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Preservation effect of *Lippia citriodora* and *Laurus nobilis* nanoemulsions incorporated with polylactic acid composite film for rainbow trout fillet packaging

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Abstract

The present study was conducted to characterize poly (lactic acid) -*Lippia citriodora* and *Laurus nobilis* essential oils (EOs) and Nanoemulsion-based films. Following Gas chromatography–mass spectrometry and *in vitro* antimicrobial activity test, 12% of Nanoemulsions of both herbs were selected for further evaluations. Accordingly, Atomic Force Microscopy and Dynamic light scattering analysis showed that the scale sizes of Nanoemulsions of *L. citriodora* and *L. nobilis* were 24-60 and 15-28 nm, respectively with no significant changes in their PDI up to 90 d. The inhibition zone of both herbs against *E. coli* and *Staphylococcus aureus* alongside MIC and MBC tests showed higher efficiency in their nanoemulsions. In conclusion, two types of incorporated PLA/*L. citriodora* films could effectively extend the shelf life of rainbow trout fillet at refrigerated temperatures.

Keywords: essential oil; nanoemulsion; polylactic acid; rainbow trout; shelf life.

Practical Application: The emergence of nanotechnology-based approaches can increase marine food shelflife.

1 Introduction

Packaging prolongs the shelf life of food products, preserving nutritional values during transport and storage (Artiga-Artigas et al., 2017; Yang et al., 2020). Due to the worries about the migration of materials, researchers have focused on applying natural, and recyclable packaging materials (Shekarchizadeh & Nazeri, 2020). Petrochemical polymers, such as polyethylene (PE) and polypropylene (PP), are defined to be non-biodegradable and toxic for the environment; therefore, should be replaced with other natural materials (Thiounn & Smith, 2020).

Poly (lactic acid) (PLA), a recyclable compound, is a starch (or other sugars such as beet)-derived plastic with good elasticity and plasticity, being eventually consumed by microorganisms (Öz et al., 2017). PLA has been accepted by the Food and Drug Administration (FDA) for food packaging purposes (Fattahi et al., 2019), containing direct-contact utilization (Öz et al., 2017). PLA, as Generally Recognized As (GRAS), are non-aromatic polyester, initially produced from lactic acid, to improve the performance of the film in nanocomposites packaging (Nordmann, 2008; Tang et al., 2020). PLA is an appropriate compound that is capable of being fruitfully agglutinated with certain compounds, such as herbal medicine (Martins et al., 2018), carvacrol-PEI (polyethyleneimine) NPs (Niza et al., 2020), PLA/ZnO, PLA/MgO (Ghozali et al., 2020), ascorbic, and fumaric acids (Popelka et al., 2020).

Lippia citriodora (*L. citriodora*) and *Laurus nobilis* (*L. nobilis*) wildly grow in South America, the Mediterranean area, and Asia (Verdian-rizi & Hadjiakhoondi, 2008). For centuries, they have been utilized for the treatment of asthma, colds, diarrhea, rheumatic pains, and dyspepsia (Pérez Zamora et al., 2018). The main components of their EOs were geranial, neral, Limonene, cineole, eugenol, sabinene, α-pinene, and α-terpineol (Mahdavi et al., 2020). To date, a few herbal EOs based nanoemulsion have been loaded with PLA to extend the shelf life of food like carvacrol (Niza et al., 2020), green tea (Martins et al., 2018), thyme, rosemary, and oregano (Zeid et al., 2019).

Nanoemulsions with more efficacy than emulsions (Raeisi et al., 2020), are kinetically but not thermodynamically stable systems. Although emulsions and nanoemulsions entail the same components like oil, water, and surfactant, they depicted fundamental differences in their composition and quantity. Nanoemulsions can carry higher percentages of the dispersed phase (oil) along with a lower amount of surfactant (Matthieu, 2008; Pavoni et al., 2020).

Seafood has degradable polyunsaturated fatty acids (PUFA), keeping sensory properties, and preventing the food from spoilage during food handling, processing, storage, and distribution (Hanif et al., 2020). Due to rapid spoilage in fish products, their packaging is of particular importance (Kumar et al., 2020). *Oncorhynchus mykiss* (Rainbow trout), a member of *Salmonidae,* is one of the most popular and freshly sold species in the world, particularly in Turkey, and Iran (Khanipour et al., 2020).

According to the significance of fish preservation through the usage of nanotechnology-based approaches, this research aimed at characterization of antimicrobial efficiencies of PLA biodegradable film loaded with nanoemulsions of *L. citriodora* and *L. nobilis*, to extend the shelf life of fresh rainbow trout preserved at refrigerated temperature.

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2 Materials and methods

2.1 Lippia citriodora/Laurus nobilis EO preparation

L. citriodora and *L. nobilis* leaves were purchased from their habitat in Iran. Their EOs were prepared by the method of Choobkar et al. (2010) with a minor modification. Primarily, the leaves were washed with adequate distilled water, and left to be dried at room temperature for 3-7 days. 100 g of the dried leaves was grounded, and then hydrodistilled with 800 mL of distilled water for 3-4 hours, using a Clevenger apparatus. The extracted oils were dried over anhydrous sodium sulfate and preserved in sealed vials at refrigerated temperature until further analysis (Figure 1).

2.2 Identification of EO contents

The prepared EO was injected into the GC-MS (HP 6890, Japan) equipped with an HP-5 column (30 m x 0.25 mm i.d. x 0.25 µm). To achieve optimized temperature, the initial temperature was assigned as 70 °C with pausing at 2 °C/min, augmented up to 220 °C at 15 °C/min, and finally, a 300 °C enhancement with pausing at 2 °C/min following the method of Agah & Najafian (2012). The retention indices (RI) for all the components were determined based on n-alkanes as standard, obtained RI with relative mass spectra stored in the database of the Wiley8 GC/MS Library and published literature (Kaskoos, 2019).

2.3 Preparation of oil-in-water (O/W) Nanoemulsion

The best percentage of the NE (12%), showed better antimicrobial efficacy (the data were not given). Then, 12% of Tween 80 as a surfactant, was initially homogenized with 75.5% of deionized water at 650 rpm for 5 min, simultaneously, 0.5% of lecithin (as a ripening inhibitor) was added to the former combination. For reaching a homogeneous mixture, it was then exposed to ultrasound with a maximum output power of 30% (power 160 watts), and a probe of 7 mm in diameter, next to an icebox (Moghimi et al., 2016; Mazarei & Rafati, 2019).

2.4 Fourier-Transform Infrared (FTIR) spectroscopy

FTIR was performed for analysis of molecular structure, functional groups, and bonds of nanoemulsions using a Bruker spectrometer (Tensor 27, Germany) in a wave number ranging from 4000-400 cm^{-1} (Pirsa et al., 2018).

2.5 Atomic Force Microscopy (AFM)

AFM (Entegra AFMNT-MDT, Russia) was carried out (Ferreyra Maillard et al., 2019) to analyze the surface topography of the nanomaterial in 2-3 dimensions. Diluted samples were placed on a clean slide, and allowed to be dried. The surface of the sample was then imaged in the dimensions of 5×5µm and photographed using NOVA_1.26.0.1443_ solver_ eng.exe software.

2.6 Dynamic Light Scattering (DLS)

To prevent multiple diffractions in the determination of the hydrodynamic diameter of the nanoemulsions, all the samples were diluted to 10% with deionized water (Sun et al., 2019). For DLS test, 5 mL of the colloidal solution was poured into the cells of SZ-100z Dynamic Light Scattering & Zeta potential analyzer (HORIBA Jobin Jyovin, Japan) equipped with a laser power of 20 mW (λ = 632.8 nm), and measured in triplicate at a 90° scattering detector angle at room temperature (Oliveira et al., 2017).

