the value of 19.00 mm inhibition zone diameter for 100% lemon essential oil (Table 2). However, lemon nanoemulsion and 100% and 10% essential oils had lower antimicrobial activities compared to tetracycline and neomycin antibiotics. Bozkurt et al. (2017) reported that inhibition zone diameters of different varieties of lemon essential oils (meyer and interdonato) on *E. faecalis* were 9 mm and 11 mm for tetracycline against this bacteria. However, in other study by Ozogul et al. (2015), inhibition zone diameters of lemon essential oil on *S. aureus* and *E. faecalis* bacteria were reported as 7.25 and 15.00 mm, respectively. Similar result were reported for Turkish Citrus peel oils which exhibited antimicrobial activity against various gram negative and gram positive bacteria, ranging from 10 to 16 mm inhibition zone diameter (Kirbaşlar et al., 2009).

Among the groups, 10% lemon essential oil exhibited the highest inhibition effect on *S.* Paratyphi A (24.25 mm), followed by *S. aureus* (16.25 mm). Although nanoemulsion also showed higher inhibition zone with the value of 17.88 mm on *S.* Paratyphi A than that of 100% lemon essential oil (13.38 mm), 10% lemon essential oil showed the highest inhibition zone (24.25 mm). Nanoemulsion and 10% lemon essential oil exhibited higher inhibition zones with values of 17.88 and 24.25 mm, respectively against this bacteria than streptomycin. However, this bacteria showed the values of 23.38 and 21.00 mm inhibition zone for tetracycline and neomycin respectively. Even though *Klebsiella pneumoniae* was demonstrated as a strong resistance against 100% lemon essential oil, less resistance against nanoemulsion and 10% essential oil was observed (Table 2). Both of essential lemon oil (100% and 10%) and its nanoemulsion showed strong antimicrobial activity on *Klebsiella pneumoniae* compared to positive control group of Streptomycin. However, other positive control tetracycline and neomycin antibiotics showed higher inhibition zone against *K. pneumoniae* than other treatment groups. These results are consistent with the research of Ozogul et al. (2015) who tested the antimicrobial activity of various essential oils against *E. coli*, *S.* Paratyphi A, *K. pneumoniae* and *Yersinia enterecolitica* bacteria. Their results indicated that lemon essential oil showed antimicrobial activity with diameter zone values of 5.25, 6.25, 10.75, 6.25 mm for *E. coli*, *S.* Paratyphi A, *K. pneumoniae* and *Yersinia enterecolitica*, respectively. In addition, it has been reported that lemon oil exhibited stronger antimicrobial activity against two groups of bacteria (maximum zone of inhibition against *Bacillus mycoides* and other *Bacillus cereus* 33 mm and 29 mm, respectively) (Gupta, 2017). It was reported that cold pressed lemon oil, orange and grapefruit seed oils showed inhibition zones ranging from 6.62 to 11 mm against fifteen tested pathogenic bacteria (Guneser et al., 2018). According to Donsi and Ferrari (2016), the antimicrobial ability of nanoemulsion based on essential oil strongly depends on emulsion formulation, droplet size, viscosity, chemical composition of the essential oil used in nanoemulsion and tested microbial strain.

3.4. Antimicrobial activity of spoilage bacteria

The antimicrobial activities of different concentrations of lemon essential oil and its nanoemulsion against fish spoilage bacteria were presented in Table 3. Both lemon essential oil (100% and 10%) and its nanoemulsion displayed a strong antimicrobial activity on fish spoilage bacteria tested. In particular, *P. damselae* bacterium showed a high sensitivity for both of 100% essential oil and nanoemulsion with 25.00 and 19.25 mm inhibition zone respectively. However, the lowest inhibition zone was obtained from 10% lemon oil against this bacteria. The higher zone diameter was observed in 100% lemon essential oil against this bacterium compared to those of neomycin and streptomycin positive controls. Nanoemulsion showed a statistically similar antimicrobial effect compared to the positive control of streptomycin antibiotic. However, the lower inhibition zone was exhibited in nanoemulsion group against *P. damselae* than tetracycline and neomycin used as reference antibiotics.

