Influence of oil phase, surfactant on nano-emulsion based on essential oil from orange using phase inversion temperature method

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Abstract. Essential oils are incredibly effective natural antimicrobials, and they have the potential for substitute synthetic antimicrobials in the food industry, but the use of essential oils is restricted by their little solubility in water. In this research, the phase inversion temperature (PIT) method was employed to produce essential oil from orange nano-emulsions. The effects of oil phase composition, surfactant, and storage condition on the formation and stability of the nano-emulsions were investigated. Study results showed that the surfactant and oil phase constitution highly influenced the thermal property of the nano-emulsions. The transparent nano-emulsions system with the least average droplet size (46.5 nm) was created in the conditions: 10 wt% tween 80, 8 wt% essential oil from orange, 2 wt% coconut oil, and distilled water. The mean droplet diameter of these nano-emulsions raised over time and depended on the composition of surfactants. The nano-emulsion systems contained 10% tween 80 gained small size and stability after 30 days of storage, while systems with 20% tween 80 showed instability, and particle size increased rapidly after 30 days of storage. These outcomes of this investigation could be useful for the fabrication and use of nano-emulsions as distribution systems in food technology.

1. Introduction

The antimicrobial activities of essential oil from orange (C. Sinensis) have been known for a long time, and essential oil from orange has the potential to replace synthetic antimicrobials in food applications. However, its use is restricted by low water-solubility. Therefore, nano-emulsion formulations are suitable for the efficient delivery of essential oil in food applications.

The essential oil from an orange shell is commonly utilized in the food as flavouring agents and natural antimicrobials [1]. The essential oil from orange is a multiplex organic admixture, including more than 200 constituents [2]. It contains a big portion of monoterpene hydrocarbons (70–95%) with D-limonene is the major component. In addition, there is a small number of sesquiterpene hydrocarbons, which are in charge of a typical flavor of essential oil [3]. Lately, essential oil from orange has received special attention in food owing to increasing demand for food safety of consumers[4]. It has been researched for various food applications [5] such as food conservation; antimicrobial wrapper for food goods; esculent thin films [6]; conservation of vegetables and fruits[7]; flavouring element in carbonated colas, soft drinks; meat, fish and seafood conservation [6, 8]

One of the principal difficulties of utilizing essential oils in foods is that they contain a big proportion of hydrophobic molecules. Therefore, they are hard to unite into aqueous-based food products directly.
Consequently, a colloidal conveyance system is normally needed to encapsulate, keep, and deliver them [9]. The colloidal conveyance systems for this object include oil-in-water (O/W) microemulsions, nano-emulsions, and emulsions [9]. In which one of the most potential systems for encapsulating essential oils is oil-in-water nano-emulsions [10, 11]. These formations comprise of minor emulsifier-coated small oil drops dissolved within a water-bearing ambiance [12]. The droplets in nano-emulsions mostly have average size smaller than 100 nm and high kinetic stability [13]. The little diameter of droplets in nano-emulsions creates special properties for commercial applications in food [13, 14]. Firstly, nano-emulsions appear transparent, which gives the ability to incorporation lipophilic bioactive compositions into optically transparent goods such as some foods and drinks [9]. Secondly, they commonly have high steadiness for gravitational separation and flocculation, which increases the shelf-life of the products [15]. Finally, bioavailability often rises as the droplet size in the system reduces, which offers the big potential of rising bioavailability of lipophilic compounds like essential oils[16-18]. Nano-emulsions which have been produced to contain essential oil from orange have been reported in several studies [1, 2, 19, 20].

Generally, nano-emulsions can be fabricated by either high energy method or low energy method [21]. The high energy methods commonly consist high-speed homogenization [22], high-pressure homogenization[23], micro-fluidization, and sonication[24]. In these methods, mechanical equipment types are used to create strong mechanical force leading to the generation of tiny oil droplets into aqueous phase [22]. In low energy approaches, tiny oil droplets are generated spontaneously within the oil-water-surfactant blend when the constitution or condition of the system is changed in a definite way. The low energy techniques comprise phase inversion temperature (PIT), phase inversion composition (PIC), spontaneous emulsification (SE), and emulsion inversion point (EPI) [25-26]. In the present study, we use the low-energy PIT method for making transparent nano-emulsion, which is suitable for food applications[27-29]. The PIT technique is based on a change in the hydration features of non-ionic surfactants when the temperature alterations [30]. When standardized, this method makes nano-emulsions containing tiny droplets. The PIT of most surfactant-oil-water formations has been investigated to be under 90°C [29], and it is suitable for fabricating essential oil nano-emulsions.

