Research Article

Omid Ahmadi and Hoda Jafarizadeh-Malmiri*

Intensification process in thyme essential oil nanoemulsion preparation based on subcritical water as green solvent and six different emulsifiers

<https://doi.org/10.1515/gps-2021-0040>

received March 03, 2021; accepted June 17, 2021

Abstract: In order to alter the solubility and bioavailability of various functional lipids and plant essential oils (EOs), it is possible to prepare their oil in water (O/W) nanoemulsions. Thyme O/W nanoemulsions were prepared under subcritical water conditions (at 120°C and pressure of 1.5 atm for 2 h), using Tween 20, Tween 80, saponin, Arabic gum, xanthan gum, and sodium caseinate as emulsifiers. Results indicated that nanoemulsions with minimum mean droplet size of 11.5 and 12.6 nm were produced using Tween 20 and 80, respectively. Moreover, nanoemulsions with minimum polydispersity index (0.139) and maximum mean value of zeta potential (−24.5 mV) were provided utilizing xanthan gum and saponin, respectively. Results also revealed that the prepared nanoemulsions using saponin had maximum antioxidant activity based on percentage of scavenging ability (40.6%) and bactericidal effects against *Streptococcus mutans* as manifested in the formed clear zone (diameter of 21 mm). Morphological assessment of all the prepared nanoemulsions demonstrated that spherical thyme nanodroplets were formed in the colloidal solutions which revealed that all the prepared nanoemulsions had high thermodynamic stability due to the minimum surface energy level of the formed nanodroplets. This can increase applications of the prepared thyme O/W nanoemulsions in the aqueous food and pharmaceutical formulations.

Keywords: emulsifiers, process intensification, oil in water nanoemulsions, subcritical water conditions, thyme essential oil

1 Introduction

Thyme (*Thymus vulgaris* L.) belongs to the family of *Thymus* of Lamiaceae and is an important medicinal plant with desirable aromatic flavor and odor. Thyme and its derivatives contain phenolic compounds such as thymol and carvacrol which are known as natural antimicrobial and antioxidant agents, and have been widely used in veterinary, agriculture and food, medicine, and control of pest. However, its essential oil (EO), as a natural preservative agent, due to its lower solubility in aqueous formulation and higher sensitivity to oxygen, light, and heat, limits its applications. On the other hand, thyme EO, in high concentrations, is capable of interacting with the main components of food formulations, including lipids, proteins, and carbohydrates, and negatively altering their organoleptic characteristics [1]. Thymol (5-methyl-2-propan-2-ylphenol) and its isomer, namely carvacrol (2-methyl-5-propan-2-ylphenol), are two main components of the thyme EO, which have unique biological activities. In fact, thymol is the key monoterpene phenol compound of thyme EO, and both thymol and carvacrol have shown high anti-inflammatory, immunomodulator, antioxidant, antibacterial, and antifungal activities [2].

In the last two decades, numerous techniques based on nanotechnology have been developed to alter dispersity and bioavailability of various functional lipids and plant EOs in the aqueous systems [3–6]. In fact, by reducing the active components' particle size into the nanoscale ranges and increasing their surface area to volume ratio, it is possible to disperse poorly water soluble compounds in the water-based formulation. In other words, saturation, solubility, and dissolution rate of the components in water can be increased by reduction in their size [4]. Numerous techniques have been developed and used to prepare different immiscible components in nanoscale ranges and shapes, such as nanoliposomes, nanoparticles, nanodroplets, nanocapsules, nanodispersions, and nanoemulsions [5]. Oil in water (O/W) nanoemulsion is

* Corresponding author: Hoda Jafarizadeh-Malmiri, Department of Food Engineering, Faculty of Chemical Engineering, Sahand University of Technology, Tabriz 51335-1996, East Azarbaijan, Iran, e-mail: h_jafarizadeh@sut.ac.ir, h_jafarizadeh@yahoo.com, tel: +98-413-345-9099, fax: +98-413-344-4355

Omid Ahmadi: Department of Food Engineering, Faculty of Chemical Engineering, Sahand University of Technology, Tabriz 51335-1996, East Azarbaijan, Iran

prepared by dispersion of lipid-based liquid nanodroplets, such as plant EO, into an immiscible liquid, such as water, in the presence of emulsifiers [6]. O/W nanoemulsions have droplet size ranging from 100 to 1,000 nm and have been used widely in cosmetics, agrochemicals, personal care commodities, pharmaceutical and food supplements, and many commercial products composed of low water-dispersed bioactive compounds [4,7]. O/W nanoemulsions are often studied in food formulations, as delivery systems of functional lipophilic compounds such as flavors, EOs, vitamins, and antimicrobial agents [8]. Generally, in food nanoemulsions, natural and food grade emulsifiers are used. Commonly, polysaccharides, amphiphilic proteins, and small molecule surfactants, which are non-toxic, have been used in food products [9]. For example, different emulsifiers, such as saponin, xanthan gum, Tween 20, Tween 80, sodium caseinate, Arabic gum, lecithin, and other natural emulsifiers, have been used to prepare numerous O/W nanoemulsions, such as thyme O/W nanoemulsions [9–14].

