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Permeation kinetics of nano-emulsified tarragon essential oil in fermented pickled cucumbers by FTIR, antioxidant capacity potentiometry, and antimicrobial properties

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Abstract

The permeation kinetics of nano-emulsified tarragon essential oil (TEO) into fermented pickled cucumber texture was measured by Fourier transform infrared spectrometry (FT-IR) and antioxidant capacity potentiometry. The objective was to evaluate how nano-emulsion affect the sensory, antioxidant, physicochemical, and microbial flora characteristics of the pickled cucumbers. Oil-in-water nano-emulsions (100, 200, 400 and 800 mg L^{-1}) were prepared by ultrasonic waves and were added to the brine/vinegar and cucumber mixture. The analysis of chemical compounds by gas chromatography showed that the main components of TEO were methyl chavicol, Z-β-ocimene, E-β-ocimene, limonene, and methyl eugenol. The average particle size of nano-emulsions was ranging from 19.03 to 218.6 nm. The sensory evaluation of pickled cucumber containing nano-emulsified TEO showed that higher TEO concentrations increased the sensory characteristics significantly in the pickled cucumbers, such as their color, aroma, taste, mouthfeel, texture crispness, and overall acceptance. The microbial test proved the inhibitory effect of TEO on *Penicillium expansum* and *Aspergillus flavus* species. According to the results of FTIR, the nano-emulsified TEO penetrated well into the pickled cucumber texture. The antioxidant capacity of the pickled cucumbers revealed that by increasing the concentration of TEO, the antioxidant capacity of the pickled cucumber texture increased significantly ($p < 0.05$). Using nano-emulsified TEO can be a good replacement for dried vegetables in producing fermented pickled cucumbers.

Keywords Nano-emulsion, Pickled cucumber, Microbial flora, Permeation kinetics

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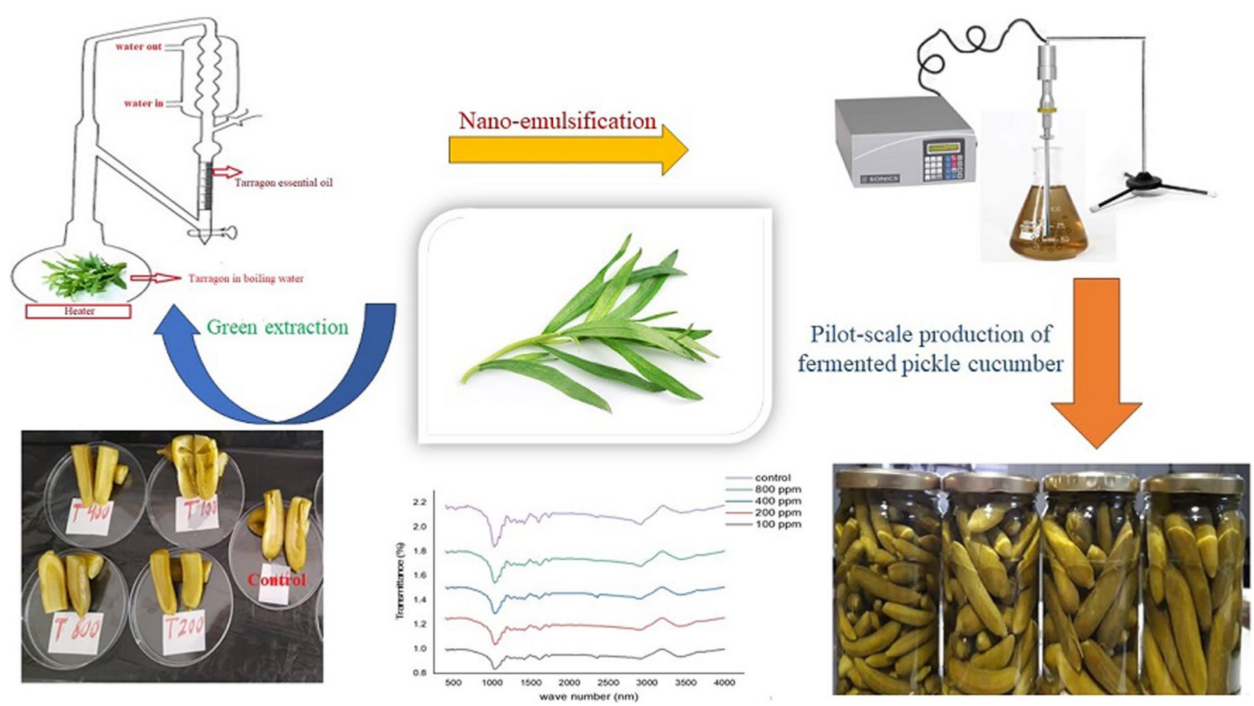
Graphical Abstract



Introduction

Flavoring agents are valuable ingredients in food formulations. They have significant roles in consumer satisfaction and largely affect food consumption (Madene et al., 2006). Most existing flavoring agents are produced through chemical synthesis or extraction from natural compounds, which are usually expensive, delicate, and volatile, and food manufacturers are usually concerned about conserving these precious additives (Milanovic et al., 2010).

Fermentation and bulk storage of pickled cucumber in tanks is a method for preparing fermented product that allows the preservation of cucumber for a long time and lead to the production of a unique food product called fermented pickled cucumber. Fermented pickled cucumber belongs to products stabilized with salt and lactic acid produced by lactic acid bacteria spp. (Di Cagno et al., 2013). Sometimes, fermented pickled cucumber load is susceptible to spoilage. This spoilage leads to an increase in production costs by applying more control over the fermentation process. Generally, the source of pickled cucumber spoilage is fermented raw materials such as aromatic vegetables such as dill and tarragon, which are heavily contaminated with mold spores, yeasts, spore-forming microbial agents, and soil Enterobacteriaceae, and can easily lead to product spoilage (Rawat, 2015).

The tarragon plant (*Artemisia dracunculus*) is a member of the Asteraceae family and has many properties (Ayoughi et al., 2011). The aromatic compounds of the tarragon plant generally include methyl chavicol, trans-ocimene, Z-β-ocimene, limonene, and α-pinene, which, in addition to the pleasant aroma and taste, have antimicrobial, antioxidant, and anti-cancer properties (Chaleshtori et al., 2011; Di Cagno et al., 2013; Osanloo et al., 2017). Adding aromatic vegetables such as tarragon and dill in the form of fresh or dried plants to fermented pickled cucumber is associated with microbial and fungal contamination and can cause product spoilage (Rawat, 2015). To solve the contamination problem, these vegetable essential oils (EOs) can be used. However, another problem is related to the solubility and stability of EOs in aqueous fermentation environments. Also, due to the hydrophobic nature of EOs, their permeation into fermented pickled cucumber is done slowly, and it seems that the solution to this problem can be achieved by emulsifying EOs.

