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IMPROVING ANTIFUNGAL EFFECT OF PEPPERMINT ESSENTIAL OIL

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ABSTRACT

Nanoencapsulation of essential oils is a promising strategy for extending their antifungal activity and addressing evaporation and decomposition in unfavorable environmental conditions. This research aimed to synthesize and compare the physical properties of solid lipid nanoparticles (SLNs) containing peppermint essential oil (PE) during 12 months of storage at various temperatures (4°C, 25°C, 27°C with 60% relative humidity, 37°C, and 40°C with 75% relative humidity), and to investigate their antifungal activity compared to free PE. The SLN formulations were prepared using high-shear homogenization and ultrasound techniques and were analyzed using a particle size analyzer, differential scanning calorimetry, transmission electron microscopy, and microscopic images of fungal mycelium to assess encapsulation efficacy. The results showed that the PE-SLNs had a size of 164.2 ±5.8 nm, a PDI value of 0.176 ±0.01, a zeta potential value of –11.3 mV, and an encapsulation percentage of approximately 75 ±0.5%. Overall, the physical properties of the formulations showed a slight and acceptable increase over the 12-month storage period at all investigated temperatures. Furthermore, the in vitro inhibition percentage of free PE at a concentration of 2000 μL L^{-1} against *Penicillium italicum* and *P. digitatum* was 66.7% ±2.6 and 66.8% ±0.8, respectively, while for PE-SLNs it was 88.8% ±0.9 and 89.9% ±1.4. These results demonstrate the potential of SLNs as an effective carrier for sustained delivery of PE with improved antifungal activity during storage.

Key words: encapsulation percentage, inhibition percentage, *Penicillium*, physical properties, solid lipid nanoparticles, synthesis

INTRODUCTION

Fungal contamination is a major cause of food spoilage, resulting in significant financial losses [Aslan et al. 2023]. Controlling the fungi *Penicillium italicum* and *P. digitatum*, which cause blue and green mold on fruits and vegetables, is challenging due to their high spore production and resistance to chemical fungicides [Papoutsis et al. 2019]. As a result, controlling food pathogens and improving food hygiene and safety have become pressing concerns for consumers. It has led to a growing interest in using natural plant materials, particularly essential oils, as an alternative to chemical fungicides. Chemical fungicides have been found to have adverse effects, such as the emergence of fungal resistance and the presence of chemical residues in food [Lehotay et al. 2008, Wu et al. 2017, Baibakova et al. 2019, Palfi et

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al. 2019, Ons et al. 2020, Senosy et al. 2020, Khorram and Ramezanian 2021]. The biological effects of essential oils, particularly their antifungal properties, have been the focus of numerous studies [Palfi et al. 2019, Shahbazi 2019, Tariq et al. 2019, Pongsumpun et al. 2020]. However, their application is limited due to evaporation, decomposition, and instability in unfavorable environmental conditions, such as humidity, light, oxygen, and pH [Gundewadi et al. 2018, Antonioli et al. 2020, Kelidari et al. 2021]. To address these issues, nano-encapsulation of essential oils has been identified as a potential solution and a way to apply new technology in food products [Campos et al. 2015, McDaniel et al. 2019, Mirtalebi et al. 2020, Kelidari et al. 2021].

One effective method for nanoencapsulation involves using solid lipid nanoparticles (SLNs) composed of solid, biodegradable, and physiological lipids [Lingayat et al. 2017]. These SLNs act as controlled-release carriers for lipophilic bioactive substances, protecting them from chemical degradation. They possess several desirable characteristics, including low toxicity, small size, large surface area, high drug loading, prolonged drug release, and high effectiveness [Lingayat et al. 2017, Talarico et al. 2021, Yu et al. 2023]. These properties make SLNs suitable for various food products, such as desserts, beverages, juices, creams, yogurts, sauces, and soups [McClements and Rao 2011, Yu et al. 2023]. However, the stability of SLN formulations is dependent on several factors, including storage temperature, types and amounts of solid physiological lipids and emulsifiers, which can lead to particle growth and subsequent gelation [Freitas and Müller 1998, Radomska-Soukharev 2007, Wu et al. 2011, Bagul et al. 2018, Shetta et al. 2019, Pongsumpun et al. 2020].

Compritol® 888 ATO is a mixture of different esters of behenic acid (CH3(CH2)20COOH) and glycerol [Chawla and Saraf 2011]. Compritol® 888 ATO is listed in both European Pharmacopoeia and United States Pharmacopoeia. The US Food and Drug Administration accepts Compritol® 888 ATO as a food additive and considers it generally recognized as safe [Aburahma and Badr-Eldin 2014]. Polysorbate 80 is a non-ionic emulsifier [Nielsen et al. 2016] that can effectively spread at the oil-water interface and combine polar and non-polar groups [Jin et al. 2008, Tang et al. 2014]. Due to its non-ionic nature, this emulsifier can stabilize solid lipid nanoparticles (SLNs) by creating steric repulsion [Mirtalebi et al. 2020]. The Food and Drug Administration (FDA) states polysorbate 80 is approved as a food additive [U.S. Food and Drug Administration 2021]. It is a surfactant and lubricant in various food items, including ice cream. In ice cream, polysorbate 80 is added in concentrations of up to 0.5% to enhance its softness and prevent quick melting [Lu et al. 2014].

Peppermint (*Mentha* × *piperita* L.) is a perennial plant from the Lamiaceae family, known for its medicinal and aromatic properties. Its essential oil (E) has been extensively researched for its potential antifungal activity [Plavšić et al. 2017 and Vakili-Ghartavol et al. 2022]. In addition, it has been traditionally used as a food flavoring and has shown promising biological effects such as antioxidant, antimicrobial, and antiviral properties [Mahendran and Rahman 2020]. There is limited understanding of how different temperatures and relative humidities during storage affect the physical stability of solid lipid nanoparticles (SLNs) incorporating peppermint essential oil. Therefore, the purpose of this study was to synthesize and compare the physical properties of SLNs containing peppermint essential oil (PE) for 12 months at various temperatures (4°C, 25°C, 27°C with 60% relative humidity, 37°C, and 40°C with 75% relative humidity). Additionally, the study aimed to investigate the correlation between the physical characteristics of the SLNs, specifically particle size, particle distribution, and surface charge. Furthermore, the antifungal activity of the PE-SLN formulations was compared to the free form of the essential oil against *Penicillium italicum* and *P. digitatum*.

MATERIAL AND METHODS

Essential oil characterization. Identification and quality-quantitative analysis of PE were carried out using gas chromatography/mass spectrometry (GC/MS), as previously described by Vakili-Ghartavol et al. [2022]. It involved comparing their retention index (RI) to a series of n-hydrocarbons and using computer matching against the NIST library [Adams 2007]. The analysis used a gas chromatograph (Finnigan-Thermo) coupled with a mass spectrometer (Trace DSQ Mass

Spectrometer) detector and a splitless injector. A fused silica capillary HP-5MS column (30 m × 0.25 mm, film thickness of 0.32 μm; J and W Scientific) was used for the analysis. The oven temperature was programmed to increase from 60°C to 220°C at a rate of 3°C/min, and the transfer line temperature was set at 250°C. Helium was used as the carrier gas at a 1 mL/min flow rate. A sample volume of 1 μL was injected at a split ratio 1:100, and electron impact MS was operated at 70 eV [Vakili-Ghartavol et al. 2022]. Table 1 displays the most prominent compositions of PE.

