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Effect of Different Hydrophilic-lipophilic Balance (HLB) on Droplet Size, Polydispersity Index and Stability of Lemon Myrtle Nanoemulsion

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ABSTRACT

Using mixed non-ionic surfactants Tween/Span, we determined the required hydrophilic-lipophilic balance (HLB) value on the oil in water nanoemulsion of lemon myrtle essential oil. In this study, Span 80 mixed with Tween 80, and Span 20 mixed with Tween 20 were used as mixed non-ionic surfactant. The droplet size, polydispersity index and stability of the lemon myrtle nanoemulsion at different HLB values were obtained over a period of 28 days. Both mixed surfactants have similar required HLB value at HLB 14 due to their smallest droplet size and lowest polydispersity index during the early nanoemulsion preparation. However, after a period of 28 days, nanoemulsion with Span 80-Tween 80 surfactant mixture was more stable compared to Span 20-Tween 20 surfactant mixture. Therefore, HLB 14 using Span 80-Tween 80 surfactant mixture has been selected as the required HLB for lemon myrtle nanoemulsion due to its stability and consistency of droplet size in 28 days.

Keywords: Essential oil, Hydrophilic-lipophilic balance, Lemon myrtle, Nanoemulsion

1. INTRODUCTION

Essential oils are natural phytochemicals, hydrophobic, volatile, and complex compounds that give scent or characteristic odor. Essential oils produced as plant secondary metabolites and known for their rich sources of biologically active compounds. They contain a multitude of bioactive compounds such as alkaloids, glucosinolates, cyanogenic glycosides, phenolics, lipids, terpenes, polythienyls and polyacetylenes [1]. Many essential oils have demonstrated interesting activity against a wide range of microbes from an agricultural consideration. The use of essential oils to combat plant pathogenic bacteria is not a minor issue, as bacteria can cause different plant diseases that can have a significant economic impact. An increasing number of studies on the antibacterial properties of essential oil against plant pathogens have been published in the last 5 years, indicating a strong interest of essential oil based for biocontrol approaches [2], [3]. Although essential oils demonstrated a wide range of biological activities, their practical application is frequently hampered by several drawbacks. Essential oils have poor physicochemical properties such as high volatility, water insolubility and a short half-life, making them difficult to handle and use [2].

Nanoemulsion technology is one approach to formulation and management, and it's cheap and accessible [4]. Nanoemulsion required the presence of low amounts of surfactant [5] where a low surfactant to oil ratio (SOR) is necessary for their production, and their water content is typically around 10%, whereas macroemulsions can reach nearly 15% [6]. For dispersal in aqueous media

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of lipophilic or low water soluble chemicals, such as essential oils used in insecticides and food additives, nanoemulsion is an ideal choice [7], [8]. In conclusion, a nanoemulsion strategy may lead to enhanced physicochemical characteristics. Many essential oils have demonstrated promising antimicrobial action from a farming standpoint. Microorganisms can cause a variety of plant illnesses, which can have a substantial economic impact, making the usage of essential oils to battle these bacteria a serious concern. Essential oil's antibacterial effects against plant diseases have been the subject of a growing number of research in the last 5 years, suggesting a significant interest in essential oil-based biocontrol strategies and stability of essential oils by reducing volatility, enabling water dispersibility and protecting them from environmental interaction.

Nanoemulsion consist of 3 main components: surfactant, oil, and water. Surfactants are classified into 4 types based on their chemical properties: anionic, non-ionic, cationic and zwitterionic. The selection of suitable surfactant agents is a critical aspect in the formulation of essential oils. The molecules of non-ionic surfactants combine hydrophilic and lipophilic groups. The HLB value quantifies the relative abundance of hydrophilic and lipophilic groups. The stable nanoemulsion droplets in emulsions are formulated by achieving the best HLB and surfactant concentration. A higher HLB value indicates increased surfactant solubility in water, which favours oil in water (O/W) formulations for pesticide formulation. HLB values 10-16 is commonly used in agriculture to produce good O/W formulations, as HLB values below 10 are typically considered oil soluble surfactants. When trying to build kinetically stable nanoemulsions, one of the most crucial factors to take into account is the surfactant's HLB value. Single or mixed surfactants can achieve a wide range of HLB values [9]. Mixed surfactants are usually being formulated in nanoemulsion formulation to improve stability due to broad chain length distribution which has a synergistic effect on nanoemulsion stability.

