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Research Article

# The Forging of Dispersed Nano-Emulsion for Potential Feed Additive from Black Soldier Fly Essential Oil by Ultrasonication and Its Biological Efficacy

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**Abstract**
Encapsulation technology was developed to address the issues of limited bioavailability and instability associated with black soldier fly oil (BSFO). This research synthesized a nano-emulsion of BSFO utilizing the ultrasonication method, while varying the sources of antioxidants (black seed oil (BSO) and curcumin) and emulsifiers (polyethylene glycol (PEG), whey protein isolate, and Tween 80). The FTIR analysis revealed that the nanoemulsion samples displayed peaks at 3500–3000 $cm^{-1}$ (O–H stretching), 2900–2700 $cm^{-1}$ (N–H stretching), and 1700–1500 $cm^{-1}$ (C=O stretching), suggesting the presence of BSFO, curcumin, BSO, and the characteristics of the emulsifiers. Particle size analysis (PSA) indicated that the emulsion had an average particle size (Z-average) of approximately 229–686 nm. The nanoemulsion containing PEG showed reduced particle sizes of 218 and 229 nm compared to those with other emulsifiers, attributable to the inherently smaller size of PEG. The HB (BSO with PEG emulsifier) showed a reduced particle size due to the smaller molecular size of the antioxidant BSO compared to curcumin. The polydispersity index (PI) values for HB and CB (curcumin with PEG emulsifier) were 0.3 and 0.2, respectively, indicating relatively homogeneous particles, consistent with the criterion of a PI value below 0.4. Biological assays showed that CB had the highest DPPH inhibition at 83%, while curcumin exhibited 90%, exceeding that of BSO. The inhibition zones of HB are 2.45 cm for *Staphylococcus aureus* and 2.70 cm for *Escherichia coli*, representing the highest levels of inhibition. In this study, PEG is the best emulsifier for achieving a smaller nanoemulsion particle size. PEG facilitates the incorporation of BSFO with antioxidants, enhancing stability, efficacy, and bioavailability in various applications, particularly in the medical and food sectors.

**Keywords:** black seed oil, black soldier fly oil, curcumin, nano-emulsion, ultrasonic-assisted extraction

## 1. INTRODUCTION

The increasing global population raises concerns regarding the food supply chain and its implications for climate change. The anticipated global demand for agricultural products is projected to rise by 15% within the next decade, leading to an expected increase in the environmental footprint of the food system by 60–90% from 2010 to 2050 [1]. Locating alternative protein sources, particularly from insects, is crucial for manufacturing sustainable goods globally in the current context. Due to their high protein content and efficient feed conversion rates, insects are increasingly recognized as sustainable and nutritionally valuable sources of animal feed [2][3]. Insect proteins may substantially replace conventional soybean and fish meal in animal feed [4]. The beneficial characteristics of one of order *Diptera*, *Hermetia illucens Linnaeus*, black soldier fly (BSF), are particularly notable, particularly the larvae's high protein and fat content [5]. Moreover, the application of BSF larvae in animal feed is enhanced by the substantial amounts of bioactive substances they contain, such as antimicrobial peptides and cellulose [6]. For oil chemicals, encapsulation techniques are useful tactics that are necessary to preserve their effectiveness during preparation and preservation [7][8]. While enabling tailored distribution to particular areas, including the digestive system, these methods are essential for reducing the negative impacts of pH level changes, heat-induced stress, water level, and the oxidation process [5][8]-[10]. BSF oils (BSFO) can also be supplemented with antioxidant compounds such as curcumin or black seed oil (BSO) [11][12]. On the other hand, curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl) 1,6 -heptadiene-3,5-dione) is a polyphenolic compound of low molecular weight, derived from the rhizome of *Curcuma longa L.*, and is well known for its antibacterial, anti-inflammatory, and antioxidant

**Table 1**. Samples composition.

| Samples Code | Emulsifier Type | BSFO (v/v) | BSO (v/v) | Curcumin (w/v) | NaCl Solution (%) |
|---|---|---|---|---|---|
| HT | Tween 80 (10% v/v) | 7.5 | 7.5 | - | 75 |
| HW | WPI 2% (w/v) | 7.5 | 7.5 | - | 85 |
| HB | PEG 2% (w/v) | 7.5 | 7.5 | - | 85 |
| CT | Tween 80 (10% v/v) | 15 | - | 0.1 | 75 |
| CW | WPI 2% (w/v) | 15 | - | 0.1 | 85 |
| CB | PEG 2% (w/v) | 15 | - | 0.1 | 85 |

properties [13]-[16]. The medicinal plant *Nigella sativa*, often known as black seed oil, belongs to the family of Ranunculaceae and is mostly grown in the Middle East and Southwest Asia for its anti-diabetic, anti-allergic, antioxidant, and anti-carcinogenic qualities [11][12].