In the past years, there has been an increasing demand from consumers for natural products with microbial safety and high organoleptic properties. The tendency to use EOs as an antimicrobial and natural preservative in the food industries has increased. Nano-emulsions as carriers of EOs cause the intensification of antimicrobial activities (Echeverría & Duarte Galhardo de Albuquerque, 2019). In this way, nano-emulsion containing EOs with high dispersion in all food textures can increase the effectiveness of plant EOs as antioxidant and antimicrobial compounds in food systems. Thus, the desirable aroma of EOs in the entire food texture can be distributed equally (Donsì et al., 2012). The reviews of past research showed that no research aimed at discerning the use of nano-emulsified EOs and investigating their permeation kinetics in fermented pickled cucumber texture. Therefore, this research aimed to investigate the physicochemical, antioxidant properties, sensory and microbial properties of fermented pickled cucumbers containing nano-emulsified TEO. Also, it dealt with the permeation kinetics of this nano-emulsified TEO in the structure of fermented pickled cucumber.

Materials and methods

Royal variety green cucumber was from Jahrom's local market, and De Man–Rogosa–Sharpe (MRS) agar and Sabro dextrose agar from Merck Co., Germany. Emulsifier Tween 20 was from Sigma Aldrich Co.

TEO extraction

The tarragon plant sample was purchased from the local field and after washing the plants was dried in ambient temperature (28 ± 2 °C) for 72 h. TEO isolation was done by hydro-distillation method by using Clevenger type apparatus for 3 h.

Identification of phytochemical components of TEO using GC/MS technique

Identifying the components of essential oil by using the gas chromatography (GC) and gas chromatography/mass spectrometric (GC/MS) analysis. To identify the constituents of the essential oil, Agilent Technologies-7890 A gas chromatograph was used. The type, length, diameter, and thickness of the column were HP-5-MS, 30 m, 0.22 mm, and 0.25 µm, respectively. The temperature program of the column was 60–210 °C at a rate of 4 °C min^{-1}. Nitrogen carrier gas was used at a flow rate of 0.5 mL min^{-1}. Gas chromatograph connected to a mass spectrometer (GC/MS) was an Agilent Technologies-5975 C model. The column type was HP-5MS, 30 m in length, 0.25 mm in diameter, and 0.25 µm in thickness. The temperature program was 280 °C and helium carrier gas was used at a flow rate of 1 mL min^{-1}. The relative percentage of each component of essential oil was determined based on chromatogram peak area and compared with the total area by using the normalization method of the GC/FID peak areas. Retention indices were determined using retention times of n-alkanes (C_8-C_{25}) that were injected after the volatile oil under the same chromatographic conditions. The retention indices for all components were estimated according to the method using n-alkanes as standard. The compounds were identified by comparison of retention indices (RI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley GC-MS Library and Adams library (Adams, 2001).

Nano-emulsion production

The production of TEO nano-emulsion involved using ultrasound waves (Hielscher up 200ht ultrasound machine), according to Azizkhani et al. (2021). The TEO/water emulsion had ratios of 100, 200, 400, and 800 mg L^{-1} TEO, 0.2% Tween 20 emulsifier, and the rest was distilled water. For this purpose, the TEO, water, and surfactant were mixed in the mentioned proportions and stirred by the IKA RW20 Ultra Thorax high-speed stirrer at 2100 rpm for 2 min. Then, we sonicated the mixture by an ultrasound machine with an amplitude of 100 and a power of 40 Watts for 4 min to reduce the size of the emulsion particles sufficiently. The samples remained in a water and ice bath to prevent the temperature of the emulsion from increasing during the sonication.

Flow behavior properties of TEO nano-emulsion

A rotary viscometer model MCR 302 SN82492533 (USA) evaluated the flow behavior of nano-emulsions containing TEO at 25 °C. For each test, 6.8 mL of nano-emulsion sample was transferred into a special cup and subjected to an initial shear rate of 100 S^{-1} for 2 min for uniformity. An appropriate shear rate became applicable according to the viscosity of the samples in the range of 0.1–100 S^{-1}. We used the power law model to investigate the effect of TEO concentration on the flow behavior of TEO nano-emulsion. The shear stress data fit against the shear rate. We calculated the model's parameters, including consistency coefficient (k) and flow behavior index (n).

Particle size distribution and stability of nano-emulsions

The average and size distribution of nano-emulsion particles were determined by laser light diffraction with a DLS device (model sz-100, Horiba-Japan). The experiment temperature was 25 °C. Emulsion particles entered the water tank of the machine until the appropriate concentration was reached. The emulsion in the device was continuously stirred by the mixer of the device to make uniformity. Then, the sample was transferred to the laser chamber and the particle size distribution was determined.

Also, to check the stability of each sample, 20 g of emulsion was poured into the test tube. The test tubes were placed in a hot water bath at 80 °C for 30 m and then centrifuged at 323 × g. Finally, the height of the remaining emulsion was measured from the test tube and the stability of the emulsion was determined from the following equation (Hematian Sourki, Koocheki, et al., 2022).

$$Emulsion\ stability = \frac{v_1 - v_0}{v_0} \times 100 \quad (1)$$

Where V_0 and V_1 were the primary volume and secondary volume of the emulsion, respectively.

Zeta potential

Zeta potential was measured based on electrophoretic movement and by dynamic light scattering technique. This parameter was measured for emulsions at 25 °C and pH 7 using a Microtrac Zetasizer (ZC007, Germany) equipped with a 4mW HeNe Laser (λ 633 nm). Analysis results were also obtained based on automatic video analysis of about 100 particles and in an electric field of 7 V cm^{-1}.

Preparation of fermented pickled cucumber containing nano-emulsified TEO

Fermented pickled cucumber production was done traditionally in 0.75 L glass containers (Di Cagno et al., 2013). Nano-emulsified TEO (0, 100, 200, 400 or 800 mg L^{-1}) was mixed with 10% brine and 5% vinegar solution that had been boiled before and added to the jars containing cucumber. A jar of pickled cucumber without any additives was produced as a control sample under the same conditions. Degassing was done for 2 d. Then, jar lids were tightly closed and the jars containing pickled cucumber were kept at ambient temperature in a dark place for 14 d.

Physicochemical, microbial and sensory characteristics of fermented pickled cucumbers

pH and acidity measurement

The brine pH value of each sample was measured using the H500 Clean pH meter after calibrating the device. Lactic acid was measured as a determinant of acidity which, in turn, was determined by titration using 0.1 M NaOH (Yoo et al., 2006).

Salt concentration determination

Salt concentration was measured using Mohr's method (Dixon, 1965). Accordingly, 5 g of pickled cucumber juice was sampled and weighed. Then, its volume was increased to 100 mL with distilled water. The solution was filtered with filter paper and reached a volume of 250 mL. Then, 50 mL of the solution was sampled. It was titrated with 0.1 M $AgNO_3$ after adding 1 mL of AgCr detector (Avramiuc, 2016).