Tweens and Spans are nonionic surfactants. They do not interact with ions and maintain their stability in weak acids, bases, and electrolytes. Tweens and Spans can be used together to create a wide range of oil/water and water/oil emulsions. Sorbitol can be dehydrated to produce spans, which are sorbitan esters. The HLB value of Spans decreases as the degree of esterification increases, resulting in enhanced solubility in lipophilic substances. On the other hand, Tweens possess hydrophilic properties and can be dispersed or dissolved in water as well as in diluted electrolyte solutions. The degree of ethoxylation of a Tween predicts how well it dissolves in water. There are various benefits to using either Spans or Tweens, such as greater stability and formulation versatility [10]. Due to these advantages, many studies have been conducted using a combination of Span and Tween surfactants. Tween 80 (HLB 16.7) and Span 80 (HLB 8.6), are the most widely used [4], [11].

We formulated a nanoemulsion of lemon myrtle essential oil using varied ratios of Span 80 and Tween 80 and Span 20 and Tween 20 as the combined surfactants. Surfactants' molecular structures are seen in Figure 1. We made a nanoemulsion of oil and water. The goal of this study is to identify the optimal HLB value and surfactant combination for preparing nanoemulsions of lemon myrtle. Droplet size, polydispersity index, and nanoemulsion stability were all measured as a function of HLB in this study of lemon myrtle.

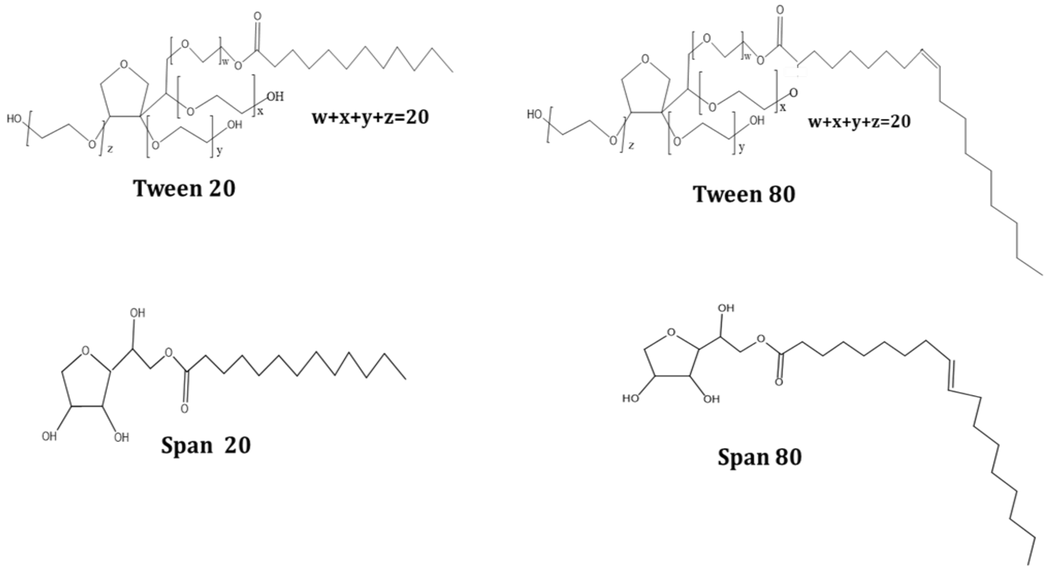

Figure 1 Structural formula of Tween 80, Span 80, Tween 20 and Span 20 [12].

2. MATERIAL AND METHODS

The lemon myrtle essential oil used here was distilled from *Backhousia citriodora* leaves in Linggi, Negeri Sembilan, Malaysia at the Malaysian Agricultural Research and Development Institute (MARDI). Tween 80, Tween 20, Span 80, and Span 20 (all non-ionic surfactants) were acquired from Sigma-Aldrich (USA). All of the tests were conducted with water that had been filtered twice using a Milli-Q system with 0.2 M filters (Milipore Co., Bedford, MA, USA).