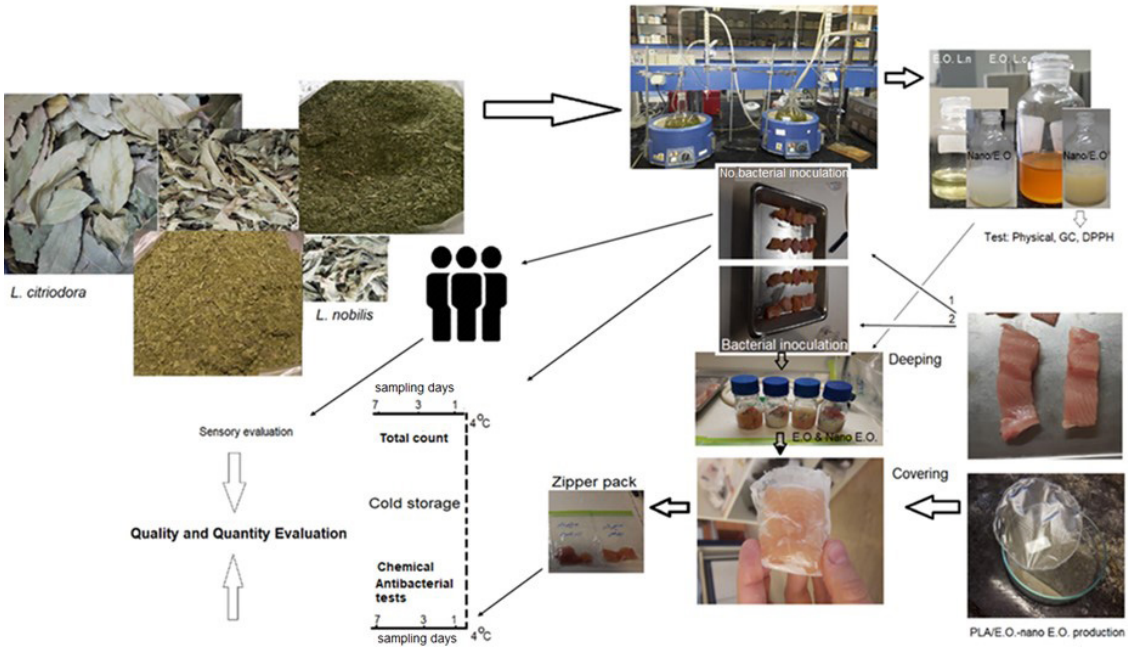

Figure 1. An overview of the casting-method synthesis of PLA coating loaded with *Laurus nobilis*/*Lippia citriodora*-Nanoemulsions was used for extension of the shelf life of *Oncorhynchus mykiss* fillets preserved at refrigerated temperature.

2.7 PLA/Nanoemulsion film preparation

PLA film was prepared with a solvent casting method following the description of Javaherzadeh et al. (2020) with a critical modification. In this study, 2 g of PLA powder (1.33 g/cm^3; Sigma-Aldrich, USA) was dissolved in 100 mL of dichloromethane using a magnetic stirrer at room temperature for 8h. Then, nanoemulsions (12%) were added (w/w), homogenized at 12000 rpm for 2 min, poured on a glass petri dish (diameter 100 mm), and placed under a chemical hood so that for evaporation (dichloromethane) for 60 min. The produced films were removed from the glass mold, and placed in a desiccator containing silica gel (Figure 1). The thickness of the produced PLA was measured using a digital caliper (Titan, China).

2.8 Antioxidant Activity (AA): DPPH method

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals (2 mL, 0.1 mM) were added to the EOs or nanoemulsions (0.1 mL) of *L. citriodora* or *L. nobilis* following the method of Farahmandfar et al. (2018) with a minor modification. Hydroxyl group of antioxidant compounds reduce DPPH by transferring the hydrogen cations to free radical DPPH, and change the color of reaction solution from dark purple to bright yellow (Santos-Sánchez et al., 2019). The EOs were exposed to the DPPH radical in methanol solution for 60 min in the dark, and the absorbance was measured at 517 nm using a spectrophotometer (Rayleigh UV-1601, China). The inhibition or reduction value (*I*) of DPPH free radical was calculated using the following Equation 1:

$$I\% = \frac{(A0 - A1)}{A0} \times 100 \quad (1)$$

A0 and A1 are the absorbances of the blank, and the sample, respectively.

2.9 Preparation of a bacterial suspension

Staphylococcus aureus (S. aureus) (ATCC 25923), *Escherichia coli* (*E. coli*) (ATCC 25922), and *Pseudomonas aeruginosa (P. aeruginosa)* (ATCC 27853) were prepared from the Iranian Research Organization for Science and Technology. 0.1% (w/v) peptone water (0.05 mM, PH 7) was added to each vial of freeze-dried bacteria to rehydrate the dried suspension, in a water bath setting at 30 °C. Cultured media were incubated in Tryptic Soy Broth (TSB) at 37 °C for 24h twice due to prolonged log phase at this condition (Xu et al., 2018).

0.5 McFarland standard of each bacteria was prepared (1.5×10^8 CFU/mL). Afterward, 1 mL of each suspension was inoculated to 100 mL of Brain Heart Infusion (BHI) broth to gain 10^6 CFU/mL. This final bacterial suspension was used for the bacterial analysis. For Minimum Inhibitory Concentration ((MIC) as the lowest concentration with no apparent growth), and Minimum Bactericidal Concentration ((MBC) with no turbidity) evaluation, *Salmonella enterica (S.enterica)* (ATCC 13076) was also added to our study.

2.10 In vitro antimicrobial activity

MIC/MBC assay

The antimicrobial activities for 0.45 µm-syringe filtrated pure EO and nanoemulsions, were evaluated by determining MIC and MBC against *S aureus*, *E. coli*, *P. aeruginosa*, and *S.enterica*. MIC was assessed using a 96-well flat microplate. 100 µL of each bacterial suspension (10^6 CFU/mL), 100 µL of BHI broth, and 100 µL of each aforementioned EOs were inoculated and incubated for 24 h at 37 °C (Shokri et al., 2020). To keep the conditions of experiments, in the same manner, 100 µL of both pure EOs, and 100 µL of 12% v/v nanoemulsion were used.

Agar diffusion bioassay

Primarily inoculated BHI broth were incubated for 24 h. Subsequently, 8 mL of the Grove–Randall's 1 culture medium (Difco) was poured into the plate, until reaching temperature 45 °C. 2 mL of each bacterial suspension was poured into the first layer. Five cylinders were located on the external layer of the second medium. Five stainless steel cylinders (0.25 cc in volume) containing EOs, nanoemulsions, and an antibiotic were placed on four points and in the center of the plate. After incubation at 37 °C for 24 h, the inhibition zones were measured (Cazedey & Salgado, 2011) and compared with Gentamicin (10µg/mL) and Neomycin (30 µg/mL). To keep the conditions of experiments, in the same manner, 250 µL of both pure EOs, and 250 µL of 12% v/v nanoemulsion.