Antibacterial and antifungal effects of the nano-emulsion

Antimicrobial properties of the nano-emulsified TEO were determined by disc diffusion microbial culture. The minimal growth-inhibiting concentration of microorganisms, including *Penicillium expansum* (ATCC 7861) and *Aspergillus flavus* (ATCC 16883) fungi as moldy spoilage indicator and *Lactiplantibacillus plantarum* (ATCC BAA-793), as natural flora of fermented pickled cucumber were determined by measuring the diameter of the growth inhibition zone using a caliper on above mentioned microorganisms that have been incubated at 25 °C for 48 h (Behbahani et al., 2017). All antimicrobial activity assays were performed in triplicates.

Sensory evaluation

Sensory evaluation was designed according to the five-point hedonic scale. There were 10 panelists, including 5 men and 5 women. The panelists consumed the pickled cucumbers and evaluated the taste, odor, color, mouthfeel and overall acceptability. They scored the samples based on a 5-point hedonic scale. Accordingly, score 1 was considered very bad, 2 bad, 3 average, 4 good and 5 very good (Yoo et al., 2006). One centimeter from the ends of the pickled cucumbers was cut off, and the middle section was soaked in water for 20 min before the sensory test. Because pickled cucumbers have very strong salty taste, they need to be served after soaking to decrease the fatigue of sensory panels (Yoo et al., 2006). The samples were coded with 3-digit random numbers, and panelists were asked to wash their mouths with water after testing each sample.

Permeation kinetics of nano-emulsified TEO into fermented pickled cucumbers by FTIR

This evaluation was performed by Fourier transform infrared spectrometer (model Tensor II, Bruker-Germany). A drop of pure TEO and pickled cucumber extract were placed between a pair of polished plates containing KBr. The mentioned pair of plates were gently compressed and placed in the device holder so that the infrared radiation passed through them. The FT-IR operated using a Bruker Tensor II FT-IR device at the frequency range of 400–4000 cm^{-1} at a resolution of 1.43 cm^{-1}.

Antioxidant capacity potentiometry for permeation kinetics determination

In order to determine the antioxidant activity of pickled cucumber brine, pickled cucumber brine was used directly, while in order to measure the antioxidant activity of pickled cucumber texture, at first, extraction was done (2:1 with water solvent) and then the antioxidant activity by spectrophotometric method based on the reduction of a methanol solution of DPPH (1,1-diphenyl-2-picryl-hydrazyl) by using the method of Oke et al. (2009) was done. In order to prepare the pickled cucumber extract, the ends of the cucumbers were first removed by one centimeter, and then the cucumber tissue was thoroughly crushed and homogenized using a laboratory mixer grinder. Finally,10 g of each sample was weighed and 20 mL of water was added, and the suspension was stirred slightly. The samples were placed on shaker (200 rpm in room temperature, 28 ± 2 °C) for 6 h. The extract was centrifuged for 10 min (8000 × g), and supernatants fluid were collected and stored at 4 °C to measure antioxidant activity.

Statistical analysis

Statistical analysis was done in a completely randomized design (CRD), with five treatments and three replications. Data analysis was done via JMP (v. 8) software and mean comparison was done based on Tukey test.

Results

Gas Chromatography/Mass Spectrometry (GC/MS) analysis of TEO

GC-MS analysis resulted in the identification of 25 components in TEO (Table 1). The main components of TEO were methyl chavicol (79.95%), Z- β-ocimene (5.12%), E- β-ocimene (5.51%), limonene (2.34%), and methyl eugenol (1.62%). The results showed that the main components of TEO in this study consisted of terpenes and terpenoids and other aromatic and aliphatic components. The constituents of TEO in this research were similar to previous results by Fraternale et al. (2015) and Chaleshtori et al. (2011), indicating that the major volatile compounds in tarragon (aerial parts) were methyl chavicol (84.83%), trans-ocimene (3.86%), Z-β-ocimene (3.42%), limonene (1.79%), and α-pinene (0.57%).

Plant variety, weather conditions, growth stage, harvest date, and habitat have significant effects on the type and amounts of components that comprise EOs extracted from plant tissues (Hematian Sourki, Pasalar et al., 2022). This issue is important because the compounds identified in plants are different from each other in different regions. The amount of compounds in plant EOs can vary depending on ecological differences, such as geographical latitude and longitude, altitude, temperature, humidity, climate and soil conditions. The metabolic pathways and biosynthesis of secondary metabolites in these plants are affected accordingly, and various secondary metabolites are biosynthesized at different rates under different environmental conditions (Behbahani et al., 2017).

Table 1 Chemical composition analysis of tarragon (*Artemisia dracunculus*) essential oil (TEO) by using GC/MS technique

No.	Compounds	RI	Percentage
1	α-Pinene	934	0.89
2	Camphene	949	0.06
3	Sabinene	974	0.32
4	β-Pinene	978	0.07
5	Myrcene	992	0.12
6	Limonene	1029	**2.34**
7	(Z)-β-ocimene	1038	**5.12**
8	(E)-β-ocimene	1049	**5.51**
9	Terpinolene	1089	0.14
10	Linalool	1099	0.17
11	Allo-ocimene	1129	0.62
12	Methyl chavicol	1196	**79.95**
13	p-Anisaldehyde, dimethyl acetal	1255	0.06
14	Bornyl acetate	1285	0.17
15	δ-Elemene	1336	0.05
16	Eugenol	1357	0.94
17	(E)-Methyl cinnamate	1381	0.65
18	Methyl eugenol	1405	1.62
19	(E)-Caryophyllene	1417	0.14
20	γ-Decalactone	1466	0.12
21	Germacrene D	1481	0.17
22	(E)-β-Ionone	1484	0.06
23	Bicyclogermacrene	1494	0.30
24	β-Sesquiphellandrene	1522	0.11
25	Spathulenol	1574	0.30
26	Caryophyllene oxide	1580	0.05
	Total		**99.88**

RI: The retention Kovats index were determined on HP-5 capillary column. Components less than 0.05 % were not reported

Flow behavior characteristics of nano-emulsified TEO

Consistency coefficient (k) and flow behavior index (n) of nano-emulsions are presented by fitting the power law model for shear stress data against shear rate at different concentrations of TEO at 25 °C (Table 2). The results showed that in concentrations of 100, 200 and 400 mg L^{-1} of TEO, the apparent viscosity remained constant despite an increase in shear rate, which means that the apparent viscosity of nano-emulsions has no dependence on shear rate, indicating the Newtonian behavior of TEO nano-emulsions. Also, the presence of a linear relationship between shear stress and shear rate in nano-emulsions with different concentrations of TEO indicates the Newtonian behavior of these nano-emulsions at different concentrations.