Two different approaches for preparation of O/W nanoemulsions, including low-energy and high-energy consuming methods, have been developed. The high-energy consuming techniques include ultrasonic emulsification, high pressure homogenization, high shear stirring, and membrane emulsification. On the other hand, most widely used low-energy consuming methods in preparation of O/W nanoemulsions are spontaneous emulsification, emulsion inversion point, and phase inversion composition and temperature [14–16]. Recently, an innovative approach using water in its subcritical conditions has been developed to prepare O/W nanoemulsions with a narrow distribution of droplet size and using minimum amount of emulsifier, to provide more soluble bioactive compounds with higher bioavailability, like plant and herbal EO, which are easier to access for the body [4,7]. Pressurized water at high temperature is known as subcritical water, which is maintained in liquid form due to the pressure [5]. Subcritical water has also been used as a solvent for the extraction of EO and other substances, at temperatures between 100°C and 374°C and pressure higher than 1 bar [17].

The main objectives of the present study were as follows: (i) to make thyme EO nanoemulsions via subcritical water method and six different emulsifiers, namely saponin, xanthan gum, Arabic gum, sodium caseinate, Tween 20, and Tween 80, (ii) to assess influences of different emulsifiers on physicochemical attributes of the prepared nanoemulsions including shape and size of nanodroplets, zeta potential, polydispersity index (PDI), turbidity, and color intensity (appearance), (iii) to study the bactericidal and antioxidant activities of the prepared nanoemulsions,

and (iv) to screen efficient emulsifier for preparation of thyme EO nanoemulsions with valuable and desired physicochemical and biological attributes.

2 Materials and methods

2.1 Materials

Dried thyme plant was provided from the local markets in Tabriz, East Azerbaijan province of Iran. Various emulsifiers including saponin, Arabic gum, xanthan gum, sodium caseinate, Tween 20, and Tween 80 were purchased from Merck Company (Darmstadt, Germany). 2,2-Diphenyl-2-picrylhydrazyl (DPPH) was bought from Sigma Company (St. Louis, Missouri, USA). Bacteria strain of *Streptococcus mutans* (PTCC 1683 1112) was provided from microbial Persian type culture collection (PTTC; Tehran, Iran). Plate count agar (PCA) was purchased from Oxoid (Oxoid Ltd., Hampshire, England).

2.2 Thyme EO extraction

There are several methods to extract EO of the plants. One of the most popular methods for extraction of the natural bioactive compounds is the Clevenger method [18]. Dried thyme leaves were milled using a domestic miller and 100 g of its powder was placed into a Clevenger glassware and heated using steam of the water boiling at 100°C for 2 h. Finally, 2.6 mL of transparent thyme EO with pale yellow color was collected and stored in a refrigerator (at 4°C) for further processes and analyses.

2.3 Thyme O/W nanoemulsion preparation

In order to prepare thyme nanoemulsions, according to the preliminary experiments and literature studies, a constant and defined amount of each emulsifier (Table 1) was selected and added to 45 mL of distilled water and mixed under a constant stirring rate of 300 rpm for 15 min [9–14]. Tweens are most common emulsifiers (small molecule surfactants) which have been used widely in the nanoemulsion preparation. On the other hand, emulsifiers with polymeric structure as macromolecules, have been used to prepare stable nanoemulsions. Therefore, these two groups of emulsifiers have been selected in the present work to evaluate their effects on the properties of the formed nanoemulsions. After that, 0.5 mL of the extracted thyme

Table 1: Amount of aqueous phase, organic phase, and six different emulsifiers for preparation of thyme essential oil nanoemulsions

	Type of emulsifier	Amount of emulsifier (g/mL)	Amount of thyme essential oil (mL)	Amount of distilled water (mL)
1	Tween 80	4.5 mL	0.5	45
2	Tween 20	4.5 mL	0.5	45
3	Saponin	4.5 g	0.5	45
4	Sodium caseinate	0.1 g	0.5	45
5	Arabic gum	0.75 g	0.5	45
6	Xanthan gum	0.25 g	0.5	45

EO was added to the aqueous phase and the samples were put into a 100 mL completely sealed Teflon hydrothermal autoclave and heated in oven (Behdad Medical Production Co., SP88, Tehran, Iran) at a temperature of 120°C and a pressure of 1.5 atm for 2 h.