2.11 Study design and procedure

Sixteen rainbow trout with an average weight of 863.3 ± 41.3 g were purchased from a fish farm located in the northern parts of Tehran. They were gutted, washed with distilled water, and transferred in a Styrofoam box close to crushed ice. After washing, they were filleted and cut into 30-g pieces. 90-minced fillets were immersed in the final bacterial suspension for 30 seconds and then divided into 10 groups. In the control group (1), fillets were placed into Plastic Zipper Packs (PZP) (Figure 1). EOs of *L. citriodora,* and *L. nobilis*, was inoculated to groups 2, and 3, respectively. Groups 4 and 5, were covered with nanoemulsions for each herb and placed into PZPs. Group 6 included the fish wrapped with PLA and then placed into PZPs. The PLA/nanoemulsions of *L. citriodora*, and *L. nobilis* were utilized for fish in groups 9, and 10, respectively, then being placed into PZPs (Figure 1). 60- fillets were employed for sensory evaluation (inviting three candidates) as well as total viable bacterial count. The fish fillets were preserved at refrigerated temperature and tested on the first, 3rd, and 7th day. The samples were unwrapped in front of them and the evaluation was started.

2.12 Microbiological analysis

10 g of each fillet sample was homogenized using a stomacher (STOMACHER®, UK) along with 90 mL of 0.1% peptone water for 2 min. The obtained mixture was serially diluted (1:10) with 0.1% peptone water. Subsequently, 0.1 mL of the dilution was pour plated with Plate Count Agar (Sigma-Aldrich, Germany) and incubated at 30-35 °C for 48-72 hours to calculate Total

Viable Bacteria Count (TVBC). Baird–Parker (BP) agar (Sigma-Aldrich, Germany) supplemented with egg yolk–tellurite emulsion was used to count *S. aureus*, and incubated at 37 °C for 24-48 h (Khoshbou Lahijani et al., 2019). The Tryptone Bile X-Glucuronide was utilized to count *E. coli*, and incubated at 44 °C for 24 h. Cetrimide Agar (Sigma-Aldrich, Germany) was applied to count *P. aeruginosa,* incubated after a 30-min pause, and then incubated at 37 °C for 24-48 h (Choi et al., 2018; Khoshbou Lahijani et al., 2019).

2.13 Sensory evaluation

The sensory analysis was carried out using the Quality Index Method (QIM) following the technique of Diler & Genç (2018) with minor modifications. Scores ranging from 0 to 2 were assumed for skin appearance (shining to dull), and fillet color (pink to dark pink). Scores from 0 to 3 were assumed for odor (freshness, seaweed, sour and rancid), and texture (firmness, elastic, soft, and very soft). Scores from 0 to 1.5, 1.5 to 3.0, and 3.5 to 5.0, were expressed as excellent, good, and moderate-quality, respectively.

2.14 Statistical analyses

One-Way ANOVA (for DLS, MIC, MBC, and DPPH), mixed-mode repeated-measurement of ANOVA (for bacteriological results), and Bonferroni test (for comparison of values), Kruskal-Wallis H test, and Mann-Whitney U test (for coating scores in the sensory evaluation), were measured out through SPSS, version 25 (SPSS Inc., Chicago, IL).

3 Results and discussion

3.1 EO composition

The hydrodistilled *L. citriodora* and *L. nobilis* leave formed an amber and light yellow, respectively. GC–MS analysis of the *L. citriodora* EO exhibited 15 compositions with 94.21% of the total EO (Table 1A) (majorly: E.citral (21.19%), Z.citral (18.63%), spathulenol (10.56%), and limonene (9.40%)). These results (Table 1) were in agreement with another finding (Fitsiou et al., 2018), showing that the major components of *L. citriodora* EO were E.citral (26.40%), and Z.citral (17.16%); or another semi-similar results (Kaskoos, 2019). GC–MS analysis of the *L. nobilis* EO indicated 24 compositions with 97.53% of the total EO (Table 1B) (majorly: 1,8- cineol (33.88%), α-terpinyl (15.50%), and sabinene (7.99%)). Verdian-rizi & Hadjiakhoondi (2008) reported that the major components of *L. nobilis* EO were sabinen (5.8-6.5%), cineol (31.4-35.7%), and terpinyl acetate (9.3-12.1%). These differences could be related to the time, place of harvesting, and solvent: sample ratio.

3.2 Fourier-transform infrared spectroscopy

FTIR spectra of *L. citriodora* and *L. nobilis* nanoemulsion, displayed a broad peak at 3417 cm^{-1} (Figure 2A) and 3470 cm^{-1} (Figure 2B), corresponding to free O-H vibration of alcoholic esters (probably geraniol, spathunol, nerolidol, and α–teripenol as well as tween-80) incorporating in the production of the nanoemulsion (Nazari et al., 2019).

Meanwhile, the peaks that appeared around 2925 cm^{-1} in both spectra were attributed to alkane groups (asymmetric-CH_2-), symmetric –CH_3-, and -CH_2- stretching (Figure 2AB) (Khani et al., 2012). According to the percentage of transmission at this wavelength, the components were more in *L. citriodora* (96%) than *L. nobilis (80%),* which were similarly expressed by other researchers (Nazari et al., 2019). The peaks around 1730 cm^{-1} in both spectra, was due to carbonyl groups (C = O ester) vibration (Figure 2AB). Furthermore, the characteristic bonds at 1673 cm^{-1} (Figure 2A), and 1635 cm^{-1} (Figure 2B) could be attributed to C = O stretching of carbonyl (Modnicki et al., 2007). The appeared peak around 1250 cm^{-1} in both spectra can be considered for N-C group in lecithin, respectively (Figure 2AB) (Dehghani et al., 2020).

3.3 Average particle size and Polydispersity Index (PDI)

Emulsions with greater particle size, have higher PDI values (Masarudin et al., 2015), showing the inconsistency of particles (Clayton et al., 2016). A greater PDI value than 0.7, exhibits a wide distribution for particle size, and the inappropriacy of DLS method (Danaei et al., 2018). No significant difference was observed amongst the PDI values for the *L. citriodora* nanoemulsions (Table 2). PDI values for *L. nobilis* were more than that of *L. citriodora* with no significant difference.

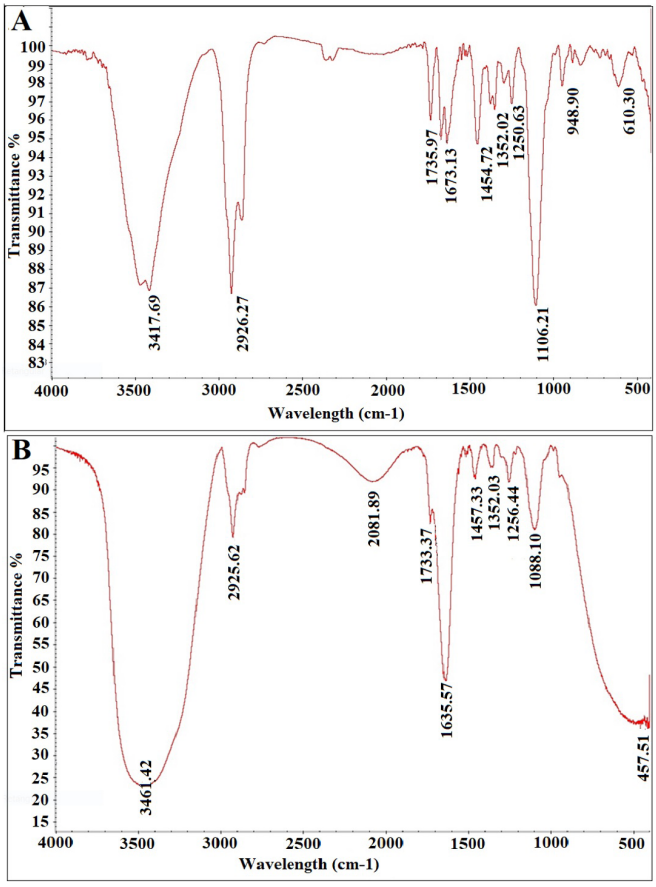

Figure 2. Fourier transform infrared spectroscopic (FTIR) spectra of spectrum of Nanoemulsions-*Lippia citriodora* (A) and *Laurus nobilis* (B) after 90 d of refrigerated storage.