There are several different encapsulation techniques, including micro- and nano-encapsulation, which are classified according to the size of the materials being encapsulated [17][18]. Encapsulation technologies play a critical role in overcoming the limitations of active compound delivery [19]. Protein-based micro- and nano-encapsulation, particularly those utilizing elastin-derived materials, offer notable advantages including enhanced chemical adaptability, biodegradability, and targeted delivery efficiency [20]-[22]. Colloidal systems known as nanoemulsions are made up of ultrafine particles that are typically smaller than 500 nm in diameter. Oil-in-water (O/W) nanoemulsions are created by dispersing tiny oil droplets in an aqueous phase that is continuous. The encapsulation of physiologically active substances is made possible by a surfactant, which separates the lipid core from the aqueous phase and integrates them into the oil [23][24]. The durability of the resulting nanostructure, the size of the particles, and the effectiveness of the created protective barrier are all significantly impacted by the emulsifier selection [25]. Additionally, it can increase the antioxidant capacity of compounds that are encapsulated, protect them from deterioration, and increase their bioavailability. Nanoemulsions are widely applied in cosmetics, pharmaceuticals (particularly in drug delivery), and the food industries [26]. The implementation of nanostructure acquisition techniques in food technology enables the development of foods with novel chemical, biological, and physical attributes [27][28]. This innovation has positive social and economic effects in addition to expanding the selection of "smart" and "functional" meals. Nevertheless, this technology is complex, and its industrial application requires a multidisciplinary approach and consideration of numerous factors that influence product properties.

A nanoemulsion system is composed of multiple components, including surfactants, oil phase, and water phase [29]-[32]. A crucial factor to consider is the selection of the suitable emulsifier, such as tween 80 [33][34], whey protein [35]-[38], and polyethylene glycol (PEG) [39]. Tween 80, a non-ionic surfactant, is extensively utilized for its significant hydrophilicity and capacity to decrease interfacial tension, thereby facilitating the formation of stable small droplets [33]. Whey protein functions as a natural biopolymer emulsifier, forming a viscoelastic interfacial film that enhances physical stability and provides a more biocompatible alternative [37]. It was reported that PEG contributes to steric stabilization via its polymeric structure, which aids in preventing droplet aggregation and improving the long-term stability of nanoemulsions [39].

Sonication is a commonly utilized method that uses high-frequency sound waves and significant vibrational energy to disrupt and disperse particles, thereby facilitating the formation of nanoemulsions [29][40]. Extending sonication duration and adjusting the wave amplitude effectively reduce particle size in the nanoemulsion system, leading to more uniform droplet distribution and enhanced stability [4][37]. Treatment of a mixture within its ideal time range can improve the homogeneity of

particle size. The sonication method was selected due to its superior efficiency in generating nano-sized particles when compared to traditional techniques. This study investigates novel encapsulation techniques for BSFO-based nanoemulsions created with different emulsifiers, highlighting the role of bacterial activity in their development.

Sonication is a commonly utilized method that uses high-frequency sound waves and significant vibrational energy to disrupt and disperse particles, thus aiding in the formation of nanoemulsions [29] [40]. Increasing the duration of sonication and modifying wave amplitude significantly decreases particle size in the nanoemulsion system, resulting in a more uniform droplet distribution and improved stability [4][37]. Particle-size homogeneity is further improved when a mixture is treated within its ideal time range. In contrast to other nanoemulsification methods like high-pressure homogenization, which necessitates expensive equipment and may result in thermal degradation, and micro fluidization, which frequently requires multiple passes and high operational energy, the sonication method was chosen because of their effectivity at producing nano-sized droplets. Sonication, on the other hand, provides precise control over droplet production and is a more accessible and energy-efficient method. In this work, new encapsulating techniques for BSFO-based nanoemulsions made using various emulsifiers are examined. The biological activity of BSFO, especially its antibacterial actions against *Escherichia coli* and *Staphylococcus aureus*, along with its antioxidant capabilities, constitutes the principal justification for encapsulation. Nano emulsification can augment the bioavailability of these bioactive constituents, safeguard them against degradation, and raise their functional efficacy during application. Therefore, an understanding of how emulsifier type and sonication settings affect the stability and preservation of antioxidant and antibacterial properties in BSFO is necessary to support the overall purpose of this work.