2.1 Determination of Hydrophilic-Lipophilic Balance (HLB)

To determine the required HLB value of lemon myrtle nanoemulsion, different surfactant mixtures were prepared. Tween 80 was mixed with Span 80, and Tween 20 was mixed with Span 20. Both surfactant mixtures were prepared using different HLB values ranging from 8 to 15 for the Tween 80-Span 80 mixture and from 9 to 16 for Tween 20-Span 20. The HLB value of the mixed surfactant system was calculated based on the following equation:

$$HLB = \frac{m_A \; x \; HLB_A + m_B \; x \; HLB_B}{m_A + m_B} \qquad (1)$$

where m_A and m_B are the mass of the surfactants *A* and *B*, respectively. HLB_A and HLB_B are the *HLB* number of surfactants *A* and *B*, respectively [11]. Table 1 and Table 2 shows the concentration of mixed surfactants needed to prepare the HLB value based on the equation.

Table 1. Concentration of the Tween 80 and Span 80 mixtures for the preparation of different HLB values

HLB	Tween 80 (%)	Span 80 (%)
8	34.6	65.4
9	43.9	56.1
10	53.3	46.7
11	62.6	37.4
12	72.0	28.0
13	81.3	18.7
14	90.7	9.3
15	100	0

Table 2. Concentration of the Tween 20 and Span 20 mixtures for the preparation of different HLB values

HLB	Tween 20 (%)	Span 20 (%)
9	4.9	95.1
10	17.3	82.7
11	29.6	70.4
12	58	42
13	54.3	45.7
14	66.7	33.3
15	79	21
16	91.4	8.6

2.2 Preparation of Nanoemulsion

The organic phase was made by combining 10 percent (w/w) of surfactant with 10 percent (w/w) of lemon myrtle essential oil by vortexing after the corresponding surfactant mixtures had been prepared. The aqueous phase is made up of 80% (w/w) of double-distilled water. A coarse emulsion was then formed by vortexing the organic and aqueous phases together at 2000 rpm for 2 minutes. The coarse emulsion was then sonicated in an ultrasonic processor at high power (Q125 Sonicator, QSonica, USA) with up to 125 watts of output power at 20 kHz to produce a fine emulsion. The 3 mm microtip probe made of titanium alloy was included with the ultrasonicator. Sonication was performed for 2 minutes with the probe positioned 20 mm from the bottle's base. In order to avoid heating of the sample, the sonication process was done with pulses of 5 s ON and 7 s OFF [13]. The nanoemulsion samples were then kept for 24 hours at room temperature before characterization. Each sample with different HLB value was prepared with 3 replicates.

2.3 Droplet Size and Polydispersity Index Measurement

Droplet size and polydispersity index were evaluated on all samples after 24 h. Dynamic light scattering (DLS) measurements were taken with a Zetasizer Nano ZS (Brookhaven Instrument, USA). Multiple scattering effects were mitigated by diluting nanoemulsion samples 1:20 with ultrapure deionized water. The measurements were carried out at a scattering angle of 90 ° and a temperature of 25 °C. [14]. The average diameter of nanoemulsion droplets was calculated from three separate measurements (nm). The droplet size distribution is quantified by the polydispersity index (PDI).

2.4 Stability

The stability of the prepared lemon myrtle nanoemulsions was observed visually at room temperature for 28 days for any instability changes, such as phase separation or color changes. The droplet size and polydispersity index measurement were also repeated every 7 days to assess the consistency of the droplet size and its distribution in the emulsion system.

3. RESULTS AND DISCUSSION

In order to formulate a successful nanoemulsion of lemon myrtle essential oil, it is necessary to first determine the HLB value and then choose an acceptable surfactant agent. A surfactant's amphiphilic properties are represented by its HLB value. In order to formulate a successful nanoemulsion of lemon myrtle essential oil, it is necessary to first determine the HLB value and then choose an acceptable surfactant agent [4]. Essential oils can be formulated with the desired HLB by combining high and low HLB values with a variety of surfactant concentrations. Different HLB values of surfactant mixtures ranging from 8 to 15 were prepared by mixing Tween 80 and Span 80, while HLB values of 9 to 16 were obtained using Tween 20 and Span 20 surfactant mixtures in various proportions. Non-ionic surfactants like Tweens and Spans are employed as emulsifiers to create stable emulsion systems, and they are often utilized in a wide variety of mixtures to obtain the appropriate emulsification stability in oil-water systems [15].

