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Effective Droplet Size Reduction and Excellent Stability of Limonene Nanoemulsion Formed by High-Pressure Homogenizer

Marcel Jonathan Hidajat [1], Wantaek Jo [2], Hyeonhyo Kim [2] and Jongho Noh [2,*]

[1] Department of Research and Development, Hiflux Co. Ltd., 361-33, Gapcheon-ro, Daejeon 34037, Korea; marcel.hidajat@gmail.com

[2] Department of Research and Development, Ilshin Autoclave Co. Ltd., 255, Techno 2-ro, Daejeon 34026, Korea; jowt@suflux.com (W.J.); hh@suflux.com (H.K.)

* Correspondence: njh@suflux.com; Tel.: +82-42-9316100

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Abstract: Limonene as an interesting bioactive material that has great benefits due to its antimicrobial and anti-carcinogen properties. However, it has several limitations such as its oxidative and oily nature. In order to overcome these limitations, a high-pressure homogenizer (HPH) was utilized to produce limonene nanoemulsion, which enhances its dispersibility while preventing oxidation with great stability. Limonene was pre-mixed with soybean oil as carrier oil prior to emulsification. The effect of soybean oil to limonene ratio, number of pass, homogenization pressure, emulsifier concentration and homogenization method were observed. A stability test was also conducted for 28 days at room temperature. The result revealed that soybean oil and limonene demonstrated a certain ratio to produce the most stable nanoemulsion. Meanwhile, emulsion size could be reduced from 327.8 nm to 55.5 nm in five passes at 1000 bar. Increasing the emulsifier concentration could reduce the droplet size to 40 nm. A comparison with other emulsification method showed that HPH was the best emulsification technique due to its intense emulsification power resulted from shear, cavitation, and droplet impacts. This study reveals that HPH is a great and simple way to produce stable limonene nanoemulsion for the cosmetic, pharmaceutical, and food industries.

Keywords: limonene; high-pressure homogenizer; nanoemulsion; emulsifier; emulsification power

1. Introduction

Limonene is a natural compound obtained from citruses peels, such as lemon, orange, tangerine, mandarin, and is the major compound of the essential oil [1–3]. Limonene has a lemon/orange fragrance and is usually used as perfume in cosmetics and in other areas such as foods and pharmaceuticals. In food sectors, limonene can be used as a flavoring to mask bitter taste, while it also has anti-oxidant, anti-microbial, anti-carcinogen, chemo-preventive, and antidiabetic properties which can be used for pharmaceutical purposes [2]. Furthermore, limonene is listed in the federal regulation as generally regarded as safe (GRAS), meaning that it is safe to be consumed [4].

Although limonene presents great benefits to human, limonene has several drawbacks to be overcome in order to be consumed. The drawbacks are listed as follows: (1) it can be oxidized in normal environment through air oxidation, which changes its structure to carvone, carveol, and limonene-oxide [5]. The oxidation will result in the loss the lemonish flavor and also the pharmaceutical properties [3,6,7]. Further, (2) limonene is an oily substance, therefore its dispersion in water is quite limited [2]. To overcome these drawbacks, limonene should be protected from outside environment before reaching the target area through the usage of nanoemulsion.

Nanoemulsion is an emulsion with the droplet size below 500 nm. Since the droplet size is so small, it can resist various de-stabilization phenomena such as aggregation, coalescence, and flocculation due to the brownian motion that dominates over gravitational forces [3,8,9]. The only factor that can de-stabilize the nanoemulsion is the Ostwald ripening phenomena, which can be prevented by using proper emulsifier and surface modificators. The advantages of nanoemulsion including easy manufacturing, controllable particle sizes, and increased bioactive compounds availability and stability due to its small size [7,10–12].

There are two ways in order to produce nanoemulsion, low-energy and high-energy methods [13]. Low-energy methods can be divided into several different methods, for example, phase inversion temperature (PIT), phase inversion composition (PIC), and spontaneous emulsification. Although the formation of nanoemulsions through these methods might produce stable nanoemulsion, these methods have several limitations. The PIT method needs specific thermal energy requirement and specific temperature-sensitive surfactants while spontaneous emulsification has a limitation in the amount of oil and solvent that can be used in the system. Furthermore, it is not applicable for large-scale production [14,15].

Meanwhile, high-energy methods, including high-pressure homogenizer (HPH), microfluidization, high-speed homogenizer (HSH), and ultrasonication are more flexible in terms of surfactant selection, and they have the ability to produce nanoemulsions in a very short time [16–18]. In addition, high-energy methods are able to produce fine emulsion from various materials [18].