2.4 Physicochemical analysis

After preparation of nanoemulsions, all the physicochemical analyses have been completed, instantly. Presence of the main functional groups and their types in the provided thyme EO were studied and characterized using Fourier transform infrared spectroscopy (FTIR 8400S, Shimadzu Co., Kyoto, Japan) with KBr pellets, at a ranging wavelength of 4,000–400 cm^{-1} .

Nanodroplet size (z -average hydrodynamic diameter) and its distribution, PDI, and zeta potential values of the resulted nanoemulsions were characterized using a dynamic light scattering (DLS) particle size analyzer (Malvern Instruments, Zetasizer Nano ZS, Worcestershire, UK) at 25°C.

UV-vis spectrophotometer (Jenway UV-Vis spectrophotometer 6705, Staffordshire, UK) was used to measure the color intensity and turbidity of the prepared thyme nanoemulsions, based on absorbance unit (% a.u.). In fact, aliquots of the samples were added into a 1 cm optical path quartz cuvette and placed in the spectrophotometer that was set at wavelengths of 420 nm (color intensity) and 625 nm (turbidity) [19]. Antioxidant activity of the samples was evaluated using the method described by Sayyar and Jafarizadeh-Malmiri [7]. For this analysis, 100 μL of the samples and 5 mL of 50% (v/v) methanol having 1 mM DPPH radicals were mixed vigorously and stored in dark bottles (at 27°C for 30 min). Control sample was prepared by mixing the pure DPPH and methanol at a ratio of 1:1. Then, using UV-Vis spectrophotometer (250–800 nm; PerkinElmer's Co., Rodgau, Germany), maximum absorbance of the samples (A_{sample}) and control (A_{control}) were recorded at a wavelength of 517 nm. Antioxidant activity of the samples based on the percentage of scavenging ability ($I\%$) was obtained by the following Eq. 1 [5]:

$$I\% = \frac{(A_{\text{Control}} - A_{\text{Sample}})}{A_{\text{Control}}} \times 100. \quad (1)$$

2.5 Bactericidal assay

Bactericidal activity of the prepared thyme (O/W) nanoemulsions was assessed using well diffusion method, described by Sayyar and Jafarizadeh-Malmiri, toward *S. mutans*, a Gram-positive bacteria strain that causes dental caries [20]. For this analysis, 0.1 mL of *S. mutans* suspension, containing 1.5×10^8 colony forming units per mL, was spread on the plates (90 mm in diameter) containing PCA. Several holes, each with a diameter of 5 mm, were created in the PCA and 10 μL of the prepared nanoemulsions were poured into them and the plates were then incubated at 37°C for 24 h. Antibacterial activity of the samples was manifested in diameters of the formed clear zones, around the holes.

2.6 Morphological study

Microstructure and shape of the resulted nanoemulsions were monitored using transmission electron microscopy (TEM, CM120, Philips, Amsterdam, Netherlands) with an acceleration voltage of 120 kV. The sample was diluted 100 times with deionized water, and one drop of it was placed on the grid and then stained by a 1% aqueous solution of phosphotungstic acid and left to dry at room temperature for imaging.

2.7 Statistical analysis

Physicochemical properties, bactericidal, and antioxidant activities of the resulted thyme EO nanoemulsions were replicated three times. The variance was analyzed using Minitab v.16 statistical package (Minitab Inc., PA, USA) to interpret the resulted data. Tukey's comparison test was used to compare the mean values of the obtained data with p -value less than 5% ($p < 0.05$).

3 Results and discussion

3.1 FTIR spectra analysis

To identify the main functional groups present in thyme EO, FTIR spectra of the extracted EO were obtained (Figure 1). As can be seen from the figure, several highlighted picks can be observed in the FTIR spectra of which six were the main ones. These main bonds were centered at 3423.72 cm^{-1} (OH vibration of hydroxyl group of thyme EO), 2961.06 and 2870.44 cm^{-1} (C–H stretching of methyl and isopropyl groups on the phenolic ring that could be related to the two main bioactive compounds, carvacrol, and thymol, in thyme EO), and 1459.06 , 1421.32 , and 811.43 cm^{-1} (C–C in ring of aromatics group). In our previous study, GC-MS chromatogram of the extracted thyme EO indicated that carvacrol and thymol existed in the provided extract, these two components being the main bioactive compounds of the thyme EO [21]. The peaks centered between $1,600$ and $1,660\text{ cm}^{-1}$ wavenumber were related to the C=C bonds, which was related to the thymol.