The effect of different HLB values on droplet size and polydispersity of lemon myrtle essential oil after 24 hours of preparation is presented in Figure 2 and Figure 3. Based on the graph, HLB 9 and 10 produced droplets above 100 nm for both combinations of the surfactant mixtures, whereas HLB 11 to 16 produced droplets below 100 nm. The droplets showed a decreased in size from HLB 10 to HLB 14 for the Tween 80-Span 80 surfactant mixture (Figure 2) with a decrease in polydispersity. The lowest droplet size and polydispersity index for Tween 80-Span 80 mixture were obtained at HLB 14 (66.07 ± 1.02 nm, 0.230 PDI). For the Tween 20-Span 20 mixture (Figure 3), a decreased pattern of droplet size and polydispersity was exhibited at HLB 10 to 14 and started to increase at HLB 15. The smallest droplet size and polydispersity index were at HLB 13 and HLB 14.

Different HLB values have caused different sizes of droplets and polydispersities of lemon myrtle nanoemulsion due to the effect of different hydrophilic and lipophilic balances on the structure, composition, and charge of the interfacial layer surrounding the oil droplets [12]. A study by Somala et al., 2022 [16] also found that nanoformulation of *Cymbogun nardus* essential oil nanoemulsion with different HLB values showed different diameters of droplets and polydispersities. The average droplet size polydispersity was decreasing as the HLB value increased from HLB 9 to HLB 14.

A surfactant or surfactant mixture with HLB values similar to those required by the oil is ideal for formulating stable emulsions, particularly whenever synthetic surfactants are utilized. Surfactants with HLB values close to those needed for oils are the most stable because they generate droplets with the smallest possible size and the narrowest possible spread [13], [17]–[19]. In this context, the Tween 80-Span 80 mixture showed the required HLB value at HLB 14, whereas Tween 20-Span 20 showed the required HLB value at HLB 13 and 14 due to their smallest droplet size and lowest polydispersity. The result is similar to the study done by Nirmal et al., 2018 [13] where they also obtained HLB 14 as the required HLB value for lemon myrtle essential oil nanoemulsion by using a Tween 80-Span 80 surfactant mixture.

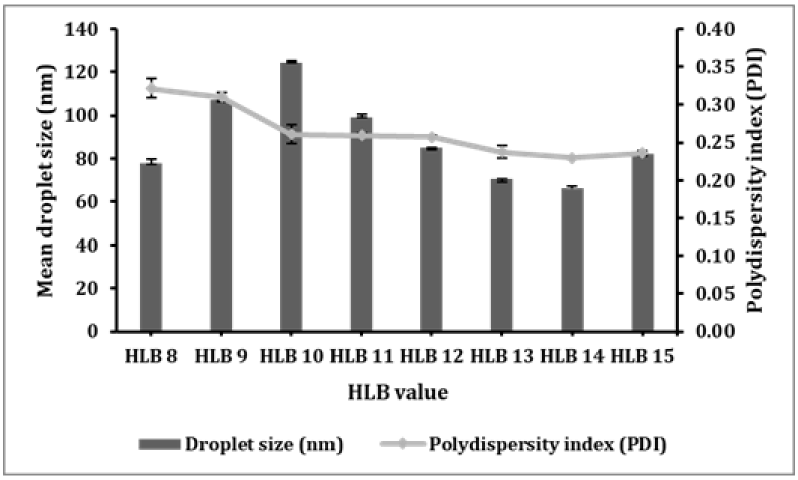

Figure 2. Effect of different HLB values on mean droplet size and polydispersity index using Tween 80-Span 80 as a surfactant mixture. Data are mean ± standard deviation of 3 replicates.

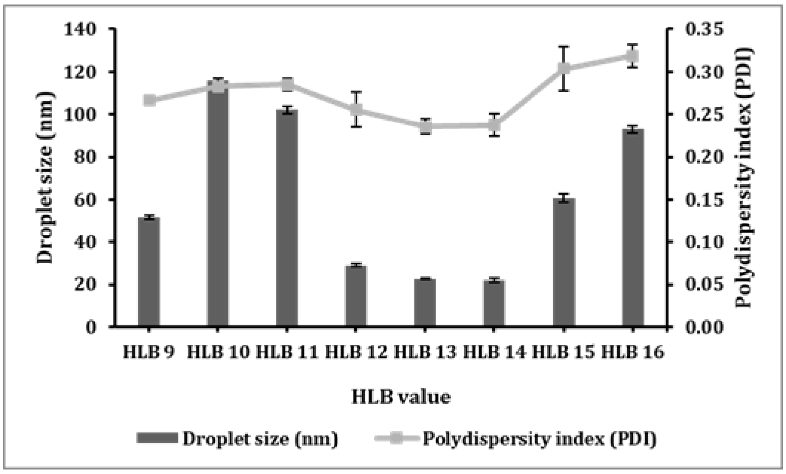

Figure 3. Effect of different HLB values on mean droplet size and polydispersity index using Tween 20-Span 20 as a surfactant mixture. Data are mean ± standard deviation of 3 replicates.