Table 1. GC-MS based analysis for Identification the chemical compounds of EOs of *Lippia citriodora* (A) and *Laurus nobilis* (B).

1A: *L. citriodora* content	Composition (%)	Retention time (min)	Retention index (KI)
6-Methyl-5-hepten-2-one	7.40	15.20	974.00
Sabinen	1.28	15.37	981.00
Limonene	9.40	16.74	1,045.00
3-Methyl-2-methyl-2-butenyl	0.73	17.95	1,102.00
α-Terpineol	1.87	20.26	1,209.00
Z-Citral (Neral)	18.63	21.17	1,252.00
E-Citral (Geranial)	21.19	21.77	1,280.00
Geranyl acetate	2.66	23.77	1,373.00
Trans-Caryophyllene	1.92	25.28	1,443.00
α-Curcumene	4.36	25.99	1,476.00
γ- Cadinene	1.62	26.83	1,516.00
Nerolidol	3.10	27.30	1,537.00
Spathulenol	10.56	27.98	1,569.00
caryophyllene oxide	6.72	28.10	1,575.00
δ-Cadinene	2.77	28.89	1,612.00
Kovalts index = KI			
1B: *L. nobilis* content	Composition (%)	Retention time (min)	Retention index (KI)
Alpha. Thujene	0.66	14.28	930.00
Alpha. Pinene	5.23	14.52	942.00
Camphene	0.51	14.88	958.00
Sabinene	7.99	15.39	982.00
Beta. Pinene	5.05	15.57	991.00
Para Cymene	1.22	16.50	1,034.00
1,8-Cineole	33.88	16.82	1,049.00
Gama. Terpinene	0.92	17.34	1,073.00
Cis- Sabinene hydrate	0.88	17.52	1,082.00
Linalool	4.12	18.11	1,109.00
Trans- Sabinene hydrate	0.72	18.23	1,115.00
Terpenene-1-ol	0.69	18.77	1,140.00
Trans- Pinocarveol	1.29	19.22	1,161.00
3-Cyclohexen	1.03	19.68	1,182.00
Terpinen-4-ol	4.94	20.04	1,199.00
Alpha. Terpineol	3.16	20.24	1,208.00
Beta. Myrcene	0.44	20.89	1,239.00
Thymol	1.96	22.14	1,297.00
Phenol	0.85	22.36	1,307.00
Alpha. Terpinyl	15.50	23.49	1,350.00
Eugenol	3.22	24.05	1,366.00
1,2-Benzenedi Carboxylic acid	1.38	24.58	1,411.00
Caryophyllene Oxide	1.27	28.04	1,426.00
Beta. Eudesmol	0.62	29.17	1,525.00

Kovalts index = KI.

In this study, PDIs of *L. citriodora,* and *L. nobilis* nanoemulsions were respectively 0.39 and 0.44, following 3 months (Table 2) with no significant differences ($p > 0.05$), confirming the stability of nanoparticles preserved at refrigerated temperatures up to 90 days.

Table 2 illustrates the increasing trend of particle size in *L. citriodora* and *L. nobilis* nanoemulsions during time storage from 24.8 ± 0.1 to 60.80 ± 1.07 nm, and from 15.5 ± 0.1 to 28.30 ± 0.20 nm, respectively, is occurred due to the Ostwald ripening (Liu & Hu, 2020), changes in the concentration of the main ingredients, and carbon chain length. However, the largest particle size belonged to *L. citriodora* after 90 days, and the smallest belonged to *L. nobilis* on the first day, indicating the higher PDI value of *L. nobilis* than *L. citriodora,* being very low to change the PDI and finally the stability.

3.4 AFM Images

Figure 3 illustrates chemical-based differences on the surface of the materials with different colors. The particle size of *L. citriodora*

and *L. nobilis* nanoemulsion ranged from 30-60 and 15-25 nm, respectively (with a negligible number of larger particles) after 90 days of refrigerated storage, indicating the stability of *L. nobilis* nanoemulsion. The observed less inharmonious surface in the 3D images indicated uniform size distribution. These results were in agreement with DLS results (Table 2), demonstrating that the average particle size of the *L. citriodora* and *L. nobilis* nanoemulsion were 60.80 and 28.3 nm, respectively. The smaller particle sizes make the emulsion resist more against droplet aggregation (Khoshbou Lahijani et al., 2019).

Table 2. The *Dynamic light scattering* analysis of *Lippia citriodora* and *Laurus nobilis* Nanoemulsions on droplet size and polydispersity index (PDI). Each measurement represents mean ± SD, n = 3.

EO	D	Particle size (nm)	PDI
L. citriodora	1	24.80 ± 0.10[a]	0.37[a]
	3	26.30 ± 0.25[a]	0.39[a]
	7	28.60 ± 0.55[a]	0.40[a]
	14	36.70 ± 0.45[b]	0.40[a]
	90	60.80 ± 1.07[c]	0.39[a]
L. nobilis	1	15.50 ± 0.10[a]	0.39[a]
	3	16.80 ± 0.10[a]	0.40[a]
	7	19.60 ± 0.10[b]	0.41[a]
	14	21.60 ± 0.05[b]	0.42[a]
	90	28.30 ± 0.20[c]	0.44[a]

PDI, polydispersity index. Different lowercase superscripts for each plant and column indicate a significant difference between d ($p < 0.05$).

3.5 Determination of the antioxidant capacity of L. citriodora and L. nobilis

The DPPH radical inhibition of *L. citriodora* and *L. nobilis* Nanoemulsion compared to their pure EOs, and BHT. The concentration of the treatment affects the antioxidant activity (100%, and 12%, for EOs, and nanoemulsions, respectively). The ratio of EOs in pure oil to those in the nanoemulsions was 8.33, demonstrating higher antioxidant activity of pure oils. However, the lowest and highest DPPH inhibition were related to *L. citriodora* EOs, and *L. nobilis* nanoemulsion, respectively. *L. nobilis* nanoemulsion revealed a significant difference compared to other groups (specifically to BHT as a commercial antioxidant). The DPPH radical inhibition of *L. citriodora* nanoemulsion was