The comparison of high-energy methods for production of nanoemulsion has been done by several researchers. Jafari et al. compared the production of nanoemulsion by microfluidization and sonication. They reported that both methods can be used for nanoemulsion production with nearly almost the same average droplet size but different size distributions, in terms of which microfluidization produced a better nanoemulsion dispersion [19]. Pinnamaneni et al. analyzed the difference between HSH and microfluidization for the production of oil-in-water emulsion. It is reported that microfluidizer produced better emulsion stability than HSH. At low concentration of emulsifier, they discovered that microfluidizer could produce stable emulsion whereas emulsion produced by HSH was quick to cream [20]. Mao et al. analyzed the differences between microfluidizer and high-pressure valve homogenizer. They concluded that microfluidizer was capable to produce slightly smaller nanoemulsion with less temperature rise [21]. These results indicate that microfluidizer seems to be the best method to produce nanoemulsions with high homogeneity than others.

In this paper, production of limonene nanoemulsion was conducted by utilizing HPH. Limonene combined with soybean oil was selected as the oil phase. Soybean oil was used as carrier oil because it contains lots of long-chain triglycerides (LCTs) such as oleic acid, linoleic, and linolenic acid, which have high hydrophobicity that might help stabilizing formed nanoemulsion [22]. Here, the formed nanoemulsion by HPH was compared to other methods (HSH and ultrasonication) and several operating conditions (homogenization pressure, number of passes, emulsifier concentration and limonene to soybean oil ratio) were optimized in order to produce highly stable and small nanoemulsion.

2. Materials and Methods

2.1. Materials

D-Limonene (or R-(+)-Limonene, 97% purity) was purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Soybean oil was purchased from Ottogi Co. Ltd. (Pyeongtaek, Korea). Polysorbate 80 (Tween 80) was purchased from Daejung Chemicals Co. Ltd. (Siheung, Korea) and DI water was provided by Merck™ Millipore Direct-Q™ 5 water purification system (Merck KGaA, Darmstadt, Germany). All materials were used without any pre-processing beforehand. The viscosity of the materials used in this study was measured by AND SV-10 Sine Wave Vibro Viscometer (A&D Co. Ltd., Tokyo, Japan).

2.2. Nanoemulsion Synthesis

Limonene nanoemulsion was synthesized by high-pressure homogenization method. The high-pressure homogenizer used in this study is a motor-driven homogenizer (220 VAC, 1 PH, 60 Hz, 1 HP) (NLM100, Ilshin Autoclave Co. Ltd., Daejeon, Korea) with capacity of 500 mL and maximum operating pressure of 2000 bar. The equipment has a dimension of 583 × 576 × 435 mm (D × W × H) with maximum flow rate of 120 mL/min. The homogenizer used in this experiment is shown in Figure 1. The homogenization chamber used in this homogenizer is called DiaCell® Z-type nozzle (Figure 1b). In order to prevent a high temperature increases during homogenization, the nozzle is equipped with cooling jacket connected to the chiller. Prior to the experiment, the chiller was set to −2 °C to maintain the temperature of the nanoemulsion at 25–35 °C.

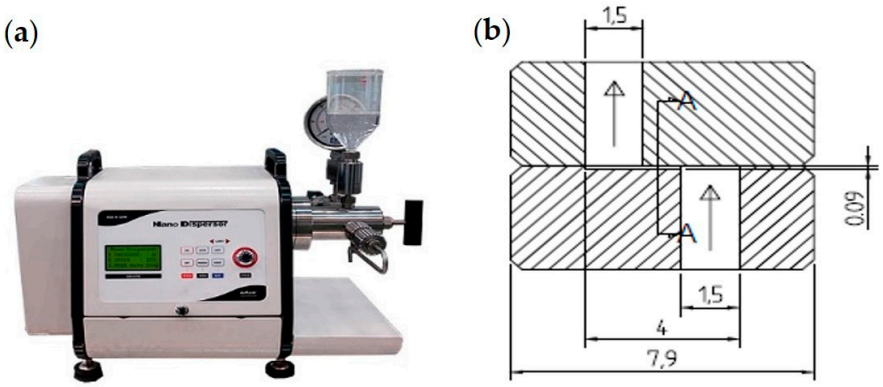

Figure 1. Scheme of the equipment used in this study: (**a**) electric motor-driven high-pressure homogenizer, and (**b**) Diacell® Z-type nozzle (unit in mm).

The method to synthesize limonene nanoemulsion in detail is described as follows. Limonene was mixed with soybean oil at a certain ratio to make total oil content of 10 wt%. The purpose of mixing limonene with soybean oil is to enhance the properties of the nanoemulsion formed. First, the mixture was formed by conventional magnetic stirring in order to enhance solubilization of limonene in soybean oil. Next, DI water and Tween-80 were prepared and mixed together in a separate beaker. The oil phase was then poured into the aqueous phase and mixed again with conventional magnetic stirring for several minutes to form pre-emulsion before subjected to high-shear mixer (X 10/20-E3 high-shear mixer, Ystral GmbH, Ballrechten-Dottingen, Germany) at 12,000 rpm for 5 min. Coarse emulsion was formed and then subjected into HPH at certain pressure and number of passes. The sample was then collected and stored under room temperature condition. Figure 2 shows the experimental procedure of nanoemulsion preparation.