Fungal microorganisms. The fungal isolates *Penicillium italicum* and *P. digitatum* were obtained from the Nikooraee® Company. Then, these isolates were cultured on Potato Dextrose Agar (PDA, Zona Industriale 64026, and Roseto Degli Abruzzi, Italy) and stored at a temperature of 25 ±3°C.

Preparation of SLN. SLNs with or without PE were prepared using high-shear homogenization and ultrasound methods, as previously described by Sarhadi et al. [2020], with slight modifications. In summary, the lipid phase, consisting of Compritol® 888 ATO (Gattefossé, France; 5%) and polysorbate 80 (Sigma, Germany; 2.5%), and the aqueous phase, consisting of double-distilled water up to 100%, were separately heated in a Ben Mai at 80–85°C. Once the lipid had melted, the essential oil was added to the Lipid Falcon to prevent evaporation. The hot aqueous and molten lipid phases were combined at the same temperature. The mixture was homogenized using a Diax 900 homogenizer (Heidolph, Germany) for 4.5 minutes. Next, the resulting emulsion was ultrasonicated using a probe sonicator (Bransonic, USA) in 5 cycles of 60 seconds of sonication with intervals of 15 seconds. Finally, the emulsion was cooled to room temperature, and SLN solutions were obtained. SLNs without PE were also synthesized using the same techniques (see Table 2 for details).

Encapsulation efficiency. The actual concentration of menthol in PE-SLNs was calculated by taking the mean area under the GC/MS calibration curve of menthol, which serves as an indicator for PE, at various concentrations ranging from 2 to 100 μg/ml. The concentration of menthol encapsulated in the PE-SLN formulations was measured using GC-MS after purification. To do this, 500 μL of the PE-SLN dispersions were centrifuged at 10 000 rpm for 30 minutes using an Amicon (Ultra-15, PLHK Ultracel-PL Membrane, 100 kDa, Millipore). A suitable dilution with chloroform : methanol (2 : 1 v/v) was then prepared for GC-MS analysis, with one μL injected [Nasseri et al. 2016]. The encapsulation efficiency was calculated using the following equation:

$$EE\% = \frac{atctual\ Menthol\ concentration\ in\ sample}{input\ Menthol\ concentration} \times 100$$

Size, PDI, and Zeta Potential analyses. The average diameter (nm), polydispersity index (PDI), and zeta potential (mV) of SLNs were assessed using a nanoparticle analyzer based on dynamic light scattering (DLS), ZetaSizer Nano-ZS; Malvern Instruments Ltd., United Kingdom [Torrisi et al. 2021].

Table 1. Main ingredients of the peppermint essential oil as determined by GC-MS

Component	Retention time (RT)	Quantity (%)
l-Menthone	11.51	27.7
Isomenthone	11.74	4.2
γ-Terpineol	11.82	5.5
d-Menthol	12.08	36.4
Pulegone	13.78	5.9
Carvone	13.94	2.2
Menthyl acetate	15.07	11.2
Caryophyllene	18.56	2.1

Table 2. The ingredients of PE-SLNs and Reference SLNs without PE*

Formulation	Ingredients	% (wt/wt)
PE-SLNs	Compritol® 888 ATO	5
	Peppermint essential oil	0.1
	Polysorbate 80	2.5
	water	92.4
SLNs without PE	Compritol® 888 ATO	5
	Polysorbate 80	2.5
	water	92.5

* SLN and PE indicate solid lipid nanoparticles and peppermint essential oil, respectively

Stability tests. Samples of PE-SLN were stored in 2 ml tubes covered with aluminum foil at different temperatures and humidity levels (4°C, 25°C, 27°C with 60% relative humidity, 37°C, and 40°C with 75% RH) for 12 months. The purpose of this study was to assess the stability of the samples by measuring their Z-average diameter (nm), polydispersity index (PDI), and zeta potential at fixed intervals (24 hours, 6 months, and 12 months after synthesis), with slight modifications to the method used by Montenegro et al. [2017].

Differential Scanning Calorimetry (DSC). DSC studies were conducted on bulk lipid, PE-SLN formulations, and free PE using a Mettler DSC 822e from Mettler Toledo in Gießen, Germany [Fazly Bazzaz et al. 2018]. Approximately 5 mg samples were weighed into standard aluminum pans, with an empty pan used as a reference. The samples were then heated under specified thermal conditions, ranging from 20 to 220°C at a heating rate of 10°C/min, under an 80 ml/min nitrogen atmosphere to determine their melting points.

Transmission Electron Microscopy (TEM). The morphological characteristics of PE-SLN formulations were evaluated using TEM equipment, CEM 902A; Zeiss, Oberkochen, Germany [Khameneh et al. 2015]. To begin, diluted samples were coated onto a carbon-coated copper grid and left for 1 minute. The excess water was then removed using filter paper. Next, 2% uranyl acetate in 20 µl of water was added to the samples for 1 minute, followed by another round of excess water removal with filter paper. After drying the grids at room temperature, the TEM equipment observed the samples.

Antifungal activity investigation. The antifungal activity of free PE, PE-SLNs, and SLN without PE was evaluated in vitro against *P. italicum* and *P. digitatum* using the agar diffusion method on PDA medium at different concentrations of 0, 750, 1000, 1500, and 2000 µL L^{-1} [Barros Gomes et al. 2019]. The samples were mixed with the PDA medium at 40–45°C and poured into 10 cm diameter plates. After solidification, a 2 mm disc of fungal mycelium from each isolate was placed in the center of the plates and incubated at 25 ±3°C. The radial growth of each fungal colony was measured daily using two orthogonal axes. This experiment was done with three replications for each treatment. The percentage of mycelium growth inhibition (IP) was then calculated using the following equation:

IP % = [(dc – dt)/dc] × 100, where dc is the mycelium growth diameter in the control plate, and dt is the mycelium growth diameter in the treated plate.

Statistical analysis. The statistical analysis was performed using SPSS 24.0 software. Duncan's multiple range test (P = 0.05) was used to compare means, while Spearman's correlation test was employed for non-normally distributed data. Microsoft Excel 2013 was used to generate the figures. The data was presented as the mean ± standard deviation (SD).

RESULTS

Characterizations of SLN formulations. The results of the DLS analysis indicated that the PE-SLN formulations had a size of 164.2 ±5.8 nm, a PDI value of 0.176 ±0.01, and a –11.3 mV Zeta-potential value after just one day of production. However, after 12

Table 3. Z-average diameter (nm), polydispersity index (PDI), and zeta potential of the formulations analyzed using DLS over 12 months at different temperature conditions*