## 2. MATERIALS AND METHODS

### *2.1. Materials*

The materials used in this study included physiological saline solution (0.9% w/v NaCl; PT Emjebe Pharma); BSFO (7.5–15% v/v); curcumin (0.1% w/v); BSO (7.5% w/v); Tween 80 (polyoxyethylene sorbitan monooleate, polysorbate 80; Merck); whey protein isolate (WPI); PEG (polyethylene glycol hexadecyl ether; Merck); 2,2-diphenyl-1-picrylhydrazyl (DPPH, analytical grade, Sigma-Aldrich), toluene (analytical grade, Merck); Gram-positive *S. aureus* ATCC 25923 and Gram-negative *E. coli* ATCC 25922 along with nutrient agar (NA, HiMedia) as the culture medium for

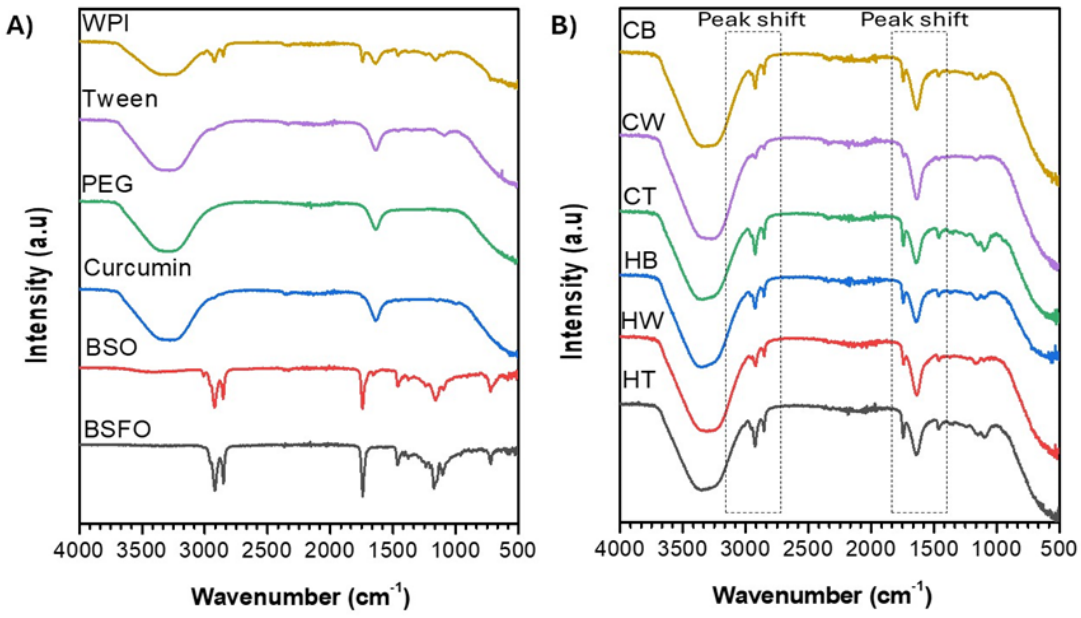


**Figure 1.** FTIR analysis of (A) precursor and (B) nanoemulsion with different emulsifiers.

antibacterial analysis.

### 2.2. Methods

#### 2.2.1. Formulation of Nanoemulsion

The emulsifier constituted 2% of the total formulation by weight and was solubilized in an 85% physiological NaCl solution through stirring under 60 °C for 30 min. The solution was supplemented with 7.5% BSFO and 7.5% BSO, and then sonicated using an ultrasonic homogenizer (Sonicator, Labtron Equipment Ltd, UK), with the probe positioned between the two phases to expedite the creation of nanoemulsions based on the formulation in Table 1. The process was carried out for 30 min.

#### 2.2.2. Characterization of Nanoemulsion

A Fourier-transform infrared (FTIR-8400S, Shimadzu, Japan) spectrophotometer was used to analyze the functional groups in the nanoemulsion in the 400–4000 $cm^{-1}$ frequency range. A dynamic light scattering (DLS) particle size analyzer (PSA, Zeta sizer Pro, Malvern Panalytical, UK) was used to measure droplet size under optimal conditions. A cleaned cuvette was filled with 12 µL of the sample to eliminate potential contaminants. The average particle size, ranging from 10–300 µm, was calculated to evaluate particle size distribution. The second cumulant of the polynomial fitting parameters was normalized to calculate the polydispersity index (PDI). Zeta potential (ELS with M3-PALS, Zetasizer Pro, Malvern Panalytical, UK) was measured using the constant current zeta mode within a size range of 100–3,800 µm.