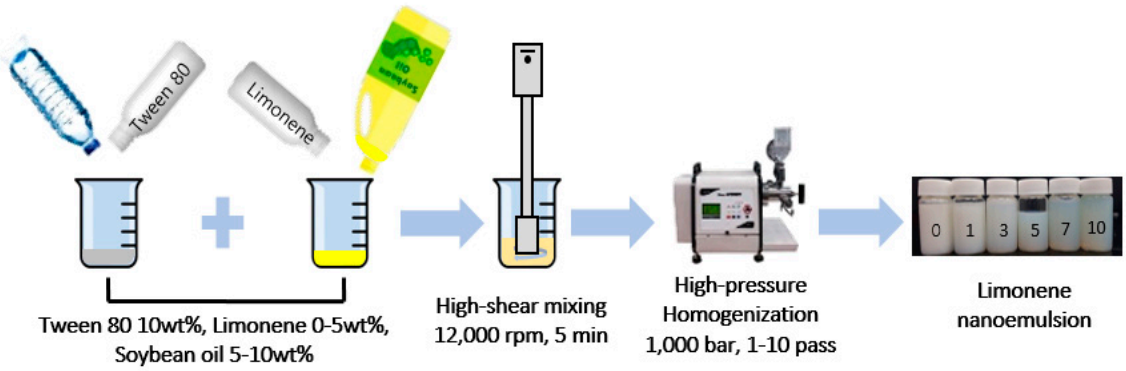

Figure 2. Experimental scheme.

In order to observe the effect of homogenization passes and pressure, the number of passes was varied from 1 to 10 passes whereas the pressure was varied from 500 to 1500 bar. When comparing the nanoemulsion synthesis method, nanoemulsion synthesized by ultrasonication method was synthesized by following method. Prior to ultrasonication, the emulsion was pre-mixed by high-shear mixer at 12,000 rpm for 5 min and then sonicated by using ultrasonic cleaner (UC-10, Jeio Tech, Daejeon, Korea) for 30 min under high frequency (40 kHz). In case of nanoemulsion synthesized by high-shear mixing, coarse emulsion was mixed at 12,000 rpm for 30 min. To observe the effect of emulsifier concentration, the amount of DI water used was adjusted to complement the changing content of the emulsifier. Sample size was measured right after formation for all samples.

2.3. Droplet Size Analysis

Droplet size of the nanoemulsion was measured by dynamic light scattering (DLS) method (Malvern Nano ZS, Malvern Panalytical Ltd., Malvern, Worcestershire, UK) at a measurement angle of 90° using a 633 nm laser with average of 13 runs for each measurement. Prior to analysis, the sample was diluted by putting 3 drops of each sample into 10 mL of water to prevent any multi-scattering phenomena. Sample was measured at room temperature and the results were reported as average of 3 measurements. The intensity-based average diameter (Z-average diameter) was obtained by a correlation function provided by the software.

2.4. Stability Test

Stability test was performed on the nanoemulsion by observing the droplet size of the nanoemulsion every 7 days for 28 days. The nanoemulsion was kept at room temperature during this test. Similar to the method described earlier, three drops of sample were diluted into 10 mL of water before droplet size analysis was conducted. Observation by naked eye was also conducted to see whether creaming process occurred or not.

3. Results and Discussion

3.1. Effect of Soybean Oil-Limonene Ratio

Addition of carrier oil into the system should provide better properties of nanoemulsion in terms of its bioaccessibility and digestibility [23–25]. In order to study the effect of encapsulation of limonene in soybean oil, ratio of carrier oil and bioactive materials, soybean oil content in the oil phase was varied from 0% (only limonene) to 100% (only soybean oil). The total oil content was maintained at 10 wt%. Emulsifier concentration was set at 10%, homogenization pressure was set at 1000 bar, and number of passes was set at five passes. The results can be seen in Figure 3.

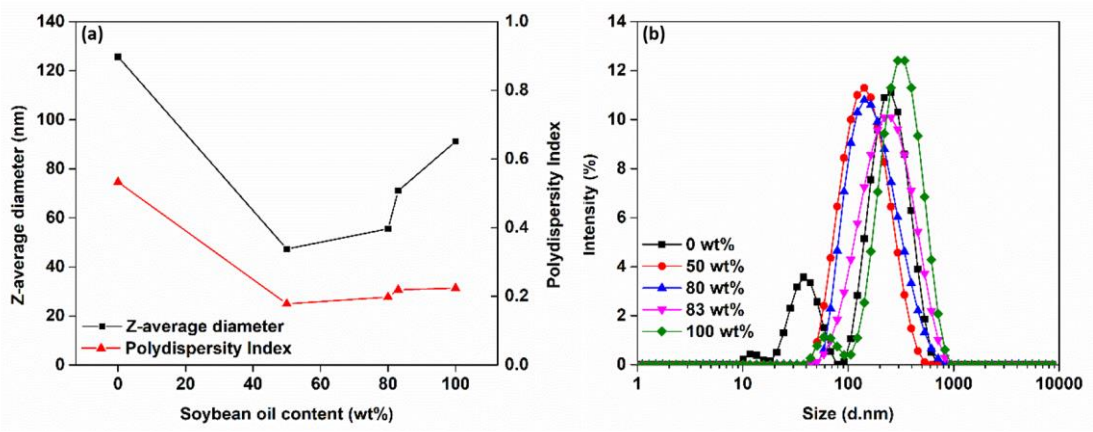

Figure 3. Effect of limonene-soybean oil concentration ratio on: (**a**) average droplet size and (**b**) size distribution at five passes.

