



Spontaneous nanoemulsion of black cumin seed (*Nigella sativa*) extract: Effects on physical and chemical properties

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ABSTRACT

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This research aims to optimize the composition of black cumin (*Nigella sativa*) seed oil nanoemulsion based on the spontaneous emulsification technique. Two factors were studied: (1) oil-surfactant ratios (1:1, 1:2, 1:3) and (2) the type of surfactants (Tween 60, Tween 80). Physical properties measured included particle size, polydispersity index (PDI), zeta potential, conductivity, absorbance, transmittance, pH, and viscosity, while chemical properties measured included antioxidant activity and total phenolic content. The results showed particle sizes of the nanoemulsion ranging between 29.77 nm (for 1:3, Tween 60) and 442.63 nm (for 1:2, Tween 80). ANOVA revealed that oil-surfactant ratio and the type of surfactants significantly impacted droplet size and PDI. Smaller and more uniform droplets (PDI 0.3–0.5) were obtained with Tween 60, while Tween 80 resulted in larger, more heterogeneous droplets (PDI 0.4–0.7). Zeta potential values (–1.91 to –17.94 mV) were all below the threshold value provided in the literature, indicating inadequate electrostatic stabilization. Conductivity values (0.0427–0.1033 ms/cm) followed the expected range. Higher oil-surfactant ratios for nanoemulsions made with Tween 60 reduced absorbance and increased transmittance, indicating enhanced optical clarity. Antioxidant activity was highest (5.70%) in nanoemulsions with a 1:1 oil-surfactant ratio using Tween 60, compared to the lowest (3.30%) in nanoemulsions with a 1:3 ratio using Tween 80. Total phenolic content varied (0.193–0.852 mg GAE/g) significantly ($p < 0.05$) as influenced by composition. Generally, Tween 60 resulted in better black cumin seed nanoemulsions with improved physical and chemical characteristics.

Contribution/Originality: This study contributes to existing literature by optimizing black cumin seed oil nanoemulsions using Tween 60 and Tween 80 surfactants. It offers new insights into how oil-surfactant ratios affect droplet size, stability, and bioactivity, enhancing understanding of formulation strategies for functional food and nutraceutical applications.

1. INTRODUCTION

Nigella sativa L., commonly known as black cumin or habbatussauda, is a medicinal plant traditionally used across the Middle East, Africa, and South Asia for centuries. Its seeds and oil are highly valued for their therapeutic potential,

supported by numerous pharmacological studies reporting antidiabetic, anticancer, anti-inflammatory, antimicrobial, hepatoprotective, and wound-healing properties (Ahmad et al., 2013; Habib & Choudhry, 2021). In addition, black cumin oil has been widely recognized for improving respiratory conditions such as asthma, bronchitis, and emphysema (Atta, 2003). These biological activities of *N. sativa* could mainly be attributed to its high bioactive compounds, including thymoquinone, polyphenols, tocopherols, and unsaturated fatty acids (Dar et al., 2024; Naderi et al., 2024).

The lipid in black cumin seed is dominated by unsaturated fatty acids such as linoleic and oleic acid. Unsaturated fatty acids have been widely attributed to lowering low-density lipoprotein (LDL) cholesterol while increasing high-density lipoprotein (HDL) cholesterol (Albakry et al., 2022). LDL and HDL balance is crucial for preventing cardiovascular disease, as high LDL levels are often associated with arterial plaque formation, and HDL may facilitate reverse cholesterol transport (Hernández et al., 2019). Moreover, thymoquinone, the major bioactive constituent in black cumin seed, has been well-documented for its antimicrobial, anti-inflammatory, antioxidant, and antineoplastic effects (Kmail, Said, & Saad, 2023), further reinforcing the health-promoting potential of black cumin seed oil.

Despite its promising health benefits, the application of *N. sativa* oil in food and pharmaceutical industries remains limited. Several challenges may hinder its utilization, including the oil's sharp and bitter flavor, dark color, and oxidative instability, which may cause negative sensory perception and short storage time (Edris, 2011). Furthermore, thymoquinone is a large non-polar molecule, which causes poor aqueous solubility and low bioavailability and restricts efficient dispersion in the gastrointestinal tract and limits systemic absorption (Li, Zheng, Xiao, & McClements, 2012; Odeh, Ismail, Abu-Dahab, Mahmoud, & Al Bawab, 2012). These challenges necessitate the development of more efficient delivery systems that can enhance the stability and bioavailability of *N. sativa* oil.

Nanoemulsion technology is a promising strategy to improve the solubility, stability, and bioavailability of bioactive hydrophobic compounds (Odriozola-Serrano, Oms-Oliu, & Martín-Belloso, 2014). Nanoemulsions are kinetically stable dispersions of oil and water stabilized by surfactants, with droplet sizes typically ranging from 20 to 200 nm. The small droplet size enhances surface area, thereby improving solubilization and absorption of bioactive compounds in biological systems. Previous studies on black cumin oil nanoemulsions have mainly utilized high-energy methods such as ultrasonication and high-pressure homogenization, yielding stable nano-sized droplets with particle sizes ranging from 37 to 175 nm (Khorami, Nasr-Esfahani, Rahimi, & Aarabi, 2024; Periasamy, Athinarayanan, & Alshatwi, 2016). However, these approaches present limitations, including high processing costs, scalability issues, and thermal degradation of thermo-sensitive bioactives during processing (Peshkovsky, Peshkovsky, & Bystryak, 2013).

In contrast, low-energy methods such as spontaneous emulsification offer several advantages, i.e., low mechanical input and typically operate at low temperatures. Thus, this method is suitable for formulating thermo-sensitive compounds such as thymoquinone, proteins, and enzymes. Moreover, low-energy techniques are cost-effective and more adaptable to small- and medium-scale industrial applications (Jamoussi et al., 2021; Salem & Ezzat, 2019). Nevertheless, spontaneous emulsification requires precise optimization of formulation, more specifically, oil-to-surfactant ratios and surfactant types, to achieve desirable physicochemical properties such as particle size distribution, polydispersity index (PDI), and stability (Handa, Ujjwal, Vasdev, Flora, & Shukla, 2021; Tayeb, Felimban, Almaghrabi, & Hasaballah, 2021).

