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Original Article

# PREPUPAE OIL FOR COSMETIC APPLICATION AND ANTI-HYALURONIDASE ACTIVITY-NANOEMULSIONS OF BLACK SOLDIER FLY (*HERMETIA ILLUCENS*)

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**ABSTRACT**

**Objective:** This study aimed to develop and evaluate Black Soldier Fly (BSF) (*Hermetia illucens*) prepupae oil nanoemulsions for cosmetic applications, focusing on their ability to inhibit the hyaluronidase enzyme.

**Methods:** The formulation process involved optimizing the combination of surfactant (Tween 80) and cosurfactant (PEG 400) using Simplex Lattice Design (SLD), with transmittance and pH as crucial parameters. A coarse oil-in-water (o/w) emulsion was prepared by mixing the oil and aqueous phases using a high-shear homogenizer. This emulsion was then subjected to ultrasonication with a probe sonicator to achieve the desired droplet size and stability. The physicochemical properties of the resulting nanoemulsions were characterized using appropriate analytical instruments, including physical appearance, pH value, particle size, polydispersity index (PDI), and zeta potential. Stability testing was conducted through heating-cooling cycling and centrifugation, monitoring changes in the formulations over time. Anti-hyaluronidase activity was assessed for anti-aging.

**Results:** The selected nanoemulsion, PP2, consisting of 53.5% Tween 80, 24.5% PEG 400, 6% distilled water, and 16% BSF prepupae oil, exhibited a droplet size of 307.8 nm, a polydispersity index of 0.656, and a zeta potential of-40 mV. The BSF prepupae oil has an $IC_{50}$ value of 0.173%, while the $IC_{50}$ values of BSF prepupae oil in nanoemulsion PP1 and PP2 are 0.053% and 0.066%.

**Conclusion:** PP2 demonstrated superior stability and anti-hyaluronidase activity ($IC_{50}$: 0.066%) compared to BSF prepupae Oil ($IC_{50}$: 0.173%), making it the most effective formulation.

**Keywords:** Nanoemulsion, Linoleic acid, Black soldier fly prepupae, Anti-hyaluronidase


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## INTRODUCTION

*Hermetia illucens,* also known as Black Soldier Fly (BSF), is a fly native to the Americas but has spread to many parts of the world [1]. In recent years, there has been growing interest in the potential applications of BSF and its oils in various industries due to its sustainable sourcing, which aligns with the growing demand for eco-friendly and natural ingredients, including the cosmetic industry [2, 3]. BSF oil can potentially be used in cosmetic formulations for moisturizing, anti-aging, and skin-repairing benefits because of its rich composition of fatty acids, proteins, vitamins, and other bioactive compounds [4, 5].

Skin aging is characterized by a decline in physiological function and structural changes influenced by intrinsic factors such as genetics and specific enzymes [6]. One key enzyme in this process is hyaluronidase, which plays a crucial role in the degradation of hyaluronic acid, a major component of the skin's extracellular matrix that helps maintain moisture and elasticity [7]. Research has demonstrated that BSF prepupae oil is rich in fatty acids, including linoleic acid (25.08%), lauric acid (33.75%), palmitic acid (13.66%), and oleic acid (13.40%) that may inhibit hyaluronidase [8]. Linoleic acid is also known for its ability to protect the skin from UV radiation, reduce melanin, and improve skin barrier function [3]. The potential of BSF oil to serve as a source of such bioactive compounds presents an exciting opportunity for developing novel skincare products. Previous studies have indicated that incorporating these oils into formulations can improve skin hydration and elasticity [9]. However, the stability and bioavailability of these oils in cosmetic formulations remain critical factors that influence their efficacy. Nanoemulsion technology has emerged as a promising approach to enhance the delivery and stability of bioactive compounds in skincare products [10]. By reducing the particle size of the active ingredients, nanoemulsions can improve skin penetration and ensure a more uniform distribution of the active compounds on the skin surface. Despite the promising attributes of BSF oil and nanoemulsion technology, limited research has explored their combined effects in cosmetic applications.

Because nanoemulsion is thermodynamically unstable, it needs emulsifiers to maintain stability while being prepared and stored. This study aims to optimize a nanoemulsion of BSF oil using Tween 80 as a surfactant and PEG 400 as a cosurfactant and evaluate the nanoemulsions in inhibiting hyaluronidase enzyme while assessing their physical stability. By conducting the *in vitro* tests, this research aims to provide insights into the potential of these formulations as effective anti-aging products. The findings from this study could pave the way for developing innovative skincare solutions that harness the power of natural ingredients, ultimately contributing to the growing demand for safe and effective cosmetic products in the market.