By increasing the TEO concentration, the flow behavior index (n) did not change significantly ($p < 0.05$). The value of this index was close to one at all concentrations, which indicates the Newtonian behavior of TEO

Table 2 Flow behavior properties and stability characteristics of nano-emulsions under different concentration of TEO and salt

Treatments	Flow behavior index (n)	Consistency coefficient (pa.s^n)	Emulsion Stability (%)	Span	Zeta potential (mV)
T100	1.02 ± 0.03	0.0012 ± 0.0001	100	0.604 ± 0.10	-3.23 ± 0.55
T200	0.99 ± 0.07	0.0012 ± 0.0007	100	0.989 ± 0.12	-10.77 ± 1.14
T400	0.97 ± 0.009	0.0013 ± 0.0004	100	0.978 ± 0.09	-11.7 ± 1.5
Ts100	1.03 ± 0.06	0.0011 ± 0.00008	-	-	-
Ts200	1.07 ± 0.06	0.0009 ± 0.0005	-	-	-
Ts400	1.02 ± 0.07	0.0012 ± 0.0001	-	-	-

T100, T200, and T400 were nano-emulsions contain 100, 200, and 400 mg L^{-1} TEO respectively. Ts100, Ts200, and Ts400 were nano-emulsions contain 100, 200, and 400 mg L^{-1} TEO respectively + 10 % salt

nano-emulsions. Also, adding salt did not significantly affect the values of flow behavior index and consistency coefficient (Table 2). Therefore, there was no significant difference in the values of consistency coefficient and flow behavior index in nano-emulsions without salt and nano-emulsions containing salt. Thus, it can be concluded that adding salt has almost no effect on the flow behavior characteristics of nano-emulsions. These results confirm the stability of nano-emulsion particles in terms of aggregation and cohesion against changes in salt concentration. The dimensional repulsion between the emulsion droplets compensated for the decrease in electrostatic repulsion caused by the increase in ion concentration. Thus, their sensitivity to salt decreased (Mohammadzadeh et al., 2016). Therefore, high amounts of salt may not have an adverse effect on nano-emulsion stability when fermented pickled cucumbers are ready for use. In fact, salt may not affect nano-emulsified TEO performance, including its permeation into the pickled cucumber texture.

Thermal stability of nano-emulsified TEO

The emulsifying capacity is the extent that the components of the emulsion can form and stabilize the emulsion. The results showed no change in sample volume after applying heat and centrifugal force to divide the nano-emulsions into two phases and separate them (Table 2). All nano-emulsions of TEO had very high stability, despite the different concentrations. One of the factors affecting the stability of emulsions is the average size of their particles. Considering the low average particle size of emulsions prepared from TEO (in nano dimensions), we can justify their high stability.

Span number

The stability of an emulsion against water-oil separation increases by reducing the size of the emulsion particles (McClements, 2004). The smaller the diameter of the particles, the more the interfacial area of the particles comprise a major share of the particle volume. The interfacial area plays a crucial role in determining the physicochemical and organoleptic characteristics of food emulsions. Many characteristics of emulsions, i.e., stability, rheology, appearance, color and texture, are related to particle size distribution and the average diameter of emulsion particles. Therefore, knowledge of emulsion particle size and particle size distribution plays an important role in predicting the behavior and characteristics of food emulsions.

Emulsions containing 100 mg L^{-1} TEO were more uniform in terms of emulsion particle size distribution (span 0.604), thereby indicating that the dispersed phase particles were more uniform in size (Table 2). The span number reflects particle size distribution. The lower the span number, the more uniform the emulsion particle size. The results showed that the highest span number or particle size non-uniformity was observed in nano-emulsions containing 200 and 400 mg L^{-1} TEO. Considering the constant amount of emulsifier (Tween 20) in all samples, increasing the TEO concentration decreased the amount of emulsifier necessary to create interfacial spaces between TEO particles and water molecules in the system. Therefore, with the increase in TEO concentration, particle uniformity decreased. The lower ability of the emulsifier to cover all TEO particles was the main reason that the particle sizes became more non-uniform in samples that contained higher TEO concentrations (200 and 400 mg L^{-1}).

Nano-Emulsion particle size average and distribution

With the increase in TEO concentration, the average particle size of the nano-emulsions increased significantly and the amplitude of the particle size distribution of nano-emulsions became broader (Fig. 1). The lowest average particle size for nano-emulsions containing 100 mg L^{-1} TEO was 19.03 nm. The highest average particle size was observed in nano-emulsions containing 400 mg L^{-1} TEO, averaging 218.6 nm. The emulsifier (Tween 20) assisted in emulsification and its stability in two ways. The emulsifier reduced the interfacial tension between oil and water droplets. Also, it formed a protective layer on the surface of the dispersed phase molecules in the emulsion (EO particles), thereby preventing them from reattaching to each other, decreasing the emulsion particle size, and increasing the emulsion stability (Hu et al., 2003). Considering the constant percentage of emulsifiers in emulsions with different EO percentages, the only factor that increased the particle size and expanded the amplitude of the uniform distributions, regarding average particle size, was attributable to the decrease in the emulsifier role while the EO concentration increased. This event destabilized the particles and their connection to each other, thereby increasing the average particle size and forming diverse-dimension particles which intensified the non-uniformity of the nano-emulsion particles.

Zeta potential

Zeta potential is an index that can determine the electric charge on the surface of emulsion particles (Jindal et al., 2013). According to the negative zeta potential theory, it creates electrostatic repulsion and prevents the emulsion particles from aggregation with each other, thereby stabilizing the emulsion (Echeverri, 2012). The high electric charge of the particle surface (zeta potential) increases the electrostatic repulsion force between the emulsion particles, so that this repulsion force overcomes the interactions of the van der Waals force and hydrophobic bonds that bind the particles together (Kim et al., 2002).

With different levels of TEO, the zeta potential of all nano-emulsions ranged from −3.23 to -11.7 mV, which indicates suitable conditions that prevent cohesion and phase separation (Table 2). The lowest zeta potential occurred in samples containing 100 mg L^{-1} TEO (-3.23) and the highest zeta potential in samples containing 400 mg L^{-1} TEO (-11.7). With the increase in TEO concentration, the zeta potential increased (became more negative), however, this amount is not sufficient for the stability of the nano-emulsion particles. If the zeta potential is too small (typically less than about 25 mV in magnitude), the repulsive force will not be strong enough to overcome the Van der Waals attraction between the particles, and they will begin to agglomerate. The suspension is said to be unstable when this happens, and other repulsive forces, such as hydration, are insignificant (Filippi et al., 2008). In this regard, Azizkhani et al. (2021) evaluated the TEO nano-emulsion and reported nano-emulsion particle diameters of 50 nm on average, with the zeta potential −30 mV. Also, Zhang et al. (2020) reported the zeta potential of nano-encapsulated TEO as -36 mV.