Lemon essential oil (100%) also showed high inhibition zones with

Table 3
Antimicrobial activity of lemon essential oil and its nanoemulsion against fish spoilage bacteria.

Fish spoilage bacteria	Inhibition zone (mm)						
	Lemon essential oil (100%)	Lemon essential oil (10%)	Lemon nanoemulsion	Tween 80	Tetracycline	Streptomycin	Neomycin
Photobacterium damselae	25.00 ± 0.82^{b}	17.75 ± 0.50^{e}	19.25 ± 0.87^{d}	0.00 ± 0.00^{f}	29.00 ± 0.82^{a}	19.13 ± 0.48^{d}	22.13 ± 0.85^{c}
Enterococcus faecalis	8.75 ± 0.65^{d}	24.75 ± 0.50^{a}	17.75 ± 0.50^{c}	0.00 ± 0.00^{f}	17.00 ± 0.71^{c}	18.88 ± 0.75^{b}	6.00 ± 0.41^{e}
Vibrio vulnificus	15.38 ± 0.48^{e}	15.00 ± 0.00^{e}	16.75 ± 0.87^{d}	0.00 ± 0.00^{f}	28.50 ± 0.58^{a}	22.00 ± 0.58^{c}	25.00 ± 0.41^{b}
Proteus mirabilis	18.38 ± 0.75^{b}	15.00 ± 0.82^{c}	12.38 ± 0.48^{d}	0.00 ± 0.00^{e}	18.13 ± 0.48^{b}	0.00 ± 0.00^{e}	21.25 ± 0.65^{a}
Serratia liquefaciens	22.00 ± 0.82^{c}	26.25 ± 0.96^{a}	14.75 ± 0.50^{e}	0.00 ± 0.00^{f}	16.75 ± 0.87^{d}	24.25 ± 0.50^{b}	22.25 ± 0.96^{c}
Pseudomonas luteola.	20.50 ± 0.58^{b}	17.75 ± 0.50^{d}	17.25 ± 0.87^{d}	0.00 ± 0.00^{e}	18.88 ± 0.48^{c}	22.38 ± 0.25^{a}	21.25 ± 0.65^{b}

Values represents mean ± SD. The same superscript (a–f) in the same row were not significantly different (P > 0.05).

Table 4
MIC and MBC determination of Lemon essential oil and its nanoemulsion on tested microorganisms.

Food borne pathogen bacteria	Lemon nanoemulsion		Lemon essential oil (100%)		Lemon essential oil (10%)		Tween 80	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Staphylococcus aureus	3.125	> 25	12.50	> 25	25	25	> 25	> 25
Klebsiella pneumoniae	6.25	> 25	12.50	> 25	25	> 25	> 25	> 25
Salmonella Paratyphi A	12.50	12.50	1.56	3.125	6.25	> 25	> 25	> 25
Enterococcus feacalis	12.50	> 25	12.50	> 25	> 25	> 25	> 25	> 25
Fish spoilage bacteria								> 25
Photobacterium damselae	> 25	> 25	≥25	> 25	25	> 25	> 25	> 25
Enterococcus feacalis	> 25	> 25	≥25	> 25	12.5	25	> 25	> 25
Vibrio vulnificus	25	> 25	> 25	> 25	25	> 25	> 25	> 25
Proteus mirabilis	> 25	> 25	> 25	> 25	6.25	> 25	> 25	> 25
Serratia liquefaciens	6.25	25	6.25	25	1.56	25	> 25	> 25
Pseudomonas luteola	6.25	25	3.125	1.56	> 25	25	> 25	> 25