	Temperatures (°C)	Relative humidity (%)	After 24 hours	After 6 months	After 12 months
Z-average diameter (nm)	4	–	164.2 ±5.8^{g}	168.8 ±0.1^{g}	176 ±2^{f}
	25	–	164.2 ±5.8^{g}	196.5 ±1.3^{e}	232.1 ±2^{c}
	27	60	164.2 ±5.8^{g}	220.46 ±2^{d}	253.77 ±0.7^{a}
	37	–	164.2 ±5.8^{g}	232.1 ±2^{c}	248.7 ±1.3^{ab}
	40	75	164.2 ±5.8^{g}	234.6 ±0.2^{c}	246.73 ±0.6^{b}
PDI (intensity)	4	–	0.176 ±0.01^{f}	0.183 ±0.001^{f}	0.181 ±0.002^{f}
	25	–	0.176 ±0.01^{f}	0.281 ±0.002^{a}	0.279 ±0.002^{ab}
	27	60	0.176 ±0.01^{f}	0.269 ±0.003^{b}	0.286 ±0.001^{a}
	37	–	0.176 ±0.01^{f}	0.197 ±0.001^{e}	0.234 ±0.004^{d}
	40	75	0.176 ±0.01^{f}	0.244 ±0.001^{d}	0.255 ±0.001^{c}
Zeta potential (mV)	4	–	–11.3 ±0.05^{h}	–18.1 ±0.1^{i}	–9.1 ±0.07^{f}
	25	–	–11.3 ±0.05^{h}	–8.03 ±0.01^{e}	–6.84 ±0.02^{c}
	27	60	–11.3 ±0.05^{h}	–8.05 ±0.03^{e}	–11 ±0.0^{g}
	37	–	–11.3 ±0.05^{h}	–4.7 ±0.01^{b}	–4.4 ±0.05^{a}
	40	75	–11.3 ±0.05^{h}	–7.6 ±0.03^{d}	–6.9 ±0.01^{c}

* Mean comparisons of various parameters (such as Z-average diameter (nm), polydispersity index (PDI), and zeta potential at specific intervals) were conducted individually. Treatments labeled with distinct letters showed statistically significant differences based on Duncan's multiple range test (P = 0.01)

months of storage at different temperatures, there was a significant increase in the average particle size and a slight increase in the PDI values of the PE-SLN formulations. The sizes were smaller than 300 nm and the PDI was below 0.28 ($P \leq 0.01$, Tab. 3). Additionally, the average Zeta-potential value of the PE-SLNs increased slightly and remained negative. Overall, the results suggest that all tested temperatures, particularly 4°C, are suitable for long-term storage of SLN formulations with satisfactory physical properties.

Encapsulation efficiency. The PE-SLNs exhibited an encapsulation efficiency (EE) of 75 ±0.5% (n = 3), with menthol, the selected index component, being the predominant ingredient in PE.

Crystallinity of SLN. Thermal analyses were conducted on PE-SLNs, SLNs without PE, bulk lipids used in SLNs (Compritol® 888 ATO), and PE to determine the crystallinity of the formulations using DSC (Fig. 1). The results showed that the melting peak of PE-SLNs, SLNs without PE, Compritol® 888 ATO, and PE occurred at 68°C, 66°C, 71°C, and 171°C, respectively (Fig. 1, D, C, B, and A). The DSC diagrams revealed a slight shift towards lower temperatures for the melting peak of PE-SLNs compared to the bulk lipid. Furthermore, no peak was observed at 171°C for any PE-SLN formulations.

Transmission Electron Microscopy (TEM). The microscopic pictures of the PE-SLNs nanoparticles show

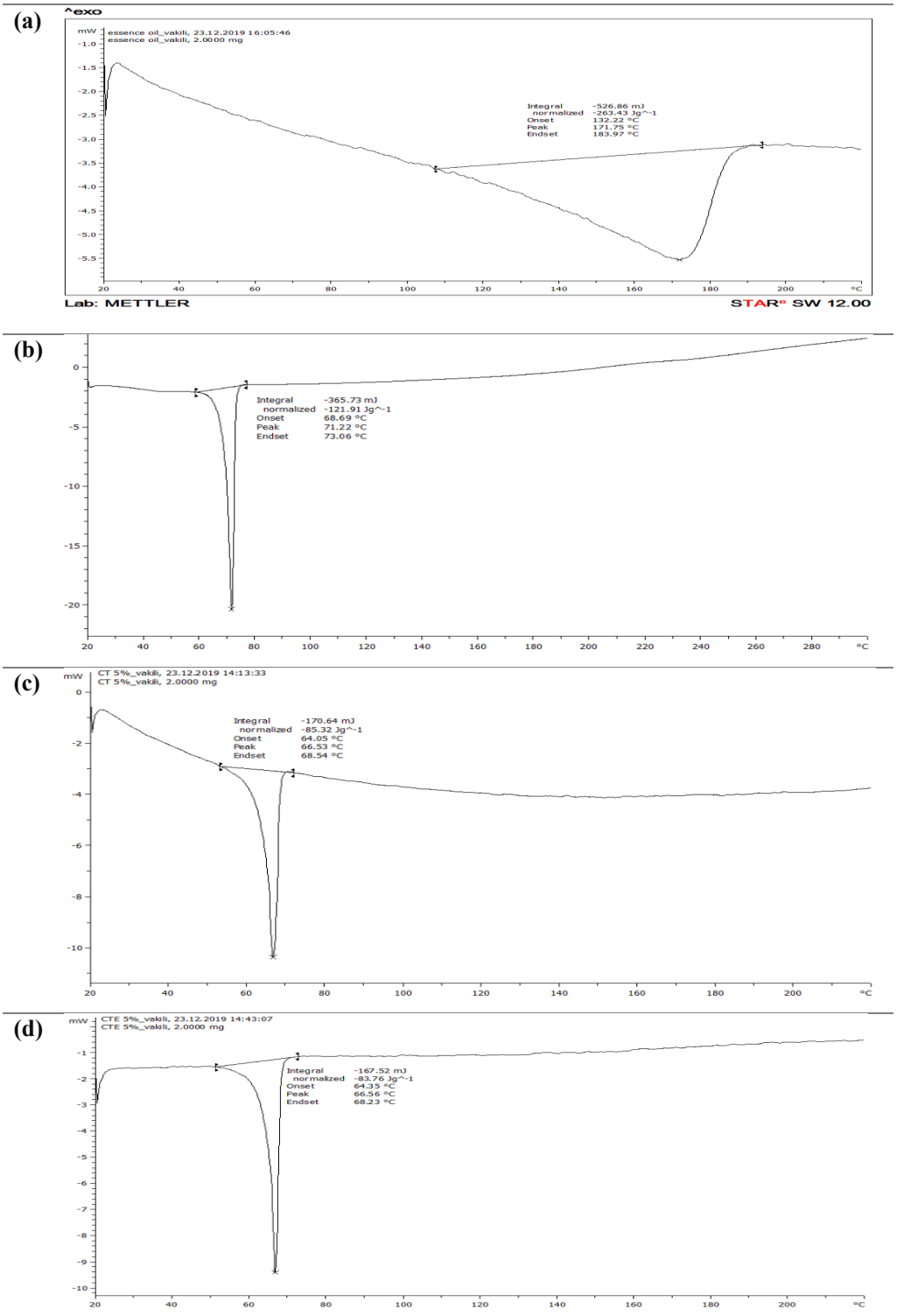

Fig. 1. Differential scanning calorimetry (DSC) thermograms: A) PE, B) Compritol® 888 ATO bulk, C) SLNs without PE, D) SLNs Containing PE, prepared by high shear homogenization and ultrasound technique. 2 mg of each sample was used in each run

that they were primarily round and uniform, with a size varying from 100 to 400 nm (Fig. 2). It is supported by the information in Table 3, which presents the particle size analysis data.

The correlation of the physical characteristics of SLN formulations

At the 0.01 level, a significant positive correlation was found between the physical properties of the PE-SLN formulations (Tab. 4). Specifically, as the average particle size increased, the zeta potential and particle dispersion values also increased.