3.2 Droplet size of the prepared thyme nanoemulsions

Mean value of the droplet size (mean value of the *z*-average hydrodynamic diameter) of the resulted thyme EO nanoemulsions using six different emulsifiers, is shown in Table 2. As can be seen in this table, minimum mean value of *z*-average hydrodynamic diameter of the resulted nanoemulsions were obtained using Tween 20 (11 nm),

Tween 80 (12 nm), xanthan gum (170 nm), saponin (230 nm), sodium caseinate (245 nm), and Arabic gum (275 nm). Figure 2a–f shows nanodroplet size distribution of the prepared thyme nanoemulsions. Tween 20 and Tween 80 are small surfactant molecules as compared to other studied emulsifiers which have polymeric structure. Therefore, *z*-average hydrodynamic diameter of the formed thyme nanodroplets in the colloidal systems, in which the droplets were covered with emulsifiers, had direct relation with the size and structure of the emulsifier molecules [12]. Achieved results were in agreement with the findings of Xue and Zhong [22] and Ziani et al. [23]. They reported that the prepared thyme EO nanoemulsions using Tween 80 and lecithin, and utilizing 5% corn oil, via high pressure homogenizer, lead to the formation of nanoemulsions with mean value of droplet size of 12 and 105 nm, respectively. Furthermore, Niu et al. prepared thyme EO nanoemulsions with droplet size ranging from 250 to 400 nm, using 0.6% emulsifier containing ovalbumin and Arabic gum, and 5% commercial sunflower oil as a co-solvent. In the meantime, using subcritical water in the present study, thyme (O/W) nanoemulsions with droplet size ranging from 11 to 275 nm were prepared without utilizing co-solvent and carrier oils. This indicated that under subcritical water conditions, nanoemulsion preparation method could be effectively intensified [10].

3.3 PDI of the prepared thyme nanoemulsions

PDI, as a value of the width of the droplet size distribution, shows the homogeneity of the resulted nanodroplets

Figure 1: FTIR spectrum of thyme essential oil.

Table 2: Physicochemical properties of prepared thyme nanoemulsions using six different nanoemulsions

Type of emulsifier	Mean value of z-average hydrodynamic diameter (nm)	PDI	Zeta potential (mV)	Color intensity (% a.u.)	Turbidity (% a.u.)
Tween 80	12 ± 1 ^e	0.317 ± 0.02 ^e	-3.22 ± 0.13 ^d	0.051 ± 0.02 ^f	0.011 ± 0.001 ^e
Tween 20	11 ± 1 ^e	0.327 ± 0.02 ^d	-2.91 ± 0.16 ^e	0.089 ± 0.02 ^e	0.006 ± 0.001 ^f
Saponin	230 ± 4 ^c	0.912 ± 0.02 ^a	-24.5 ± 0.48 ^a	1.411 ± 0.06 ^c	0.095 ± 0.009 ^d
Sodium caseinate	245 ± 7 ^b	0.842 ± 0.03 ^b	-8.41 ± 0.46 ^c	3.972 ± 0.08 ^a	1.863 ± 0.011 ^b
Arabic gum	275 ± 7 ^a	0.645 ± 0.03 ^c	-22 ± 0.54 ^b	0.62 ± 0.04 ^d	0.374 ± 0.010 ^c
Xanthan gum	170 ± 4 ^d	0.139 ± 0.01 ^f	-8.88 ± 0.51 ^c	3.142 ± 0.09 ^b	2.096 ± 0.011 ^a

Different small letters represent significant differences ($p < 0.05$) in column.

in the nanoemulsions and ranges from 0 to 1 [7]. PDI values of the prepared nanoemulsions using six different emulsifiers are indicated in Table 2. As can be seen from the table, minimum PDI was achieved for nanoemulsions prepared using xanthan gum, Tween 20, and Tween 80, with values of 0.139, 0.327, and 0.317, respectively indicating the homogeneity of the formed nanodroplets in these three systems. Small droplet size and PDI values of the prepared thyme EO nanoemulsions using Tween 20, Tween 80, and xanthan gum, indicate that these three nanoemulsions could effectively limit unwanted phenomena such as creaming, coalescence, and Ostwald ripening in nanoemulsions. In Ostwald ripening, smaller droplets submit themselves to the bigger ones and start growing bigger and eventually cause destabilization of nanoemulsions [3].