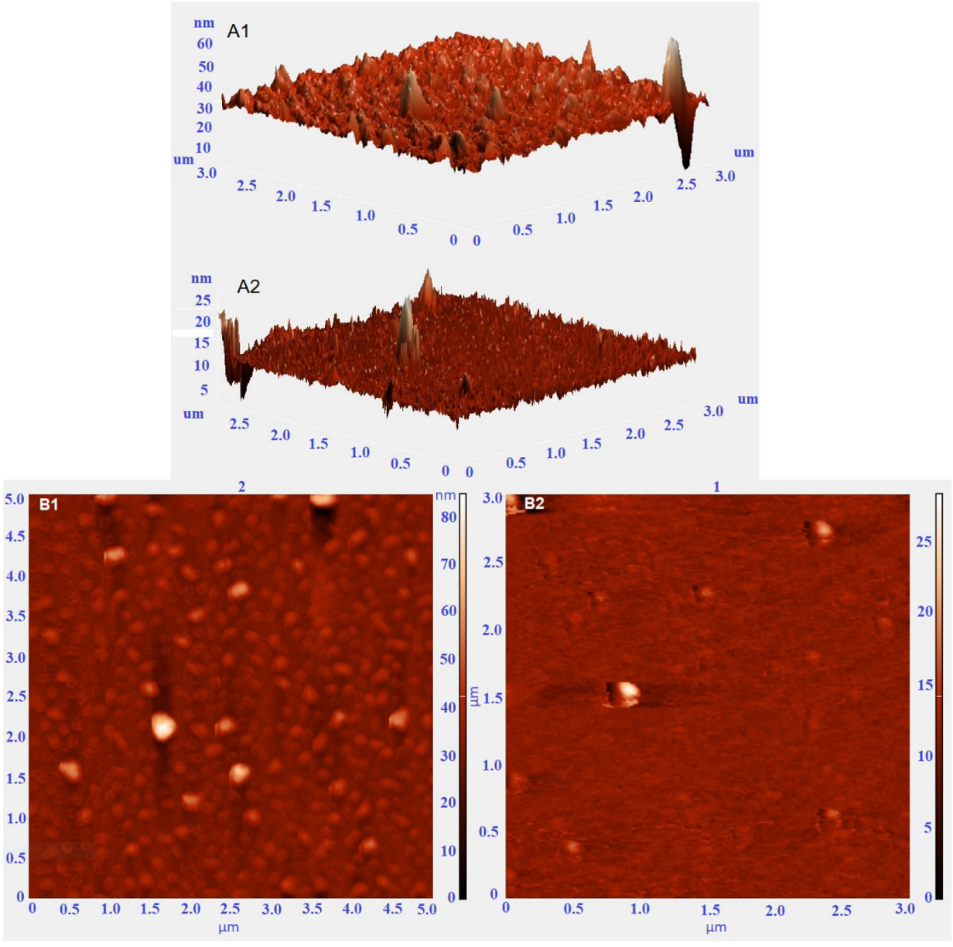

Figure 3. Atomic Force Microscope (AFM) images. The 3-dimension (A) and 2-dimension (B) AFM micrographs of Nanoemulsion-*Lippia citriodora* (1) and *Laurus nobilis* (2) were taken at room temperature. The heights of 35-60 and 15-25 nm of NPs were observed after 90 d of refrigerated storage, respectively, for *Lippia citriodora* and *Laurus nobilis*.

75.85%, significantly ($p < 0.05$) greater than that of *L. citriodora* EO (62.72%), but lower ($p < 0.05$) than that in the BHT (92.66%). Due to a higher surface active area in a form of tiny droplets and larger surface area in *L. citriodora*, it could better scavenge the DPPH radicals when compared to the pure EO. While the concentration of the nanoemulsion was too low when compared to the pure EOs. Accordingly, it was hypothesized that a greater concentration of nanoemulsion of *L. citriodora* results in further antioxidant activities through an increased number of phenolic compounds and inhibitory activities of free radicals.

Similarly, there was a higher antioxidant capacity of *L. nobilis* nanoemulsion than its free form. The DPPH inhibition for it was 98.37%, which was significantly more than that of BHT ($P < 0.05$). Whereas, *L. nobilis* EOs did not depict a significant difference ($P > 0.05$). In a research, it was reported that the DPPH inhibition by *L. nobilis* EO was approximately 60% for concentrations greater than 1.0 µL/mL (Basak & Candan, 2013), whereas, in this study, it was shown 83.84%. Figure 4 illustrates that nanoemulsions of both *L. citriodora* and *L. nobilis* had more DPPH inhibition activity than their free forms. The justification for this can be the presence of EOs in the form of nanodroplets.

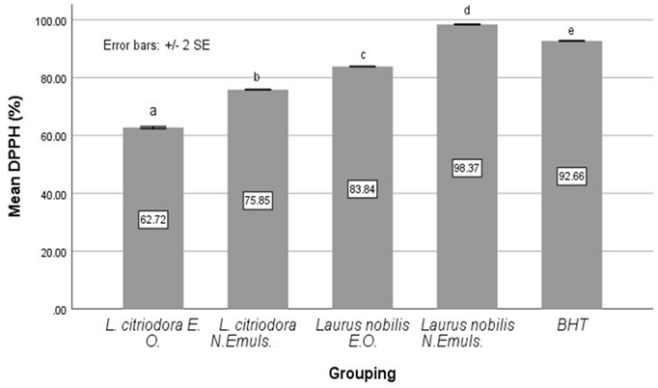

Figure 4. Values (%) of the DPPH radical inhibition obtained from *L. citriodora* and *L. nobilis* (EO and Nanoemulsion) in comparison to BHT. Different small-scripts above each column indicate a significant difference ($p < 0.05$). N.Emuls: Nanoemulsion.

By decreasing the size of nanodroplets, their surface area is increased. Previous studies have reported that the smaller nanodroplets, the more antioxidant activity (Nabil et al., 2020). It can also be hypothesized that nanoemulsions with smaller nanodroplets show higher stability, thereby, the nanodroplets always have the high surface area and are ready to act as an antioxidant (Erdmann et al., 2015). To sum up, indicating that their excellent antioxidant potential could be a good candidate for fish packaging engineering.

3.6 Antibacterial analysis

The impact of nanoemulsions and EOs on the inhibition of four public health pathogens was carried out by considering the ratio of MIC value per the amount of the employed EOs (Real MIC/MBC) (signed as "Ψ"), being defined temporarily as "MIC or MBC × *C*" (the concentration of the EO in each treatment).

Comparatively, nanoemulsions showed lower Ψ ($P < 0.01$) for *E. coli, S. aureus, Salmonella spp.,* and *P. aeruginosa,* and more antibacterial activity (Table 3). *L. citriodora* EO gained lower Ψ, and greater antibacterial performance compared to the *L. nobilis* EO ($P < 0.05$). Nanoemulsion of *L. citriodora*, showed better antibacterial activity (lower Ψ) against all bacteria based on MIC results ($P < 0.05$), which means that the less amount of the *L. citriodora* will show stronger inhibition, being attributed to the differences in the main components concluded from the FTIR analysis.

Based on MBC results, the lowest Ψ was recorded for *L. citriodora* nanoemulsion against all bacteria ($P < 0.05$). Similarly, *L. citriodora* EO showed lower Ψ against all bacteria compared to *L. nobilis* EO ($P < 0.05$). The *L. citriodora* nanoemulsion showed the lower Ψ against *E. coli, Salmonella spp.,* and *S. aureus* in contrast with its free form ($P < 0.05$). *L. nobilis* nanoemulsion depicted the lower Ψ against all bacteria in contrast with their pure EOs.

Based on MBCs results of previous studies, *L. citriodora* EO against *E. coli* and *S. aureus* was 12.48, and 9.73 mg/mL, respectively (Oukerrou et al., 2017). These lower efficiencies are attributed to the usage of less aqueous extract or calibrated extract methodology rather than EOs being applied in this study.

Table 3. Minimum inhibitory concentration (µg/mL) and Minimum bactericidal concentration (MBC) performed with EOs and Nanoemulsions of *Lippia citriodora* and *Laurus nobilis* against selected bacteria 48 h ($n = 3$).