#### 2.2.3. Product Quality Assessment

The density of each sample was determined using pycnometer (Pycnometer 25 mL, Duran, Germany), followed by viscosity measurement using an Ostwald viscometer (Ostwald Capillary Viscometer, Cannon Instrument Company, USA). A 10 mL portion of the sample was placed into the larger bulb, then drawn into the smaller bulb until it surpassed the upper calibration mark. Once suction was released, the sample was allowed to flow downward, and the time required to pass from the upper to the lower mark of the smaller bulb was recorded. Repeated measurements ensured

**Table 2.** Physical characteristics of nanoemulsion.

| Samples | Antioxidant | Emulsifier Type | pH | Density (g/mL) | Viscosity (cPs) | Specific gravity (g/mL) |
|---|---|---|---|---|---|---|
| HT | BSO | Tween 80 | 6.33±0.04 | 1.11±0.01 | 1.54±0.02 | 1.04±0.01 |
| HW | BSO | WPI | 6.51±0.02 | 1.23±0.02 | 1.53±0.03 | 1.09±0.02 |
| HB | BSO | PEG | 6.24±0.04 | 1.05±0.02 | 1.42±0.02 | 1.10±0.02 |
| CT | Curcumin | Tween 80 | 6.43±0.01 | 1.17±0.02 | 1.40±0.01 | 1.09±0.01 |
| CW | Curcumin | WPI | 6.61±0.02 | 1.25±0.02 | 1.51±0.02 | 1.10±0.01 |
| CB | Curcumin | PEG | 6.15±0.02 | 1.12±0.02 | 1.30±0.02 | 1.05±0.02 |

consistent viscosity values. A total of 6 nanoemulsion formulations were tested, with three replicates conducted for each, and viscosity was calculated using Equation (1);

$$\eta_x = \frac{\eta_0 t_x \rho_x}{t_0 \rho_0} \quad (1)$$

where $t_o$ represents the flow time of the reference liquid (s), $\rho_o$ is the density of the reference liquid (g/mL), $\rho_x$ refers to the density of the sample liquid (g/mL), $t_x$ denotes the flow time of the sample liquid (s), $\eta_o$ = indicates the viscosity of the reference liquid (cP), and $\eta_x$ corresponds to the viscosity of the sample liquid (cP).

Nanoemulsion stability was assessed using a centrifugation test. For 30 min, 40 mL of the sample were centrifuged at 1,000 rpm in tubes. Separation, creaming, aggregation, cracking, and precipitation were all monitored in the samples. A calibrated pH meter was used to measure the pH using a reference solution with a pH of 7. The pycnometer was used to measure specific gravity. Equation (2) was used to calculate specific gravity after the empty pycnometer was weighed, loaded with the nanoemulsion sample, and reweighed;

$$Specific\ gravity = \frac{W_s - W_0}{V_{pycnometer}} \quad (2)$$

where $V_{pycnometer}$ is pycnometer volume (mL), $W_s$ is sample-filled pycnometer weight, and $W_0$ is empty pycnometer weight.

### 2.2.4. Antioxidants Activity

For antioxidant activity, 100 mg of sample was placed in a test tube and mixed with 1 mL of toluene. This was combined with 3.9 mL of 0.1 mM DPPH in toluene and vortexed for 30 s until homogeneous. The mixture was incubated at room temperature in the dark for 1 h. Absorbance was measured at 515 nm using a UV–Vis spectrophotometer. Toluene served as the blank control, and vitamin C as the positive control. DPPH scavenging activity was calculated using Equation (3).

$$DPPH\ (\%) = \frac{(Abs\ control) - (Abs\ sample)}{Abs\ control} x\ 100\% \quad (3)$$

### 2.2.5. Antibacterial Activity

The antibacterial assay sought to assess the efficacy of the nanoemulsions against Gram-positive *S. aureus* and Gram-negative *E. coli*. Samples (5–10%) were produced in a 10 mL volumetric flask. The bacterial suspensions were calibrated to an inoculum concentration of approximately $1\times10^6$ CFU/mL before testing. NA was dispensed into sterile Petri plates, and upon solidification, the prepared suspensions of *S. aureus* and *E. coli* were uniformly distributed across the surface using a sterile inoculating loop. Sterile disc papers were immersed in the samples and placed on the agar surface. The plates were incubated at 37 °C for 24 h, after which inhibition zones were measured in mm.