3.4 Zeta potential of the prepared thyme nanoemulsions

Zeta potential, as a surface charge density of the formed nanodroplets, has a direct relation to electrostatic stability and tendency of the formed nanodroplets to aggregate with each other [24]. Zeta potential of the prepared nanoemulsions using six different emulsifiers is shown in Table 2. As can be observed from the table, maximum zeta potential values for the prepared nanoemulsions were obtained using Arabic gum, xanthan gum, sodium caseinate, Tween 80, and Tween 20. Obtained results indicate that the zeta potential of nanoemulsion prepared by saponin has the highest value (-24.5 mV), followed by Arabic gum (-22 mV) and xanthan gum (-8.88 mV). Obtained results were in line with the findings of Lue et al. [25]. They studied the effects of the three emulsifiers on the stability of the prepared O/W nanoemulsions and found that zeta potential values of the prepared nanoemulsions using saponin, lecithin, and Tween 80 were -70, -26, and -10 mV, respectively. In fact, in the absence of hydrophobic emulsifiers, the zeta potential of the droplets in the nanoemulsions stabilized by saponin, was highly negative which can be attributed to anionic functional groups on the saponin molecules. Our previous studies related to preparation of thyme O/W nanoemulsions using saponin and xanthan gum as two different emulsifiers, under subcritical water conditions, indicated that the prepared nanoemulsion using saponin had higher zeta potential value (-22.51 mV) as compared to that prepared using xanthan gum (-10.1 mV) [21,26]. Zeta potential value other than -30 mV to +30 mV is generally considered to

Figure 2: Nanodroplet size distributions of the prepared thyme nanoemulsions using Tween 80 (a), Tween 20 (b), saponin (c), sodium caseinate (d), Arabic gum (e), and xanthan gum (f).

have sufficient repulsive force to attain better physical colloidal stability. On the other hand, a small zeta potential value can result in particle aggregation and flocculation due to the van der Waals attractive forces acting upon them. These may result in physical instability [23,24].

3.5 Color intensity and turbidity of the produced thyme nanoemulsions

Appearance and color of a commercial product is an important factor for the customers to select that product. In the case of nanoemulsions, their appearance can be manifested in their homogeneity, opacity, turbidity, and color, and strong influences by the size and concentration of the oil droplets [27]. Therefore, appearance of a nanoemulsion is determined by a combination of light scattering (e.g., turbidity and opacity) and absorption (e.g., color) phenomena [28,29]. Due to the importance of nanoemulsion appearance, the color intensity and

turbidity of the prepared thyme EO nanoemulsions using six different emulsifiers are shown in Table 2. As can be seen from the table, minimum color intensity of the prepared nanoemulsions was obtained using Tween 80, Tween 20, and Arabic gum. Furthermore, the prepared thyme nanoemulsions using Tween 20, Tween 80, and saponin had minimum turbidity values. Droplet size of nanoemulsion plays an important role in the turbidity and color of the prepared nanoemulsions. Diameter of the formed nanodroplets in nanoemulsions may change between 20 and 500 nm, causing variation in the appearance of the resulting nanoemulsions, from transparent (for small droplet size), which remain stable for a long period, to opaque (for large droplet size) due to unwanted phenomena such as creaming, coalescence, flocculation, aggregation, and Ostwald ripening [23,26]. The color intensity of the nanoemulsions is also increased by increasing droplet concentration in the colloidal systems [30]. Appearance of the prepared thyme nanoemulsions using different emulsifiers is shown in Figure 3. As can be observed from the figure, the droplet size has a direct relation to the color

Figure 3: Appearance (color and turbidity) of the prepared thyme nanoemulsions using six different emulsifiers.

intensity and turbidity of the prepared samples; nanoemulsions using Tween 20 and Tween 80 had minimum droplet size and the lowest color intensity and turbidity. McClements [28] indicated that color of the emulsion systems is strongly related to their composition and microstructure of the formed droplets.

3.6 Antioxidant assay of the produced thyme nanoemulsion

Antioxidant activity of the bioactive compounds is related to their hydrogen-donating capacity in the DPPH test, where it is assumed that the control is completely oxidized [31]. Obtained results indicated that the antioxidant activities of the provided thyme nanoemulsions using six different emulsifiers and the extracted thyme EO ranged from 16% to 95.1%. While pure thyme EO had antioxidant activity of 95.1%, prepared nanoemulsion using saponin had maximum antioxidant activity (40.6%), as compared to the Tween 80 (25.8%), xanthan gum (22.4%), Tween 20 (22%), sodium caseinate (20%), and Arabic gum (16%). Due to the dilution of the nanoemulsion and the low amount of EO present in the thyme nanoemulsion, the antioxidant activities of the prepared thyme nanoemulsions using different emulsifiers, were lower than that of the pure thyme EO. In fact, antioxidant ability of thyme EO is due to the presence of the main phenolic compounds namely, thymol and carvacrol in the thyme leaves [21]. In general, during autoxidation of lipids, thymol is a more effective and more active antioxidant than carvacrol. Thymol radicals do not participate in chain propagation during lipid oxidation. However, carvacrol radicals take part in one reaction of chain propagation in the lipid systems [32]. Obtained results also indicated that the control solutions containing emulsifiers, with defined amounts, in the water

had no antioxidant activity as compared to the prepared nanoemulsions.