At present, high energy methods often are utilized for the production of nano-emulsions in the food industry. However, these methods require high energy input and big equipment costs. Therefore, the use of low energy methods that are suitable for industrial production and feasible economically is of interest. In the low energy techniques, the PIT method is simple to implement and has the potential for industrial application. To the best of our knowledge, the establishment of essential oil from orange nano-emulsions by the PIT technique has not yet been reported. Therefore, the object of the present work was to consider the vital factors affecting the construction and stability of nano-emulsions using the PIT method. The nano-emulsions were formed using essential oil from the orange as a bioactive ingredient and coconut oil as a ripening inhibitor. The outcomes of this work could be useful for the design and use of nano-emulsions as distribution systems in the food and other industries.

2. Materials and Methods

2.1 Materials
The essential oil from orange (C. Sinensis) was purchased from Aota International Company (VietNam). Coconut oil, whose ingredients include 47.5% lauric acid, 18.1% myristic acid, and 8% caprylic acid, was also a product of Aota International Company. The non-ionic surfactants (Tween 20, 40, 80, and Span 80) were supplied from Xilong Scientific Co., Ltd (China). Double-distilled water was used in the preparation of all solutions and emulsions.

2.2 Nano-emulsion preparation
The essential oil from orange nano-emulsions was produced according to the technique described by Rao and McClements [31], with some small adaptations. In brief, essential oil from orange and coconut oil blended together for 3 min, and then surfactant and double-distilled water were added. All
components were mixed together and stirred (500 rpm) for 30 min to produce a coarse emulsion, and each system was then heated to 15°C above the phase PIT. Then, a two-step cooling procedure was performed. Firstly, the temperature was reduced to the PIT to allow a stable microemulsion to form. Secondly, a rapid cooling step was performed by immersing in an ice bath at 5°C with continuous stirring. Factors affecting the formation of nano-emulsions were investigated. The process was carried out at different ratios of orange oil-to-coconut oil (2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 10:0 wt%); ratio of surfactant-to-oil (0.5:1, 1:1, 1.5:1, 2:1) and surfactant type (tween 20, 40, 80, and span 80). Except for investigating the effect of surfactant concentrations, other tests were performed using standardized setups: a total oil phase (orange oil + coconut oil) proportion of 10 wt% (5g), surfactant proportion of 10 wt% (5g), and aqueous phase content of 80 wt% (40g). The PIT, droplet size, particle size distribution, and stability of all samples were investigated and reported.

2.3 Turbidity determination
The nano-emulsion turbidity was evaluated by measuring the absorbance at 600 nm with a UV-Vis spectrophotometer (UV1800, Shimadzu Scientific Instruments, Japan) without dilution. Double-distilled water was used as a reference (blank) sample.

2.4 Particle size measurements
The droplet sizes and particle size distribution of orange oil nano-emulsions were analyzed using a dynamic light scattering device (Nanoparticle SZ-100; HORIBA Ltd, Kyoto, Japan). The samples were diluted with deionized water (1:20 v/v) prior to analysis to avoid multiple scattering effects.

2.5 Determination of nano-emulsion stability
The physical stability of the essential oil from orange nano-emulsions was determined by measuring the alteration in the turbidity and droplet size throughout 30 days of storage at 5°C and 30 °C. The stability of the orange oil nano-emulsions was monitored in two different systems with oil phase/surfactant/water ratios of 10%/10%/80% and 10%/20%/70%, respectively.

2.6 Statistical Analysis.
All tests were performed 2 or 3 times, and the outcomes are presented as the computed mean and standard deviation of these measurements. Means were subjected to Duncan's experiment, and a p-value of <0.05 was confirmed statistically significant.

3. Results

3.1. Effect of the Oil Composition on nano-emulsion properties
Firstly, we evaluated the effect of the oil-phase constitution on the size of droplets. The components of the system are fixed (10 wt% oil phase, 10 wt% tween 80, and 80 wt% double-distilled water). The oil-phase constitution was mixed by altering different the mass ratios of orange essential oil to coconut oil. The samples were measured turbidity and particle size. According to previous studies, the turbidity of the emulsion was closely related to particle size. As the droplet size increased, the turbidity increased [32]. Besides, many studies indicated that absorbance could be measured at 600nm in order to assess the turbidity of nano-emulsion [29].

Figure 1 showed that the ratio of orange oil-to-coconut oil in the oil phase had a crucial effect on the droplet sizes of nano-emulsions. The results showed that transparent nanoparticles only were created by mixing orange oil and coconut oil in a certain proportion. The smallest size droplets were created when the ratio of orange oil in the oil phase was 70% (63.8 nm) and 80% (58.7 nm). When the composition of the orange oil in the oil phase was less than 60%, there was a notable rise in the droplet sizes, creating non-transparent nano-emulsions.