Test	Coatings	*E. coli*	Ψ(µg/mL)	*S. aureus*	Ψ(µg/mL)	*P. aeruginosa*	Ψ(µg/mL)	*Salmonella* spp.	Ψ(µg/mL)
MIC 48 h	EO of *L. citriodora*	0.39 ± 0.05	0.39 ^{Aa}	1.56 ± 0.05	1.56 ^{Ab}	1.56 ± 0.05	1.56 ^{Ab}	0.78 ± 0.05	0.78 ^{Ac}
	Nanoem. *L. citriodora* 12%	0.78 ± 0.05	0.09 ^{Ba}	1.56 ± 0.05	0.19 ^{Bb}	6.25 ± 0.05	0.75 ^{Bc}	1.56 ± 0.05	0.19 ^{Bb}
	EO of *Laurus nobilis*	1.56 ± 0.05	1.56 ^{Ca}	6.25 ± 0.05	6.25 ^{Cb}	6.25 ± 0.05	6.25 ^{Cb}	3.13 ± 0.05	3.13 ^{Cc}
	Nanoem. *L. nobilis* 12%	1.56 ± 0.05	0.19 ^{Da}	3.13 ± 0.05	0.38 ^{Db}	25.00 ± 0.05	3 ^{Dc}	6.25 ± 0.05	0.75 ^{Ad}
MBC 48 h	EO of *L. citriodora*	0.78 ± 0.04	0.78 ^{Aa}	1.56 ± 0.04	1.56 ^{Ab}	1.57 ± 0.04	1.57 ^{Ab}	0.78 ± 0.04	0.78 ^{Aa}
	Nanoem. *L. citriodora* 12%	0.78 ± 0.04	0.07 ^{Ba}	1.57 ± 0.04	0.19 ^{Bb}	12.50 ± 0.04	1.5 ^{Ac}	1.57 ± 0.04	0.19 ^{Bb}
	EO of *Laurus nobilis*	1.57 ± 0.04	1.57 ^{Ca}	6.33 ± 0.04	6.33 ^{Cb}	6.33 ± 0.04	6.33 ^{Cb}	3.13 ± 0.04	3.13 ^{Cc}
	Nanoem. *L. nobilis* 12%	1.57 ± 0.04	0.19 ^{Da}	3.13 ± 0.04	0.38 ^{Db}	25.00 ± 0.04	3 ^{Dc}	12.50 ± 0.04	1.5 ^{Dd}

Different lowercase superscripts for each attribute indicate a significant difference between bacterial species ($p < 0.05$). Different uppercase superscripts for each attribute indicate a significant difference between coatings ($p < 0.05$). nanoem.: Nanoemulsion

For *L. nobilis* EO and nanoemulsion, MIC and MBC values showed 1.5 µg/mL against *E. coli*, which was higher than those of *L. citriodora*. Meanwhile, Tomar et al. (2020) exhibited that MIC and MBC values were respectively 0.75 and 0.50 mg/L, which were slightly lower than those in this study (Table 3).

EOs and nanoemulsions of *L. citriodora* showed no significant differences ($p>0.05$) in MIC and MBC against *S. aureus* (1.56 µg/mL). *Tomar* and his colleagues reported lower values for *L. citriodora* (0.75 and 0.25 µg/L, respectively) (Tomar et al., 2020), while *Fitsiou* and his coworkers reached greater results (Fitsiou et al., 2018) equal to 923.0 µg/mL. Considering *L. nobilis*, *Silveria* and her colleagues reported the MIC and MBC of approximately 10.0 µg/mL against *S. aureus* (Silveira et al., 2012), while in our study, the proportional value was 1.5 µg/mL (Table 3). Varieties in the number of active ingredients are deeply rooted in the plant origin, method of extraction, and operational parameters.

According to Table 3, it can be concluded that just 12 µL of the EOs (*L. citriodora and L. nobilis*) in the form of nanoemulsion can show acceptable antibacterial activity in comparison with 100 µL of their pure EOs. It was hypothesized that enhanced concentrations of both nanoemulsions will result in better MIC and MBC values compared to their pure EOs. The reason might turn back to the nano-dimension of the droplets of the EOs in nanoemulsions, (24, and 15 nm, respectively). EO has an easy passive transport through the bacterial cell membrane, being generalized to the previous studies using EOs in the form of nanoemulsions (Moghimi et al., 2016; Prakash et al., 2020). The nanodroplet in the nanoemulsion can deliver the EO on the surface of the cell membrane, while due to little solubility in water, pure EO cannot penetrate easily within the cell membrane.

Although the size of the nanodroplets in *L. nobilis* nanoemulsion was smaller than that of *L. citriodora*, nanoemulsion of *L. citriodora* showed better antibacterial activity than that of the *L. nobilis* nanoemulsion. It can be hypothesized that *L. citriodora* inherently owns higher antibacterial activity probably due to differences in the amount of the active ingredients (Figure 2). Considering the EOs, it is vital to detect the main antibacterial agents for purification. This strategy will enhance the antibacterial activity of EOs. The same can be considered for antioxidant activity.

Table 4 shows the inhibition zones induced by the interactions between the *L. citriodora*, *L. nobilis* EOs, and nanoemulsions with *E. coli* and *S. aureus*. Due to the different concentrations, the parameter of đ (mm/µL) was defined to show the diameter of the inhibition zone per one µL of the EOs in each form (free or nanodroplet). The more đ is related to higher antibacterial activity.

In the case of *E. coli*, it was observed that the most and least đ was attributed to the nanoemulsion of *L. citriodora* (0.64 mm/µL), and *L. nobilis* EOs (0.1 mm/µL), respectively. On contrary to the comparison between Nanoemulsions of *L. citriodora and L. nobilis,* EO of *L. citriodora* and *L. nobilis* did not show a significant difference ($P > 0.05$). In the case of *S. aureus*, the higher antibacterial activity belonged to the nanoemulsion of *L. citriodora* (đ = 0.69 mm/µL), and the weakest reported for both of the EOs in their free form (đ = 0.12 mm/µL), showing no significant difference compared with each other ($P > 0.05$). Similar to *E. coli*, the nanoemulsion of *L. citriodora* obtained higher đ in comparison with *L. nobilis* Nanoemulsion against *S. aureus*.

The Nanoemulsion of *L. citriodora* depicted a significant difference in antibacterial activity compared with Neomycin against *E. coli* ($P < 0.05$), while no significant difference was observed between them against *S. aureus* ($P > 0.05$). The Nanoemulsion of *L. nobilis* showed an insignificant difference in antibacterial efficacy compared to that of Neomycin ($P > 0.05$). The free form of the EOs did not reveal a significant difference compared with that of Neomycin ($P > 0.05$). Regarding Gentamycin, against whether *E. coli* or *S. aureus, a* significant difference was observed in contrast with other treatments including EOs and their nanoemulsion form ($P < 0.05$). Nanoemulsion of both EOs illustrated better results than their free form. It was hypothesized that an increment in the concentration of nanoemulsions could increase their antibacterial activity as well as gentamicin. For instance, based on Table 4, $đ_{Gentamycin}$/ $đ_{NanoEm.}$ *L. citriodora* equals 2.82 which means by increasing the concentration of the *L. citriodora* to 34% (2.82 × 12% = 34%) one can expect similar efficacy to gentamicin. However, in this case, the size of the nanodroplets and also their PDI and stability index should not be neglected.

Previous studies reported that inhibition zones were 10.0 and 2.1 mm, respectively (Yilmaz et al., 2013) for *L. nobilis* EO and Erythromycin against *S. aureus* (far lower than those in our study) while those of *E. coli* were 33.0 and 0.0 mm (*L. nobilis* and Erythromycin, respectively), which was relatively similar to the results herein (Table 4). Other researchers (Fidan et al., 2019) found different results. The inhibition zone was 8.0 and

Table 4. Inhibition zone (mm) measured with essential oil and Nanoemulsion of *Lippia citriodora* and *Laurus nobilis* (*n*=3)

Disk	*E. coli*	đ (mm/µL)	*S. aureus*	đ(mm/µL)
EO *L. citriodora*	29.12 ± 0.02^{aA}	0.12^{Aa}	30.65 ± 0.02^{aA}	0.12^{Aa}
Nanoem. *L. citriodora* 12%	19.46 ± 0.02^{aB}	0.64^{Ba}	20.79 ± 0.02^{aB}	0.70^{Ba}
EO *Laurus nobilis*	26.40 ± 0.02^{aA}	0.10^{Aa}	30.43 ± 0.02^{bA}	0.12^{Aa}
Nanoem. *L. nobilis* 12%	15.96 ± 0.02^{aB}	0.53^{Ca}	16.14 ± 0.02^{aC}	0.53^{Ca}
gentamicin	18.10 ± 0.02^{aB}	$1.81\ mm/\mu g^{DA}$	22.41 ± 0.02^{bB}	$2.24\ mm/\mu g^{DB}$
Neomycin	17.11 ± 0.02^{bB}	$0.57\ mm/\mu g^{CA}$	21.39 ± 0.02^{aB}	$0.71\ mm/\mu g^{BB}$

In order to compare two bacteria, small superscripts in each row indicate a significant difference ($p < 0.05$). Different capital superscripts in each column indicate a significant difference ($p < 0.05$). nanoem.:Nanoemulsion.