Given these considerations, the present study was designed to optimize the nanoemulsion formulation of black cumin seed oil by investigating the influence of oil-to-surfactant ratios (1:1, 1:2, and 1:3) and surfactant types (Tween 60 and Tween 80) on the physicochemical and functional characteristics of the nanoemulsion. This research is expected to provide insights into developing stable and bioavailable nanoemulsion systems for enhancing the applicability of *N. sativa* oil in food and pharmaceutical industries.

2. MATERIALS AND METHODS

2.1. Materials and Chemicals

Black cumin seed oil was obtained from PT Habbasyi Niaga Utama (Indonesia). Ethanol 96%, methanol 99%, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent, Tween 60, and Tween 80 were purchased from Merck (Germany). Aquadest and aquabidest were purchased from PT Ikhapharmindo Putramas (Indonesia). The instruments used in this research were UV-Vis spectrophotometer (Shimadzu, UV-1280, Kyoto, Japan), Zetasizer Pro (Malvern Instruments, LT., Malvern, United Kingdom), Dip cell kit (Malvern Instruments, LT., Malvern, United Kingdom), vacuum pump filter 0.22 μm (Sartorius Stedim 11694-2-50-06, France), micropipette (Socorex, Switzerland), blue tip (1 mL pipette tips, Biologix, Hong Kong), hotplate (Cimarec Thermo Scientific SP142025Q, United States), vortex (Thermolyne, Maxi Mix Plus, United Kingdom), aluminum foil, wrap paper, filter membrane 0.22 μm (Whatman), beaker glass (Pyrex), Erlenmeyer (Pyrex), volumetric flask (Pyrex), vial, test tube, and digital scale.

2.2. Research Design

An empirical study was carried out following an experimental approach design of a 3×2 factorial completely randomized design (CRD). The first independent variable was the ratio of oil and surfactant (1:1, 1:2, and 1:3), while the second independent variable was the type of surfactant, namely Tween 60 and Tween 80 (Table 1). Nanoemulsions of black cumin oil were prepared by a spontaneous nanoemulsion method. Once the nanoemulsion was formed, tests were conducted on physical characteristics, including particle size, polydispersity index, zeta potential, and conductivity using Zetasizer Pro (Malvern Instruments, Ltd., Malvern, United Kingdom). Absorbance values, turbidity, and percent transmittance (%T) were also measured using UV-Vis spectrophotometry assay (Shimadzu, UV-1280, Kyoto, Japan). Subsequently, chemical characteristic tests were performed, including antioxidant activity using the DPPH assay and total phenolics using the Folin-Ciocalteu assay. Microsoft Excel software was used for data tabulation. Furthermore, SPSS version 27 statistical software was used for normality and homogeneity tests of the data. If a normal and homogeneous distribution of the data was indicated, the analysis continued with the two-way analysis of variance (ANOVA) test to determine the influence of each independent variable and its interactions on the dependent variable. When significant differences were found, further analysis was conducted using the Duncan post-hoc test to identify which treatment groups were significantly different.

Table 1. Experimental variable design of a 3×2 factorial completely randomized design (CRD).

Run	Independent variable		Sample code
	Oil: surfactant ratio	Surfactant type	
1	1 : 1	Tween 60	P1
2	1 : 2	Tween 60	P2
3	1 : 3	Tween 60	P3
4	1 : 1	Tween 80	P4
5	1 : 2	Tween 80	P5
6	1 : 3	Tween 80	P6

2.3. Fabrication of Nanoemulsion from Black Cumin Seed Oil

Nanoemulsion of black cumin seed oil was prepared following variations in oil-to-surfactant ratios (1:1, 1:2, and 1:3). In addition, two types of surfactants were used (Tween 60 and Tween 80). Meanwhile, the concentration of black cumin seed oil was maintained at 3% (Periasamy et al., 2016) across all treatments to ensure that the observed effects were due to the variation of oil-to-surfactant ratios. The composition of nanoemulsion with Tween 60 and Tween 80 surfactant is shown in Table 2 and Table 3, respectively. Nanoemulsion was prepared following a spontaneous nanoemulsion method, which refers to the process of emulsion formation that occurs spontaneously due to interfacial instability when an oil phase containing surfactants mixes with an aqueous phase. The oil and surfactants were

weighed in a glass beaker. Subsequently, water was weighed according to the specified composition as shown in Table 2 and Table 3. The mixture of oil and surfactants was then heated to 75°C while being stirred using a magnetic stirrer at a speed of 1100 rpm. Heating was also conducted for the aqueous phase until the temperature reached 75°C. Once both phases reached a uniform temperature, the mixture of oil and surfactant was added to the liquid phase slowly (drop by drop) using a 500 µl micropipette while a continuous stirring was maintained using a magnetic stirrer at a speed of 1100 rpm for 30 minutes. Afterwards, sonication was performed for 30 minutes (45 kHz and power of 100%) to produce stable nano-sized particles. Once the sonication process was completed, the sample was stored in a refrigerator at 4°C before analysis.

Table 2. Composition of nanoemulsion with Tween 60 surfactant.

Oil: Surfactant ratio	Black cumin seed oil (g)	Tween 60 (g)	Water (g)	Total (g)
1 : 1	0.9	0.9	28.2	30
1 : 2	0.9	1.8	27.3	30
1 : 3	0.9	2.7	26.4	30

Table 3. Composition of nanoemulsion with Tween 80 surfactant.

Oil: Surfactant ratio	Black cumin seed oil (g)	Tween 80 (g)	Water (g)	Total (g)
1 : 1	0.9	0.9	28.2	30
1 : 2	0.9	1.8	27.3	30
1 : 3	0.9	2.7	26.4	30

2.4. Physical Characteristics Analysis

2.4.1. Particle Size, Polydispersity Index, Zeta Potential, and Conductivity

The physical characteristics of the black cumin oil nanoemulsion were analyzed using a Zetasizer Pro (Malvern Instruments, Ltd., Malvern, United Kingdom), and data were analyzed using the ZS Explorer application. The principle of the Zetasizer Pro was based on the dynamic light scattering (DLS) method, which measured fluctuations in light intensity caused by particles or molecules in suspension that move randomly due to Brownian motion. Measurements were conducted at a wavelength of 633 nm at 25°C. The detector was positioned at an angle of 173° to minimize the effects of multiple scattering. The values of zeta potential and conductivity were automatically measured following an equilibration process lasting 120 seconds at 25°C.