When only limonene was added into the system (0% soybean oil content), the resulted droplet size from HSH at 12,000 rpm was fairly small (152.8 nm) even before homogenization (see Supplementary Table S1). Interestingly, HPH at 1 pass did not result in any reduction of the droplet size but after 5 passes, the nanoemulsion droplet size was able to be reduced to 125.7 nm (Figure 3a). The size distribution peak at five passes was divided into three different peaks located around 10, 40, and 300 nm, showing that various sizes of droplets were observed (Figure 3b). This also indicates that the tendency of Ostwald ripening phenomena to occur is high due to the difference in Laplace pressure between bigger and smaller droplets [26,27]. This is confirmed by the increase in nanoemulsion size after 14 days as shown in Supplementary Table S2 and the phase separation that can be seen in Supplementary Figure S1.

On the other hand, when only soybean oil (100% soybean oil) was put into test, the droplet size was reduced from 481.6 nm (0 pass) to 90 nm (5 pass) with polydispersity index (PDI) value slightly more than 0.2, which indicates good homogeneity of the sample (Figure 3a). Note that the starting point of the droplet size between limonene only and soybean oil only was so different. When only limonene was used as the oil phase, the droplet size was already 153 nm before HPH whereas in only soybean oil case, the starting droplet size was 480 nm (Supplementary Table S1). The difference between this two might be caused by the difference in the viscosity of limonene and soybean oil (see Supplementary Table S3). According to Wooster et al., there is an optimum viscosity ratio between dispersed and continuous phase in a turbulent dispersion which effectively breaks the droplets. This viscosity ratio range is about 0.1 to 5. According to Supplementary Table S3, soybean oil has a really high viscosity ratio as compared to limonene and this makes soybean oil more difficult to break as compared to limonene because it can glide and rotate during HSH process instead of breaking into smaller droplets [28]. After five passes of the HPH process, the soybean nanoemulsion droplets were found stable at 90 nm possibly due to the LCTs content which have no solubility in water [29].

Interestingly, when limonene and soybean oil were combined together at a certain ratio, smaller size of nanoemulsion droplets can be formed. Lowering the soybean oil content in the system from 100% up to 50% resulted in smaller nanoemulsion size and PDI value (Figure 3a). The droplet size was 90, 70, and 55 nm for 100%, 83%, and 80% soybean content at five passes, respectively, but when the soybean concentration reduced to 50% (1:1 soybean-limonene ratio), the size of the nanoemulsion reached the smallest size at 47 nm. The PDI value of this nanoemulsion was below 0.2, which indicates high homogeneity. Only one peak was observed for these three cases, in which the 50% soybean oil content produced the left-most peak (red line in Figure 3b). As explained before, the amount of soybean oil used affects the size of the nanoemulsion due to its high viscosity. Therefore, the size of the nanoemulsion will be bigger as the soybean oil content increases at the same energy applied.

The nanoemulsion was also stable when tested for 28 days with no cream or any aggregation formed (Supplementary Figure S1). This shows that when soybean oil and limonene were mixed together, limonene might be covered by the soybean oil so that the stability of the nanoemulsion improved. In this study, using 1:1 ratio of soybean oil to limonene oil produced smallest nanoemulsion size, but considering that limonene should be diluted in the soybean oil in order to prevent oxidation of limonene, the ratio of 8:2 was used for the rest of the study. The ratio of soybean oil and limonene at 8:2 was selected because, at this ratio, the resulted nanoemulsion was the second smallest among other formulation and this ratio ensures the covering of limonene in soybean oil.

3.2. Effect of Number of Passes and Homogenization Pressure

In this section, the mechanism related to the droplet disruption in the HPH device will be discussed in detail. There are two important parameters that will be discussed in this section, which are number of homogenization passes and homogenization pressure. Both parameters affect the efficiency in producing nanoemulsion. The desired criteria is to be able to produce nanoemulsion as fast as possible with low number of passes and low pressure. The number of passes was varied from 1 to 10 passes and the pressure was varied from 500 bar to 1500 bar.

Figure 4 shows the effect of homogenization passes and pressure on the size of the nanoemulsion. It can be clearly seen that after one pass in all pressure tested the nanoemulsion droplet size was able to be reduced significantly from 327.8 to 151, 123.9, and 107.2 nm after 1 pass at 500, 1000, and 1500 bar, respectively (Figure 4a). After five passes, both 1000 bar and 1500 bar pressure resulted in almost similar droplet size at 50–55 nm whereas at 500 bar the droplet size was about 96 nm. Increasing the passes to more than five passes resulted in an insignificant reduction of nanoemulsion size for both 1000 and 1500 bar cases.