15.1 mm, respectively for *E. coli* and *S. aureus* in the case of EO of *L. nobilis*. Some researchers (Fitsiou et al., 2018) showed that *S. Enteritidis*, *Salmonella typhimurium*, and *Pseudomonas fragi* were sensitive to *L. citriodora* EO. All bacteria were sensitive to citral, which involved approximately 40% of the *L. citriodora* EO. Thus, its antibacterial activity seems to be efficient.

Table 5 shows the count of the selected bacteria, which were separately inoculated to rainbow trout fillets on the zero of the study. Considering the first day of the experiment, no significant difference was observed between the control group and the treated groups ($p > 0.05$). The EO and Nanoemulsion of *L. citriodora* and *L. nobilis* alone or incorporated with PLA could retard the count of *S. aureus* about 0.4-0.7 log CFU/g on the first day and 0.8-0.12 on the 3rd day in comparison to the control group. On the 7th day of the cold storage, PLA/*L. citriodora* nanoemulsion film could be the strongest compound against *S. aureus* in the fish samples (1.68 log CFU/g) with no significant differences ($p > 0.05$) compared to that of PLA/*L. nobilis* EO and other treatments ($p>0.05$). In the control group, *E. coli* could not be stable in the refrigerated temperature since its count was less than 1 log CFU/g on the third and seventh days. *E. coli* count was not determined until the third day in the rainbow trout samples wrapped with EO or Nanoemulsion of PLA/*L. citriodora* or *L. nobilis*. It reached less than 1.0 log CFU/g in the bacterial-inoculated fillets wrapped with PLA loaded with EOs or Nanoemulsions of *L. citriodora* and *L. nobilis* after the seventh day. PLA was hypothesized to affect the barrier properties by reducing oxygen penetration, moisture removal, and volatile substances release resulting in preventing oxidation and disturbing the required conditions for microorganism growth (Lagarón, 2011). Our findings (Table 4) showed that both EO and nanoemulsion of *L. citriodora* had higher antibacterial potentials. When they were integrated with PLA (Table 5), they exhibited that PLA/*L. citriodora* nanoemulsion owns significant antibacterial activity ($p < 0.05$). This could be due to the size of the nanodroplets and their appropriate stability up to day 90 (Figure 3) as well as the role of PLA in the refrigerated temperature (Sotiriou & Pratsinis, 2010). The small nanodroplets show higher potential against microorganisms that the rationale behind this is the

Table 5. The effect of different types of coatings of *Lippia citriodora* or *Laurus nobilis* on the count of selected bacteria (log CFU/g) in the refrigerated rainbow trout during the 7-day evaluation. Each measurement represents mean ± SE, $n = 3$.

Coatings	D	*S. aureus*	*E. coli*	*P. aeruginosa*
Control	1	3.00 ± 0.00^{aA}	nd	<2.0
	3	3.11 ± 0.00^{aA}	<1.0	<2.0
	7	2.89 ± 0.00^{aA}	<1.0	<2.0
L.C. EO	1	2.30 ± 0.00^{aA}	<1.0	<2.0
	3	2.30 ± 0.01^{aB}	<1.0	<2.0
	7	2.30 ± 0.00^{aB}	<1.0	<2.0
L.C. Nanoemulsion	1	2.46 ± 0.00^{aA}	<1.0	<2.0
	3	2.30 ± 0.01^{aB}	<1.0	<2.0
	7	1.99 ± 0.00^{aB}	<1.0	<2.0
L.N. EO	1	2.46 ± 0.00^{aA}	<1.0	<2.0
	3	2.30 ± 0.01^{aB}	<1.0	<2.0
	7	1.99 ± 0.00^{aB}	<1.0	<2.0
L.N. Nanoemulsion	1	2.46 ± 0.00^{aA}	<1.0	<2.0
	3	2.30 ± 0.01^{aB}	<1.0	<2.0
	7	1.99 ± 0.00^{aB}	<1.0	<2.0
PLA	1	2.45 ± 0.00^{aA}	nd	<2.0
	3	1.92 ± 0.01^{bB}	<1.0	<3.0
	7	1.80 ± 0.00^{bB}	<1.0	<2.0
PLA/ L.C. EO	1	2.64 ± 0.00^{aA}	nd	<2.0
	3	2.75 ± 0.01^{aAB}	nd	<2.0
	7	2.18 ± 0.00^{aB}	<1.0	<2.0
PLA/L.C. Nanoemulsion	1	2.54 ± 0.00^{aA}	nd	<2.0
	3	2.25 ± 0.01^{aB}	nd	<2.0
	7	1.68 ± 0.00^{bB}	<1.0	<2.0
PLA/ L.N. EO	1	2.64 ± 0.00^{aA}	nd	<2.0
	3	2.17 ± 0.01^{aB}	nd	<2.0
	7	1.73 ± 0.00^{bB}	<1	<2.0
PLA/L.N. Nanoemulsion	1	2.50 ± 0.00^{aA}	nd	<2.0
	3	2.56 ± 0.01^{aB}	nd	<2.0
	7	2.25 ± 0.00^{aB}	<1.0	<2.0

L.C.: *Lippia citriodora*, L.N.: *Laurus nobilis*, nd: Not determined, Different lowercase superscripts at each coating group indicate a significant difference ($p < 0.05$). To compare between all groups, uppercase superscripts in each column and same d indicate a significant difference ($p < 0.05$).

enhancement in the surface area of droplets (Erdmann et al., 2015). *P. aeruginosa* revealed less than 2 log CFU/g in the rainbow trout samples after the seventh day of the cold storage with no significant differences ($p > 0.05$) compared to those of the other sampling days (Table 6). EO nanoemulsions, or PLA/EO, PLA/ nanoemulsions could control the growth of *P.aeuroginosa* on fish fillets since there was no bacterial growth observed during storage. However, to compare the antimicrobial activity of those treatments, the initial concentration of *P.aeuroginosa* on fish fillets should be increased. Generally, psychotropic bacteria, particularly for specific spoilage organisms (SSOs) such as *Pseudomonas spp*., a gram-negative strictly aerobic bacterium, could be used as a good indicator for refrigerated fish quality (Mai & Huynh, 2017). The results observed in this study suggested that EO and Nanoemulsions of the selected herbs in combination with PLA (decrease available oxygen to bacteria; (Heydari-Majd et al., 2019)(, could be used as a package film to control the growth of *P. aeruginosa* in fish fillets during the cold storage.

Table 6. The effects of different types of *Lippia citriodora* and *Laurus nobilis* coatings on the total viable bacteria count (log CFU/g) isolated from fish fillets without bacterial inoculation during the 7-day evaluation. Each measurement represents mean ± SE, $n = 3$.