***In vitro* antifungal activity.** Figure 3 illustrates the mycelial growth of fungi in response to various concentrations of treatments after 15 days of storage at 25 ±3°C. The different isolates showed varying levels of mycelial growth when exposed to the treatments (PE, PE-SLNs, and SLNs without PE) at different concentrations. As the concentration of treatments increased, there was a decrease in fungal mycelium growth. The results revealed that the *in vitro* inhibition percentage of free PE at a concentration of 2000 μL L^{-1} against *P. italicum* and *P. digitatum* was 66.7% ±2.6 and 66.8% ±0.8, respectively. In comparison, the PE-SLN formulations showed a higher inhibition percentage of 88.8% ±0.9 and 89.9% ±1.4 (Tab. 5; $p < 0.05$). These findings indicate that the SLN formulations had significantly improved antifungal activity compared to free PE during this period. The fungal pathogens showed no sensitivity to formulations without essential oils (Tab. 5).

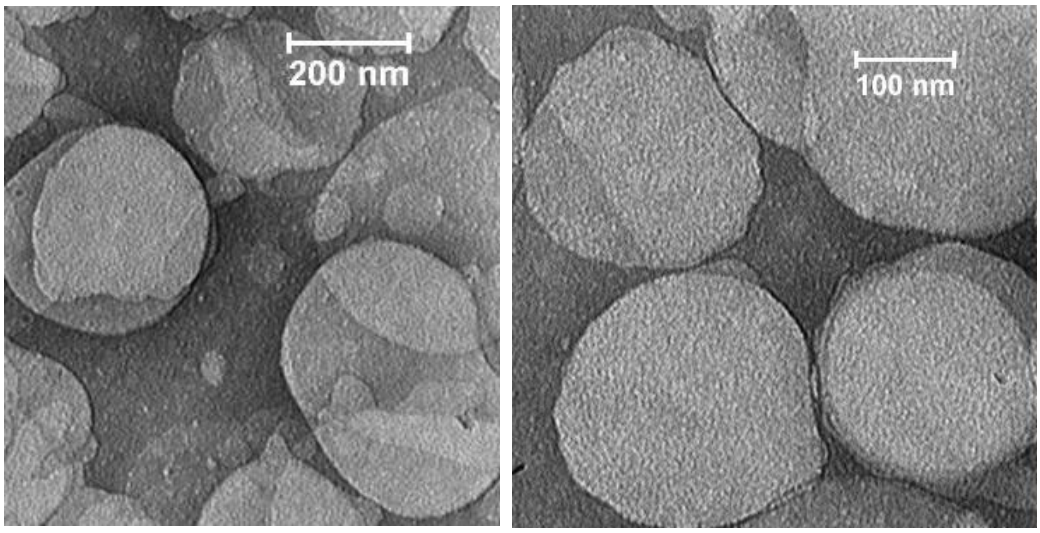

Fig. 2. The transmission electron microscopy (TEM) images of PE-SLNs. a) a size of 200 nm, and b) a size of 100 nm

Table 4. Spearman's correlation coefficients of the SLN physical characteristics

	Z-average	Zeta potential	PDI
Z-average	1		
Zeta potential	0.765**	1	
PDI	0.807**	0.622**	1

** Correlation is significant at the 0.01 level (2-tailed)

Table 5. The results of the mean comparison of the effect of various concentrations (μL L^{-1}) and various formulations (PE, PE-SLN, SLNs without PE, and control) on the inhibition percentage of the growth of the fungi pathogens

Formulation	Concentration (μL L^{-1})	*P. italicum*	*P. digitatum*
Control	0	0 ±0 ^{k}	0 ±0 ^{k}
SLN without PE	0	0 ±0 ^{k}	0 ±0 ^{k}
PE	750	23.7 ±2.1 ^{i}	16.46 ±1.7 ^{j}
	1000	28.2 ±2.8 ^{h}	22.4 ±2.2 ^{i}
	1500	43.9 ±2.6 ^{g}	48.6 ±1.9 ^{f}
	2000	66.7 ±2.6 ^{c}	66.8 ±0.8 ^{c}
PE-SLNs	750	56.2 ±2 ^{e}	51.2 ±1.7 ^{f}
	1000	69.5 ±1.9 ^{c}	60.6 ±2.8 ^{d}
	1500	76.1 ±1.4 ^{b}	74.1 ±4 ^{b}
	2000	88.8 ±0.9 ^{a}	89.9 ±1.4 ^{a}

Above columns indicate significant differences according to Duncan's multiple range tests at $P \leq 0.05$

DISCUSSION

The essential oil contained several components, with menthol as the primary component. It is consistent with the findings of Mahboubi and Kazempour [2014] but differs from the results of Yadegarinia et al. [2006], who found that α-terpinene and pipertitinone oxide were the dominant compounds in the essential oil. These variations in the chemical composition of the essential oil may be attributed to factors such as the plant's geographic location during harvest, developmental stages, and environmental conditions [Li et al. 2020]. To determine the encapsulation percentage, menthol was used as the standard composition of the essential oil.

To address the challenges associated with the instability of PE and its practical use as a natural antifungal, antioxidant, and food flavoring in the food industry, researchers have turned to nano-encapsulation of PE in SLNs [Rolim and Ramalho 2020, Tavassolirajaee et al. 2022]. This method has shown an encapsulation efficacy of 75 ±0.5%, considered high and acceptable. This success can be attributed to the high lipophilicity of the essential oil and its strong affinity with lipids [Bashiri et al. 2020, Naseri et al. 2020].

The size and distribution of particles are crucial factors that affect the physical stability, delivery efficiency, and bioavailability of poorly soluble drugs [Bahari and Hamishehkar 2016, Danaei et al. 2018, Kolluru et al. 2021]. The results showed that after 12 months of storage at various temperatures, the average particle size and PDI values of the PE-SLNs increased slightly but significantly, remaining below 300 nm and 0.28, respectively. It is consistent with the findings of Lai et al. [2007], who reported that SLNs formulated with *Arborescens arborescens* and Compritol® 888 ATO lipid were highly efficient in entrapping essential oil, with a PDI value lower than 0.350 and only a minimal increase in the average particle size after two years of storage. A PDI of 0.3 or lower indicates an optimal dispersion and homogeneous distribution of SLNs [Shah et al. 2014, Vijayakumar et al. 2017]. Piran et al. [2017] also found that lower particle size was associated with higher clarity and a slower sedimentation

rate, while larger particle sizes had the opposite effect. These results demonstrate the formulations' narrow particle size distribution and high physical stability. Another critical factor that affects physical stability is the zeta potential, which reflects the surface charge of the particles and the level of repulsion between particles with the same charge in the formulation [Shah et al. 2014, Yu et al. 2019]. Our results showed that the zeta potential of the PE-SLNs ranged from –4.4 to –11.3 mV, indicating a negative charge. This shift in zeta potential value could be attributed to changes in the emulsifier composition and particles at the oil -in-water interface [Milsmann et al. 2018]. A higher zeta potential indicates less particle aggregation due to stronger electric repulsion, resulting in increased stability of the formulation [Chawla and Saraf 2011]. The negative zeta potential in our formulations could be attributed to the anionic group (-COO-) of the lipids, Compritol® 888 ATO, which has an ester chemical structure [Chawla and Saraf 2011], as well as the anionic groups of essential oil compounds [Ghodrati et al. 2019] such as menthol, menthone, and other compounds in our study. It prevented particle aggregation in the SLNs by creating electrostatic repulsion [Salminen et al. 2016, Pereira et al. 2018]. Polysorbate 80, due to its non-ionic nature, can stabilize SLNs by creating steric repulsion [Liu et al. 2007, Mirtalebi et al. 2020].