The stability of an emulsion can be affected by the tendency for droplets of the dispersed phase to coalesce, leading to larger particles. The surface tension of two phases, the aqueous and oil phases, can be lowered by using the right combination of surfactants and their concentration, which in turn reduces droplet coalescence. Emulsion stability may be misinterpreted if the first days of research are focused on droplet size [18]. Nirmal et al., 2018 [13] observed the mean droplet size of lemon myrtle essential oil on different HLBs after one day and after a week in determining the best HLB value for the oil. The same approach was also done by Mazarei and Rafati, 2019 [20], analysed the mean droplet size of *Satureja khuzestanica* essential oil a day after preparation and after a week time to determine the suitable surfactant mixtures for HLB using Tweens and Spans emulsifiers. Meher et al., 2013 [18] analysed the mean droplet diameter of citronella oil over a period of 30 days to determine the oil required for HLB. In this study, droplet size and polydispersity of lemon myrtle nanoemulsion using Tween 80-Span 80 mixture and Tween 20-Span 20 mixture as surfactant, at different HLB values were analysed over 28 days for their physical stability assessment. Figure 4 shows macroscopic observations on the physical stability of lemon myrtle nanoemulsion used within 28 days. Generally, there is no observable separation for Tween 80-Span 80. In contrast, instability was observed as early as day 7 for the Tween 20-Span 20 mixture and exhibited a progressive increase in instability over time. The stability of nanoemulsions refers to their ability to resist changes in their physicochemical properties over time. These include creaming or sedimentation, coalescence, flocculation, Oswald ripening and phase separation [21]. The result indicated that a surfactant mixture of Tween 80-Span 80 is the most suitable surfactant for the formulation of lemon myrtle essential oil nanoemulsion compared to Tween 20-Span 20 as surfactant mixtures.

The droplet size analysis of lemon myrtle essential oil nanoemulsion using Tween 80-Span 80 over a period of 28 days for different HLB values is presented in Figure 5. Although no separation was observed, the droplet analysis of HLB value 8, 9, 10 and 11 showed decreased size over time. Droplet size reduction in nanoemulsions of lemon myrtle essential oil indicated a destabilizing process. Micellarization is the process by which individual surfactant molecules form a colloidal cluster. Micelles are constantly breaking apart and rejoining until they approach kinetic stability, where they remain in a state of dynamic equilibrium [13], and when stability cannot be reached, separation may occur [22]. HLB 12 to 15 have shown an increase in droplet size. Among them, HLB 14 has shown the lowest droplet size with minimal increase, which seemed more stable and consistent in droplet size compared to other HLB values over a period of 28 days. A suitable mixture of surfactants or emulsifiers will result in a minimal increase in droplet diameter, and this is another useful indicator for assessing nanoemulsion stability [20]. Analysis of polydispersity also showed changes between the different HLB values. Based on the data presented in Figure 6, it can be observed that the polydispersity index exhibited a declining trend as the HLB values increased. During the 28-day period, it was observed that HLB 14 for the Tween 80-Span 80 surfactant mixture, consistently demonstrated low polydispersity in comparison to other HLBs (Figure 6). The lower polydispersity index refers to the higher uniformity of droplets in distinct size classes of the nanoemulsion [23]. While for Tween 20-Span 20, even though no separation occurred for HLB 8 and 9, droplet size analysis over 28 days exhibited destabilisation by the decreased droplet diameter.