Physicochemical, microbial and sensory characteristics of fermented pickled cucumber containing nano-emulsified TEO

Changes in pH and acidity of fermented pickled cucumber samples

In the fermented pickled cucumber containing nano-emulsified TEO, the highest pH value occurred in control samples. The lowest pH was observed in samples containing 800 mg L^{-1} nano-emulsified TEO. With the increase nano-emulsified TEO concentration, the pH of pickled cucumber samples decreased significantly after 14 days of storage (Table 3). The lowest acidity was observed in the control sample and the highest in samples with 800

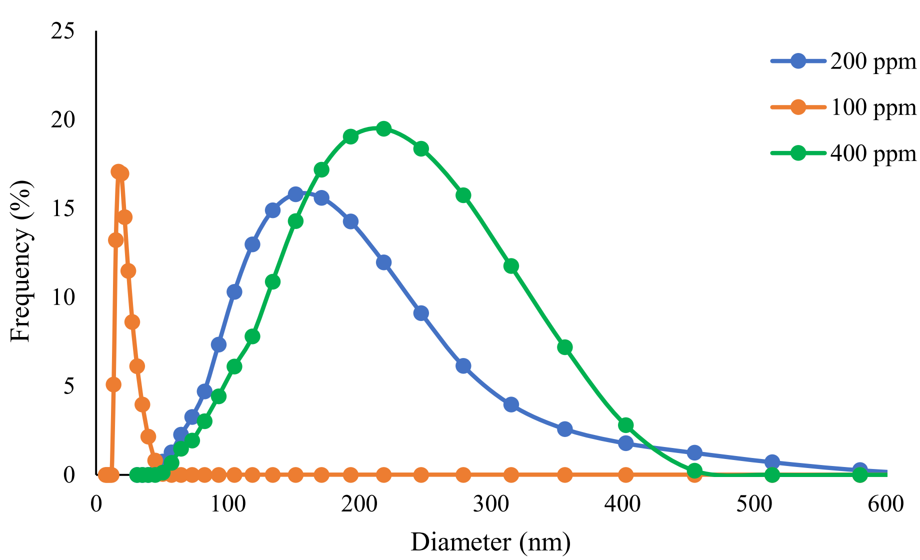


Fig. 1 Average particle size distribution of nano-emulsion contains 100, 200 and 400 ppm of TEO

mg L^{-1} nano-emulsified TEO, indicating statistically significant differences ($p < 0.05$).

During the first stage of pickled cucumber fermentation, the microbial flora included different types of bacteria, yeasts, and molds. Under these conditions (high salt concentration), the environment had facultative conditions for lactic acid bacteria and yeasts, thereby increasing their populations. In contrast, other microorganisms decreased in number (Adams & Moss, 2000). Also, according to previous results, the antimicrobial effect of TEO prevented the growth of unwanted microorganisms and provided conditions for lactic acid bacterial growth (Elgayyar et al., 2001; Rafii & Shahverdi, 2007). Tarinejad et al. (2018) and Zomorodi et al. (2021) reported that bacteriocins of lactic acid bacteria have significant antimicrobial properties against other spoilage bacteria, thereby facilitating the growth of homofermentative bacteria and lactic acid production.

Salt concentration of fermented pickled cucumber brine

Salt is commonly used in pickled products because of its effect on flavor and increasing the shelf life of food (Fleming et al., 1988). Sodium chloride contributes to two main functions in the preservation of pickled cucumbers. Salt regulated the type of microbial activity and prevented softening and other degrading changes in pickled cucumber texture (Humphries & Fleming, 1989). The results showed that the salt concentration of fermented pickled cucumber brine increased with the increase in nano-emulsified TEO (Table 3). These results showed that the increase in nano-emulsified TEO concentration to 800 mg L^{-1} decreased the amount of salt absorption by pickled cucumbers. In fact, the permeability of cucumbers to salt decreased at higher nano-emulsified TEO concentrations. Actually, with the increase of penetration of nano-emulsified TEO into the texture of pickled cucumber, the permeability of cucumber to salt decreased. Probably, the penetration and accumulation of nano-emulsion under the skin of the cucumber prevented the penetration of brine into it. Nasri et al. (2021) reported that *Echinophora platyloba* powder (contain EOs) prevented the permeation of salt into cucumbers, and the amount of salt permeation decreased with increasing EO concentration, thereby confirming the current results.

Table 3 Changes in pH, acidity, and salt concentration of fermented pickled cucumbers under the influence of different nano-emulsified TEO concentrations

TEO concentration (mg L^{-1})	pH	Acidity (%)	Salt concentration (%)
0	4.32 ± 0.04^{a}	0.61 ± 0.06^{c}	7.02 ± 0.18^{cd}
100	4.11 ± 0.12^{b}	0.65 ± 0.07^{bc}	6.84 ± 0.21^{d}
200	4.01 ± 0.15^{ab}	0.71 ± 0.09^{ab}	7.19 ± 0.22^{c}
400	4.05 ± 0.13^{ab}	0.66 ± 0.04^{bc}	7.66 ± 0.12^{b}
800	4.02 ± 0.14^{ab}	0.77 ± 0.05^{a}	8.07 ± 0.17^{a}

Common letters in each column indicate no significant difference based on Tukey's test

Sensory evaluation of fermented pickled cucumber

By increasing the TEO concentration in the nano-emulsion samples up to 800 mg L^{-1}, the overall consumer acceptance of pickled cucumber increased significantly (Table 4). The highest overall acceptance score (3.93) occurred in pickled cucumber samples containing 800 mg L^{-1} nano-emulsified TEO. The lowest overall acceptance score (2.10) occurred in the control sample, thereby showing statistically significant differences ($p < 0.05$).

Sensory evaluations showed that the highest texture crispness score (4.09) occurred in fermented pickled cucumbers containing 800 mg L^{-1} nano-emulsified TEO (Table 4). The lowest texture crispness score (2.37) was observed in the control sample, showing statistically significant differences ($p < 0.05$). Regarding texture crispiness, the same trend was observed with the overall acceptance score. Increase the nano-emulsified TEO concentration up to 800 mg L^{-1} improved the textural crispiness.

Table 4 Organoleptic properties of fermented pickled cucumbers under different concentration of nano-emulsified TEO

TEO concentration (mg L^{-1})	overall acceptability	texture crispness	mouthfeel	taste	odor	color
0	2.10 ± 0.65^{c}	2.37 ± 0.93^{b}	2.02 ± 0.51^{c}	2.00 ± 0.74^{b}	1.91 ± 0.67^{c}	1.75 ± 0.72^{c}
100	2.98 ± 0.57^{b}	3.46 ± 0.75^{b}	2.75 ± 0.72^{bc}	2.50 ± 0.65^{b}	2.29 ± 0.81^{bc}	3.38 ± 0.53^{b}
200	3.56 ± 0.47^{ab}	3.81 ± 0.61^{a}	3.35 ± 0.57^{ab}	3.33 ± 0.64^{a}	3.35 ± 0.68^{a}	3.88 ± 0.71^{ab}
400	3.81 ± 0.47^{a}	3.94 ± 0.47^{a}	3.82 ± 0.74^{a}	3.67 ± 0.53^{a}	3.58 ± 0.59^{a}	$4.170.72^{a}$
800	3.60 ± 0.64^{ab}	3.96 ± 0.54^{a}	3.40 ± 0.91^{ab}	3.78 ± 0.86^{a}	3.15 ± 0.97^{ab}	3.96 ± 0.69^{ab}

Means with the same letters in each column have not significant difference. Mean comparison was done according to Tukey's test.

Statically analysis was done by JMP software (Version 8) with ten replications.