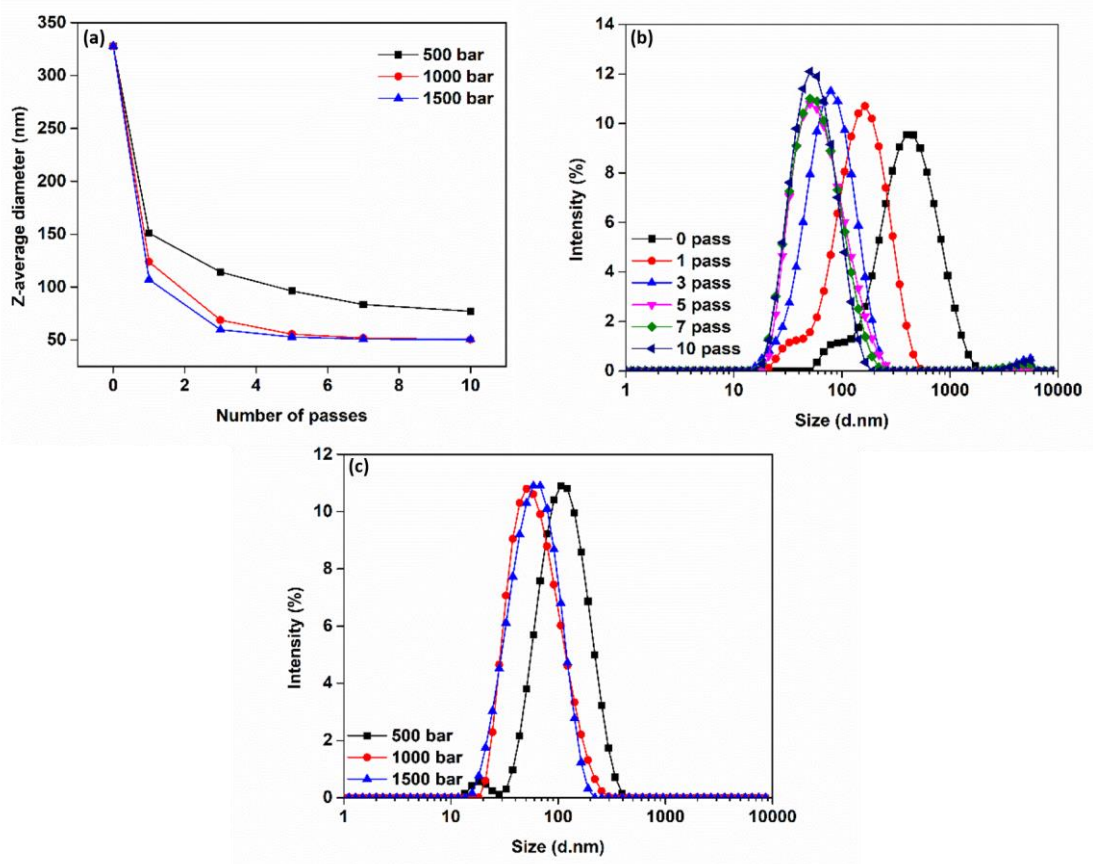

Figure 4. Effect of homogenization pressure and passes on nanoemulsion size: (**a**) average droplet size according to number of pass and pressure, (**b**) size distribution of nanoemulsion at 1000 bar, and (**c**) size distribution of nanoemulsion at five passes.

The size distribution according to the number of pass at 1000 bar of pressure is shown in Figure 4b. Here, from zero to three passes, it can be seen that there were one main peak and one shoulder peak which indicates that homogenization at one and three passes did not produce homogenous droplets. As the number of pass increased, the shoulder peak disappeared which indicates the mono-modality of the droplets and the droplet distribution peak was more shifted to the left, showing that the size of the droplets got smaller. The droplet size can be reduced possibly due to intense phenomena of cavitation, shear, and droplet impact that occurs in the homogenizing chamber (see Figure 5) [8,30]. However, above five passes, the peak position remained same except for its intensity. Above five passes, the peak intensity rose higher, indirectly stating that the concentration of the droplet size in that range increased.