**Table 3.** PSA results of samples.

| Samples | Antioxidant | Emulsifier Type | Particle size (nm) | Polydispersity Index (PI) |
|---|---|---|---|---|
| HT | BSO | Tween 80 | 264.4± 26.0 | 0.3993±0.050 |
| HW | BSO | WPI | 686.6±34.3 | 0.7227±0.060 |
| HB | BSO | PEG | 218.3±17.5 | 0.2713±0.030 |
| CT | Curcumin | Tween 80 | 275.5±16.5 | 0.4133±0.040 |
| CW | Curcumin | WPI | 551.7±55.2 | 0.5233±0.070 |
| CB | Curcumin | PEG | 229.2±11.0 | 0.3491±0.040 |
| BSO | - | - | 1.402±0.120 | 0.8101±0.045 |
| BSFO | - | - | 395.2±39.5 | 0.7364±0.050 |
| Curcumin | - | - | 125.6±18.8 | 0.8841±0.030 |
| PEG | - | - | 0.5212±0.050 | 0.1936±0.020 |
| WPI | - | - | 12.58±1.26 | 0.3065±0.025 |
| Tween 80 | - | - | 0.7227±0.110 | 0.4719±0.030 |

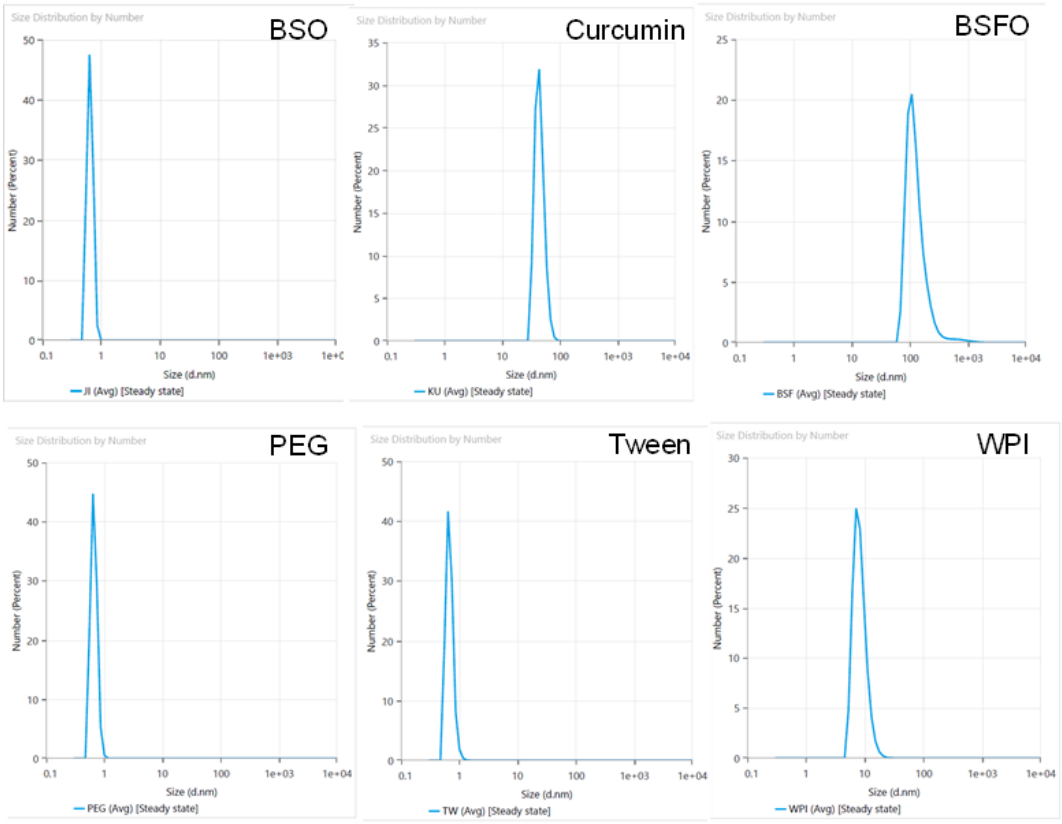


**Figure 2.** PSA results for the raw materials.

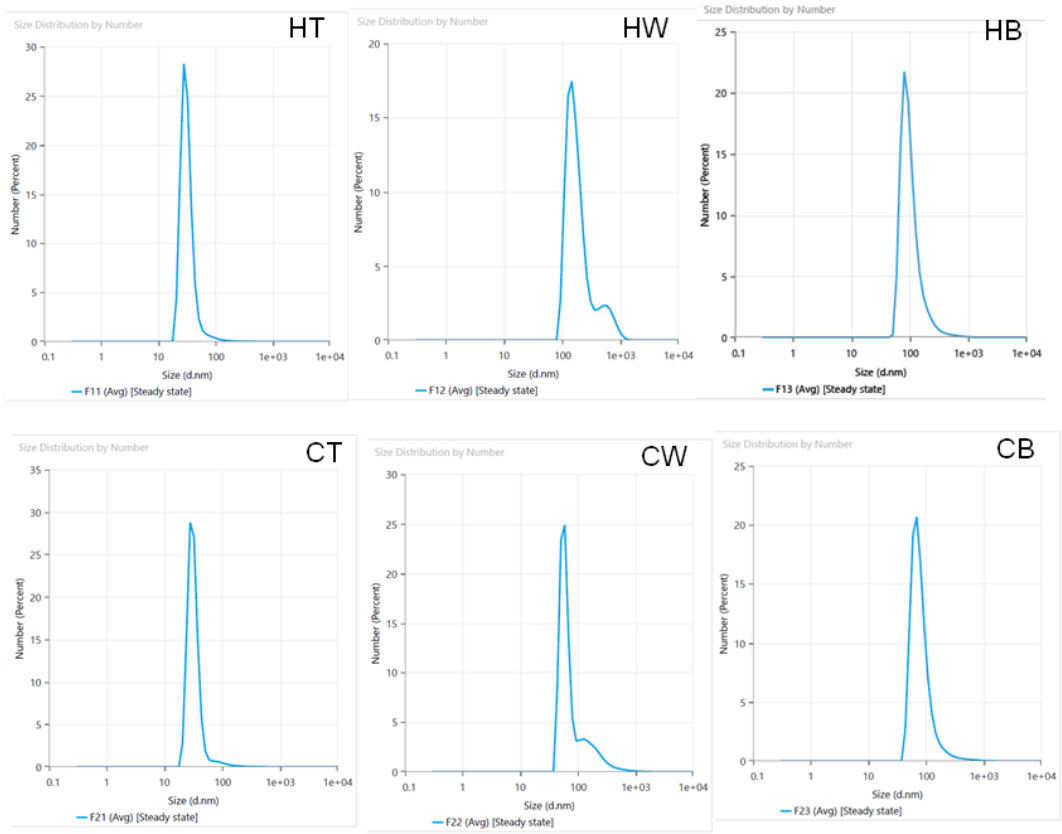


**Figure 3.** PSA results for the nano materials.

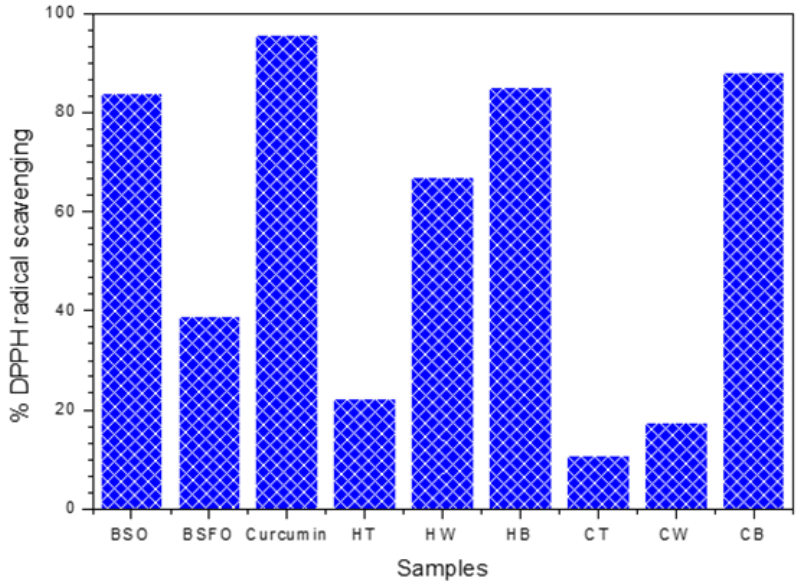


**Figure 4.** Antioxidants activity of sample by DPPH analysis.

*2.2.6. Analysis Data*

The data analysis utilized descriptive statistics to interpret the results of antibacterial activity, which was evaluated by measuring the inhibition zone against bacteria. Inferential analysis utilized one-way ANOVA and Tukey's post hoc test at a significance level of $\alpha = 0.05$ to differentiate among the formulations.

## 3. RESULTS AND DISCUSSIONS

BSFO is gaining recognition as a sustainable lipid source, attributed to its significant concentration of medium-chain fatty acids, especially lauric acid, which enhances antimicrobial activity and improves interfacial properties for emulsion formation. BSFO demonstrates potential as a carrier oil for nanoemulsion-based delivery systems. In addition, BSO is recognized as an effective herbal remedy, primarily due to thymoquinone (TQ), a compound known for its significant antioxidant, anti-inflammatory, and antimicrobial properties. Curcumin, a hydrophobic polyphenol derived from turmeric, is extensively researched for its potent antioxidant and anti-carcinogenic properties; however, its therapeutic efficacy is constrained by low solubility and bioavailability [19].