3.7 Antibacterial activity of the produced thyme nanoemulsion

Thyme EO is more active against Gram-positive bacteria than Gram-negative bacteria strains [33]. Therefore, in the present study, antibacterial activity of the prepared thyme nanoemulsions was evaluated on Gram-positive bacteria, namely *S. mutans*. Bactericidal activity of the samples against *S. mutans* is shown in Figure 4. As clearly observed from the figure, diameters of the created clear zones around the holes containing prepared thyme nanoemulsion using saponin, xanthan gum, Tween 80, Tween 20, sodium caseinate, and Arabic gum were 21 ± 1 , 19 ± 1 , 16 ± 1 , 14 ± 1 , 11 ± 1 , and 8 ± 1 mm, respectively. Obtained results revealed that the control solutions containing emulsifiers, with defined amounts, in the water had no bactericidal effect against *S. mutans*, as compared to the prepared nanoemulsions. Antibacterial activity of the prepared thyme nanoemulsions was related to the presence of two active substances in thyme EO, namely carvacrol and thymol [26,34]. These two compounds are phenolic compounds (a group of secondary metabolites) which can alter

Figure 4: Antibacterial activity of the resulted thyme nanoemulsion using six different emulsifiers against *Streptococcus mutans* (incubated at 37°C for 24 h).

cytoplasmic membrane of the bacteria strains and inhibit protein and RNA synthesis processes [35]. Obtained results were in line with the findings of Mostafa [36]. In that study, high antibacterial activity of the prepared ginger EO nanoemulsion using Tween 80 was reported against *S. mutans*, due to the the phenolic compounds of ginger oil, including gingerols and shogaols.

3.8 Morphological characteristics

Morphology of the prepared thyme EO nanoemulsions using six different emulsifiers is shown in Figure 5a–f. As can be seen from the figure, using all these six

different emulsifiers and subcritical water conditions, spherical thyme nanodroplets were formed in the colloidal system. This spherical shape of the thyme droplets in the resulted nanoemulsions could be related to their minimum surface energy level in the formed nanoemulsions, which reduced the rate of some unwanted phenomena, such as creaming and flocculation, in the prepared nanoemulsions [37]. Obtained high values of zeta potential of all the prepared thyme nanoemulsions (Table 2) indicate that there was strong repulsion between the formed nanodroplets in the samples, which help nanodroplets to preserve their spherical shape. Achieved results show that the resulted thyme nanoemulsions using Tween 20 and Tween 80 had an approximate droplet size mean value of 4 and 5 nm, respectively, while DLS analysis

Figure 5: Morphological attributes (size and shape) of the prepared thyme nanoemulsions using Tween 80 (a), Tween 20 (b), saponin (c), sodium caseinate (d), Arabic gum (e), and xanthan gum (f).

shows the values as 11 and 12 nm, respectively. In fact, TEM measures droplet size of nanodroplets in dry state and DLS measures nanodroplets surrounded with solvent molecules [5,38].

4 Conclusion

Emulsifiers are amphiphilic compounds with high potential activity to reduce surface tension between two immiscible fluids and have key role in the nanoemulsions preparation. Emulsifiers, depending on their nature, molecular weight, size, and chemical structure, have different effects on the physicochemical, morphological, appearance, antioxidant, and antimicrobial properties of the prepared nanoemulsions. In the present study, thyme EO nanoemulsions were prepared using six different emulsifiers and obtained results indicated that Tween 20 and Tween 80 were more suitable for preparation of clear thyme EO nanoemulsions with minimum droplet size, color intensity, and turbidity. Furthermore, preparation of thyme EO nanoemulsions using xanthan gum led to the formation of monodispersed oil nanodroplets in the nanoemulsions. Using saponin, as a natural emulsifier, could result in preparation of thyme oil nanoemulsions with maximum zeta potential value, antioxidant, and antibacterial activities. However, sodium caseinate and Arabic gum did not show desirable effects on the studied properties of the prepared thyme EO nanoemulsion. Furthermore, polarity of water in its subcritical conditions alters some organic solvents including methanol, ethanol, and acetone, which makes dissolution of thyme EO in the water easy, as a solvent, and decreases the number of emulsifiers in the process of nanoemulsions preparation. Preparation of plant oils nanoemulsions using novel, simple, fast, and cost-effective technique of subcritical water, using combination of suitable emulsifiers can be used to prepare nanoemulsions with high stability, lowest droplet size, and uniformity in their droplet size.

Acknowledgements: The authors appreciate the support of Sahand University of Technology to accomplish this research.

Funding information: The authors appreciate Sahand University of Technology (grant no. 30.5592) for its financial support.