Based on the DSC diagrams, the bulk lipid melting peak was observed at 7°C, while the PE-SLN formulations produced with this lipid shifted to a lower temperature of 68°C. These changes in the lipid melting peak in the formulations were consistent with previous studies [Keck et al. 2014, Naseri et al. 2020], which could be attributed to the different forms of lipids, their interaction with drugs, or both [Fouad et al. 2015, Nahr et al. 2018]. The PE-SLN formulations did not exhibit a melting peak for the essential oil, which may be due to the incorporation of the essential oil into the lipids in the SLN formulation. These lipids act as a suitable protection system for the essential oil [Nasseri et al. 2016, Dzulhi et al. 2018, Nahr et al. 2018]. The TEM image of the PE-SLN formulations showed spherical particles of approximately 200 nm, which was consistent with the data obtained from the particle size analysis (Tab. 3). It was also consistent with the results of previous studies [Piran et al. 2017, Dzulhi et al. 2018, Kelidari et al. 2021].

We conducted a study to evaluate the effectiveness of PE-SLN formulations compared to free essential oil on the mycelial growth of the fungal pathogens *P. italicum* and *P. digitatum*. The treatments were applied at different concentrations, and the mycelial growth was measured after 15 days of storage at 25 ±3°C (Fig. 3). The fungal pathogens showed varying reactions to the treatments, with a decrease in mycelial growth as the treatment concentration increased. Our results demonstrate that the PE-SLN formulations were significantly more effective in inhibiting fungal mycelium growth than the free PE (Tab. 5). Interestingly, the fungal pathogens showed no reaction to the SLNs without PE, indicating that the essential oil in these formulations was responsible for the observed distinction (Tab. 5). Additionally, the more effective antifungal activity of PE-SLNs compared to free PE may be attributed to their improved accessibility and even distribution of the antimicrobial compound, as well as the expulsion of the active compounds from the droplet core to the surface of the droplets through crystallization of the carrier lipids [McDaniel et al. 2019]. Furthermore, the small size of nanoparticles and their larger surface-to-volume ratio have the potential to increase their antimicrobial activity by enhancing mechanisms such as passive cellular absorption, penetration into the cell membrane, disruption of the cell membrane, and leakage of intracellular content [Ghodrati et al. 2019, McDaniel et al. 2019, Vakili-Ghartavol et al. 2024].

Indeed, SLNs possess enhanced antimicrobial activity due to their solid structure and targeted release of active ingredients, potentially extending the biological properties of essential oils. When applied as a food coating, they control microbial agents and improve other biological aspects of the food. Furthermore, these nanocarriers protect active substances from evaporation and degradation in unfavorable environments [Zhao et al. 2017, Souto et al. 2020, Tavassolirajaee et al. 2022].

CONCLUSIONS

In this study, we successfully encapsulated PE within SLNs with high yields. The physical characteristics of PE-SLNs were significantly affected by different temperatures and 12 months of storage, but the impact was slight. We observed a positive correlation

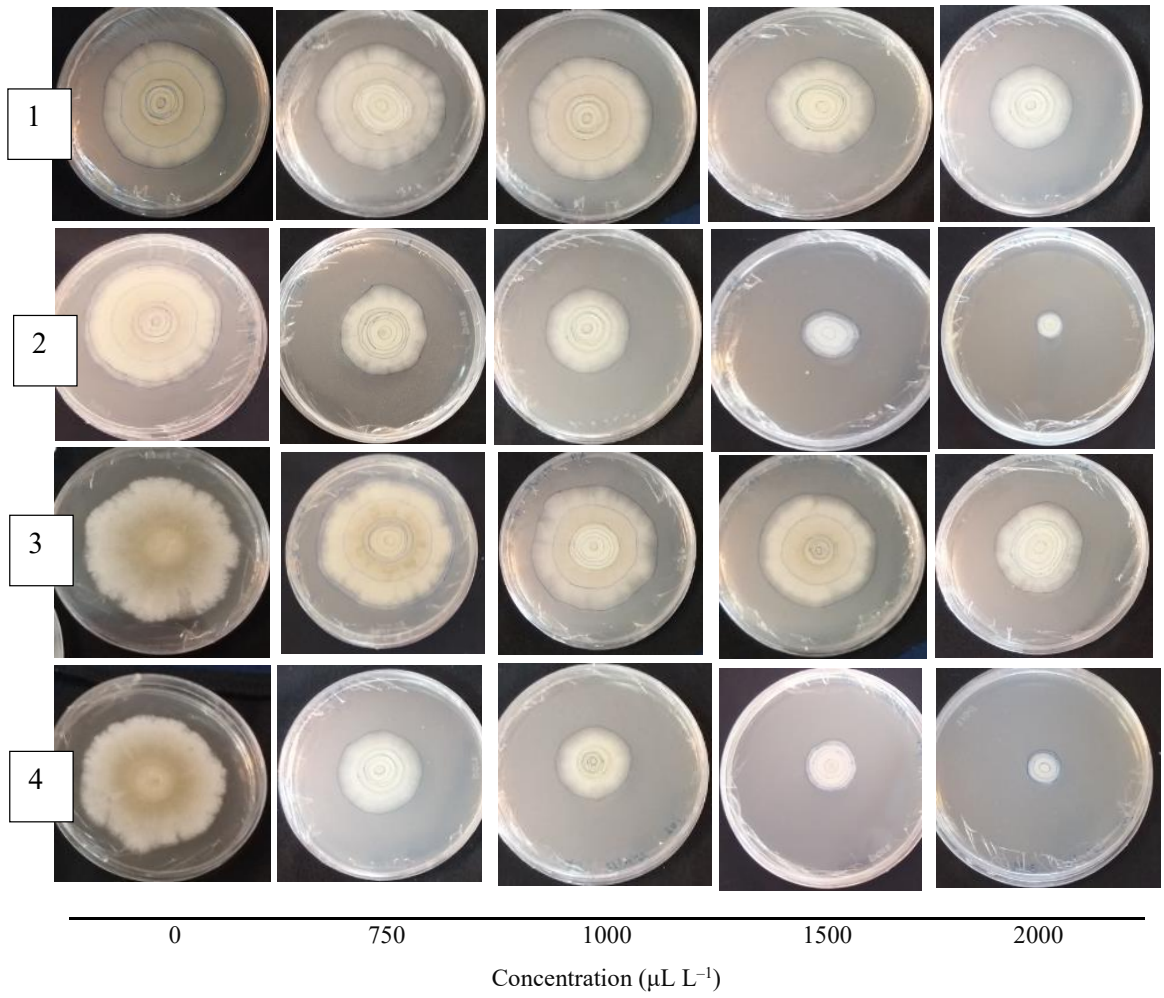

Fig. 3. The antifungal activity of PE, PE-SLNs, and SLNs without PE on the mycelial growth of the fungal pathogens. The colony diameter of fungi was measured daily during incubation at 25 ±3°C. 1 and 3 rows: peppermint essential oil, and zero represents control, 2 and 4 rows: PE-SLN formulations and zero in this row represent SLNs without PE

between the physical characteristics of the SLN formulations. Additionally, the SLN formulations showed significantly more effective antifungal activity than free PE. It suggests that SLNs can serve as a protective system to enhance the effectiveness of essential oils. The promising results of this study suggest potential applications of carrier systems and essential oils in various industries, such as pharmaceuticals, flavorings, functional food additives, food, cosmetics, and beverages. However, further *in vivo* studies are needed to evaluate the effects of solid lipid nanoparticles containing essential oils on taste and sensory characteristics.

