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# Process optimization and stability of D-limonene nanoemulsions prepared by catastrophic phase inversion method

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ABSTRACT

The aim of this study was to optimize the process and stability of D-limonene nanoemulsions. The nanoemulsions were prepared by catastrophic phase inversion method using Tween 80 as surfactant. According to the results, the SOR value would considerably affect the turbidity and the mean particle diameter of emulsions. At a high concentration of surfactant (S/O = 1.5), D-limonene nanoemulsions could be obtained. In addition, the formation of nanoemulsions may be primarily related to the viscosity of oil phase. When the oil phase contained less than 15% (w/w) olive oil, the nanoemulsions could be prepared. The turbidities and the mean particle diameters of D-limonene nanoemulsions with adding the same concentration of different plant oils were similar. Furthermore, it can be found that adding olive oil could enhance the stability of D-limonene nanoemulsion system and decrease Ostwald ripening rate, and the Ostwald ripening rate of D-limonene nanoemulsion at 4 °C was higher than that at 28 °C.



## 1. Introduction

D-limonene (4-isopropenyl-1-methylcyclohexene), a natural and functional monoterpene, is the major component of all citrus essential oils. It has been reported that D-limonene had bactericide, antioxidant, chemo-preventative and therapeutic activities (Gerhäuser et al., 2003). Since D-limonene has oxidative degradation under normal storage condition and is not soluble in water, it is difficult to keep the lemon-like flavor and be active in those areas of food, such as water-rich phases or liquid–solid interfaces (Soottitantawat et al., 2003). Furthermore, transparent D-limonene aqueous solution would be preferred in commercial application, such as food and beverages. Therefore, it is very important to utilize a delivery system to protect D-limonene from chemical degradation and improve its water-solubility.

Oil-in-water (O/W) nanoemulsion technology is probably a good method to protect D-limonene from chemical degradation and improve its ease of handling and utilization. Nanoemulsions have their potential advantages over other types of colloidal delivery systems for food applications: high optical clarity, good physical stability, enhanced bioavailability, improved water-solubility (Fanun, 2009; Flanagan and Singh, 2006; Huang et al., 2010; Lee and McClements, 2010; Weiss et al., 2006). Therefore, they can be used to incorporate non-polar functional components into transparent aqueous-based food and beverage products (Chen et al., 2006). Some literatures provided information that the nanoemulsions of essential oils could enhance their biological activity, increase their water-solubility and improve their chemical stability (Gaysinsky et al., 2008; McClements, 2011).

Two main methods are currently used to prepare the nanoemulsions: high-energy method and low-energy method. High-energy methods typically involve the methods of microfluidization, high-pressure homogenization, or ultrasonic (McClements, 2011; Wooster et al., 2008). All of these methods require the input of a considerable amount of mechanical energy (Bilbao-Sáinz et al., 2010). In contrast, low-energy methods are less expensive and energy efficient alternative that takes advantage of the chemical energy stored in the systems (Jahanzad et al., 2009; Solè et al., 2006a,b). Catastrophic phase inversion (CPI) method is a kind of low-energy methods. And it is the process whereby an oil-in-water system (O/W) inverts into a water-in-oil system (W/O) and vice versa (Züge et al., 2013).

D-limonene nanoemulsions were usually prepared by high-energy methods. Li and Chiang (2012) systematically investigated the influence of ultrasonic emulsification conditions on the droplet size of D-limonene nanoemulsions, and to optimize the conditions for preparing D-limonene nanoemulsions with smallest droplet size using response surface methodology (RSM) and examined the stability of D-limonene nanoemulsions. Jafari et al. (2012) used a high pressure homogenization to prepare D-limonene nanoemulsions. Lemon oil nanoemulsions were prepared by Microfluidics 110L (Ziani et al., 2012). However, there are few reports describing

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the formation and the stability of physically stable transparent nanoemulsions by CPI method.

This study aimed to optimize the process and improve the stability of D-limonene-in-water nanoemulsions prepared by CPI method. In this approach, nanoemulsions were prepared by CPI method using Tween 80 as the surfactant. Our objective explored the formation and stability of D-limonene nanoemulsions from turbidity and mean particle diameter. In addition, the stability of D-limonene nanoemulsions under different storage times and temperatures were also examined.

## 2. Materials and methods

### 2.1. Materials

D-limonene was purchased from Florida Worldwide Citrus Products Group Inc. (United States of America, USA). Sorbitan monooleate (Tween 80), propylene glycol and sodium chloride (purity > 99.5%) were purchased from the Sinopharm Chemical Reagent Co., Ltd. Olive oil, corn oil, sunflower oil and soybean oil were purchased from China Oil & Foodstuffs Corporation. Double distilled and deionized water was filtered prior to use.