The results showed a complex interaction between essential oil from orange and coconut oil. The systems in which components of the orange oil in the oil phase was less than 60% did not lead to the
creation of a bicontinuous microemulsion during heating, so tiny oil droplets were not created during
the later cooling procedure. These may be due to the increase in coconut oil content, which increases
the viscosity of the system and affect the phase inversion process according to some previous studies
[33]. On the other hand, when the ratio of orange oil in the oil phase was too high (greater than 80%), it
was not able to create stable and transparent nano-emulsions. This can be interpreted by the phenomenon
of Ostwald ripening [34], which small particles come together and become the larger one, which makes
the emulsion system less stable. To solve this problem, a non-polar compound, such as triglyceride, is
added to inhibit this phenomenon, which increases the durability of the nano-emulsion system. This is
one of the reasons when adding a certain amount of coconut oil to the orange oil nano-emulsions, the
system was more stable than when not using coconut oil. Previous studies had shown that both type and
amount of the oil phase had an important effect [2]. This research indicated that the orange oil nano-
emulsions with tiny droplet size (58.7 nm) could be created by the PIT method using a tiny amount of
coconut oil in the oil phase (20%). This result is quite favorable; it is only necessary to use a small
number of ripening inhibitors that create a stable nano-emulsion when compared with some other studies
[29]. In our study, the system containing 20 wt% coconut oil and 80 wt% essential oil from orange in
the oil phase created stable nano-emulsions.

Figure 1. Effect of the ratio of essential oil and coconut oil on nano-emulsion.

3.2. Effect of the Surfactant Concentration on the on nano-emulsion
The PIT method is based on variations in the hydration feature of non-ionic surfactants when the
temperature variation. Therefore, surfactant concentration plays a major role in the foundation of nano-
emulsions. To evaluate the effect of surfactant concentration on the size of the nano-emulsions, the
concentration of surfactant (Tween 80) was varied (5, 10, 15, 20 wt%), while keeping the total content
of the oil phase (2% coconut oil and 8% orange oil) constant, the remainder is double-distilled water.

Figure 2 showed that the concentration of the surfactant had a great effect on the size of the droplets.
When the tween 80 concentration raised, the particle size dropped. Specifically, the tween 80
concentration of 5, 10, 15, and 20 wt%, the mean particle sizes were 49, 46, 35, and 31 nm, respectively.
At a concentration of 5%wt, although the average particle size was small, the particle size distribution
was wide, so the system had a non-transparent state. When tween 80 levels of 10% or more, the system
became optically transparent.
These results were in agreement with prior investigations that have also presented that smaller sizes are created in essential oil nano-emulsions at higher surfactant proportions. High surfactant levels at the oil–water boundary lead to a reduction in the interfacial tension, and the oil–water interface can be stabilized [2, 29]. When creating a nano-emulsion system, we always want to use a minimum of surfactant and still achieve the desired effect due to cost, savor, and toxicity concerns. In this study, nano-emulsion systems with tween 80 concentration of 10% and 20% were selected to monitor its stability over time.

Figure 2. Effect of the surfactant concentration on the mean particle diameter.

3.3. Effect of the surfactant Type on nano-emulsion properties
In the low energy methods for creating nano-emulsion systems, surfactants play a significant role in the production of nano-emulsion systems. Each type of surfactant has a different hydrophilic-lipophilic balance (HLB) index, which is suitable for creating different o/w or w/o nano-emulsion systems. In these tests, the effect of non-ionic surfactants on the size of nanoparticles has also been considered. In order to prepare nano-emulsion systems, the surfactant types were varied (Tween 20, 40, 80, and Span 80), while keeping the content of the oil phase (2% coconut oil and 8% orange oil), surfactant (20 wt%) and water phase (70 wt% double-distilled water) constant.

Figure 3 showed that the type of surfactant greatly affected the formation of orange essential oil nano-emulsions. Transparent nano-emulsions holding tiny droplets were created when using tween 40 or tween 80 (d = 32 nm or d = 38 nm, respectively), whereas non-transparent emulsions holding quite large and unstable droplets were created when using tween 20 (d = 217 nm). Specifically, for the emulsion system containing span 80, although the average particle size was small, the particle size was distributed over a wide range, from 5 nm to 10000 nm (Figure 4). Therefore, the emulsion system did not achieve transparency and stability.