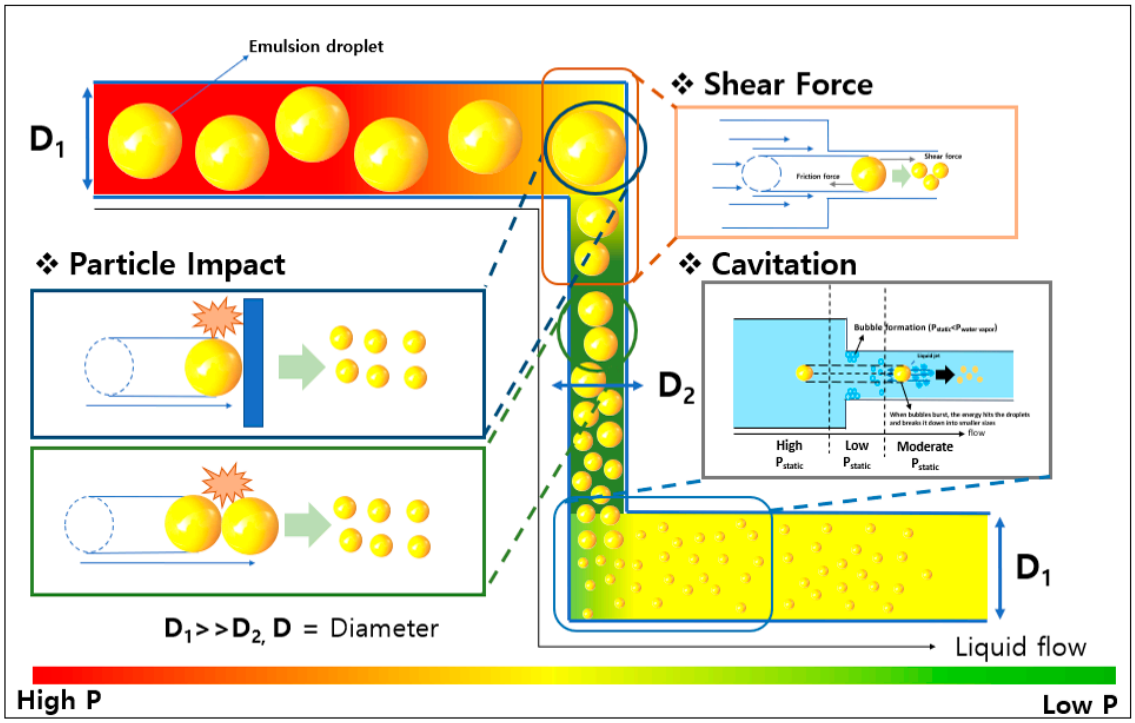

Figure 5. Phenomena in homogenization chamber.

According to the result shown in Figure 4a, the effectiveness of nanoemulsion homogenization is related to the applied pressure. As explained earlier, after 1 pass the droplet size was different for each pressure applied, in which the highest pressure produced the smallest size at 107.2 nm. Furthermore, pressure also affects the number of pass needed to reach minimum droplet size. The minimum droplet size is defined as the smallest droplet size that can be achieved in a nanoemulsion system where any disruption on the nanoemulsion droplet could not result in smaller droplets. High pressure brings more energy to disrupt the emulsion droplets. In our case, 1500 bar of pressure surely could reduce the droplet size faster than 1000 bar until three passes, as can be seen by the gap that exists between them (Figure 4a). However, at five passes, both pressure almost produced similar droplet size at 50–55 nm, which indicates that number of passes needed to reach minimum droplet size for both pressure was similar.

The size distribution for the effect of pressure at 5 passes is shown in Figure 4c. Pressure of 1000 bar and 1500 bar almost produced similar distribution at similar peak location, whereas only the 500 bar case that was different. The result indicates that both 1000 and 1500 bar could reach the minimum droplet size at 5 passes and there was almost no difference in terms of size and droplet distribution. Based on these results, further experiments will be limited to five passes and 1000 bar of pressure.

3.3. Effect of Emulsifier Concentration

Emulsifier plays a crucial role in encapsulating the oil in water during emulsification process. The number of passes and pressure plays the role in determining how fast a nanoemulsion can be synthesized while emulsifier determines the droplet size and stability of nanoemulsion. In an oil-in water emulsion, emulsifier has a role to adsorb on the oil molecules so that the oil and water could form one phase. In order to see the effectiveness of the emulsifier in encapsulating the oils in the nanoemulsion system, series of test by variating the amount of emulsifier from 5% to 15% were conducted.

Table 1 shows the size and the PDI of the nanoemulsion before and after homogenization. Before HPH, the droplet size and the polydispersity index were different for each sample. The 5% has the

biggest emulsion size of 434 nm with high PDI value, followed by 10% and 15% case with 327.8 and 264.5 nm, respectively. The PDI value decreased with increasing emulsifier content. Droplet size was reduced significantly after HPH. At 5 pass, the droplet size for both 10% and 15% case reached 50.4 and 40.9 nm, respectively, whereas the 5% case droplet size was still above 100 nm. All samples showed low PDI value around 0.20, indicating the samples have good size uniformity. Furthermore, a higher emulsifier concentration resulted in the distribution curve being shifted to the smaller size (Figure 6a).

Table 1. Effect of emulsifier on the nanoemulsion size and polydispersity index before and after homogenization.