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ship No. 97015381) for its partial financial support. The results of this study are some parts of the Ph.D. thesis of the first author.

CONFLICT OF INTEREST

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 paper.

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CREDIT AUTHOR STATEMENT

Masoumeh Vakili-Ghartavol has designed and performed research, writing, review, and editing. Hossein Arouiee, Shiva Golmohammadzadeh, and Mahboobeh Naseri designed the research and editing. All authors read and approved the final manuscript.

REFERENCES

Ahmed, M.J., Singh, Z., Khan, A.S. (2009). Postharvest Aburahma, M.H., Badr-Eldin, S.M. (2014). Compritol 888 ATO: a multifunctional lipid excipient in drug delivery systems and nanopharmaceuticals. Exp. Op. Drug Del., 11(12), 1865–1883. https://doi.org/10.1517/17425247.2014.935335.

Adams, R.P. (2007). Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing, Carol Stream, IL. U.S. Food and Drug Administration. (2021). Polysorbathe 80. Available: https://www.govinfo.gov/content/pkg/CFR-2021-title21-vol3/pdf/CFR-2021-title21-vol3-sec172-840.pdf

Antonioli, G., Fontanella, G., Echeverrigaray, S., Delamare, A.P.L., Pauletti, G.F., Barcellos, T. (2020). Poly (*lactic acid*) nanocapsules containing lemongrass essential oil for postharvest decay control: in vitro and in vivo evaluation against phytopathogenic fungi. Food Chem., 326, 126997. https://doi.org/10.1016/j.foodchem.2020.126997

Aslan, M., Ertaş, N., Demir, M.K. (2023). Storage stability, heat stability, controlled release and antifungal activity of liposomes as alternative fungal preservation agents. Food Biosci., 51, 102281. https://doi.org/10.1016/j.fbio.2022.102281

Bagul, U,S., Pisal, V.V., Solanki, N.V., Karnavat, A. (2018). Current status of solid lipid nanoparticles: a review. Mod. Appl. Bioequiv. Bioavailab., 3(4), 1–2.

Bahari, L.A.S, Hamishehkar, H. (2016). The impact of variables on particle size of solid lipid nanoparticles and nanostructured lipid carriers; a comparative literature review. Adv. Pharm. Bull., 6(2), 143. https://doi.org/10.15171/apb.2016.021

Baibakova, E.V., Nefedjeva, E.E., Suska-Malawska, M., Wilk, M., Sevriukova, G.A., Zheltobriukhov, V.F. (2019). Modern fungicides: mechanisms of action, fungal resistance and phytotoxic effects. Annm Res. Rev. Biol., 1–16. https://doi.org/10.9734/arrb/2019/v32i330083

Barros Gomes, P.R. et al. (2019). Chemical study and antifungal activity of the essential oil of the branches of Aniba duckei Kostermans. J. Essent. Bear. Plants, 22(6), 1554–1561. https://doi.org/10.1080/0972060X.2019.1700829

Bashiri, S., Ghanbarzadeh, B., Ayaseh, A., Dehghannya, J., Ehsani, A., Ozyurt, H. (2020). Essential oil-loaded nanostructured lipid carriers: the effects of liquid lipid type on the physicochemical properties in beverage models. Food Biosci., 35, 100526. https://doi.org/10.1016/j.fbio.2020.100526

Campos, E.V.R. et al. (2015). Polymeric and solid lipid nanoparticles for sustained release of carbendazim and tebuconazole in agricultural applications. Sci. Rep., 5(1), 1–14. https://doi.org/10.1038/srep13809

Chawla, V., Saraf, S.A. (2011). Glyceryl behenate and its suitability for production of aceclofenac solid lipid nanoparticles. J. Am. Oil Chem. Soc., 88(1), 119–126. https://doi.org/10.1007/s11746-010-1618-6

Danaei, M. et al. (2018). Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics, 10(2), 57. https://doi.org/10.3390/pharmaceutics10020057

Dzulhi, S., Anwar, E., Nurhayati, T. (2018). Formulation, characterization and in vitro skin penetration of green tea (*Camellia sinensis* L.) leaves extract-loaded solid lipid nanoparticles. J. Appl. Pharm. Sci., 8(8), 57¬62. https://doi.org/10.7324/JAPS.2018.8809

Fazly Bazzaz, B., Khameneh, B., Namazi, N., Iranshahi, M., Davoodi, D., Golmohammadzadeh, S. (2018). Solid lipid nanoparticles carrying *Eugenia caryophyllata* essential oil: the novel nanoparticulate systems with broad-spectrum antimicrobial activity. Lett. Appl. Microbiol., 66(6), 506–513. https://doi.org/10.1111/lam.12886

Fouad, E.A., Yassin, A.E.B., Alajami, H.N. (2015). Characterization of celecoxib-loaded solid lipid nanoparticles formulated with tristearin and softisan 100. Trop. J. Pharm. Res., 14(2), 205–210. https://doi.org/10.4314/tjpr.v14i2.3

Freitas, C., Müller, R.H. (1998). Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLN™) dispersions. Int. J. Pharm., 168(2), 221–229. https://doi.org/10.1016/S0378-5173(98)00092-1

Ghodrati, M., Farahpour, M.R., Hamishehkar, H. (2019). Encapsulation of peppermint essential oil in nanostructured lipid carriers: *in vitro* antibacterial activity and accelerative effect on infected wound healing. Colloids Surf. A Physicochem. Eng. Aspects, 564, 161–169. https://doi.org/10.1016/j.colsurfa.2018.12.043

Gundewadi, G., Sarkar, D.J., Rudra, S.G., Singh, D. (2018). Preparation of basil oil nanoemulsion using *Sapindus mukorossi* pericarp extract: physico-chemical properties and antifungal activity against food spoilage pathogens. Ind. Crops Prod., 125, 95–104. https://doi.org/10.1016/j.indcrop.2018.08.076

Jin, X., Streett, D.A., Dunlap, C.A., Lyn, M.E. (2008). Application of hydrophilic–lipophilic balance (HLB) number to optimize a compatible non-ionic surfactant for dried aerial conidia of *Beauveria bassiana*. Biol. Control, 46(2), 226–233. https://doi.org/10.1016/j.biocontrol.2008.03.008

Keck, C.M., Kovačević, A., Müller, R.H., Savić, S., Vuleta, G., Milić, J. (2014). Formulation of solid lipid nanoparticles (SLN): the value of different alkyl polyglucoside surfactants. Int. J. Pharm., 474(1–2), 33–41. https://doi.org/10.1016/j.ijpharm.2014.08.008

Kelidari, H.R., Moemenbellah-Fard, M.D., Morteza-Semnani, K., Amoozegar, F., Shahriari-Namadi, M., Saeedi, M., Osanloo, M. (2021). Solid-lipid nanoparticles (SLNs) containing Zataria multiflora essential oil with no-cytotoxicity and potent repellent activity against Anopheles stephensi. J. Parasit. Dis., 45(1), 101–108. https://doi.org/10.1007/s12639-020-01281-x

Khameneh, B., Halimi, V., Jaafari, M.R., Golmohammadzadeh, S. (2015). Safranal-loaded solid lipid nanoparticles: evaluation of sunscreen and moisturizing potential for topical applications. Iran. J. Basic Med. Sci., 18(1), 58.