2.4.2. Nanoemulsion Absorbance

Absorbance measurement was performed to identify the initial size of the nanoemulsion droplets. The higher the absorbance value, the more light is absorbed, indicating the presence of larger droplets or particle agglomeration within the system. The nanoemulsion sample was diluted tenfold using aquabidest. Subsequently, the absorbance of the solution was measured at a wavelength of 650 nm using a UV-Vis Spectrophotometer (Shimadzu, Kyoto, Japan) with aquabidest as the blank.

2.4.3. Nanoemulsion Turbidity (τ)

The turbidity measurement of the nanoemulsion was conducted according to the method of Xu et al. (2024), which conducted the analysis using a UV-Vis Spectrophotometer at 650 nm wavelength. The nanoemulsion was prepared by diluting it tenfold prior to measurement. The turbidity was then calculated using the following equation.

$$T = 2.303 A D / L \quad (1)$$

Where T is the turbidity (cm⁻¹) of the nanoemulsion, A is the absorbance value of the dilution at a wavelength of 650 nm, D is the dilution factor (10), and L is the path length of the cuvette (cm).

2.4.4. Nanoemulsion Transmittance (%)

The measurement of % transmittance was performed to determine the self-emulsification ability of the nanoemulsions. This parameter was measured using a UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) at a wavelength of 650 nm after 10-fold dilution (Wiwiek et al., 2017).

2.5. Chemical Characteristics Analysis

2.5.1. Antioxidant Activity Measurement

Antioxidant activity was measured using a DPPH solution at a concentration of 24 ppm and a 99% methanol solvent. A sample stock solution of 10,000 ppm was first prepared by weighing 0.1 grams of the sample and dissolving it in 10 mL of methanol. Subsequently, 0.1 mL of the stock solution was taken and placed into a test tube. An aliquot (3.9 mL) of 24 ppm DPPH solution was added to the test tube, initiating a reaction between DPPH and the antioxidant compounds in the sample. The solution was then incubated in the dark for 90 minutes. As a control, a blank solution was prepared by reacting 0.1 mL of 99% methanol with 3.9 mL of a 24 ppm DPPH solution without undergoing incubation in the dark. After the reaction process was completed, absorbance measurements were taken using a UV-Vis spectrophotometer at a wavelength of 517 nm, starting with the blank and followed by the samples.

$$\text{Antioxidant activity (\%)} = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100\% \quad (2)$$

3. RESULTS

3.1. Particle Size

The results of the particle size of black cumin nanoemulsion are shown in Figure 1. Based on Figure 1, the smallest average size (29.767 nm) of black cumin seed nanoemulsion droplets is found at an oil-to-surfactant ratio of 1:3 using Tween 60. Conversely, the largest average size (442.633 nm) of black cumin seed nanoemulsion droplets is observed at an oil-to-surfactant ratio of 1:2 using Tween 80.

Statistical analysis indicates that the difference between these minimum and maximum values is significant ($p < 0.05$), suggesting the influence of the oil-to-surfactant ratio and surfactant type on nanoemulsion droplet formation.

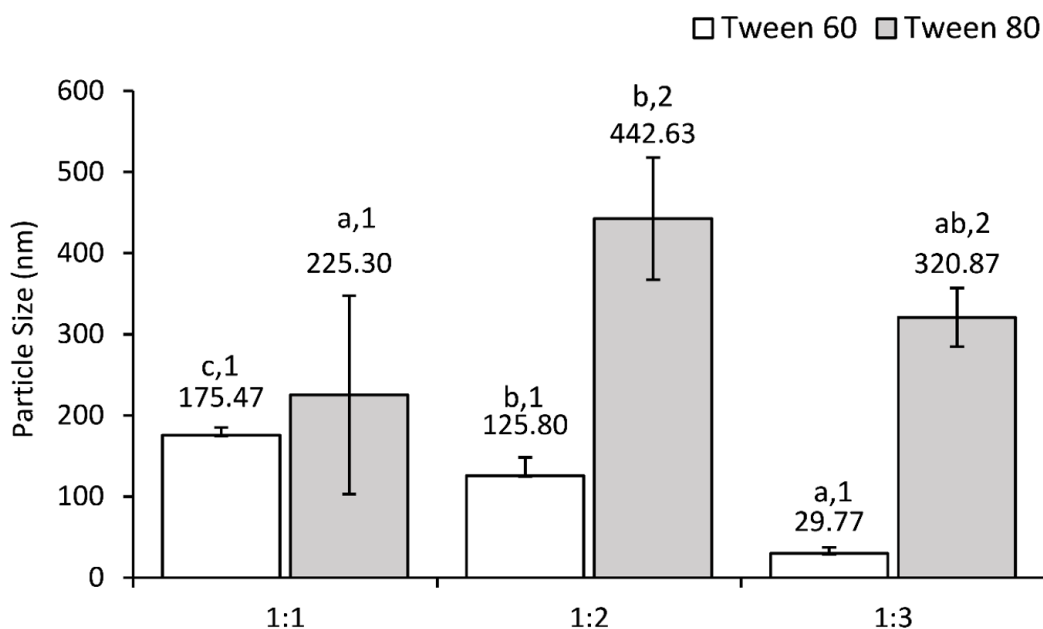


Figure 1. Particle size of black cumin seed nanoemulsion.
Note: The lowercase superscript indicates a one-way ANOVA test of oil-to-surfactant ratio (1:1, 1:2, and 1:3) in the Tween 60 and Tween 80 groups ($p \leq 0.05$). The superscript numbers indicate the results of the independent t-test for the surfactant type (Tween 60 and Tween 80) at each ratio ($p \leq 0.05$).

3.2. Polydispersity Index

The results of the polydispersity index of black cumin nanoemulsion are shown in Figure 2. Based on Figure 2, the smallest polydispersity index value of black cumin nanoemulsion is found at an oil-to-surfactant ratio of 1:1 using Tween 60, which is 0.328. The largest polydispersity index value is at an oil-to-surfactant ratio of 1:3 using Tween 80, which is 0.749. Statistical analysis shows that the difference between the smallest and largest values is significant ($p < 0.05$), indicating the effect of the oil-to-surfactant ratio and surfactant type on nanoemulsion uniformity.