A statistical comparison of averages regarding the sensory characteristics showed that the highest mouthfeel score (3.88) occurred in pickled cucumber samples containing 800 mg L^{-1} nano-emulsified TEO (Table 4). The lowest mouthfeel score (2.02) was observed in the control sample, which was significantly different ($p<0.05$). Changes in the mouthfeel score in fermented pickled cucumber samples was similar to changes in the overall consumer acceptance score and texture crispness characteristics. The mouthfeel score increased in response to higher nano-emulsified TEO concentrations.

Among the pickled cucumber samples, the highest taste score (3.84) was observed in pickled cucumber samples containing 400 mg L^{-1} TEO and the lowest score (2) in the control sample, indicating a statistically significant difference ($p<0.05$) (Table 4). Samples containing 800 mg L^{-1} nano-emulsified TEO were in the second place regarding the taste score, followed by samples containing 100 mg L^{-1}, 200 mg L^{-1} and the control. It seemed that adding high amounts of nano-emulsified TEO rendered the sample taste unpleasant.

Regarding the aroma index in fermented pickled cucumber samples, the highest score (3.75) occurred in pickled cucumber samples containing 400 and 800 mg L^{-1} nano-emulsified TEO (Table 4). The lowest score (1.91) was observed in the control sample, showing a statistically significant difference ($p<0.05$). With higher EO concentrations, the volatile compounds in pickled cucumbers increased, i.e., limonene, carvone, and phellandrene, thereby increasing the aroma score (Yazdani et al., 2004).

Among the fermented pickled cucumber samples, the highest color score (4.19) was observed in the pickled cucumber sample containing 800 mg L^{-1} nano-emulsified TEO and the lowest score (1.75) in the control sample, showing statistically significant differences ($p<0.05$) (Table 4). Zomorodi et al. (2021) reported that the reason for the color stability of raisin containing EO was the antioxidant effect and the ability of EO to prevent browning reactions. Ayoughi et al. (2011) reported that TEO contained high amounts of oxygenated compounds such as D-carvone, D-apiol 4, 5 trans-dihydrocarvone, 6 trans-dihydrocarvone, with phenolic compounds such as linalool 8, terpinol 9, and thymol. Tarragon EO compounds, as natural antioxidants, have the ability to react with free radicals and reduce the oxidation rate.

In general, the statistical analysis of the sensory evaluation of fermented pickled cucumber containing nano-emulsified TEO showed that the sample containing 800 mg L^{-1} nano-emulsified TEO scored highest on sensory evaluation characteristics. No statistical difference occurred among pickled cucumber samples containing 200, 400, and 800 mg L^{-1} ($p<0.05$). The sensory evaluation showed that an increase in nano-emulsified TEO concentration increased the organoleptic properties of fermented pickled cucumber, which is attributable to limonene, carvone, and methyl chavicol (Cleary & McFeeters, 2006). According to Table 4, all samples were significantly different from the control sample in this regard.

Microbial analysis of fermented pickled cucumber containing nano-emulsified TEO

The nano-emulsified TEO had a strong inhibitory effect on *Penicillium* and *Aspergillus* species. The growth inhibition zone was observed in most of the concentrations used. The largest growth inhibition zone was observed in the culture medium containing *Aspergillus flavus* (8 mm diameter) compared to pure TEO. The growth inhibition zone formation also occurred in the culture medium containing nano-emulsified TEO (20000 mg L^{-1}) (Table 5). The strongest inhibitory action against fungal growth occurred in samples containing 50,000 mg L^{-1}TEO, causing a growth inhibition zone (2 mm) for *Penicillium expansum* and *Aspergillus flavus*. In nano-emulsified TEO, the growth inhibition zone against *Penicillium expansum* (2 and 1 mm) were observed by concentrations of 40,000 mg L^{-1} and 30,000 mg L^{-1}, respectively (Table 5).

Considering that *Aspergillus flavus* is involved in food spoilage and fermented pickled cucumber decay (Céspedes et al., 2006), pure TEO was largely effective, and nano-emulsions containing 5% TEO caused a growth inhibition zone (2 mm diameter) (Table 5). Thus, the minimum inhibitory concentration of nano-emulsified TEO was 3% regarding *Penicillium*

Table 5 Effect of different concentrations of nano-emulsified and pure TEO on fungal growth in Sabro Dextrose Agar (SDA) culture medium

Fungi type	Growth inhibition zone (mm)				
	Pure TEO	TEO 50,000 mg L^{-1}	TEO 40,000 mg L^{-1}	TEO 30,000 mg L^{-1}	TEO 20,000 mg L^{-1}
Penicillium expansum	3 ± 0.75	2 ± 0.51	2 ± 0.23	1 ± 0.32	-
Aspergillus flavus	8 ± 0.44	2 ± 0.19	-	-	-

expansum and 5% regarding *Aspergillus flavus*. The TEO had a strong effect against food spoilage agents (Table 5), which is attributable to compounds such as methyl chavicol (79.95%), β-ocimene (10.63%), and limonene (2.34%), thereby accounting for antifungal properties (Ayoughi et al., 2011).

The antibacterial effect of TEO may cause problems by affecting beneficial microorganisms in the fermentation process. Examining the extent of this problem involved preparing a pour plate culture, due to the anaerobic nature of *Lactiplantibacillus plantarum* from pickled cucumbers of the control group and samples containing 800 mg L^{-1} nano-emulsifed TEO. After counting the number of colonies, 370 ± 10 colonies appeared in the sample containing 800 mg L^{-1} nano-emulsifed TEO, and 285 ± 18 colonies appeared in the control sample (Fig. 2). Based on the statistical comparison (t-test) between the 800 mg L^{-1} nano-emulsifed TEO and control samples, a signifcant diference ($p < 0.01$) in terms of colony formation was observed. The sample containing 800 mg L^{-1} of nano-emulsifed TEO not only did not adversely afect the growth of probiotic species, such as *Lactobacillus*, but also promoted their growth and increased their populations. It provided an environment that favored the growth of benefcial bacterial species by preventing the growth of harmful species.

Bloater formation in pickled cucumbers

In the frst stage of fermentation, which lasted for 2–3 days, the microbial fora included diferent types of bacteria, yeasts, and molds (Board et al., 1995). Under these conditions, the environment had supportive conditions for lactic acid bacteria and yeasts, thereby increasing their populations rapidly, whereas other microorganisms declined in numbers. The fermentation process takes place by the brine around the cucumbers. Fermentation also takes place inside the cucumbers where the microorganisms enter through cucumber pores (Adams & Moss, 2000). In some cases, this occurrence leads to bloating in the product, causing the pickled cucumber to become hollow. Then, carbon dioxide is collected inside the fruit and cannot be released. Some of the produced gas is caused by the internal respiration of the texture, but most of it results from microbial fora as they contribute to malo-lactic fermentation and cause the heterofermentative fermentation of sugars (Adams & Moss, 2000). The antibacterial efect of the nano-emulsifed TEO and the selective preparation of the environment for the growth of highly homofermentative species, such as *Lactobacillus plantarum* and *Pediococcus pentozaceus*, which replace heterofermentative species, the amount of carbon dioxide production decreased (Fig. 3). Thus, it is possible to decrease the bloater formation and hollow occurrence in pickled cucumbers by increasing the nano-emulsifed TEO concentration (Fig. 3).

