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Physical Characteristics of Clove Essential Oil Microemulsions: Comparison of PIC and Spontaneous Techniques with High Energy and Low Energy Methods

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ABSTRACT

Clove flower essential oil (*CFEO*), which is useful as an anti-inflammatory, has characteristics that make it easily oxidised and sensitive to heat, so it is formulated in the form of a microemulsion preparation. This study aims to compare the effects of the Phase Inversion Composition (PIC) technique and spontaneous emulsification on the physical characteristics of microemulsions made using high and low-energy methods. The research began with the formulation of clove flower essential oil using the PIC technique and spontaneous emulsification based on the optimal composition from previous studies. The microemulsion was prepared at a stirring speed of 3600 rpm for high-energy and 360 rpm for low-energy. The parameters analysed included globule size, polydispersity index, viscosity, and physical stability against phase separation using centrifugation at 3500 rpm for 30 minutes. The results showed that in the high-energy method, the globule size and viscosity between the two techniques were not significantly different ($p > 0.05$), but the polydispersity index value in the PIC technique was lower than in spontaneous emulsification ($p < 0.05$), indicating a more uniform droplet distribution. In the low-energy method, the PIC technique significantly produced smaller droplet size, lower polydispersity index, and higher viscosity ($p < 0.05$), indicating better kinetic stability. Physical stability testing showed that all formulations remained homogeneous without phase separation. Overall, the PIC technique demonstrated superiority and consistency in producing more homogeneous and stable microemulsions. The low-energy method remains an economical and efficient alternative with proper surfactant composition optimisation.

INTRODUCTION

Clove (*Syzygium aromaticum* L. Myrtaceae) is an aromatic plant that is a leading agricultural commodity in Indonesia (Suparman et al., 2017). One part of the plant that is often used is the clove flower. Clove Flower Essential Oil (*CFEO*) is a yellow to dark brown liquid with distinctive physical and chemical properties, such as a refractive index of 1.52–1.54 and a specific gravity of 1.03–1.07 g/mL (Haro-González et al., 2021). *CFEO* is dominated by eugenol compounds (70-85%), which are key to its various biological benefit(Sohilait, 2015). Eugenol has been scientifically proven to have anti-inflammatory properties by suppressing the COX-2 enzyme(Ardiawijianti et al., 2017 ; Sugihartini et al., 2020), antibacterial against *Staphylococcus aureus* and *Propionibacterium acnes* by damaging the bacterial cell membrane and disrupting metabolic function (Cui et al., 2018), and antioxidants that help ward off free radicals that cause premature ageing (Haro-González et al., 2021). Its effectiveness in reducing inflammation and treating minor skin infections has been supported by various studies, including animal testing, which showed positive results with low risk of irritation (Sugihartini et al., 2018; Misra et al., 2024).

Despite its great potential, the formulation of *CFEO* in pharmaceutical and cosmetic preparations faces particular challenges due to its instability, susceptibility to oxidation, and sensitivity to exposure to heat and light (Sohilait et al., 2023). To overcome this obstacle, advanced formulation technologies such as microemulsions and nanoemulsions are strategic options(Szumała, 2022). The formulation of clove flower essential oil microemulsion has also been carried out by Khiromah et al., (2023), where optimisation of a 10% microemulsion of clove flower essential oil with a surfactant and cosurfactant ratio of 2:1 produces a microemulsion with globule sizes <100 nm, which is homogeneous, stable and has a transmittance close to 100%.

In terms of preparation techniques, microemulsions can be classified based on their formation mechanisms, which include spontaneous emulsification, phase inversion composition (PIC), and phase inversion temperature (PIT). The spontaneous emulsification method utilises the transfer of surfactants between phases during low-speed stirring to form small droplets. This process is known to be simple and energy-efficient, although the stability of the resulting system is highly dependent on the accuracy of the formulation(Aswathanarayan et al., 2019). Meanwhile, the PIC technique relies on changes in system composition to induce phase inversion by manipulating the hydrophilic-lipophilic properties of surfactants. The main advantage of this method lies in the simplicity of its equipment, although it often requires high surfactant concentrations. Similarly, the PIT technique achieves phase inversion through temperature manipulation that affects the spontaneous curvature of the surfactant film. However, its application requires strict temperature control and is less suitable for materials that are unstable to heat. (Gonçalves et al., 2018).

Based on the energy approach, the microemulsion manufacturing process can be classified into two main methods, namely high energy and low energy, each of which has its own characteristics. The high energy method relies on mechanical disruption forces generated by special equipment such as ultrasonicators, microfluidizers, or high-speed homogenisers to break down the dispersed phase into nano-sized droplets (Gonçalves et al., 2018). Conversely, the low-energy method prioritises the combination and selection of appropriate surfactants and cosurfactants, relying solely on gentle agitation (<1600 rpm). Although superior in energy efficiency, this approach often requires higher surfactant concentrations, which can potentially affect the physical properties and final characteristics of the product(Kumar et al., 2019).