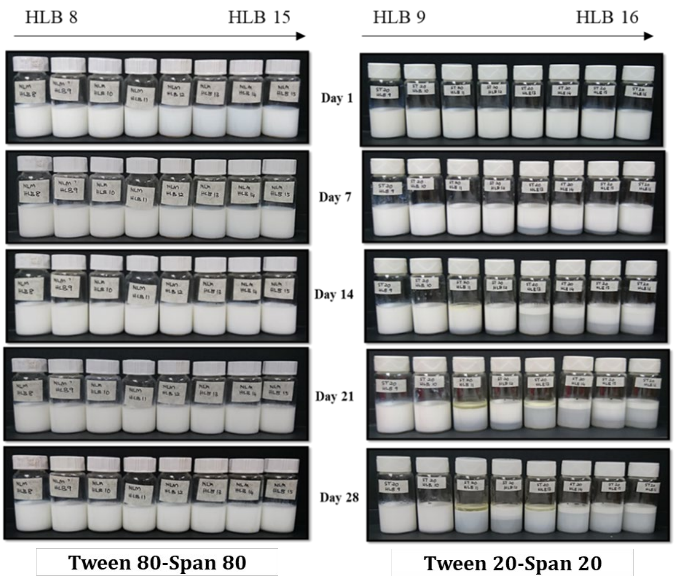

Figure 4 Macroscopic observation on the physical stability of lemon myrtle nanoemulsion using Tween 80-Span 80 and Tween 20-Span 20 as surfactant mixtures within 28 days.

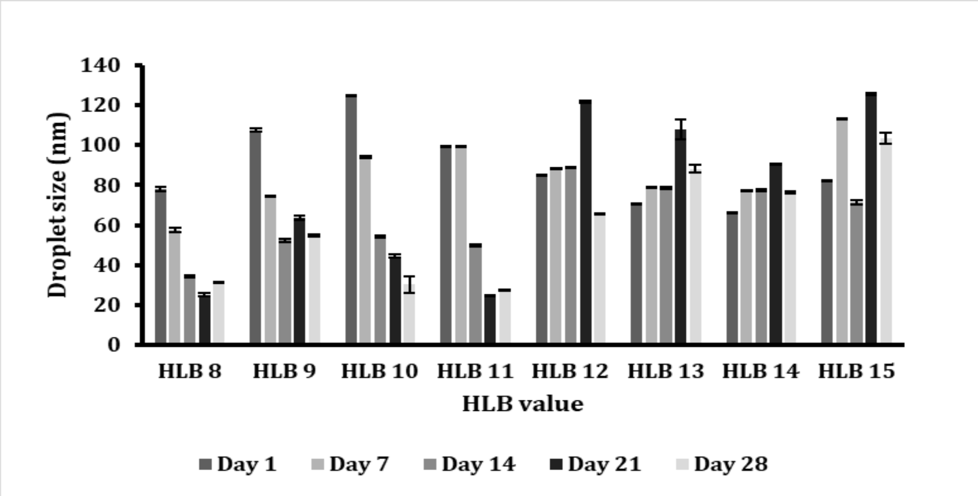

Figure 5. Effect of different HLB values on droplet size of lemon myrtle essential oil nanoemulsion using Tween 80-Span 80 mixture as surfactant over a period of 28 days. Data are mean ± SD with 3 replicates.

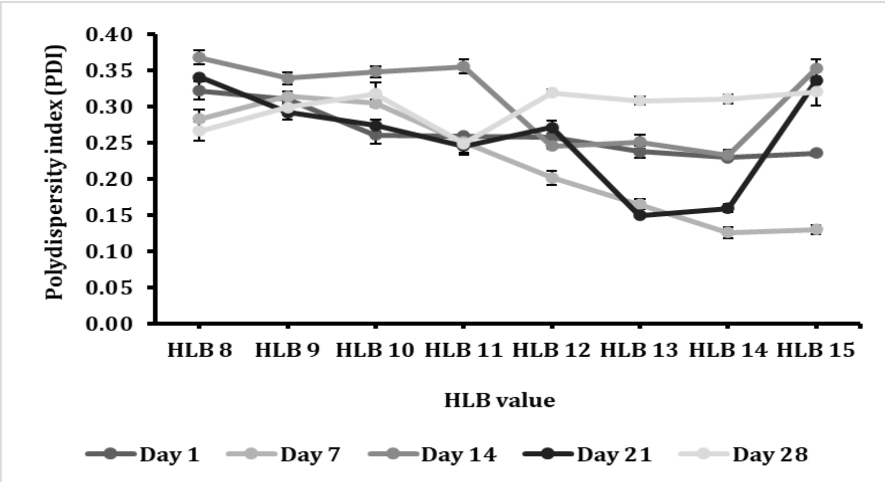

Figure 6. Effect of different HLB values on polydispersity index of lemon myrtle essential oil nanoemulsion using Tween 80-Span 80 mixture as surfactant over a period of 28 days. Data are mean ± SD with 3 replicates.