values of 22.0, 20.50, 18.38 mm for *S. liquefaciens*, *P. luteola*, *P. mirabilis* whereas 10% lemon essential oil exhibited inhibition zones of 26.25, 17.25, 15.00 mm, respectively. There were no significant differences ($P > 0.05$) between 100% lemon essential oil and neomycin for *S. liquefaciens*, *P. luteola* and also tetracycline for *P. mirabilis* (Table 3). Similarly Balkan et al. (2018) compared to antimicrobial activity of various essential oils (*Thymus sipyleus* boiss. subsp. sipyleus boiss. var. sipyleus *L.*, *Satureja thymbra* L. and *Origanum onites L.*) and indicated that *P. mirabilis* were the most sensitive against these essential oils. In the other research, lemon essential oil showed inhibition zone of 9 mm for L. *monocytogenes* and *Enterobacter cloacae*, 12.0 mm for *Escherichia coli* O157:H7, 11.0 mm for *S. typhimurium*, 13 mm for *Salmonella enteritidis*, 16 mm for *Staphylococcus aureus*, 14 mm for *S. epidermidis*, 18 mm for *Bacillus subtilis* and 19 mm for *Micrococcus flavus*, although no inhibition zone of this essential oil was observed against *P. aeruginosa* and *P. mirabilis*. Streptomycin exhibited inhibition zone from 10 to 20 mm against all tested bacteria as well (Sokovic et al., 2010).

In the current study, nanoemulsion also showed the lowest inhibition zone with the value of 12.38, 14.75, 17.25 mm on *P. mirabilis*, *S. liquefaciens*, *P. luteola*, respectively in comparison to 100% lemon essential oil. The highest inhibitory effect was obtained from 10% lemon essential oil against *S. liquefaciens* (26.25 mm), followed by *P. luteola* (17.75 mm) although no significant differences (P > 0.05) were observed between 10% lemon oil and nanoemulsion (17.25 mm) against *P. luteola*. All antibiotics used as reference also exhibited higher inhibition zone against *S. liquefaciens*, *P. luteola* than nanoemulsion. Tetracycline and neomycin also showed higher inhibition zone against *P. mirabilis*. However, streptomycin did not show any inhibition zone against *P. mirabilis*. Although nanoemulsion exhibited a lower inhibition zone against these tested bacteria (Table 3), conversion of lemon oil into nanoemulsion demonstrated effective antimicrobial effect. In general, it was indicated that the antimicrobial efficacy of EO based nanoemulsions strongly depends on EO components, tested microbial strain and emulsion formulation and size (Donsi and Ferrari, 2016).

The highest antimicrobial effect on *E. faecalis* was obtained from 10% lemon essential oil (24.75 mm). Nanoemulsion had strong antimicrobial effects with the values of 17.75 and 16.75 mm on *E. faecalis*, and *V. vulnificus* compared to 100% lemon essential oil. Among the groups, 10% lemon essential oil showed the highest antimicrobial effects on *E. faecalis* (24.75 mm), and *S. liquefaciens* (26.25 mm). It also exhibited statistically similar impact on *E. faecalis* between nanoemulsion and tetracycline. All the antibiotics exhibited strong effect with the higher zone diameter values on *V. vulnificus*.