Author contributions: Omid Ahmadi: methodology, validation, investigation, resources, data curation, writing –

original draft, visualization, and funding acquisition; Hoda Jafarizadeh-Malmiri: formal analysis, writing final manuscript, writing – review and editing, supervision, and project administration.

Conflict of interest: Authors state no conflict of interest.

Data availability statement: All data generated or analyzed during this study are included in this published article.

References

- [1] Ghaderi-Ghahfarokhi M, Barzegar M, Sahari MA, Azizi MH. Enhancement of thermal stability and antioxidant activity of thyme essential oil by encapsulation in Chitosan nanoparticles. *J Agric Sci Technol*. 2016;18:1781–92.
- [2] Boskovic M, Zdravkovic N, Ivanovic J, Janjic J, Djordjevic J, Starcevic M, et al. Antimicrobial activity of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) essential oils against some food-borne microorganisms. *Proc Food Sci*. 2015;5:18–21.
- [3] Anarjan N, Fahimdanesh M, Jafarizadeh-Malmiri H. β -Carotene nanodispersions synthesis by three-component stabilizer system using mixture design. *J Food Sci Tech*. 2017;54:3731–6.
- [4] Acosta E. Bioavailability of nanoparticles in nutrient and nutraceutical delivery. *Curr Opin Colloid Sci*. 2009;14(1):3–15.
- [5] Sayyar Z, Jafarizadeh-Malmiri H. Preparation, characterization and evaluation of curcumin nanodispersions using three different methods – Novel subcritical water conditions, spontaneous emulsification and solvent displacement. *Z Phys Chem*. 2019;10:1485–502.
- [6] Jafari SM, McClements DJ. Nanoemulsions: formulation, applications, and characterization. 1st ed. Cambridge: Academic Press; 2018.
- [7] Sayyar Z, Jafarizadeh-Malmiri H. Temperature effects on thermodynamic parameters and solubility of curcumin O/W nanodispersions using different thermodynamic models. *Int J Food Eng*. 2019;15:34–46.
- [8] Anarjan N, Jafarizadeh Malmiri H, Ling TC, Tan CP. Effects of pH, ions, and thermal treatments on physical stability of astaxanthin nanodispersions. *Int J Food Prop*. 2014;17:937–47.
- [9] Xue J, Davidson PM, Zhong Q. Antimicrobial activity of Thyme essential oil co-nanoemulsified with sodium caseinate and lecithin. *Int J Food Prop*. 2015;210:1–8.
- [10] Niu F, Pan W, Su Y, Yang Y. Physical and antimicrobial properties of Thyme essential oil emulsions stabilized by ovalbumin and gum Arabic. *Food Chem*. 2016;212:138–45.
- [11] Qiu C, Zhao M, McClements DJ. Improving the stability of wheat protein-stabilized emulsions: Effect of pectin and xanthan gum addition. *Food Hydrocoll*. 2015;43:377–87.
- [12] Chong WT, Tan CP, Cheah YK, Lajis AFB, Dian NL, Kanagaratnam S, et al. Optimization of process parameters in preparation of tocotrienol-rich red palm oil-based nanoemulsion stabilized by Tween80-Span 80 using response surface methodology. *PLoS ONE*. 2018;13:e020271.