Coatings	1	3	7
Control	4.38 ± 0.00^{aA}	4.55 ± 0.01^{aA}	6.53 ± 0.00^{bA}
EO *L. citriodora*	4.37 ± 0.00^{aA}	4.11 ± 0.01^{aA}	6.10 ± 0.00^{bB}
Nanoem. *L. citriodora*	4.55 ± 0.00^{aA}	4.23 ± 0.01^{aA}	6.04 ± 0.00^{bB}
E.O. *Laurus nobilis*	4.27 ± 0.00^{aA}	3.60 ± 0.01^{bB}	5.90 ± 0.00^{cB}
Nanoem. *L. nobilis*	4.35 ± 0.00^{aA}	4.18 ± 0.01^{aA}	6.28 ± 0.00^{bAB}
PLA	4.62 ± 0.00^{aA}	4.25 ± 0.00^{aA}	6.34 ± 0.00^{bAB}
PLA EO *L. citriodora*	3.89 ± 0.00^{aB}	4.72 ± 0.01^{bA}	5.49 ± 0.00^{cB}
PLA Nanoem. *L. citriodora*	4.04 ± 0.00^{aB}	3.28 ± 0.01^{bB}	4.95 ± 0.00^{cC}
PLA EO *Laurus nobilis*	3.85 ± 0.00^{aB}	3.66 ± 0.00^{aB}	4.82 ± 0.00^{bC}
PLA Nanoem. *L. nobilis*	4.07 ± 0.00^{aB}	3.48 ± 0.00^{aB}	4.72 ± 0.00^{bC}

Different lowercase superscripts for each row indicate a significant difference ($p < 0.05$) between d of sampling. To compare the total viable bacteria count between coatings at each sampling d, the uppercase superscripts in each column indicate a significant difference ($p < 0.05$). nanoem.: Nanoemulsion

Embedding the EO or nanoemulsion of *L. citriodora* and *L. nobilis* caused a reduction in oxygen penetration and controlled the moisture level, leading to higher antibacterial and antioxidant activities. This was the main reason for the higher efficiency of PLA-packaged films in contrast with the immersed treatments. However, previous studies depicted that the EO Nanoemulsion can improve the barrier properties like the water vapor permeability, resulting in controlling the level of moisture content (Dammak et al., 2017).

Table 6, represented the TVBC of the fish fillets treated with different produced films and coatings. There were no significant differences ($p > 0.05$) on the initial concentration of TVBC observed in the fillets treated with PLA/ EO *L. citriodora* or *L. nobilis* and PLA/ nanoemulsions of *L. citriodora* and *L. nobilis* on the first day of analysis. However, the least TVBC belonged to the groups of PLA/Nanoemulsions of *L. citriodora* (3.28 log CFU/g) and PLA/Nanoemulsion of *L. nobilis* (4.72 log CFU/g) were observed on the third and seventh day of storage. These results indicated the antimicrobial activity of the PLA/ Nanoemulsions film against bacteria in the fillets. In general, the maximum acceptable level (MAL) of the TVBC for fresh or frozen fish has been reported to be 7 log CFU/g (Raeisi et al., 2020). Since the PLA loaded with EO or nanoemulsion of both herbs could remarkably show the ability to decrease the TVBC in fish samples, it confirmed that PLA films loaded with nanoemulsion of *L. citriodora* and *L. nobilis* can be used as an effective package for prolonging the quality of fish fillets. These results were in line with several previous studies (Ma et al., 2018; Nilsuwan et al., 2020).

3.7 Sensory evaluation

Major spoilage in fish meat and its products is due to bacterial growth. Loss or change in color, texture, and odor properties with increasing refrigeration time can be caused by compounds resulting from the oxidation of fatty acids, and producing aldehydes and ketones (Tokur et al., 2006). Figure 5 demonstrates the sensory evaluation of the refrigerated rainbow trout fillets due to the application of different types of

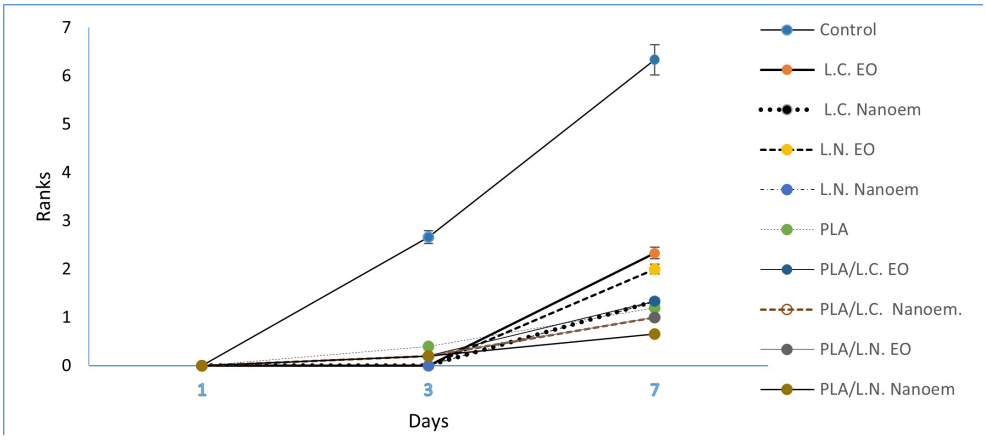

Figure 5. The effects of different types of coatings of *L. citriodora* and *Laurus nobilis* on sensory criteria during the 7-day evaluation. Each measurement represents mean ± SD, $n = 3$.

coating individually or incorporated with PLA film. Accordingly, the sum of scores for all the criteria was shown clearly on the first and third days. This situation continued up to the seventh day except for EOs of *L. citriodora* and *L. nobilis* (good score); so that they were dedicated "excellent" scores. In general, the acceptance of appearance, color, odor, and texture parameters of fillets did not change significantly over time except for the control treatment ($P < 0.05$). The undesired odor observed from the control treatment on the seventh day was probably due to the breakdown of protein and the growth of microorganisms. The use of EOs and Nanoemulsions, alone or in combination in the film matrix not only had no negative effects on the sensory properties of fish fillets but also kept the quality an acceptable level compared to control and pure PLA during storage.

4 Conclusion

The nanoemulsions incorporated with PLA, were analyzed in terms of size properties, stability, morphologically, antioxidant/antimicrobial efficacies against different bacteria (including *S. aureus*, *P. aeruginosa, Salmonella spp., E. coli*). According to the AFM analyses, both nanoemulsions had homogenous dispersity. The *L. nobilis* nanoemulsion showed antioxidant ability greater than the commercial antioxidant (BHT), and antibacterial activity less than another nanoemulsion. Notable antibacterial activities were reported for the nanoemulsions forms of both EOs compared with their free form, and the reason was attributed to the nanodroplet size. Thereafter, the type and quantity of the EOs affect the efficacy of the prepared PLA-based films/coating. Both Nanoemulsion-based PLA films kept sensory properties, leading to prolongation of shelf life for Rainbow trout up to the seventh day at the refrigerator condition. Overall, this study shows the potential of *L. citriodora* and *L. nobilis* nanoemulsions embedded within PLA for usage as active food packaging.

Ethical approval

This is an observational study. The IAU Research Ethics Committee has confirmed that no ethical approval is required.

Author contributions

Hojatoleslami. M: Preparation of the last version of manuscript, literature review, data curation, visualization, designation of images and tables, and final arrangement of the data. *Ahari. H*: Supervision, verification of the manuscript, academical and grammatical peer revision, designation of methodologies, and major conceptualization. *Larijani. K*: scientific consultations, critical revision, designation of methodologies. *Sharifan. A*: conceptualization, revision, methodology, validation of data, edition, and visualization.

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