These results indicated that the formation of orange essential oil nanoparticles in this study was unsuitable when using surfactants with too high an HLB value (Tween 20, HLB = 16.7) or too low an HLB value such as (Span 80, HLB = 8.6). On the contrary, surfactants with median HLB numbers such as (Tween 40, HLB = 15.6; Tween 80, HLB = 15) are more suited for the formation of nano-emulsions in this research. These results are consistent with previous studies that have also suggested that low HLB (below 9.0) surfactants are used in the creation of W/O nano-emulsions, whereas big HLB (above 11.0)
Surfactants are used in the formation of O/W nano-emulsions [9]. In these tests, tween 80 was the most suitable surfactant to create highly transparent and stable orange oil nano-emulsions.

![Figure 3](image1.png)

**Figure 3.** Effect of the surfactant type on the mean particle size.

![Figure 4](image2.png)

**Figure 4.** Diagram of particle size distribution using different surfactants: Tween 80 (a) and Span 80 (b).

3.4. **Effect of storage conditions and storage time on nano-emulsion properties**

An important requirement of nano-emulsion delivery systems in food applications is that they must be physically stable throughout their shelf life. In these experiments, we investigated the influence of surfactant concentration on the physical stability of orange oil nanoparticles during 30-days storage. In addition, we also investigated the influence of storage conditions (at 5°C and 30 °C) on the stability of orange oil nanoparticles. Previous experiments showed that the nano-emulsion systems which contain tween 80 concentration of 10% or more became optically transparent and contained very small droplets. Therefore, in this study, nano-emulsion systems with tween 80 concentrations of 10% and 20% were selected to investigate the physical stability of nanoparticles during 30-days storage at 5°C and 30 °C.

The results of the study showed that there was a great difference in the physical stability of orange oil nano-emulsion systems (storage time of 30 days) in two cases. In the first case, the nanosystem, which contains 8% orange oil, 2% coconut oil, 10 wt% tween 80, and distilled water, indicated physical
stability after 30-days storage both storage temperatures 5°C and 30 °C (fig 5). Particle size varied very slightly (40-50nm), and the orange oil nano-emulsions remained transparent during storage. In the other case, the nanosystems which contain 8% orange oil, 2% coconut oil, 20 %wt tweens 80, and distilled water showed physical instability after 30 days of storage both storage temperatures 5°C and 30 °C (Figures 5). Particle size increased very fast, and the orange oil nano-emulsion systems were non-transparent after 30 days of storage at both different storage conditions at 5°C and 30 °C.

The results of the study showed that a big tween 80 concentration had boosted droplet accretion through coalescence or Ostwald ripening. This has been explained in a number of previous studies that have also suggested that when the amount of surfactant is too high, the attendance of surfactant micelles in the aqueous phase creates attractive osmotic stress and changes the properties of the interfacial layer, thereby promoting coalescence [29]. In summary, these experiments showed that the nano-emulsion system, which contains 8% orange oil, 2% coconut oil, 10 wt% tween 80, and distilled water has high stability and optically transparent.

The influence of storage conditions on the stability of the nano-emulsions was also performed in this study. Overall, at storage conditions of 5 and 30 °C, there was no notable change in mean droplet size during storage for nanoparticles containing 10 wt% tween 80. However, at storage conditions of 30°C, the droplet size increased slightly after 30 days of storage, which may be due to the rate of droplet coalescence rises in the temperature range just below the PIT. The closer the PIT temperature is, the less stable the nano-emulsion system. However, the temperature of 30 °C was still quite far away PIT temperature, the coalescence effect was not significant, the nano-emulsion system was still stable, and the particle size only increased slightly after 30 days of storage.

Figure 5. Change in the mean particle size of selected nano-emulsions during 30 days of storage at 5°C and at 30°C.

4. Conclusions
In this paper, an orange essential oil nano-emulsion system was successfully produced by the phase inversion temperature method. Investigation showed that the emulsification process that gave the uniform, smallest droplets should be implemented with the optimal parameters of the system's components; details are as follows: 2% coconut oil, 8% essential oil from orange, 10% tween 80, and 80% distilled water. When created under these parameters, the dispersion phase droplets of the nano-emulsion could maintain the mean size of 46.3 nm and transparency after 30 days of storage at temperature 5°C. In addition, the nano-emulsion systems with tween 80 levels of 10% or more contained small and transparent droplets. However, nano-emulsion systems containing 10% tween 80 gained small size and stability after 30 days of storage, while nano-emulsion systems containing 20% tween 80 showed instability, particle size increased rapidly after 30 days of storage. Experimental results also showed that different storage conditions (at temperature 5°C and 30 °C) did not remarkably affect particle size and stability of orange essential oil nano-emulsion after 30 days of storage.
The production of orange essential oil nano-emulsion by phase inversion temperature method is a simplistic and inexpensive method. The results of this study could be useful for the design and use of nano-emulsions as delivery systems in the food and other industries. In future studies, we plan to study the antimicrobial activity of orange oil nano-emulsions in food applications.

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