Khorram, F., Ramezanian, A. (2021). Cinnamon essential oil incorporated in shellac, a novel bio-product to maintain quality of 'Thomson navel'orange fruit. J. Food Sci. Technol., 58, 2963–2972. https://doi.org/10.1007/s13197-020-04798-4

Kolluru, L.P., Atre, P., Rizvi, S.A. (2021). Characterization and applications of colloidal systems as versatile drug delivery carriers for parenteral formulations. Pharmaceuticals, 14(2), 108. https://doi.org/10.3390/ph14020108

Lai, F., Sinico, C., De Logu, A., Zaru, M., Müller, R.H., Fadda, A.M. (2007). SLN as a topical delivery system for Artemisia arborescens essential oil: *in vitro* antiviral activity and skin permeation study. Int. J. Nanomed., 2(3), 419-425. https://doi.org/10.2147/IJN.S2.3.419

Lehotay, S.J., Mastovska, K., Amirav, A., Fialkov, A.B., Martos, P.A., De Kok, A., Fernández-Alba, A.R. (2008). Identification and confirmation of chemical residues in food by chromatography-mass spectrometry and other techniques. TrAC Trends Anal. Chem., 27(11), 1070–1090. https://doi.org/10.1016/j.trac.2008.10.004

Li, Y., Kong, D., Fu, Y., Sussman, M.R., Wu, H. (2020). The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol. Biochem., 148, 80–89. https://doi.org/10.1016/j.plaphy.2020.01.006

Lingayat, V.J., Zarekar, N.S., Shendge, R.S. (2017). Solid lipid nanoparticles: a review. Nanosci. Nanotechnol. Res., 4(2), 67–72. https://doi.org/10.12691/nnr-4-2-5

Liu, J., Hu, W., Chen, H., Ni, Q., Xu, H., Yang, X. (2007). Isotretinoin-loaded solid lipid nanoparticles with skin targeting for topical delivery. Int. J. Pharm., 328(2), 191–195. https://doi.org/10.1016/j.ijpharm.2006.08.007

Lu, Y. et al. (2014). Food emulsifier polysorbate 80 increases intestinal absorption of di-(2-ethylhexyl) phthalate in rats. Toxicol. Sci., 139(2), 317–327. https://doi.org/10.1093/toxsci/kfu055

Mahboubi, M., Kazempour, N. (2014). Chemical composition and antimicrobial activity of peppermint (*Mentha piperita* L.) essential oil. Songklanakarin J. Sci. Technol., 36(1), 83–87.

Mahendran, G., Rahman, L.U. (2020). Ethnomedicinal, phytochemical and pharmacological updates on peppermint (*Mentha× piperita* L.) – a review. Phytother. Res. 34(9), 2088–2139. https://doi.org/10.1002/ptr.6664

McClements, D.J., Rao, J. (2011). Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. Crit. Rev. Food Sci. Nutr., 51(4), 285–330. https://doi.org/10.1080/10408398.2011.559558

McDaniel, A., Tonyali, B., Yucel, U., Trinetta, V. (2019). Formulation and development of lipid nanoparticle antifungal packaging films to control postharvest disease. J. Agric. Food Res., 1, 100013. https://doi.org/10.1016/j.jafr.2019.100013

Milsmann, J., Oehlke, K., Schrader, K., Greiner, R., Steffen-Heins, A. (2018). Fate of edible solid lipid nanoparticles (SLN) in surfactant stabilized o/w emulsions. Part 1: Interplay of SLN and oil droplets. Colloids Surf. A Physicochem. Eng. Aspects, 558, 615–622. https://doi.org/10.1016/j.colsurfa.2017.05.073

Mirtalebi, M., Rajaei, A., Bahmaei, M., Yari Khosroushahi, A. (2020). Storage stability of wheat germ oil encapsu-

lated within nanostructured lipid carriers. J. Nanostruct., 10(2), 268–278. 10.22052/JNS.2020.02.007

Montenegro, L., Pasquinucci, L., Zappalà, A., Chiechio, S., Turnaturi, R., Parenti, C. (2017). Rosemary essential oil-loaded lipid nanoparticles: In vivo topical activity from gel vehicles. Pharmaceutics, 9(4), 48. https://doi.org/10.3390/pharmaceutics9040048

Nahr, F.K., Ghanbarzadeh, B., Hamishehkar, H., Kafil, H.S. (2018). Food grade nanostructured lipid carrier for cardamom essential oil: preparation, characterization and antimicrobial activity. J. Funct. Foods, 40, 1–8. https://doi.org/10.1016/j.jff.2017.09.028

Naseri, M., Golmohamadzadeh, S., Arouiee, H., Jaafari, M.R., Nemati, S.H. (2020). Preparation and comparison of various formulations of solid lipid nanoparticles (SLNs) containing essential oil of *Zataria multiflora*. J. Hortic. Postharvest Res., 3(1), 73–84. https://doi.org/10.22077/jhpr.2019.2570.1068

Nasseri, M., Golmohammadzadeh, S., Arouiee, H., Jaafari, M.R., Neamati, H. (2016). Antifungal activity of Zataria multiflora essential oil-loaded solid lipid nanoparticles in vitro condition. Iran. J. Basic Med. Sci., 19(11), 1231.

Nielsen, C.K., Kjems, J., Mygind, T., Snabe, T., Meyer, R.L. (2016). Effects of Tween 80 on growth and biofilm formation in laboratory media. Front. Microbiol., 7, 1878.

Ons, L., Bylemans, D., Thevissen, K., Cammue, B.P. (2020). Combining biocontrol agents with chemical fungicides for integrated plant fungal disease control. Microorganisms, 8(12), 1930. https://doi.org/10.3390/microorganisms8121930

Palfi, M., Konjevoda, P., Vrandečić, K. (2019). Antifungal activity of essential oils on mycelial growth of *Fusarium oxysporum* and *Bortytis cinerea*. Emirates J. Food Agric., 31(7), 544–554. 10.9755/ejfa.2019.v31.i7.1972

Papoutsis, K., Mathioudakis, M.M., Hasperué, J.H., Ziogas, V. (2019). Non-chemical treatments for preventing the postharvest fungal rotting of citrus caused by *Penicillium digitatum* (green mold) and *Penicillium italicum* (blue mold). Trends Food Sci. Technol., 86, 479–491. https://doi.org/10.1016/j.tifs.2019.02.053

Pereira, I., Zielińska, A., Ferreira, N.R., Silva, A.M., Souto, E.B. (2018). Optimization of linalool-loaded solid lipid nanoparticles using experimental factorial design and long-term stability studies with a new centrifugal sedimentation method. Int. J. Pharm. 549(1–2), 261–270. https://doi.org/10.1016/j.ijpharm.2018.07.068

Piran, P., Kafil, H.S., Ghanbarzadeh, S., Safdari, R., Hamishehkar, H. (2017). Formulation of menthol-loaded nanostructured lipid carriers to enhance its antimicrobial activity for food preservation. Adv. Pharm. Bull., 7(2), 261. https://doi.org/10.15171/apb.2017.031