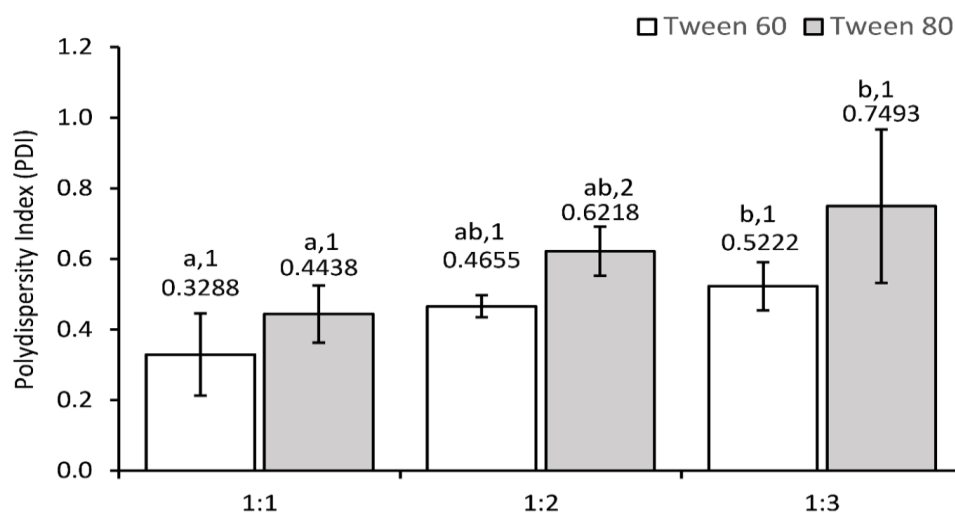


Figure 2. Polydispersity index of black cumin seed nanoemulsion.

Note: The lowercase superscript indicates a one-way ANOVA test of oil-to-surfactant ratio (1:1, 1:2, and 1:3) in the Tween 60 and Tween 80 groups ($p \leq 0.05$). The superscript numbers indicate the results of the independent t-test for the surfactant type (Tween 60 and Tween 80) at each ratio ($p \leq 0.05$).

3.3. Zeta Potential

The results of the zeta potential of black cumin nanoemulsion are shown in Figure 3. Based on Figure 3, the smallest zeta potential value of black cumin nanoemulsion (-1.911) occurs at an oil-to-surfactant ratio of 1:2 with the use of Tween 80. Meanwhile, the largest zeta potential value of black cumin nanoemulsion (-17.944) is found at an oil-to-surfactant ratio of 1:3 with the use of Tween 60. Statistical analysis indicates that the difference between the smallest and largest values is significant ($p < 0.05$), indicating the influence of the oil-to-surfactant ratio and the type of surfactant on the repulsive forces at the droplet surface.

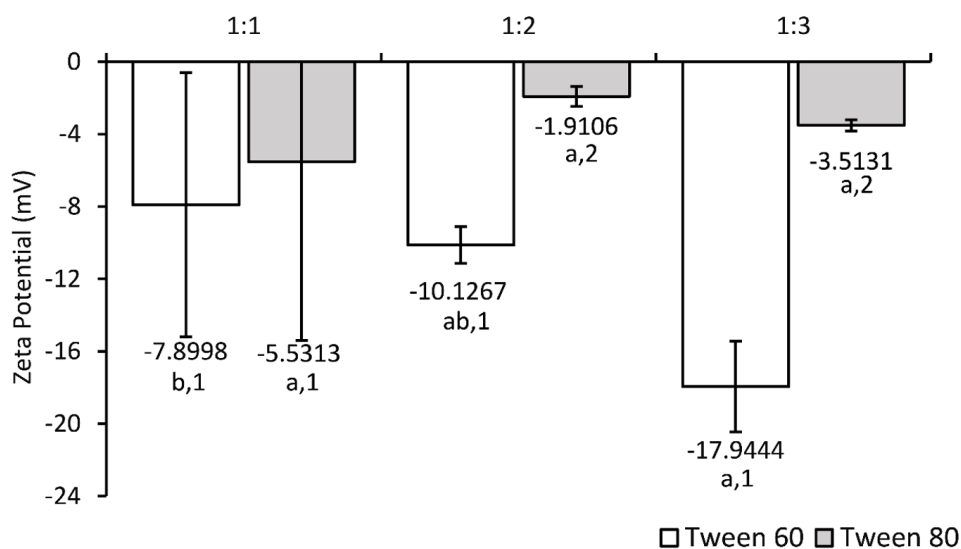


Figure 3. Zeta potential of black cumin seed nanoemulsion.

Note: The lowercase superscript indicates a one-way ANOVA test of oil-to-surfactant ratio (1:1, 1:2, and 1:3) in the Tween 60 and Tween 80 groups ($p \leq 0.05$). The superscript numbers indicate the results of the independent t-test for the surfactant type (Tween 60 and Tween 80) at each ratio ($p \leq 0.05$).

3.4. Conductivity

The results of the conductivity of black cumin nanoemulsion are shown in Figure 4. Based on Figure 4, the smallest conductivity value of the black cumin nanoemulsion is observed at an oil-to-surfactant ratio of 1:1 using Tween 60, which is 0.0427. Meanwhile, the largest conductivity value of the black cumin nanoemulsion is found at an oil-to-surfactant ratio of 1:3 with the use of Tween 80, which is 0.1033. Statistical analysis indicates that the difference between these smallest and largest values is significant ($p < 0.05$), indicating the influence of the oil-to-surfactant ratio and the type of surfactant on the system's ability to conduct electric current.