This study aims to determine the effect of PIC technique and spontaneous emulsification on the production of *CFEO* microemulsion and to determine the best method in the production of *CFEO* microemulsion using high energy and low energy methods.

METHODS

Equipments And Materials

The equipment used in this study included 100 ml and 250 ml beakers (Iwaki), 10 ml measuring flasks (Iwaki), spatulas, droppers, stirring rods, a 300 g capacity analytical balance (Quattro FH 300), a magnetic stirrer (Thermo Scientific Ciramec+), a pH meter (Lutron PH-220), a Brookfield viscometer (NDJ-8S), an homogeniser (U-Tron T30/2), a particle size analyser (PSA Malven MAL1310860) and centrifuges (800-D). The materials used in this study included clove flower essential oil (*CFEO*) (from Inbi Nusantara Sejahtera) glycerine (from KLK Oleo), Tween 80 (Elotant Psmo 120 from Elotant), PEG 400 (from Clariant), isopropyl myristate (Palmsurf IPM from IOI Oleochemical) and aqua DM (from Brataco).

Formulation

Table 1. Laboratory-scale *CFEO* microemulsion formula

Ingredients	Formula (%)
CFEO	
Tween 80	6
PEG 400	8
Isopropyl Myristate	
Aqua DM	9

The formulation in this study refers to the optimal formula in a previous study by Kiromah (2023) can be seen in **Table 1.**

Procedure

At this stage, microemulsions were produced using the PIC mixing technique and sequence, as well as spontaneous emulsification. Both production techniques were carried out at the lowest speed of each device, namely 3600 rpm for the high-energy method using a homogeniser and 360 rpm for the low-energy method using a magnetic stirrer.

In PIC, the oil phase is mixed with surfactants–cosurfactants at 25°C for approximately 1 minute, then the water phase is titrated into the mixture while mixing at a predetermined speed. Mixing is continued for 10 minutes until phase inversion occurs and an oil-in-water (O/W) emulsion is formed. In spontaneous emulsification, the aqueous phase is first mixed with surfactants and cosurfactants at room temperature, then the oil phase is slowly added while mixing at a predetermined speed. Mixing is continued for 10 minutes until an O/W microemulsion is formed.

Characterization of Microemulsion

Viscosity tests

Viscosity measurements were performed using a Brookfield viscometer with 200 ml of nanoemulgel in a beaker, with the appropriate spindle selection. Measurements were performed on all replicates of the preparation. (Gumilar et al., 2023).

Droplet size and polydispersity index

The test was conducted by diluting *CFEO* microemulsion and distilled water in a ratio of 1:10. The mixture was placed in a cuvette and analysed using a Particle Size Analyser (PSA Malven MAL1310860) (Zulfa E et al., 2019).

Physical stability test

Physical stability testing was conducted using a centrifuge (800-D) at a speed of 3500 rpm for 30 minutes (Kiromah et al., 2023).

RESULT

This study aims to compare the effects of microemulsion preparation techniques and sequences using PIC and spontaneous emulsification on the physical characteristics of microemulsions produced through high-energy and low-energy methods. The critical parameters analysed include globule size, polydispersity index, viscosity and physical stability through phase separation for each combination of techniques and methods, as shown in **Table 2.**

Table 2. Physical characteristics of clove flower essential oil microemulsions (n=5)

Techniques	Low Energy			High Energy		
	Globule size (nm)	PDI	Viscosity (cP)	Globule size (nm)	PDI	Viscosity (cP)
PIC	17.97 ± 0.13 *	0.0986 ± 0.0084 *	223.68 ± 2.69 *	16.31 ± 0.10	0.0762 ± 0.0125 *	226.53 ± 0.82
spontaneous emulsification	19.90 ± 0.04	0.1417 ± 0.0063	218.64 ± 0.68	16.48 ± 0.15	0.1196 ± 0.0155	225.39 ± 1.21

Note: * Significant (P <0.05) compared to spontaneous emulsification within the same energy level

In the high energy method, the analysis results show that the PIC technique has a smaller globule size compared to the spontaneous emulsification technique with a significance value of 0.062 ($p > 0.05$). This value indicates that the difference in globule size between the two techniques is not statistically significant. However, the PDI value shows a significant difference between the PIC technique (0.0762) and spontaneous emulsification (0.1196) with ($p < 0.05$). This is because the high-energy method utilises high shear forces generated by the homogeniser, causing the droplet breaking process to occur intensely and evenly in both systems. However, the PIC technique still affects the distribution of droplet size, which is more uniform and stable compared to the spontaneous emulsification technique (Silva et al., 2016). The viscosity values obtained from the high energy method showed results of 226.53 cP for the PIC technique and 225.39 cP for the spontaneous emulsification technique with a significance value of 0.117 ($p > 0.05$). This difference is not statistically significant, indicating that the shear force generated by the homogeniser has produced droplets of almost uniform size in both techniques, resulting in similar viscosity characteristics. The relationship between globule size, PDI, and viscosity is interrelated in emulsion systems. Smaller droplets have a larger surface area, thereby increasing viscosity and interphase interaction (Liu et al., 2016). Meanwhile, low PDI indicates a more homogeneous particle size distribution, which plays an important role in maintaining the kinetic stability of the emulsion.