3.5. Minimum inhibition and bactericidal concentration

Among food-borne pathogen bacteria tested, as previously observed with paper disc diffusion method, *S. aureus* had the highest sensitivity since the lower concentration of nanoemulsion was needed with 3.125 mg/mL MIC values in comparison to 100% lemon essential oil (12.50 mg/mL MIC). Nanoemulsion displayed strong antimicrobial effect on other tested pathogen bacteria; *K. pneumoniae*, *S.* Paratyphi A, *E. faecalis* with the MIC ranging from 6.25 to 12.50 mg/mL (Table 4). The bacterial growth of *S.* Paratyphi A was inhibited by nanoemulsion with MIC value of 12.50 mg/mL. Similarly, nanoemulsion exhibited strong bactericidal effect on the same bacteria with MBC value of 12.50 mg/mL. The result of current study indicated that nanoemulsion strongly inhibited bacterial growth of pathogen bacteria in the MIC method, but it did not have strong bactericidal effect on other three pathogen bacteria according to MBC value (> 25). However, *S.* Paratyphi A gave similar values for MIC and MBC (12.50 mg/mL). These outcomes also support to the paper disc diffusion method result for pathogen bacteria and confirms that the nanoemulsion enhanced antimicrobial effect of lemon essential oil. 10% lemon essential oil had the highest MIC value (6.25 mg/mL) on *S.* Paratyphi A among pathogens. Generally, 100% essential oil was more effective on pathogens than 10% lemon essential oil. Some studies have shown that there was no change in the antimicrobial activity of essential oils when they were emulsified (Chang et al., 2012; Xue et al., 2015; Ziani et al., 2011). Zhang et al. (2014) reported that the MIC values of nanoemulsion including D-limonene

(4% w/w) were lower than that of unprocessed D-limonene against target bacteria of *S. aureus*, *B. subtilis*, *E. coli*, *S. cerevisiae*. They also reported that the MIC values of D-limonene nanoemulsion in combination with nisin (D-limonene 4% w/w and nisin 3% w/w) were 0.0628 μg/mL, 0.25 μg/mL, 0.5 μg/mL, 0.25 μg/mL against these four target bacteria.

Among the fish spoilage bacteria tested, both *S. liquefaciens* and *P. luteola* were the most susceptible since the lower concentration of lemon nanoemulsion was needed with MIC values (6.25 mg/mL). However, the other spoilage bacteria tested including *P. damselae*, *Enterococcus faecalis*, *P. mirabilis* showed less sensitivity against the lemon nanoemulsion with the MIC values > 25 mg/mL. MIC of the nanoemulsion was 25 mg/mL for *V. vulnificus*. The bactericidal influence was seen on *S. liquefaciens*, *P. luteola* with MBC value of 25 mg/mL for lemon nanoemulsion. On the other hand, bactericidal activity was not strong on the other spoilage bacteria tested with MBC values ≥ 25 mg/mL against nanoemulsion (Table 4).

Lemon essential oil (100% and 10%) showed a wide range of inhibitory effect on all tested pathogenic bacteria (Table 4). However, among pathogen bacteria, *S.* Paratyphi A was the most sensitive against 100% lemon essential oil as the least concentrations of essential oil were needed with MIC value of 1.56 mg/mL and MBC values of 3.125 mg/mL. Although 10% lemon essential oil showed strong inhibitory effect on *S.* Paratyphi A with MIC value of 6.25 mg/mL, MBC value of 10% lemon essential oil was found as > 25 mg/mL. 100% lemon essential oil inhibited growth of all pathogen bacteria tested with MIC ranging from 1.25 mg/mL for *S.* Paratyphi A to 12.50 mg/mL for other pathogens. However, MBC values of 100% lemon essential oil were found as ≥ 25 mg/mL against *Staphylococcus aureus*, *Klebsiella pneumonia* and *Enterococcus faecalis*. MBC values of 10% lemon essential oil were found as ≥ 25 mg/mL for all pathogens. The results of previous studies were reported to be consistent with the current study. It was reported that the MIC values of lemon oil (*Citrus limon*) varied from 6.25 to 50 mg/mL against different bacteria (Gupta, 2017). They also reported that *S. aureus* and *Micrococcus luteus* were found to be highly sensitive to the essential oil with the lowest MIC value 6.25 mg/mL followed by *Bacillus cereus* and *Bacillus mycoides* with the MIC value of 12.5 mg/mL. Lemon essential oil has strong antimicrobial activity on *S. aureus*, *L. monocytogenes* and *E. faecium* with the MIC values of 2.0, 1.0 and 0.5 μl/mL respectively and has also bactericidal effects on *S. aureus* and *E. faecium* with MBC values of 5.0 μl/mL (Espina et al., 2011). On the other hand, they reported that lemon essential oil did not show bactericidal effect on L. *monocytogenes*, *Salmonella Enteritidis*, *E. coli* O157:H7, *P. aeruginosa* with MBC value > 30.0 μl/mL. In another research by Sharafati Chaleshtori and Sharafati Chaleshtori (2017), MIC values for lemon essential oil was observed as 1.41 mg/mL for the bacteria including *Shigella dysenteriae*, *L. monocytogenes*, *S. paratyphi* and *S. pyogenes* while MBC value for lemon essential oil was found as 2.81 mg/mL for the same bacteria.