### 2.2. Nanoemulsion preparation

A water phase was prepared by mixing distilled water with propylene glycol in a 2:1 mass ratio, including 0.1% NaCl (w/w). The surfactant was Tween 80, which has a hydrophilic–lipophilic balance (HLB) of 15.0. Nanoemulsions were prepared from a mixture of oil phase and Tween 80 by slowly adding water phase with the gentle agitation which speed was 1300 rpm. The addition rate of water was kept constant at approximately 1.0 mL/min. The concentration of oil phase in all emulsions was kept constant at 4.0 wt%, while the surfactant-to-oil weight ratios (S/O) were 0.5, 1, and 1.5. With slowly adding water phase in a mixture of oil phase and Tween 80, a W/O emulsion with a high oil-to-water ratio was formed, and then increasing amounts of water were added to the system with continuous stirring. The amount of water added to a W/O emulsion was progressively increased, until a phase inversion occurred and an O/W emulsion was formed with continuous stirring for 6 h.

### 2.3. Turbidity measurements

The turbidity was determined using an UV–visible spectrophotometer at 600 nm (Ultra-spec 2450, SHIMADZU Ltd., Japan). Distilled water was used as a reference to blank the cells. Each measurement was replicated twice.

### 2.4. Droplet size determination

The mean particle diameters ($Z$-averages) of samples were determined using Dynamic Light Scattering (DLS) at 25 °C (Zetasizer Nano-ZS 90, Malvern Instruments, UK). The samples were placed in vertical cylindrical cuvettes (10 mm-diameter). The analysis was performed at a scattering angle of 90°. Each individual measurement was an average of 10 runs.

### 2.5. Viscosity measurements

All kinematic viscosity measurements of liquid were performed using a kinematic viscosimeter (WFY-108D, Dalian Continental Petroleum Equipment Co. Ltd., China). All density measurements of liquid were determined using a densitometer (Shanghai Huachen Medical Instruments Co. Ltd., China). The viscosity, $\eta$ can be obtained:

$$\eta = \upsilon\rho$$

where $\upsilon$ is the kinematic viscosity of liquid and $\rho$ is the density of liquid.

### 2.6. Storage stability

The stability of nanoemulsions was determined by measuring the change of droplet size every 2 days during storage at 4 or 28 °C for 12 days.

### 2.7. Statistical analysis

All measurements were performed on two or three freshly prepared samples and were reported as means and standard deviations.

## 3. Results and discussion

### 3.1. Influence of SOR value for the formation of nanoemulsions

Table 1 shows the mean particle diameter of the nanoemulsions which adding different concentrations of Tween 80. As the concentration of surfactant increasing, the mean particle diameter and turbidity of the nanoemulsions were decreased. When S/O = 1.5, D-limonene nanoemulsions could be obtained (mean particle diameter = 40.13 nm). That is because of high surfactant concentration can make the oil phase dissolve in water completely at emulsion inversion point. But low surfactant concentration resulted in the formation of larger droplets. When S/O was large enough, the surfactant form the layer structure at phase inversion point, then the minimum interfacial tension was formed and it promoted the formation of small droplets.

Fig. 1 shows the phase diagram of D-limonene nanoemulsions. When S/O = 0.5, the oil component totally solubilized at 19% water; therefore, a region of single lamellar liquid crystals was observed from 19% to 32% water content. Phase inversion occurred when the water contents were above 32%, and then the D-limonene was redistributed, forming a single D-limonene O/W emulsion. However, when S/O value was 1.5, in the furthest zone from the water corner, a lamellar liquid crystalline phase coexisted with an excess oil phase

**Table 1**
The change of turbidity and mean particle diameter of emulsions with S/O values. Values were measured at ambient temperature.

| SOR | 1:2 | 2:2 | 3:2 |
|---|---|---|---|
| Turbidity ($cm^{-1}$) | 2.492 ± 0.015 | 0.249 ± 0.003 | 0.073 ± 0.001 |
| Mean particle diameter (nm) | 229.37 ± 6.39 | 143.29 ± 3.58 | 40.13 ± 2.37 |

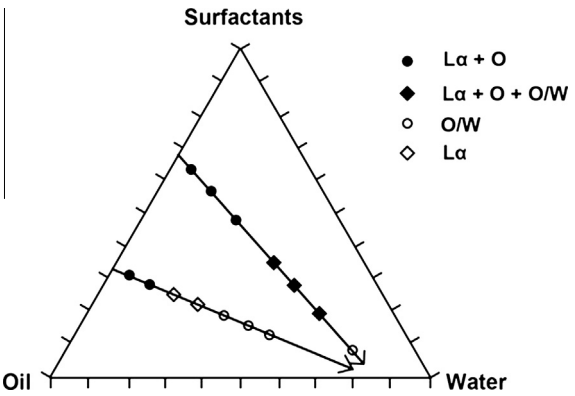


**Fig. 1.** Equilibrium phases of the D-limonene nanoemulsions system.