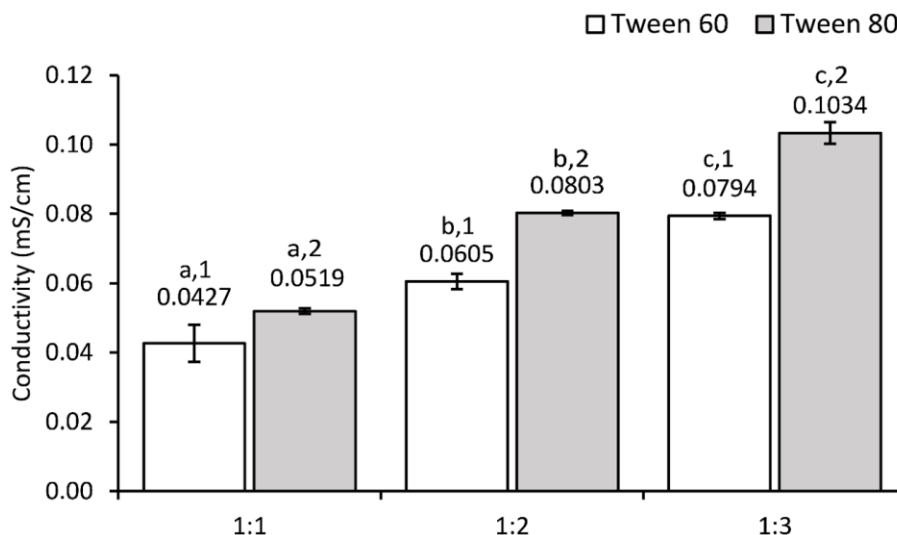


Figure 4. Conductivity of black cumin seed nanoemulsion.

Note: The lowercase superscript indicates a one-way ANOVA test of oil-to-surfactant ratio (1:1, 1:2, and 1:3) in the Tween 60 and Tween 80 groups ($p \leq 0.05$). The superscript numbers indicate the results of the independent t-test for the surfactant type (Tween 60 and Tween 80) at each ratio ($p \leq 0.05$).

3.5. Absorbance

The results of the absorbance of black cumin nanoemulsion are shown in Figure 5. Based on Figure 5, the lowest absorbance value of black cumin nanoemulsion is found at the oil-to-surfactant ratio of 1:3 with Tween 60, which is 0.0769. Meanwhile, the highest absorbance value is at the same ratio with Tween 80, which is 2.1192. Statistical analysis indicates that the difference between the lowest and highest values is significant ($p < 0.05$), suggesting the influence of the oil-to-surfactant ratio and surfactant type on nanoemulsion concentration.

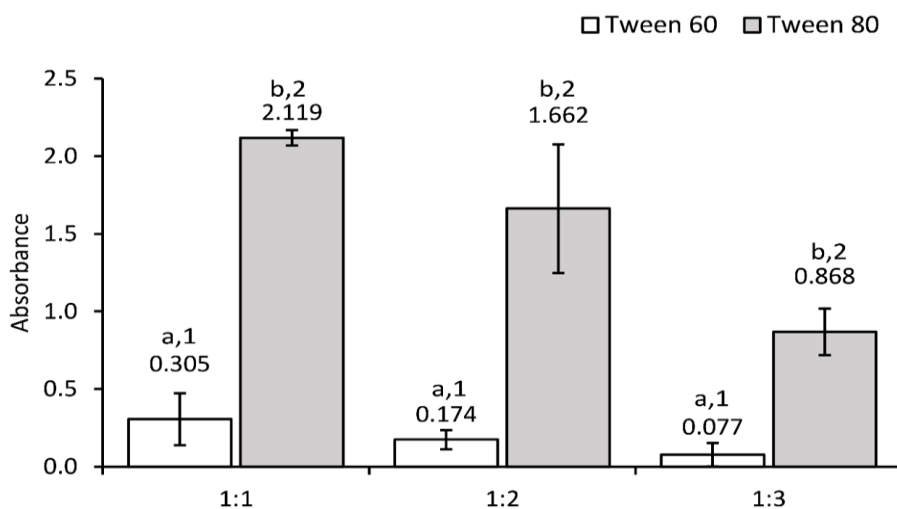


Figure 5. Absorbance of black cumin seed nanoemulsion.

Note: The lowercase superscript indicates a one-way ANOVA test of oil-to-surfactant ratio (1:1, 1:2, and 1:3) in the Tween 60 and Tween 80 groups ($p \leq 0.05$). The superscript numbers indicate the results of the independent t-test for the surfactant type (Tween 60 and Tween 80) at each ratio ($p \leq 0.05$).

3.6. Transmittance (%T)

The results of the transmittance (%T) of black cumin nanoemulsion are shown in Figure 6. Based on Figure 6, the lowest transmittance value occurs at an oil-to-surfactant ratio of 1:1 with Tween 80, which is 0.34%. The highest transmittance is at a ratio of 1:3 with Tween 60, which is 77.537%. Statistical analysis indicates that the difference between these values is significant ($p < 0.05$), showing the influence of the oil-to-surfactant ratio and surfactant type on nanoemulsion clarity.

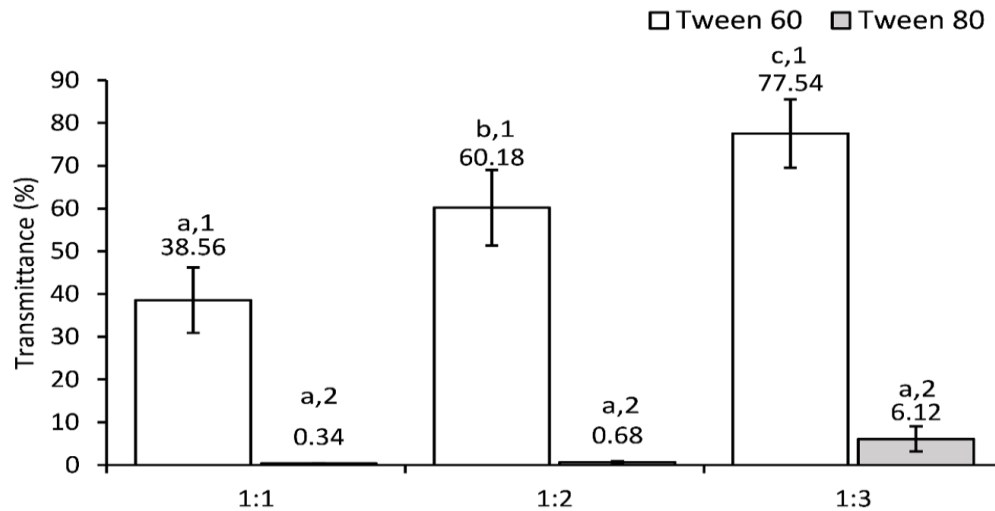


Figure 6. Transmittance (%) of black cumin seed nanoemulsion.