Among all the fish spoilage bacteria tested, *S. liquefaciens* was found to be sensitive to 100% lemon essential oil and its nanoemulsion with MIC values of 6.25 mg/mL whereas this bacteria was the most sensitive to 10% lemon essential oil with the MIC value of 1.56 mg/mL (Table 4). The bactericidal effect was only seen on *P. luteola* with MBC value of 1.56 mg/mL for 100% lemon essential oil. 10% lemon essential oil did not have strong bactericidal effect on both pathogens and spoilage bacteria (≥ 25 mg/mL). However, MIC and MBC values of nanoemulsion were found as > 25 mg/mL against *P. damselae*, *E. faecalis*, *P. mirabilis*. While the MIC values of lemon nanoemulsion against *V. vulnificus* was found as 25 mg/mL, bactericidal concentration was observed as > 25 mg/mL. On the other hand, 100% lemon essential oil had same bactericidal effect with MBC values of > 25 mg/mL on *P. damselae*, *E. feacalis*, *P. mirabilis*, *V. vulnificus*. MIC values of 100% lemon essential oil were found as ≥ 25 mg/mL against *E. faecalis* and *P. damselae*. However, MIC values of 100% lemon essential oil were exhibited as > 25 mg/mL against *P. mirabilis*, V. *vulnificus*.

Tween 80 as used negative control did not have any inhibitory and bactericidal effect on all tested pathogen bacteria in the microdilution method, since they do not have any antimicrobial properties.

4. Conclusion

The results of this research demonstrated that lemon nanoemulsion has more effective antimicrobial influence against *S. aureus*, and *E. faecalis*, than both 100% and 10% lemon essential oil. However, 10% lemon essential oil had strong antimicrobial effect on *S.* Paratyphi A, followed by its nanoform. This outcome confirms that the conversion of the essential oil into nanoemulsion enhanced its antimicrobial activity. On the other hand, assessment of the antimicrobial effects of lemon essential oil and its nanoemulsion against fish spoilage bacteria showed that 100% essential oil was more effective on especially *P. damselae*, *P. mirabilis*, *S. liquefaciens*, *P. luteola* than nanoemulsion except *E. faecalis*, *V. vulnificus*. However, 10% lemon essential oil was more effective on *E. faecalis*, and *S. liquefaciens* than 100% lemon essential oil. According to value of MIC, both nanoemulsion and 100% lemon essential oil inhibited bacterial growth of all of the pathogen bacteria tested whereas they were less effective on fish spoilage bacteria. However, 10% essential oil was more effective on spoilage bacteria than pathogens. MBC showed that nanoemulsion and 100% lemon essential oil presented a noticeable bactericidal activity against *S. paratyphi* A whereas 10% lemon essential oil was found as ≥ 25 mg/mL against pathogens and spoilage bacteria. To conclude, nanoemulsion based on lemon essential oil can have potential as a natural antimicrobial agent against food-borne pathogen and spoilage bacteria for fish processing industry. To determine effects of nano form of lemon essential oil against food-borne pathogens and fish spoilage bacteria, further studies could be conducted on fish or food products.

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References

Alfonzo, A., Martorana, A., Guarrasi, V., Barbera, M., Gaglio, R., Santulli, A., Settanni, L., Galati, A., Moschetti, G., Francesca, N., 2017. Effect of the lemon essential oils on the safety and sensory quality of salted sardines (Sardina pilchardus Walbaum 1792). Food Control 73, 1265–1274.