- [13] Vijaya R, Suresh Kumar S, Kamalakannan S. Preparation and *in vitro* evaluation of miconazole nitrate nanoemulsion using tween 20 as surfactant for effective topical/transdermal delivery. *J Chem Pharm Sci.* 2015;8:92–8.
- [14] Ryu V, McClements DJ, Corradini MG, Yang JS, McLandsborough L. Natural antimicrobial delivery systems: formulation, antimicrobial activity, and mechanism of action of quillaja saponin-stabilized carvacrol nanoemulsions. *Food Hydrocoll.* 2018;82:442–50.
- [15] Anarjan N, Jafarizadeh-Malmiri H, Nehdi IA, Sbihi HM, Al-Resayes SI, Tan CP. Effects of homogenization process parameters on physicochemical properties of astaxanthin nanodispersions prepared using a solvent-diffusion technique. *Int J Nanomed.* 2015;10:1109–18.
- [16] Anarjan N, Nehdi I, Sbihi H, Al-Resayes S, Jafarizadeh-Malmiri H, Tan CP. Preparation of astaxanthin nanodispersions using gelatin-based stabilizer systems. *Molecules.* 2014;19:14257–65.
- [17] Gamiz-Gracia L, De Castro ML. Continuous subcritical water extraction of medicinal plant essential oil: comparison with conventional techniques. *Talanta.* 2000;51:1179–85.
- [18] Clevenger J. Apparatus for the determination of volatile oil. *J Am Pharm Assoc.* 1928;17:345–9.
- [19] Ahmadi O, Jafarizadeh-Malmiri H, Jodeiri N. Optimization of processing parameters for hydrothermal silver nanoparticles synthesis using Aloe vera leaf extract and estimation of their physico-chemical and antifungal properties. *Z Phys Chem.* 2019;233:651–67.
- [20] Sayyar Z, Jafarizadeh-Malmiri H. Photocatalytic and antibacterial activities study of prepared self-cleaning nanostructure surfaces using synthesized and coated ZnO nanoparticles with Curcumin nanodispersion. *Z Kristallogr Cryst Mater.* 2018;234:307–28.
- [21] Ahmadi O, Jafarizadeh-Malmiri H. Green approach in food nanotechnology based on subcritical water: effects of Thyme essential oil and saponin on characteristics of the prepared oil in water nanoemulsions. *Food Sci Biotechnol.* 2020;26:783–92.
- [22] Xue J, Zhong Q. Thyme essential oil nanoemulsions coemulsified by sodium caseinate and lecithin. *J Agric Food Chem.* 2014;62:9900–7.
- [23] Ziani K, Chang Y, McLandsborough L, McClements DJ. Influence of surfactant charge on antimicrobial efficacy of surfactant-stabilized Thyme essential oil nanoemulsions. *J Agric Food Chem.* 2011;59:6247–55.
- [24] Chung C, Sher A, Rousset P, Decker EA, McClements DJ. Formulation of food emulsions using natural emulsifiers: Utilization of quillaja saponin and soy lecithin to fabricate liquid coffee whiteners. *J Food Eng.* 2017;209:1–11.
- [25] Luo X, Zhou Y, Bai L, Liu F, Zhang R, Zhang B, et al. Production of highly concentrated oil-in-water emulsions using dual-channel microfluidization: Use of individual and mixed natural emulsifiers (saponin and lecithin). *Food Res Int.* 2017;96:103–12.
- [26] Ahmadi O, Jafarizadeh-Malmiri H. Intensification and optimization of the process for Thyme essential oil in water nanoemulsions preparation using subcritical water and xanthan gum. *Z Phys Chem.* 2020;235:629–48.
- [27] McClements DJ. Food emulsions: principles, practices, and techniques. Florida: CRC press; 2015.
- [28] McClements DJ. Theoretical prediction of emulsion color. *Adv Colloid Interface Sci.* 2002;97:63–89.
- [29] Linke C, Drusch S. Turbidity in oil-in-water-emulsions – Key factors and visual perception. *Food Res Int.* 2016;89:202–10.
- [30] Rahn-Chique K, Puertas AM, Romero-Cano MS, Rojas C, Urbina-Villalba G. Nanoemulsion stability: experimental evaluation of the flocculation rate from turbidity measurements. *Adv Colloid Interface Sci.* 2012;178:1–20.
- [31] Youdim KA, Deans SG, Finlayson HJ. The antioxidant properties of thyme (*thymus zygis* L.) essential oil: An inhibitor of lipid peroxidation and a free radical scavenger. *J Essent Oil Res.* 2002;14:210–5.
- [32] Jukić M, Miloš M. Catalytic oxidation and antioxidant properties of thyme essential oils (*Thymus vulgaris* L.). *Croatica Chem Acta.* 2005;78:105–10.
- [33] Jouki M, Mortazavi SA, Yazdi FT, Koocheki A. Characterization of antioxidant-antibacterial quince seed mucilage films containing thyme essential oil. *Carbohydr Polym.* 2014;99:537–46.
- [34] Abdollahzadeh E, Rezaei M, Hosseini H. Antibacterial activity of plant essential oils and extracts: The role of thyme essential oil, nisin, and their combination to control *Listeria monocytogenes* inoculated in minced fish meat. *Food Control.* 2014;35:177–83.
- [35] Lucchini JJ, Corre J, Cremieux A. Antibacterial activity of phenolic compounds and aromatic alcohols. *Res Microbiol.* 1990;141(4):499–510.
- [36] Mostafa NM. Antibacterial activity of ginger (*Zingiber officinale*) leaves essential oil nanoemulsion against the cariogenic *Streptococcus mutans*. *J Appl Pharm Sci.* 2018;8:34–41.
- [37] Sayyar Z, Jafarizadeh-Malmiri H. Process intensification for curcumin nanodispersion preparation using subcritical water – Optimization and characterization. *Chem Eng Process.* 2020;153:107938.
- [38] Sayyar Z, Jafarizadeh-Malmiri H. Preparation of curcumin nanodispersions using subcritical water–Screening of different emulsifiers. *Chem Eng Technol.* 2020;43:263–72.