Note: The lowercase superscript indicates a one-way ANOVA test of oil-to-surfactant ratio (1:1, 1:2, and 1:3) in the Tween 60 and Tween 80 groups ($p \leq 0.05$). The superscript numbers indicate the results of the independent t-test for the surfactant type (Tween 60 and Tween 80) at each ratio ($p \leq 0.05$).

3.7. Turbidity (τ)

The results of the turbidity (τ) of black cumin nanoemulsion are shown in Figure 7. Based on Figure 7, the lowest turbidity occurs at an oil-to-surfactant ratio of 1:3 using Tween 60, which is 1.771 cm^{-1} . The highest turbidity is at a 1:1 ratio using Tween 80, which is 48.803 cm^{-1} . Statistical analysis indicates a significant difference ($p < 0.05$), suggesting the influence of the oil-to-surfactant ratio and surfactant type on turbidity.

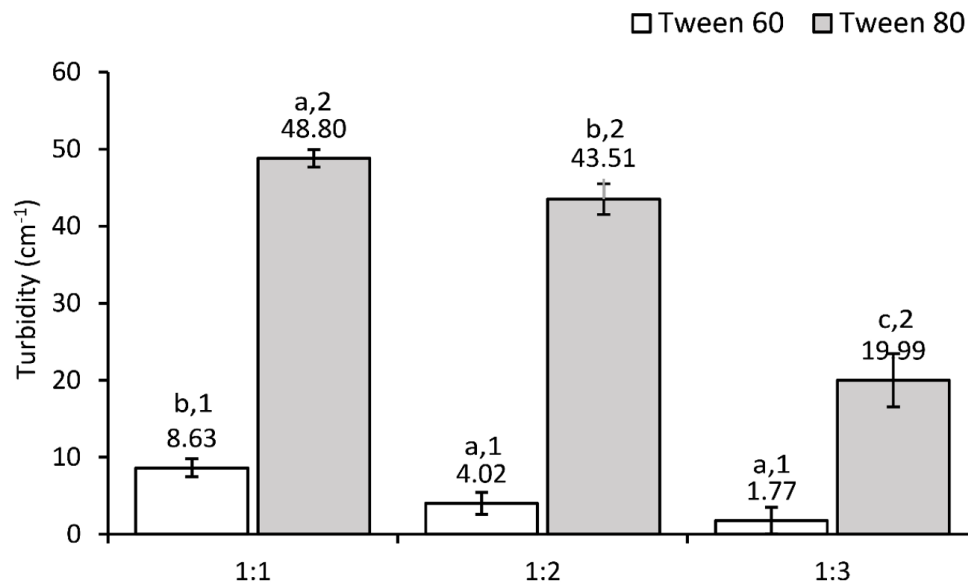


Figure 7. Turbidity of black cumin seed nanoemulsion.

Note: The lowercase superscript indicates a one-way ANOVA test of oil-to-surfactant ratio (1:1, 1:2, and 1:3) in the Tween 60 and Tween 80 groups ($p \leq 0.05$). The superscript numbers indicate the results of the independent t-test for the surfactant type (Tween 60 and Tween 80) at each ratio ($p \leq 0.05$).

3.8. Antioxidant Activity

The results of the antioxidant activity of black cumin nanoemulsion are shown in Figure 8. Based on Figure 8, the smallest antioxidant activity of black cumin nanoemulsion is found at an oil-to-surfactant ratio of 1:3 using Tween 80, which is 3.301%. Meanwhile, the largest antioxidant activity is found at an oil-to-surfactant ratio of 1:1 using Tween 60, which is 5.703%. Statistical analysis indicates a significant difference between the smallest and largest values of antioxidant activity ($p < 0.05$), suggesting the influence of surfactant type on the percentage inhibition of the nanoemulsion against DPPH free radicals.

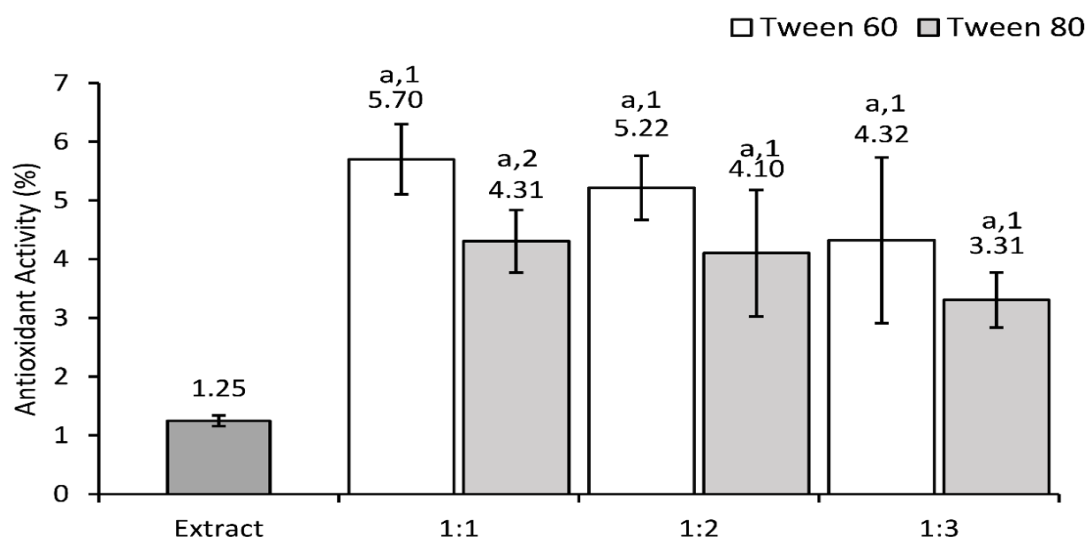


Figure 8. Antioxidant activity of black cumin seed nanoemulsion.

Note: The lowercase superscript indicates a one-way ANOVA test of oil-to-surfactant ratio (1:1, 1:2, and 1:3) in the Tween 60 and Tween 80 groups ($p \leq 0.05$). The superscript numbers indicate the results of the independent t-test for the surfactant type (Tween 60 and Tween 80) at each ratio ($p \leq 0.05$).