Al-Reza, S.M., Rahman, A., Lee, J., Kang, S.C., 2010. Potential roles of essential oil and organic extracts of Zizyphus jujuba in inhibiting food-borne pathogens. Food Chem. 119, 981–986.

Baboota, S., Shakeel, F., Ahuja, A., Ali, J., Shafiq, S., 2007. Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib. Acta Pharma. 57, 315–332.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils–a review. Food Chem. Toxicol. 46, 446–475.

Balkan, C., Kordali, S., Bozhüyük, A.U., 2018. Antimicrobial effect of plant oils against some bacterias izolated from patients samples. J. Mol. Genet. Med. 12 1747-0862.

Bourgou, S., Rahali, F.Z., Ourghemmi, I., Saidani Tounsi, M., 2012. Changes of peel essential oil composition of four Tunisian citrus during fruit maturation. Sci. World J. 2012.

Bozkurt, T., Gülnaz, O., Kaçar, Y.A., 2017. Chemical composition of the essential oils from some citrus species and evaluation of the antimicrobial activity. IOSR J. Environ. Sci. Toxicol. and Food Tech. (IOSR-JESTFT) 11, 29–33.

Chang, Y., McLandsborough, L., McClements, D.J., 2012. Physical properties and antimicrobial efficacy of thyme oil nanoemulsions: influence of ripening inhibitors. J. Agri. Food Chem. 60, 12056–12063.

Chikhoune, A., Hazzit, M., Kerbouche, L., Baaliouamer, A., Aissat, K., 2013. Tetraclinis articulata (Vahl) masters essential oils: chemical composition and biological activities. J. Essent. Oil Res. 25, 300–307.

Djenane, D., 2015. Chemical profile, antibacterial and antioxidant activity of Algerian citrus essential oils and their application in Sardina pilchardus. Foods 4, 208–228.

Donsi, F., Ferrari, G., 2016. Essential oil nanoemulsions as antimicrobial agents in food. J. Biotech. 233, 106–120.

Donsi, F., Annunziata, M., Vincensi, M., Ferrari, G., 2012. Design of nanoemulsion-based delivery systems of natural antimicrobials: effect of the emulsifier. J. Biotech. 159, 342–350.

Espina, L., Somolinos, M., Lorán, S., Conchello, P., García, D., Pagán, R., 2011. Chemical composition of commercial citrus fruit essential oils and evaluation of their