In the low energy method, the size of globules produced through the PIC technique was smaller than that produced through the spontaneous emulsification technique, with a significance value of $p < 0.001$, which means that there was a statistically significant difference between the two techniques. These results indicate that that the PIC technique has higher droplet formation efficiency under low agitation conditions due to the phase inversion mechanism controlled by the composition ratio of the oil and water phases. In addition, the PDI value in the PIC technique was also lower than that in the spontaneous emulsification technique, with a significance value of $p<0.001$, indicating a more homogeneous droplet size distribution. Thus, in the low energy method, the PIC technique proved to be more effective in producing small and uniform droplets than the spontaneous emulsification technique, which relies on spontaneous diffusion between phases. This is in line with the research by Safaya et al., (2020) where PIC allows better control over the speed of formation and phase inversion, so that droplets are formed uniformly with small sizes and low PDI compared to spontaneous emulsification.

The viscosity measurement results using the low energy method show that the PIC technique produces a viscosity of 223.68 cP, which is higher than the spontaneous emulsification technique at 218.64 cP, with a significance value of 0.004 ($p < 0.05$). This difference is statistically significant and closely related to the size of the globules and PDI produced. Smaller droplet size in the PIC technique increases the interface area and particle-particle interactions, thereby increasing the viscosity of the system. Meanwhile, a low PDI value enhances the kinetic stability of the emulsion by reducing the possibility of droplet coalescence(Liu et al., 2016). The relationship between these three parameters shows that the PIC technique is capable of producing a system with small size, homogeneous distribution, and optimal viscosity. In general, in the low energy method, differences in the droplet formation mechanism have a significant effect on the physical properties of the emulsion formed.

In physical stability testing, the results showed that the *CFEO* microemulsion production process using the PIC technique and spontaneous emulsification, both in the high energy and low energy methods, produced good physical stability without phase separation after centrifugation testing at 3500 rpm for 30 minutes, as shown in **Figure 1**. This proves that both methods are capable of producing a stable emulsion system and maintaining the homogeneity of *CFEO*.

Figure 1. Appearance and physical stability of CFEO microemulsion (1. Low energy PIC; 2. Low energy spontaneous; 3. High energy PIC; 4. High energy spontaneous)

Overall, the PIC technique shows consistent advantages over spontaneous emulsification techniques in both high-energy and low-energy methods. This is due to the phase inversion mechanism that occurs gradually through changes in the composition between phases, allowing the formation of smaller droplets and a more uniform droplet distribution with precise control, whereas spontaneous emulsification is more dependent on rapid diffusion that is highly sensitive to the composition of surfactants and cosurfactants (Feng et al., 2020).

In terms of reproducibility, high energy has a relatively stable range of values and is not affected by the manufacturing technique. This can be seen from the significance value and standard deviation (SD) for assessing the consistency of emulsification results. The emulsification process with high shear force produces a strong and uniform turbulent flow, so that the high energy method produces more homogeneous droplets because the process is easy to control (Jiang et al., 2019). In general, increasing the energy in the emulsification process can improve the homogeneity of the system and strengthen the effectiveness of the PIC technique as an efficient and stable method for microemulsion formation. However, the low-energy method can also produce good nanoemulsions if the ratio of surfactants, cosurfactants, and procedures are carried out with precise control. In addition, the use of the low-energy method requires lower investment and is considered more economical than the high-energy approach.

CONCLUSIONS

This study shows that the PIC technique provides superior results compared to spontaneous emulsification in both high-energy and low-energy methods. In the high-energy method, although the differences in globule size and viscosity are not statistically significant, the lower PDI value in the PIC technique indicates a more homogeneous and stable droplet distribution. Meanwhile, in the low energy method, the PIC technique significantly produced smaller droplets, lower PDI, and higher viscosity, indicating better kinetic stability and droplet formation efficiency compared to spontaneous emulsification. Physical stability tests also showed that all emulsion systems, both from the PIC technique and spontaneous emulsification, did not experience phase separation, indicating good physical stability. Overall, the PIC technique proved to be more efficient, stable, and controllable in microemulsion formation. In terms of production methods, the high-energy method has better reliability, but the low-energy method remains an economical and industrially viable alternative if formulation and process parameters are optimised appropriately.

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AUTHORS' CONTRIBUTIONS

Nugroho, Arif Budi Setianto, and Sugihartini Nining conducted the design study, Nugroho processed the data, Sugihartini Nining served as the project supervisor, and Nugroho and Sugihartini Nining wrote the manuscript.

CONFLICT OF INTERESTS

The author has no interest in the writing of this manuscript.

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