Permeation kinetics of EO nano-emulsion into fermented pickled cucumber by antioxidant capacity potentiometry

Changes in antioxidant activity of fermented pickled cucumber texture

Previous research showed a signifcant correlation between the antioxidant capacity and the total phenolic content of plant extracts or EOs (Albayrak et al., 2010; Chaleshtori et al., 2011). The results showed that TEO

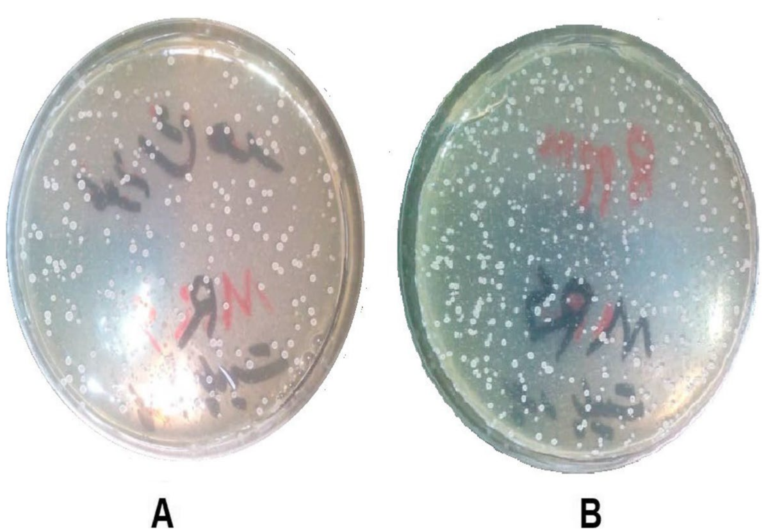


Fig. 2 Pour plate culture of pickled cucumber in MRS culture medium. A: control sample of pickled cucumber without EO. B: pickled cucumber with 800 ppm nano-emulsifed TEO

Fig. 3 Effect of nano-emulsified TEOs concentration on the bloater formation in pickled cucumbers

had a high antioxidant capacity (Fig. 4). An analysis of TEO components showed that the high methyl chavicol content (79.95%) increased its antioxidant activity. Other studies showed that linalool, limonene, spatholnol, and eugenol are the precursors of antioxidant activities in EOs (Ayoughi et al., 2011; Kirchner et al., 2010).

By increasing the TEO concentration from 200 to 800 mg L^{-1} in the nano-emulsion structure, the antioxidant capacity of the pickled cucumber texture increased significantly (Fig. 4). Up to 200 mg L^{-1} TEO, the antioxidant capacity of pickled cucumber texture did not change significantly compared to the control sample, whereas the increase over 200 mg L^{-1} caused significant changes in antioxidant activity. These changes are attributable to the permeation of nano-emulsified TEO into pickled cucumber texture. At 200 mg L^{-1} TEO, the prepared nano-emulsion was less stable due to the reduction of the non-polar component in the nano-emulsion structure, thereby not becoming able to penetrate into the pickled cucumber texture. By placing the emulsifier at the phase interface, the oil particles and aqueous phase became connected (Najaf Najafi et al., 2020). Thus, the present research showed that at concentrations less than 200 mg L^{-1} TEO, the high ratio of emulsifier to the oil portion of EO caused a decrease in the emulsion stability, thereby reducing the TEO permeation into fermented pickled cucumber texture. Thus, the antioxidant activity of pickled cucumber at 200 mg L^{-1} did not increase significantly. In this regard, Tavakkol Afshari et al. (2019) reported that increasing dill EO concentration in their nano-emulsion caused an increase in free radical inhibition percentage and antioxidant capacity.

Antioxidant capacity of fermented pickled cucumber brine

By increasing the TEO concentration from 0 to 200 mg L^{-1} and then from 200 to 800 mg L^{-1} in the nano-emulsion structure, the antioxidant capacity of pickled cucumber brine first increased and then decreased significantly compared to the control ($p < 0.05$) (Fig. 4). These changes are attributable to the EO nano-emulsions' permeation into the pickled cucumber texture, based on the antioxidant capacity of pickled cucumber pulp. At low TEO

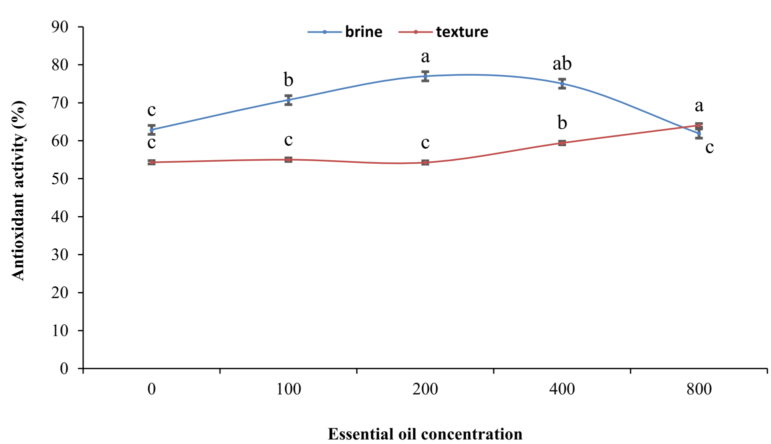


Fig. 4 Effect of Tarragon essential oil concentration on antioxidant capacity of fermented pickled cucumber and brine

concentrations (less than 200 mg L^{-1}), the high share of the emulsifier and the low portion of non-polar portion (TEO), nano-emulsions resulted in weaker stability. Since the emulsion becomes unstable, EO does not penetrate the pickled cucumber texture when used at low concentrations. Thus, the EO remains in the brine surrounding the fermented pickled cucumber and significantly increases the antioxidant capacity of the brine.

Permeation kinetics of nano-emulsified TEO into fermented pickled cucumber through FT-IR spectroscopy

IR spectroscopy determines chemical functional groups and intermolecular bonds in samples. Different chemical bonds in the polymer chain of molecules absorb specific frequencies of IR radiation. Infrared spectroscopy is a suitable tool in displaying structural changes in biopolymers. Figure 5 reveal the FT-IR spectrum of TEO samples. The position and intensity of bonds are specific regarding different components. A wave number between 400 and 4000 cm^{-1} enabled distinctions among main groups and molecular chain bonds in TEO (Omidi et al., 2018). Comparison of FT-IR spectra from the TEO with fermented pickled cucumber containing nano-emulsified TEO can confirm or negate nano-emulsified TEO permeation into the pickled cucumber texture.