antimicrobial activity acting alone or in combined processes. Food Control 22, 896–902.
Franceschi, E., Grings, M.B., Frizzo, C.D., Oliveira, J.V., Dariva, C., 2004. Phase behavior of lemon and bergamot peel oils in supercritical CO2. Fluid Ph. Equilibria. 226, 1–8.
Govaris, A., Solomakos, N., Pexara, A., Chatzopoulou, P.S., 2010. The antimicrobial effect of oregano essential oil, nisin and their combination against Salmonella Enteritidis in minced sheep meat during refrigerated storage. Int. J. Food Microbiol. 137, 175–180.
Gram, L., Huss, H.H., 1996. Microbiological spoilage of fish and fish products. Int. J. Food Microbiol. 33, 121–137.
Guneser, B.A., Zorba, N., Yilmaz, E., 2018. Antimicrobial activity of cold pressed citrus seeds oils, some citrus flavonoids and phenolic acids. Riv. Ital. Sostanze. G. XCV 119–131.
Gupta, C., 2017. Comparative study of antimicrobial effects of lemon oil and peel extract against food-spoilage microbes. J. Nutrit. Health Food Sci. 5, 1–5.
Hamedo, H.A., Abdelmigid, H.M., 2009. Use of antimicrobial and genotoxicity potentiality for evaluation of essential oils as food preservatives. Open Biotechnol. J. 3 (1).
Handford, C.E., Dean, M., Spence, M., Henchion, M., Elliott, C.T., Campbell, K., 2015. Awareness and attitudes towards the emerging use of nanotechnology in the agri-food sector. Food Control 57, 24–34.
Hosseinnia, M., Khaledabad, M.A., Almasi, H., 2017. Optimization of Ziziphora clinopodiodes essential oil microencapsulation by whey protein isolate and pectin: a comparative study. Int. J. Biol. Macromol. 101, 958–966.
Hsouna, A.B., Halima, N.B., Smaoui, S., Hamdi, N., 2017. Citrus lemon essential oil: chemical composition, antioxidant and antimicrobial activities with its preservative effect against listeria monocytogenes inoculated in minced beef meat. Lipids Health Dis. 16, 146.
Huang, J.-Y., Li, X., Zhou, W., 2015. Safety assessment of nanocomposite for food packaging application. Trends Food Sci. Tech. 45, 187–199.
Kirbaşlar, F.G., Tavman, A., Dülger, B., Türker, G., 2009. Antimicrobial activity of Turkish citrus peel oils. Pak. J. Bot. 41, 3207–3212.
Lane, D., 1991. 16S/23S rRNA Sequencing. Nucleic Acid Techniques Bacterial Systematics. pp. 115–175.
Liang, R., Xu, S., Shoemaker, C.F., Li, Y., Zhong, F., Huang, Q., 2012. Physical and antimicrobial properties of peppermint oil nanoemulsions. J. Agri. Food Chem. 60, 7548–7555.
Ligor, M., Stankevičius, M., Wenda-Piesik, A., Obelevičius, K., Ragažinskienė, O., Stanius, Ž., Maruška, A., Buszewski, B., 2013. Comparative gas chromatographic–mass spectrometric evaluation of hop (Humulus lupulus L.) essential oils and extracts obtained using different sample preparation methods. Food Anal. Method. 7, 1433–1442.
Martinez, M., Betancourt, J., Alonso-Gonzalez, N., Jauregui, A., 1996. Screening of some Cuban medicinal plants for antimicrobial activity. J. Ethnopharmacol. 52, 171–174.
McClements, D.J., Decker, E.A., Park, Y., Weiss, J., 2009. Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. Crit. Rev. Food Sci. Nutr. 49 (6), 577–606.
Moghimi, R., Aliahmadi, A., McClements, D.J., Rafati, H., 2016. Investigations of the effectiveness of nanoemulsions from sage oil as antibacterial agents on some food borne pathogens. LWT-Food Sci. Tech. 71, 69–76.
Moosavy, M., Hassanzadeh, P., Mohammadzadeh, E., Mahmoudi, R., Khatibi, S., Mardani, K., 2017. Antioxidant and antimicrobial activities of essential oil of lemon (Citrus limon) peel in vitro and in a food model. J. Food Qual. Hazards Control. 4, 42–48.
Murray, P., 1995. In: Nolte, F.S., Metchock, B. (Eds.), Manual of Clinical Microbiology, sixth edition. Asm Press, Washington DC, pp. 400–437.
Ozogul, Y., Kuley, E., Ucar, Y., Ozogul, F., 2015. Antimicrobial Impacts of Essential Oils on Food Borne-pathogens. 7. pp. 53–61.