Based on the obtained data, it can be inferred that although both combinations of surfactant mixtures possess a similar required HLB value at HLB 14, the nanoemulsion formulated with Tween 80-Span 80 exhibited greater stability. A study of surfactant mixtures effect on *S. khuzestanica* oil nanoemulsion droplets size stability, using various combinations of Tween 80, Tween 20, Span 80 and Span 20 by Mazarei and Rafati, 2019 [20] , also in line with the results of this study. Among the surfactants blended, Tween 80 mixed with Span 80 showed the most stability after a period, even though surfactants mixed with Tween 20 and Span 20 showed smaller droplets at the early preparation of the nanoemulsion.

Nanoemulsion droplet stability depends on more than just the HLB value of the surfactant mixture; the surfactant's shape is just as important [10], [12]. It has been reported that nanoemulsion production is significantly affected by the packing shape of surfactant molecules at the oil/water interface, as determined by their molecular geometry [20]. Surfactants in the Tween family share a common molecular structure, consisting of a hydrophilic polyethoxylated sorbitan ester head group and a variety of primary fatty acid tail groups. Tween 20's saturated lauric acid moiety is more straight-lined than Tween 80's unsaturated oleic acid moiety is twisted. The bonds in Tween 80 and Span 80 are unsaturated, while those in Tween 20 and Span 20 are fully saturated. In contrast to Span 80's unsaturated kinked tail group, Span 20's is linear and saturated [12]. Therefore, compared to the Tween 20-Span 20 mixture, the Tween 80-Span 80 mixture is predicted to have a larger packing parameter, leading to the generation of more stable droplets.

4. CONCLUSION

Selecting a suitable surfactant mixture and determining the required HLB are necessary, as it has been proven to influence essential oil nanoemulsion droplet characteristics and stability. In order to attain maximum stability, a surfactant or surfactant mixture should have an HLB value near to that needed for an oil. This will result in droplets that are the smallest possible size and have the lowest possible polydispersity index. Besides, the geometry of the surfactant is also critical to the stability of nanoemulsion droplets. In this study, the surfactant mixture of Tween 80-Span 80 has greater stability compared to the Tween 20-Span 20 mixture. Therefore, Tween 80-Span80 surfactant mixture was chosen as a suitable surfactant for further optimization of the lemon myrtle nanoemulsion formulation, with HLB 14 as the required HLB for lemon myrtle essential oil.

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REFERENCES

[1] Borges, D. F. Lopes, E. A. Moraes, A. R. F. Soares, M. S. Visotto, L. E. Oliveira, C. R. and Valente, V. M. M. (2018). Formulation of botanicals for the control of plant-pathogens: A review. Crop Protection. Volume: 110. 135–140.
[2] Raveau, R. Fontaine, J. and Lounès-Hadj S. A. (2020) Essential oils as potential alternative biocontrol products against plant pathogens and weeds: A review. Foods. Volume: 9 Issue 3.
[3] Alonso-Gato, M. Astray, G. Mejuto, J. C. and Simal-Gandara, J. (2021). Essential oils as antimicrobials in crop protection. Antibiotics. Volume: 10 Issue:1. 1–12.
[4] Pavoni, L. Pavela, R. Cespi, M. Bonacucina, G. Maggi, F. Zeni, V. Canale, A. Bruschi, F. and Benelli, G. (2019). Green micro-and nanoemulsions for managing parasites, vectors and pests. Nanomaterials. Vol: 9 Issue: 9.
[5] Sugumar, S. Ghosh, V. Mukherjee, A. and N. Chandrasekaran, N. (2016). Essential oil-based nanoemulsion formation by low- and high-energy methods and their application in food preservation against food spoilage microorganisms. Elsevier Inc.
[6] Mustafa, I. F. and Hussein, M. Z. (2020). Synthesis and technology of nanoemulsion-based pesticide formulation. Nanomaterials. Vol: 10 Issue: 8. 1–26.
[7] Heydari, M. Amirjani, A. Bagheri, M. Sharifian, I. and Sabahi, Q. (2020). Eco-friendly pesticide based on peppermint oil nanoemulsion: preparation, physicochemical properties, and its aphicidal activity against cotton aphid. Environment. Science Pollution Research. Vol. 27 Issue:6. 6667–6679.
[8] Ozogul, Y. Boga, E. K. Akyol, I. Durmus, M. Ucar, Y. Regenstein, J. M. and Kosker, A.R. (2020). Antimicrobial activity of thyme essential oil nanoemulsions on spoilage bacteria of fish and food-borne pathogens *Food Bioscience.* Vol: 36.
[9] Lu, W. C. Huang, D. W. Wang, C. C. Yeh, C. H. and Tsei, J. C. (2018). Preparation, characterization, and antimicrobial activity of nanoemulsions incorporating citral essential oil. Jurnal of Food and Drug Analysis. Vol: 26 Issue:1. 82–89.