The FTIR spectroscopy depicted in Figure 1 examined the functional groups in nanoemulsions with various emulsifiers. The absorption band appeared of Tween 80, curcumin, and PEG appeared in phenolic O–H (3100 $cm^{-1}$) and aromatic C=C stretching (2918 and 2846 $cm^{-1}$) [41]. The broad band at 3274 $cm^{-1}$ observed in WPI, attributed to H-bonded OH stretching vibrations, is significantly larger than that of curcumin and PEG due to the contribution of N–H stretching vibrations associated with the hydrogen bonds of the protein. Moreover, WPI showed the peaks observed between 3200 and 2500 $cm^{-1}$ as stretching vibrations of N–H and C–H bonds, then stretching vibration of the C=O at 1743 $cm^{-1}$ [42]. Two prominent amide bands at 1620 and 1550 $cm^{-1}$ were identified, corresponding to the C=O stretch (amide I) and the N–H bend and C–N stretch (amide II) for BSO and BSFO, respectively. For the nanoemulsions products, all the samples demonstrated absorption peaks in the ranges of 1700–1500 (C=O and amide I/II), 2900–2700 (C–H stretching), and 3500–3000 $cm^{-1}$ (O–H/N–H stretching) [3]. The FTIR analysis revealed distinct peak shifts, particularly in the O–H and C=O regions, which suggest hydrogen bonding and molecular interactions among the components. Kabiriyel et al. [41] also stated that the interactions improved the stability of nanoemulsions and facilitated the formation of smaller, more uniform droplets, especially in PEG-based formulations.

To determine whether the nanoemulsion could

undergo phase separation because of gravity and whether centrifugal force could displace sample components from the centre of rotation, the centrifugation test was conducted during the first week. During phase separation, the lower-density phase rose to the top of the sample, while the higher -density phase sank to the bottom. Six samples were analysed, and the results showed no phase separation. The pH measurement was conducted to assess any significant changes in the acidity levels of the six samples, using consistent procedures depicted in Table 2. The average pH for the BSO-based nanoemulsion was 6.33±0.04, while the curcumin-based nanoemulsion had an average pH of 6.36±0.01. The findings showed no appreciable changes in pH, suggesting that the samples maintained stable acidity values. A pycnometer was used to test the specific gravity of samples. The six nanoemulsion samples had average specific gravities, densities, and viscosities ranging from 1.04–1.10 g/mL, 1.05–1.23 g/mL, and 1.30–1.54 cPs, respectively, according to the test results. The findings align with previous research by Tahir et al. [3], indicating that O/W nanoemulsions generally possess densities like water, accompanied by marginally increased viscosities that improve droplet stability and mitigate creaming or coalescence during storage.

The particle size of a nanoemulsion generally falls between 1 and 100 nm. The PSA and DLS utilized in this study (Table 3) were specifically developed to assess the particle size of the final sample. The determination of particle size is achieved through the rapid detection of scattered light at a specific angle utilizing a photon detector [33]. Figures 2 and 3 depict the distribution of particle sizes (Z-average) in the nanoemulsion between approximately 229.2±11.0 and 686.6±34.3 nm. PEG can produce the smallest particle sizes among the investigated agents such as WPI and Tween 80. The HB and CB nanoemulsions made with PEG showed smaller particle size diameters than those made with other emulsifiers [43]. A PI value of less than 0.4 denotes particle homogeneity, while values exceeding this threshold indicate heterogeneity. To evaluate the stability and homogeneity of nanoemulsion formulations, the PI is a crucial measure for determining particle size distribution. Increased PI values indicate a reduction in particle homogeneity within the formulation [44]. Emulsifiers carry out by lowering surface tension, which aids in the creation of smaller droplets during homogenization, leading to nanoemulsions with significantly reduced particle sizes [43]. The PSA and PI values obtained in this study were lower than those reported by Saffarian et al. [44], who utilized Tween 80 (1% w/w) as an emulsifier and achieved an average particle size of

**Table 4.** Inhibition zone of antibacterial activity for samples.

| Samples | Antioxidant | Emulsifier Type | Concentration (%) | Inhibition Zone (mm) | |
|---|---|---|---|---|---|
| | | | | *E. coli* | *S. aureus* |
| HT | BSO | Tween 80 | 5 | 18.5±0.3 | 18.0±0.2 |
| | | | 10 | 23.0±0.5 | 19.5±0.4 |
| HW | BSO | WPI | 5 | 18.0±0.2 | 18.5±0.3 |
| | | | 10 | 20.5±0.4 | 22.0±0.5 |
| HB | BSO | PEG | 5 | 24.0±0.6 | 19.5±0.3 |
| | | | 10 | 27.0±0.7 | 24.5±0.6 |
| CT | Curcumin | Tween 80 | 5 | 18.0±0.3 | 13.5±0.2 |
| | | | 10 | 23.5±0.5 | 15.5±0.4 |
| CW | Curcumin | WPI | 5 | 14.5±0.2 | 16.0±0.3 |
| | | | 10 | 15.5±0.3 | 23.0±0.5 |
| CB | Curcumin | PEG | 5 | 14.0±0.1 | 11.0±0.2 |
| | | | 10 | 16.0±0.4 | 16.0±0.3 |

483.4 nm for *Carum copticum* essential oil.