3.9. Total Phenolic Content (TPC)

The results of the total phenolic content (TPC) of black cumin nanoemulsion are shown in Figure 9. Based on Figure 9, the lowest TPC of the nanoemulsion is found at an oil-to-surfactant ratio of 1:3 with Tween 80, which is 0.193 mg GAE/g. The highest TPC is at a 1:1 ratio with Tween 80, measuring 0.852 mg GAE/g. Statistical analysis indicates a significant difference between these values ($p < 0.05$), suggesting the surfactant type influences the inhibition percentage concerning phenolic compound content.

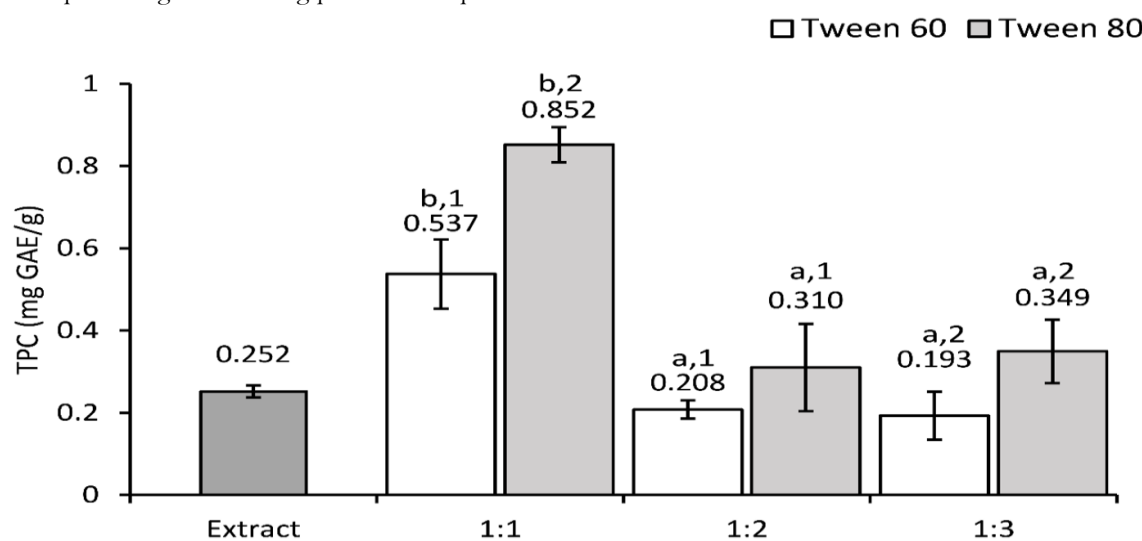


Figure 9. Total phenolic content (TPC) of black cumin seed nanoemulsion.

Note: The lowercase superscript indicates a one-way ANOVA test of oil-to-surfactant ratio (1:1, 1:2, and 1:3) in the Tween 60 and Tween 80 groups ($p \leq 0.05$). The superscript numbers indicate the results of the independent t-test for the surfactant type (Tween 60 and Tween 80) at each ratio ($p \leq 0.05$).

4. DISCUSSION

4.1. Antioxidant Activity and Total Phenolic Content

The antioxidant activity of black cumin (*Nigella sativa* L.) nanoemulsion, as evaluated by the DPPH assay, demonstrated that the formulation with Tween 60 at an oil-to-surfactant ratio of 1:1 exhibited the highest radical scavenging capacity (5.703%), whereas the lowest activity was recorded in the 1:3 ratio with Tween 80 (3.301%). These results indicate that both the type of surfactant and the ratio of oil to surfactant significantly influence the antioxidant efficiency of the nanoemulsion ($p < 0.05$). The higher scavenging activity of Tween 60-based formulations may be attributed to its ability to form more stable micelles, thereby improving the solubility and dispersion of bioactive compounds such as thymoquinone, the principal antioxidant component of black cumin. Previous studies have also highlighted that surfactant selection directly affects particle size and stability, which subsequently modulates the bioavailability and functional activity of encapsulated phytochemicals (Patra et al., 2018).

In comparison with crude oil, the antioxidant activity of nanoemulsions was generally lower, which may result from partial encapsulation of active compounds within the nanoemulsion matrix. This limits their immediate interaction with the DPPH radical during *in vitro* testing. However, the low antioxidant activity does not necessarily signify diminished functional potential, as nanoemulsion systems are specifically designed to enhance *in vivo* stability and solubility. The lipophilic bioactive compounds encapsulated within the nanoemulsion are expected to be released in a controlled manner as it enters the body. Thus, despite lower initial radical scavenging activity *in vitro*, nanoemulsions may provide physiological benefits due to improved absorption and bioavailability when applied in food or pharmaceutical systems.

The total phenolic content (TPC) analysis revealed significant differences across formulations. The highest phenolic content was observed in the 1:1 ratio with Tween 80 (0.852 mg GAE/g), while the lowest was in the 1:3 ratio with Tween 80 (0.193 mg GAE/g). These results confirm that surfactant type and concentration play a crucial role in phenolic compound retention ($p < 0.05$). Tween 80, being more hydrophilic than Tween 60, may enhance encapsulation efficiency and solubilization of phenolic constituents, leading to higher TPC in formulations using Tween 80, as seen in Figure 9. However, at higher surfactant ratios (1:3), dilution and molecular interactions could reduce extractable phenolic content, explaining the decline in TPC values.

The observed disparity between antioxidant activity and total phenolic content further suggests that phenolic compounds are not the sole contributors to antioxidant capacity. In black cumin, thymoquinone and other volatile oils may also exhibit antioxidant effects. However, their behavior and antioxidative properties within nanoemulsion may differ from phenolic compounds. This highlights the complexity of evaluating antioxidant functionality. Moreover, the encapsulation process may selectively influence the availability of different classes of antioxidative compounds, thereby modifying their measurable contribution in the resulting chemical properties analysis.