Sample	Nanoemulsion Average Size (nm)		Polydispersity Index	
	0 pass	5 pass	0 pass	5 pass
5 wt% Tween 80	434.20 ± 12.71	99.50 ± 1.01	0.49 ± 0.01	0.17 ± 0.01
10 wt% Tween 80	327.80 ± 8.27	55.53 ± 0.19	0.35 ± 0.07	0.20 ± 0.01
15 wt% Tween 80	264.50 ± 1.99	40.85 ± 0.58	0.29 ± 0.03	0.20 ± 0.01

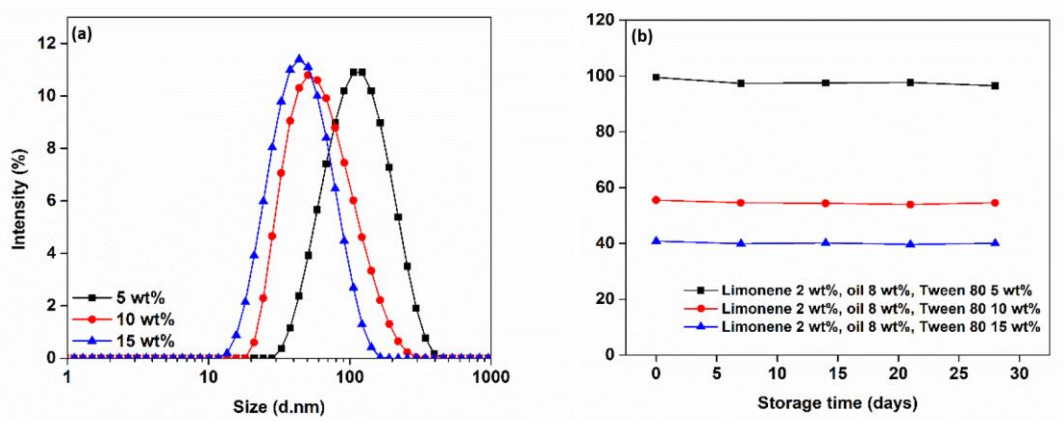

Figure 6. Emulsifier concentration effect on (**a**) nanoemulsion size distribution, and (**b**) nanoemulsion stability.

In order to form smaller nanoemulsion, there are two forces involved during droplet breakup, which are droplet disruption force and recoalescence force. To achieve the smaller nanoemulsion droplets, droplet disruption force must be higher than the recoalescence force. Here, the emulsifier plays the role in eliminating possible recoalescence force. When the new smaller droplets formed, the emulsifier quickly stabilizes the formed droplets through the Gibbs-Marangoni Effect [28]. The newly formed droplets will have uneven adsorption of emulsifier, where one side is covered with emulsifier and the other side is not. Since there is a gradient in the emulsifier concentration on the droplet, it induces gradient of surface tension which pulls more emulsifier into the uncovered spot of the droplets. In an emulsifier-rich system, all droplets formed will be covered by the emulsifier so that the droplet size could go really small. On the other hand, using less emulsifier leads to a lack of emulsifying capability, thus creating a large nanoemulsion size. When all emulsifier are used, any disruption on the nanoemulsion will not result in reduction of droplet size as they will recoalesce again [31–33].

The role of emulsifier is not only to encapsulate the oil compounds in the water, but also to protect them against instability. Therefore, the stability of these formed nanoemulsions is important to be tested. In order to prove the stability of the produced nanoemulsions, the nanoemulsions were kept at room temperature and the samples' size were checked every seven days. The result is reported in Figure 6b. It can be seen that there was no change in the droplet size even after 28 days, and observation by the naked eye revealed that there was no cream formation or even sedimenting particles on the bottom of the sample bottle.

3.4. Nanoemulsion Processing Method Comparison

Comparison with other methods such as HSH and ultrasonicator was also conducted to see how well HPH performs to produce nanoemulsion. The amount of oil, emulsifier, and DI water used were kept constant at 10 wt%, 10 wt%, and 80 wt%, respectively. Based on the results obtained in previous section, the number of pass in this case was limited to five passes and the pressure was set to 1000 bar. The result can be seen in Figure 7a.