Ozogul, Y., Yuvka, İ., Ucar, Y., Durmus, M., Kösker, A.R., Öz, M., Ozogul, F., 2017. Evaluation of effects of nanoemulsion based on herb essential oils (rosemary, laurel, thyme and sage) on sensory, chemical and microbiological quality of rainbow trout (Oncorhynchus mykiss) fillets during ice storage. LWT-Food Sci. Tech. 75, 677–684.
Prakash, B., Kiran, S., 2016. Essential oils: a traditionally realized natural resource for food preservation. Curr. Sci. 110, 1890–1892.
Rao, J., McClements, D.J., 2011. Formation of flavor oil microemulsions, nanoemulsions and emulsions: influence of composition and preparation method. J. Agric. Food Chem. 59, 5026–5035.
Rao, J., McClements, D.J., 2012. Food-grade microemulsions and nanoemulsions: role of oil phase composition on formation and stability. Food Hydrocoll. 29, 326–334.
Schieberle, P., Grosch, W., 1988. Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. J. Agric. Food Chem. 36, 797–800.
Seow, Y.X., Yeo, C.R., Chung, H.L., Yuk, H.-G., 2014. Plant essential oils as active antimicrobial agents. Crit. Rev. Food Sci. Nutr. 54, 625–644.
Settanni, L., Palazzolo, E., Guarrasi, V., Aleo, A., Mammina, C., Moschetti, G., Germanà, M.A., 2012. Inhibition of foodborne pathogen bacteria by essential oils extracted from citrus fruits cultivated in Sicily. Food Control 26, 326–330.
Severino, R., Ferrari, G., Vu, K.D., Donsì, F., Salmieri, S., Lacroix, M., 2015. Antimicrobial effects of modified chitosan based coating containing nanoemulsion of essential oils, modified atmosphere packaging and gamma irradiation against Escherichia coli O157:H7 and Salmonella typhimurium on green beans. Food Control 50, 215–222.
Shafiq, S., Shakeel, F., Talegaonkar, S., Ahmad, F., Khar, R., Ali, A., 2007. Design and development of oral oil in water ramipril nanoemulsion formulation: in vitro and in vivo assessment. J. Biomed. Nanotech. 3 (1), 28–44.
Sharafati Chaleshtori, F., Sharafati Chaleshtori, R., 2017. Antimicrobial activity of chitosan incorporated with lemon and oregano essential oils on broiler breast meat during refrigerated storage. Nutr. Food Sci. 47, 306–317.
Sharma, N., Tripathi, A., 2008. Effects of Citrus sinensis (L.) Osbeck epicarp essential oil on growth and morphogenesis of Aspergillus niger (L.) Van Tieghem. Microbiol. Res. 163, 337–344.
Sokovic, M., Glamoclija, J., Marin, P.D., Brkic, D., van Griensven, L.J., 2010. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. Molecules 15, 7532–7546.
Solans, C., Izquierdo, P., Nolla, J., Azemar, N., Garciacelma, M., 2005. Nano-emulsions. Curr. Opin. Colloid Interface Sci. 10, 102–110.
Turner, S., Pryer, K.M., Miao, V.P., Palmer, J.D., 1999. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. J. Eukaryot. Microbiol. 46, 327–338.
Walker, R.M., Gumus, C.E., Decker, E.A., McClements, D.J., 2017. Improvements in the formation and stability of fish oil-in-water nanoemulsions using carrier oils: MCT, thyme oil, & lemon oil. J. Food Eng. 211, 60–68.
Xue, J., Davidson, P.M., Zhong, Q., 2015. Antimicrobial activity of thyme oil co-nanoemulsified with sodium caseinate and lecithin. Int. J. Food Microbiol. 210, 1–8.
Yazgan, H., Ozogul, Y., Durmus, M., Balikci, E., Gokdogan, S., Ucar, Y., Aksun, E.T., 2017. Effects of oil-in-water nanoemulsion based on sunflower oil on the quality of Farmed Sea bass and Gilthead Sea bream stored at chilled temperature (2 +/- 2 degrees C). J. Aquat Food Prod. Tech. 26, 979–992.
Zhang, Z., Vriesekoop, F., Yuan, Q., Liang, H., 2014. Effects of nisin on the antimicrobial activity of d-limonene and its nanoemulsion. Food Chem. 150, 307–312.
Ziani, K., Chang, Y., McLandsborough, L., McClements, D.J., 2011. Influence of surfactant charge on antimicrobial efficacy of surfactant-stabilized thyme oil nanoemulsions. J. Agric. Food Chem. 59, 6247–6255.