The results of this research demonstrate that nanoemulsion formulation, particularly the choice of surfactant and oil-to-surfactant ratio, substantially affected the functional characteristics of black cumin oil bioactive compounds. Although the nanoemulsion in this study exhibited lower *in vitro* antioxidant activity compared to the crude oil used, their potentially enhanced phenolic retention and *in vivo* bioavailability support the application of nanoemulsion as a delivery system for the health-promoting black cumin seed oil. Further studies employing *in vivo* antioxidant assays and bioavailability measurements are warranted to fully elucidate the health-promoting potential of black cumin nanoemulsions.

4.2. Particle Size Analysis

Black cumin (*Nigella sativa* L.) nanoemulsion showed significant variations in its particle (droplet) size depending on the type of surfactant and oil-to-surfactant ratio. The smallest droplet size was achieved with Tween 60 at 1:3 ratio (29.77 nm), while the largest was obtained with Tween 80 at 1:2 ratio (442.63 nm). This result highlights the significant ($p < 0.05$) role of surfactant selection and concentration in controlling nanoemulsion droplet size.

Application of Tween 60 as emulsifier in the black cumin seed oil nanoemulsion resulted in a lower particle size most likely due to its lower hydrophilic-lipophilic balance (HLB) compared to Tween 80, which enhances droplet stabilization by improving interfacial tension reduction. Previous studies confirmed that smaller droplet size improves stability, bioactive compound solubilization, and bioavailability in food and pharmaceutical applications (McClements, 2012a).

The polydispersity index (PDI) results further support these findings. The lowest PDI value (0.328) was recorded with Tween 60 at a 1:1 ratio, indicating high homogeneity of droplet distribution. Conversely, the highest PDI (0.749) was observed with Tween 80 at a 1:3 ratio, suggesting a broader size distribution and a less stable system. A PDI value below 0.3 is generally considered indicative of monodispersity and better long-term stability (Danaei et al., 2018). Thus, formulations prepared with Tween 60, particularly at lower surfactant ratios, appear to produce more stable and uniform emulsions compared to Tween 80-based systems. The zeta potential analysis revealed values ranging from -1.91 mV (Tween 80, 1:2) to -17.94 mV (Tween 60, 1:3). Although these values are relatively low compared to the conventional threshold for electrostatically stable dispersions (± 30 mV), they still indicate some surface charge contributing to repulsive interactions between droplets ($p < 0.05$). The higher negative zeta potential in Tween 60 formulations suggests better repulsion and reduced droplet aggregation risk compared to Tween 80. Similar results are reported in nanoemulsions stabilized with nonionic surfactants, where steric stabilization plays a more dominant role than electrostatic repulsion in maintaining colloidal stability (Tadros, Izquierdo, Esquena, & Solans, 2004).

Electrical conductivity measurements also revealed consistent trends. The lowest conductivity was found in Tween 60 at a 1:1 ratio (0.0427 mS/cm), while the highest was recorded in Tween 80 at a 1:3 ratio (0.1033 mS/cm). This increase in conductivity with higher surfactant ratios reflects the stronger presence of hydrophilic domains and enhanced ion mobility in the aqueous phase. Conductivity data, therefore, provide additional insights into the structural organization of the nanoemulsion system, with Tween 80 promoting greater hydrophilic interactions compared to Tween 60. Such differences in electrical behavior may influence the suitability of these nanoemulsions for specific applications, particularly in formulations requiring controlled ionic environments.

Overall, the results in this study suggest that Tween 60 generally produces smaller, more uniform, and relatively more stable nanoemulsion droplets compared to Tween 80. However, Tween 80 may enhance conductivity and phenolic retention. These differences underline the importance of optimizing surfactant type and concentration to balance particle size, stability, and functional properties of nanoemulsions. Such optimization is crucial for designing delivery systems aimed at enhancing the stability and bioavailability of black cumin bioactives for nutraceutical and pharmaceutical applications.

4.3. Optical Properties

The optical properties of *Nigella sativa* nanoemulsions, including absorbance, transmittance, and turbidity, were strongly influenced by both the surfactant type and oil-to-surfactant ratio. Formulations prepared with Tween 60 at a 1:3 ratio showed the lowest absorbance (0.0769), highest transmittance (77.537%), and lowest turbidity (1.771 cm^{-1}), indicating the formation of smaller droplets and more transparent systems. In contrast, Tween 80 at a 1:1 ratio produced the highest absorbance (2.1192), lowest transmittance (0.343%), and highest turbidity (48.803 cm^{-1}), suggesting larger droplet sizes and greater light scattering. These results are consistent with earlier findings that smaller droplets enhance transparency, while larger droplets increase opacity in nanoemulsions (McClements, 2012b; Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005).

The superior performance of Tween 60 can be attributed to its relatively higher lipophilicity compared to Tween 80, which favors the stabilization of *N. sativa* oil rich in thymoquinone and other lipophilic compounds. Previous studies have emphasized that the hydrophilic-lipophilic balance (HLB) of surfactants plays a crucial role in droplet

size reduction, stability, and optical properties of nanoemulsions (Gupta, Eral, Hatton, & Doyle, 2016; Mahdi Jafari, He, & Bhandari, 2006).

Overall, these findings suggest that the combination of Tween 60 and higher surfactant ratios is more effective in producing transparent and concurrently smaller particle size and more stable *N. sativa* nanoemulsions. This may enhance the potential of black cumin seed oil nanoemulsion for functional food and pharmaceutical applications.

5. CONCLUSION

This study has consistently demonstrated that the physical and chemical properties of black cumin (*Nigella sativa* L.) seed oil nanoemulsions were strongly influenced by surfactant type and oil-to-surfactant ratio in the emulsification process. Tween 60 consistently produced smaller droplet sizes, narrower distributions, higher transmittance, and greater antioxidant activity compared to Tween 80, indicating superior stability and functionality. Although the nanoemulsions exhibited lower *in vitro* antioxidant activity compared to the crude black cumin oil, the *in vivo* phenolic retention and bioavailability may be enhanced due to the physical properties of the nanoemulsion. The observed differences in particle size, zeta potential, conductivity, and optical properties between the nanoemulsions in this study emphasize the importance of surfactant selection in designing effective delivery systems. Overall, Tween 60-based nanoemulsions, particularly at lower oil-to-surfactant ratios, show promise for applications in functional food and nutraceutical formulations. Further *in vivo* studies are recommended to validate their physiological efficacy and long-term stability.

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