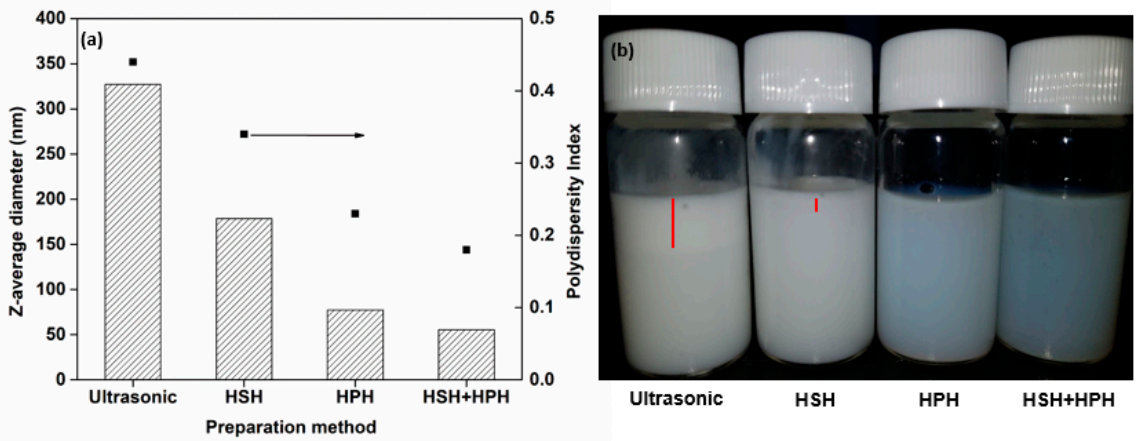

Figure 7. (**a**) Effect of nanoemulsion synthesis method on nanoemulsion size. (**b**) Images of the limonene nanoemulsion formed by various methods after 28 days. Red line indicates the cream thickness of the nanoemulsion.

Prior to ultrasonication, the coarse emulsion was first formed by mixing the oil, water, and surfactant by using conventional magnetic stirrer. This method produced an emulsion with the size of about 400 nm. After ultrasonication, the droplet size was reduced only about 70 nm to about 330 nm with almost no changes in the PDI value (Supplementary Table S4). The resulted nanoemulsion was not stable and clear cream formation could be seen (red line in Figure 7b). The phase separation could be observed 30 min after the ultrasonication process ended. Although emulsifier was present herein, the sonication power seems to be the problem because there was not enough power to mix all the phase together.

In case of HSH, pre-mixing with conventional magnetic stirring was not necessary, therefore mixing was conducted directly by HSH. Usage of HSH more than 12,000 rpm lead to overheat of the equipment, so the equipment was set to use at 12,000 rpm for 30 min. The produced droplet size was 178.5 nm with PDI value of 0.34 (Figure 7a), smaller than the droplet size produced by ultrasonication. The difference in the emulsification power between HSH and ultrasonication was quite significant, as in HSH the emulsification was occurred mainly due to the intense shear power during emulsification. However, the produced nanoemulsion was fairly stable at first, but over time, cream was formed at the top of the sample although the cream formation was lesser than ultrasonication. The picture of the sample taken after 28 days shows the cream formation (Figure 7b).

The formation of nanoemulsion by HPH was conducted without utilizing HSH for its pre-emulsification method. Instead, conventional magnetic stirring was utilized to pre-mix all the phases together. Prior to HPH, the formed nanoemulsion size was 390 nm and after five passes of HPH the droplet size was reduced to 77.3 nm with PDI value of 0.23 (Figure 7a). On the other hand, when HSH and HPH were used simultaneously, the droplet size of the nanoemulsion could be reduced to 55.5 nm with lower PDI value. This indicates that HSH is a good pre-emulsification technique. Its high shear power combined with HPH shear, cavitation, and impact effectively disrupt the droplets into smaller droplets.

4. Conclusions

In this study, the formation of limonene nanoemulsion was conducted by utilizing HPH. In order to achieve this, soybean oil and limonene were used as oil phase. The results showed that the ratio between limonene and soybean was crucial to produce stable and small nanoemulsion. It was found that at 80:20 ratio of soybean oil and limonene produce stable nanoemulsion with fairly small size. Meanwhile, the number of passes and pressure played important roles in determining how fast the nanoemulsion can be synthesized. In this case, HPH was able to produce small-sized nanoemulsion (55 nm) with high stability over the course of 28 days at 1000 bar and five passes. A higher emulsion concentration inside the emulsion will encapsulate more of the formed droplets during disruption process. Compared to ultrasonication and HSH, HPH was proven to be the best method to produce limonene nanoemulsion in fast and simple way. This result provides an insight into the production of nanoemulsion for food, cosmetic, and even pharmaceutical industries.

Supplementary Materials: The following are available online at http://www.mdpi.com/2504-5377/4/1/5/s1, Figure S1: Images of the limonene nanoemulsion formed by various ratio of limonene and soybean oil: (a) 0% soybean oil, (b) 50% soybean oil, (c) 80% soybean oil, (d) 83% soybean oil and (e) 100% soybean oil, Table S1: Limonene nanoemulsion size and polydispersity index (PDI) of number of pass and soybean oil content experiments, Table S2: Time-based observation of nanoemulsion average droplet size of 0% soybean oil sample, Table S3: Viscosity of the materials used in this study, Table S4: Limonene nanoemulsion size and PDI of various synthesis method experiment.

Author Contributions: Conceptualization, M.J.H and W.J.; methodology, M.J.H. and W.J.; software, M.J.H.; validation, W.J., H.K. and J.N.; formal analysis, M.J.H.; investigation, M.J.H. and W.J.; resources, W.J.; data curation, M.J.H.; writing-original draft preparation, M.J.H.; writing-review and editing, W.J.; visualization, M.J.H.; supervision, H.K. and J.N.; project administration, W.J. and J.N.; funding acquisition, W.J., H.K. and J.N. All authors have read and agreed to the published version of the manuscript.

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