Fourier transform spectroscopy of TEO

Fourier transform infrared spectroscopy showed that the tarragon EO had several unique absorption peaks in the two ranges of 518–1638 and 2834–3078. Strong absorption peaks at 3032 and 3063 cm^{-1} pertained to the vibrational bands of =C-H bonds. In previous research by Osanloo et al. (2017), absorption peaks in this area at 3028 and 3061 cm^{-1} were reported. The absorption peaks observed at 3076, 3001, 2933, 2906, and 2834 cm^{-1}

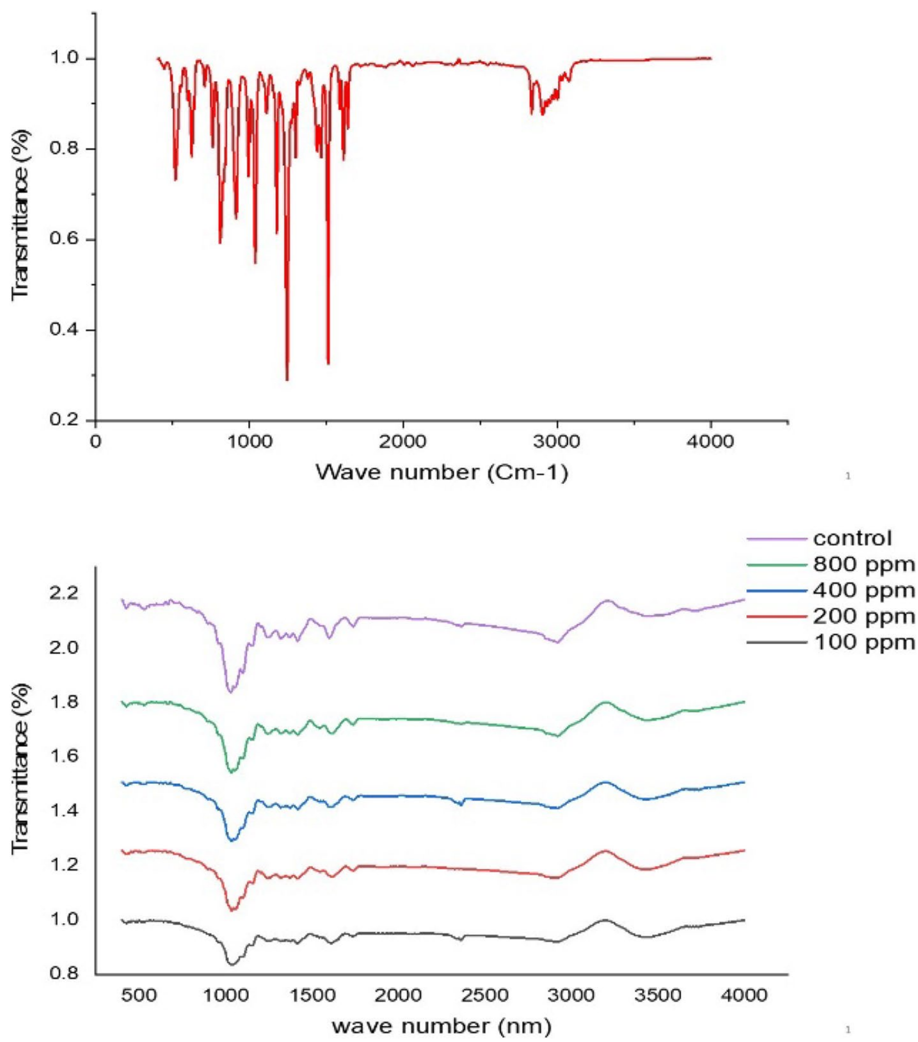


Fig. 5 FTIR spectrum of pure TEO and fermented pickled cucumbers containing different concentrations of nano-emulsified TEO

pertain to the stretching vibrations of CH-CH in SP^3 and SP^2, respectively (Osanloo et al., 2017). The absorption peak at 1638 cm^{-1} pertained to the stretching vibration of carbonyl groups (C=O). The absorption peak at 1243 cm^{-1} pertained to the stretching vibrations of C-O groups. The absorption peak at 1035 cm^{-1} pertained to the C-H absorption band. Absorption at 808 cm^{-1} pertained to the C-H absorption vibration of benzene rings, indicating various aromatic compounds in the TEO structure. Also, the absorption peak at 1611 cm^{-1} probably denoted a new C=O group (a ketone or an aldehyde).

Fourier transform spectroscopy of fermented Pickled cucumber contained nano-emulsified TEO

Pickled cucumber samples containing different concentrations of nano-emulsified TEO had absorption peaks at certain wavenumbers, similar to the wavenumbers of pure TEO FTIR spectrum. Absorption peaks were at 1034 (C-H absorption band), 1244 (C-O stretching vibration), 1616 (C=O ketone group), and 2918 (-CH stretching vibration). These values indicated the permeation of nano-emulsified TEO into fermented Pickled cucumber. Meanwhile, the Fourier transform infrared spectrum of the control sample, i.e., pickled cucumber without nano-emulsified TEO, showed no absorption bands at the mentioned wavenumbers. The spectrum of the control sample was structurally different from samples containing nano-emulsified TEO. By comparing the FTIR spectra of pickled cucumber samples containing different concentrations of nano-emulsified TEO, it appeared that an increase in nano-emulsified TEO concentration caused higher values in absorption peaks, indicating that nano-emulsified TEO penetrated the fermented pickled cucumber texture more significantly.

Conclusion

The study utilized gas chromatography to identify the main components of tarragon essential oil (TEO) as methyl chavicol (79.95%), Z- β-ocimene (5.12%), E-β-ocimene (5.51%), limonene (2.34%), and methyl eugenol (1.62%). The nano-emulsified TEO exhibited Newtonian behavior, as their viscosity remained constant regardless of the shear rate. The study also noted that TEO concentrations up to 800 mg L-1 in pickled cucumber brine reduced salt absorption due to non-polar compounds in TEO. Sensory evaluations revealed that up to 800 mg L-1 TEO enhanced various attributes of pickled cucumbers, including color, aroma, taste, mouthfeel, texture, crispness, and overall acceptance. Microbial analysis demonstrated the antifungal efficacy of nano-emulsified TEO against Penicillium expansum and Aspergillus flavus, requiring minimum inhibitory concentrations of 30,000 mg L-1 and 50,000 mg L-1, respectively. FTIR analysis confirmed the presence of TEO within the pickled cucumber structure, supporting the claim that TEO effectively penetrated the cucumber during fermentation. In conclusion, plant essential oils in nano-emulsion form are suitable, affordable, and safe options for producing fermented products such as pickled cucumbers.

Abbreviations

CRD	Completely randomized design
DPPH	1,1-diphenyl-2-picrylhydrazyl
EO	Essential oil
EOs	Essential oils
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
GC/MS	Gas chromatography/mass spectrometric
TEO	Tarragon essential oil

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Authors' contributions
MN, Conceptualization, Formal analysis, Methodology, Visualization, Data curation, Writing – original draft. AHS, Methodology, Validation, Visualization, Resources, Project administration, Investigation, Writing – review & editing. AG, Methodology, Validation, Formal analysis, Writing – review & editing.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors listed have read the complete manuscript and have approved submission of the paper.

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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