Plavšić, D.V., Dimić, G.R., Psodorov, Đ.B., Psodorov, D.Đ., Šarić, L.Ć., Čabarkapa, I.S., Košutić, M.B. (2017). Antifungal activity of *mentha piperita* and *carum carvi* essential oils. Zb. Matice Srp. Prir. Nauke, (133), 201–207. https://doi.org/10.2298/ZMSPN1733201P

Pongsumpun, P., Iwamoto, S., Siripatrawan, U. (2020). Response surface methodology for optimization of cinnamon essential oil nanoemulsion with improved stability and antifungal activity. Ultrasonics Snochem., 60, 104604. https://doi.org/10.1016/j.ultsonch.2019.05.021

Radomska-Soukharev, A. (2007). Stability of lipid excipients in solid lipid nanoparticles. Adv. Drug Del. Rev., 59(6), 411–418. https://doi.org/10.1016/j.addr.2007.04.004

Rolim, H.M.L., Ramalho, T.C. (2020). Essential oil encapsulated in nanoparticles for treatment of skin infections. In: Nanotechnology in skin, soft tissue, and bone infections, M. Rai (ed.). Springer, 121–131. https://doi.org/10.1007/978-3-030-35147-2_7

Salminen, H., Helgason, T., Kristinsson, B., Kristbergsson, K., Weiss, J. (2016). Formation of nanostructured colloidosomes using electrostatic deposition of solid lipid nanoparticles onto an oil droplet interface. Food Res. Int., 79, 11–18. https://doi.org/10.1016/j.foodres.2015.11.031

Sarhadi, S., Gholizadeh, M., Moghadasian, T., Golmohammadzadeh, S. (2020). Moisturizing effects of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) using deionized and magnetized water by *in vivo* and *in vitro* methods. Iran. J. Basic Med. Sci., 23(3), 337. https://doi.org/10.22038/IJBMS.2020.39587.9397

Senosy, I.A., Guo, H.-M., Ouyang, M.-N., Lu, Z.-H., Yang, Z.-H., Li, J.-H. (2020). Magnetic solid-phase extraction based on nano-zeolite imidazolate framework-8-functionalized magnetic graphene oxide for the quantification of residual fungicides in water, honey and fruit juices. Food Chem., 325, 126944. https://doi.org/10.1016/j.foodchem.2020.12694

Shah, R., Eldridge, D., Palombo, E., Harding, I. (2014). Optimisation and stability assessment of solid lipid nanoparticles using particle size and zeta potential. J. Phys. Sci., 25(1).

Shahbazi, Y. (2019). Antioxidant, antibacterial, and antifungal properties of nanoemulsion of clove essential oil. Nanomed. Res. J., 4(4), 204–208. https://doi.org/10.22034/NMRJ.2019.04.001

Shetta, A., Kegere, J, Mamdouh, W. (2019). Comparative study of encapsulated peppermint and green tea essential oils in chitosan nanoparticles: encapsulation, thermal stability, *in vitro* release, antioxidant and antibacterial activities. Int. J. Biol. Macromol., 126, 731–742. https://doi.org/10.1016/j.ijbiomac.2018.12.161

Souto, E.B. (2020). Croton argyrophyllus Kunth essential oil-loaded solid lipid nanoparticles: evaluation of release profile, antioxidant activity and cytotoxicity in a

neuroblastoma cell line. Sustainability, 12(18), 7697. https://doi.org/10.3390/su12187697

Talarico, L., Consumi, M., Leone, G., Tamasi, G., Magnani, A. (2021). Solid lipid nanoparticles produced via a coacervation method as promising carriers for controlled release of quercetin. Molecules, 26(9), 2694. https://doi.org/10.3390/molecules26092694

Tang, X., Huston, K.J., Larson, R.G. (2014). Molecular dynamics simulations of structure–property relationships of Tween 80 surfactants in water and at interfaces. J. Phys. Chem. B, 118(45), 12907–12918. https://doi.org/10.1021/jp507499k

Tariq, S. et al. (2019). A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant microbial pathogens. Microbial Pathogen., 134, 103580. https://doi.org/10.1016/j.micpath.2019.103580

Tavassolirajaee, M., Tatari, M., Kazemi, M.S., Taghizadeh, S.F. (2022). In vitro cytotoxicity of Cuminum cyminum essential oil loaded SLN nanoparticle. Nanomed. J., 9(3), 252–260. https://doi.org/10.22038/NMJ.2022.63943.1668

Torrisi, C., Di Guardia, M., Castelli, F., Sarpietro, M.G. (2021). Naringenin release to biomembrane models by incorporation into nanoparticles. Experimental evidence using differential scanning calorimetry. Surfaces, 4(4), 295–305. https://doi.org/10.3390/surfaces4040025

Vakili-Ghartavol, M., Arouiee, H., Golmohammadzadeh, S., Naseri, M. (2022). Antifungal activity of Mentha× piperita L. essential oil. Acta Sci. Pol. Hortorum Cultus, 21(1), 143–152. https://doi.org/10.24326/asphc.2022.1.12

Vakili-Ghartavol M., Arouiee H., Golmohammadzadeh S., Naseri M., Bandian L. (2024). Edible coatings based on solid lipid nanoparticles containing essential oil to improve antimicrobial activity, shelf-life, and quality of strawberries. Journal of Stored Products Research, 106, 102262. https://doi.org/10.1016/j.jspr.2024.102262

Vijayakumar, A., Baskaran, R., Jang, Y.S., Oh, S.H., Yoo, B.K. (2017). Quercetin-loaded solid lipid nanoparticle dispersion with improved physicochemical properties and cellular uptake. AAPS PharmSciTech, 18, 875–883. https://doi.org/10.1208/s12249-016-0573-4

Wu, L., Zhang, J., Watanabe, W. (2011). Physical and chemical stability of drug nanoparticles. Adv. Drug Del. Rev. 63(6), 456–469. https://doi.org/10.1016/j.addr.2011.02.001

Wu, S., Wang, Y., Liu, N., Dong, G., Sheng, C. (2017). Tackling fungal resistance by biofilm inhibitors. J. Med. Chem., 60(6), 2193–2211. https://doi.org/10.1021/acs.jmedchem.6b01203

Yadegarinia, D., Gachkar, L., Rezaei, M.B., Taghizadeh, M., Astaneh, S.A., Rasooli, I. (2006). Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils. Phytochemistry, 67(12), 1249–1255. https://doi.org/10.1016/j.phytochem.2006.04.025

Yu, Y., Chen, D., Lee, Y.Y., Chen, N., Wang, Y., Qiu, C. (2023). Physicochemical and *in vitro* digestion properties of curcumin-loaded solid lipid nanoparticles with different solid lipids and emulsifiers. Foods, 12(10), 2045. https://doi.org/10.3390/foods12102045

Yu, Z., Fan, W., Wang, L., Qi, J., Lu, Y., Wu, W. (2019). Effect of surface charges on oral absorption of intact solid lipid nanoparticles. Mol. Pharm., 16(12), 5013–5024. https://doi.org/10.1021/acs.molpharmaceut.9b00861

Zhao, Y., Chang, Y.-X., Hu, X., Liu, C.-Y., Quan, L.-H., Liao, Y.-H. (2017). Solid lipid nanoparticles for sustained pulmonary delivery of Yuxingcao essential oil: preparation, characterization and in vivo evaluation. Int. J. Pharm., 516(1–2), 364–371. https://doi.org/10.1016/j.ijpharm.2016.11.046