[10] Hong, I. K. Kim, S. I. and Lee, S. B. (2018). Effects of HLB value on oil-in-water emulsions: Droplet size, rheological behavior, zeta-potential, and creaming index. Journal of Industrial and Engineering Chemistry. Vol: 67. 123–131.

[11] Shahavi, M. H. Hosseini, M. Jahanshahi, M. Meyer, R. L. and Darzi, G. N. (2019). Evaluation of critical parameters for preparation of stable clove oil nanoemulsion. Arabian Journal of Chemistry. Vol: 12 Issue: 8. 3225–3230.

[12] Lindner, M. Bäumler, M. and Stäbler, A. (2018) Inter-correlation among the hydrophilic-lipophilic balance, surfactant system, viscosity, particle size, and stability of candelilla wax-based dispersions. Coating. Vol: 8 Issue 12

[13] Nirmal, N. P. Mereddy, R. Li, L. and Sultanbawa, Y. (2018). Formulation, characterisation and antibacterial activity of lemon myrtle and anise myrtle essential oil in water nanoemulsion. Food Chemistry. Vol: 254. 1–7.

[14] Kumari, S. Kumaraswamy, R. V. Choudhary, R. C. Sharma, S. S. Pal, A. Raliya, R. Biswas, P. and Saharan, V. (2018). Thymol nanoemulsion exhibits potential antibacterial activity against bacterial pustule disease and growth promotory effect on soybean. Scientific Reports. Vol: 8 Issue:1.

[15] Foo, K. S. Bavoh, C. B. Lal, B. and Mohd, A. (2020). Rheology Impact of Various Non-Ionic Surfactants on Cyclopentane Hydrates. Vol: 25. 3725.

[16] Somala, N. Laosinwattana, C. and Teerarak, M. (2022). Formulation process, physical stability and herbicidal activities of *Cymbopogon nardus* essential oil-based nanoemulsion. Scientific Reports. Vol: 12 Issue:1. 1–13.

[17] Martin, M. J. Trujillo, L. A. Garcia, M. C. Alfaro, M. C. and Munoz, J. (2018). Effect of emulsifier HLB and stabilizer addition on the physical stability of thyme essential oil emulsions of thyme essential oil emulsions. Journal of Dispersion Science and Technology. 1–8.

[18] Meher, J. G. Yadav, N. P. Sahu, J. J. and Sinha, P. (2013). Determination of required hydrophilic – lipophilic balance of citronella oil and development of stable cream formulation. Drug Development and Industrial Pharmacy. Vol: 39. 1540–1546.

[19] Lee, Y. Y. and Yoon, K. S. (2020). Determination of Required HLB Values for Citrus unshiu Fruit Oil, Citrus unshiu Peel Oil, Horse Fat and Camellia japonica Seed Oil. Journal of Cosmetic Science. Vol: 71 Issue:6. 411–424.

[20] Mazarei, Z. and Rafati, H. (2019). Nanoemulsification of *Satureja khuzestanica* essential oil and pure carvacrol; comparison of physicochemical properties and antimicrobial activity against food pathogens. LWT. Vol: 100. 328–334.

[21] Pavoni, L. Perinelli, D. R. Bonacucina, G. Cespi, M. and G. F. Palmieri. (2020). An overview of micro-and nanoemulsions as vehicles for essential oils: Formulation, preparation and stability. Nanomaterials. Vol:10 Issue:1.

[22] Barradas, T. N. and Silva, K. G. D. H. (2021). Nanoemulsions of essential oils to improve solubility, stability and permeability: a review. Environmental Chemistry Letters. vol. 19 Issue: 2. 1153–1171.

[23] Badawy, M. E. I. Saad, A.-F. S. A. Tayeb, E.-S. H. M. Mohammed, S. A. and Abd-Elnabi, A. D. (2017). Optimization and characterization of the formation of oil-in-water diazinon nanoemulsions: Modeling and influence of the oil phase, surfactant and sonication. Journal of Environmental Science and Health, Part B. Vol: 52 Issue: 12. 896–911.