The antioxidant activity of BSFO-based nanoemulsions was assessed via the DPPH method to determine the percentage inhibition value shown in Figure 4. DPPH serves as an oxidizing agent in reactions with antioxidant compounds present in a sample (Table 4). The raw materials, BSO and curcumin, are well-established herbal remedies, globally distributed and extensively utilized in traditional medicine due to their potent antioxidant properties. Findings indicate that BSO and curcumin demonstrate inhibition rates of 84% and 96%, respectively. The formation of nanoemulsions may diminish antioxidant activity, likely attributable to the stability of nanoparticles, which, according to previous data, lack sufficient homogeneity—except for HB and CB, which demonstrate inhibition percentages of 85% and 88%, respectively [45].

Table 4 presents the antibacterial data for the nanoemulsion. Curcumin is a dimeric derivative of ferulic acid. The lipophilic nature of curcumin facilitates its integration into liposome bilayers. This enhances the permeability of the bilayer. In bacterial cells, curcumin may cause 1,2-dipalmitoyl -sn-glycero-3-phosphocholine (DPPC) membranes to break down. A bacterial agent with an inhibitory zone between 140 and 230 mm was demonstrated by the formation of curcumin-based nanoemulsion (CT, CW, and CB). The findings of the study indicate that the nanoemulsion exhibits a greater value than Sayyar et al. [46] demonstrated that curcumin nano dispersion, prepared at optimal proportions, possessed substantial antibacterial properties against *S. aureus* (35 mm) and *E. coli* (31 mm). The FTIR spectra of BSO-based nanoemulsions revealed the presence of significant functional groups linked to its bioactive compounds, notably TQ, which exhibits quinone C=O vibrations at 1743 $cm^{-1}$ related to reactivity for the antimicrobial activity. BSO is rich in polyunsaturated fatty acids, particularly oleic acid, linoleic acid, omega-9, and omega-6, and is known for its notable stability against oxidative degradation. The antibacterial activity of BSO in food has been attributed to its diverse phytochemical constituents, such as thymohydroquinone, carvacrol, thymol, and thymoquinone, which have been shown to inhibit pathogens including *Shigella, Staphylococcus aureus*, *Salmonella*, and *Listeria monocytogenes* [47]. The optimal activity of BSO-based nanoemulsion (HT, HW, and HB) is observed in HB, measuring 270 mm. The increased efficacy of BSO-based nanoemulsion compared to curcumin is attributed to the presence of TQ. TQ, the primary bioactive ingredient in black cumin seed oil, possesses anti-inflammatory, anti-apoptotic, and antioxidant properties [47].

## 4. CONCLUSIONS

The formulation of nanoemulsions based on BSFO was enhanced by incorporating the antioxidants curcumin or BSO. The formulations employed various emulsifiers, including PEG, Tween 80, and WPI. According to PSA data, nanoemulsions prepared with PEG as an emulsifier exhibited smaller and more uniformly dispersed particle sizes compared to those prepared with Tween 80 or WPI due to PEG size of 0.5212±0.050 nm. The BSO-based nanoemulsion displayed smaller particle sizes than the curcumin-based nanoemulsion, as curcumin has a larger molecular size of 125.6±18.8 nm. Among the samples, HB exhibited the smallest particle diameter at 218.3±17.5 nm with their homogeneity of PI of 0.2713±0.030. The curcumin-based nanoemulsion (CB samples) demonstrated the highest antioxidant activity at 88.09%, which can be attributed to curcumin's superior antioxidant capacity (95.5%) compared to BSO's 83.9%. Meanwhile, the BSO-based nanoemulsion (HB samples) exhibited the strongest antibacterial activity, with inhibition zones of 24.5±0.6 mm against *S. aureus* and 27.0±0.7 mm against *E. coli*, attributable to TQ, the main component of black seed oil, which possesses anti-inflammatory, anti-apoptotic, and antioxidant properties. These findings demonstrate that BSFO nanoemulsions act as multifunctional bioactive carriers with significant potential in healthcare, pharmaceuticals, and functional foods. Future studies should focus on in vivo validation of bioavailability and safety, long-term stability testing under industrial conditions, and synergistic formulation strategies to enhance therapeutic and nutraceutical benefits, thereby facilitating translation into practical applications.

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### Conflicts of Interest

The authors declare no conflict of interest.

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## DECLARATION OF GENERATIVE AI

Not applicable.

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