## MATERIALS AND METHODS

BSF prepupae oil is purchased from Rumah Maggot Semen Padang (Indonesia). Other chemicals and solvents are Tween 80 (PT. Bratachem, Indonesia), PEG 400 (Sigma Aldrich, USA), NaOH (Nitra Kimia, Indonesia), phosphoric acid (Nitra Kimia, Indonesia), bentonite (T and T Chemical, Indonesia), activated charcoal (T and T Chemical, Indonesia), Tris-HCl buffer (Sigma Aldrich®), bovine testes type I-S hyaluronidase (Sigma Aldrich®), NaCl, bovine serum albumin (BSA), hyaluronic acid (Sigma Aldrich), hexane, methanol, HCl, Na2SO4, BF3, disodium phosphate, monosodium phosphate, double-distilled water, glacial acetic acid, and sodium acetate.

### Purification of BSF prepupae oil

BSF prepupae oil was purified through degumming, dewaxing, and bleaching. For degumming and dewaxing, 100 ml of BSF prepupae oil was heated to 70 °C, followed by adding 0.2 ml of 99% phosphoric acid ($H_3PO_4$) (v/v), and stirred for 30 min. Then, 10 ml of 15% sodium hydroxide (NaOH) (w/v) was added for neutralization, which proceeded for 30 min. The oil was then centrifuged at 6000 rpm for 5

min. The bleaching process involved adding 0.5 g of activated charcoal and 1.5 g of bentonite, stirring at 150 rpm for 30 min. The mixture was then filtered using filter paper [11].

**Optimization of BSF prepupae oil nanoemulsion formula**

The optimization of surfactant and co-surfactant was performed by combining Tween 80 and PEG 400 as the surfactant and co-surfactant. Tween 80, PEG 400, and BSF prepupae oil were mixed according to the ratio listed in table 1. The BSF prepupae oil and PEG 400 were stirred at 250 rpm for 10 min at 37 °C. Subsequently, Tween 80 was added, and the mixture was stirred for 20 min at 37 °C and 250 rpm while distilled water was gradually added. This formulation was then subjected to visual observation. The stirring speed was tested through several trials, including 150, 175, 200, 250, 300, and 350 rpm.

**Table 1: Surfactant ratio's impact on visual perception**

| Tween 80 (ml) | PEG 400 (ml) | BSF prepupae oil (ml) |
|---|---|---|
| 1 | 7 | 1 |
| 2 | 6 | 1 |
| 3 | 5 | 1 |
| 4 | 4 | 1 |
| 5 | 3 | 1 |
| 6 | 2 | 1 |
| 7 | 1 | 1 |
| 1 | 6 | 1 |
| 2 | 5 | 1 |
| 3 | 4 | 1 |
| 4 | 3 | 1 |
| 5 | 2 | 1 |
| 6 | 1 | 1 |

The comparison with clear visual appearance was used to establish the upper and lower limits for the surfactant and co-surfactant components. These were subsequently input into the *Design Expert* software to generate the recommended formulas. These formulas were evaluated based on the desired response criteria, % transmittance, and pH value. Transmittance measurements were performed using a UV-Vis spectrophotometer (Shimadzu AUX 220, Japan) at a wavelength of 650 nm, with distilled water as the blank.

After evaluating the responses of all the recommended formulas, data analysis was conducted to determine the most optimal formula based on the best % transmittance and pH values with the highest desirability index.

**Optimization of the preparation method for BSF prepupae oil nanoemulsion**

Method optimization was conducted using the optimal formula recommended by the Design Expert to enhance % transmittance. The methods applied included the preparation process using a heating magnetic stirrer (IKA, Germany) at 250 rpm and 37 °C for 40 min and heating magnetic stirring (250 rpm, 37 °C, 40 min) followed by sonication with a sonicator (IKA, Germany) at 40 °C for 10 min. The method that produced the preparation with the highest % transmittance will be used for nanoemulsion formulation and its characterization.

**Preparation of BSF prepupae oil nanoemulsion**

The optimal formula recommended by the Design Expert was prepared using mechanical stirring and sonication. BSF prepupae oil was first mixed with PEG 400 by stirring at 250 rpm for 10 min at 37 °C. Tween 80 was then added, and the mixture was stirred at 250 rpm for 20 min at 37 °C while deionized water was gradually added. This formulation was subsequently sonicated at 40 °C for 10 min.

**Measurement of droplet size, polydispersity index, and zeta potential (ZP) of BSF prepupae oil nanoemulsion**

The optimum formula was tested for particle size using a Particle Size Analyzer (Horiba SZ-100, Japan) by diluting the sample at a 1:100 ratio in deionized water. The sample was placed in a cuvette and positioned in the instrument holder. It was followed by analysis with the PSA to provide information on particle size, polydispersity index, and zeta potential [12].

**Measurement of viscosity of BSF prepupae oil nanoemulsion**

The viscosity of BSF prepupae oil nanoemulsion was measured in triplicate at room temperature (25 °C) using Spindle 02 at a speed of 50 rpm for 15 s [13].

**Studies on the thermodynamic stability of BSF prepupae oil nanoemulsion**

The thermodynamic stability test was performed on the selected formula using freeze-thaw and centrifugation tests. The formula was stored in a refrigerator at-5 °C for 24 h and in an oven at 40 °C for 24 h, repeated for three cycles. During each cycle, observations were made for phase separation, homogeneity, and clarity. The centrifugation test was conducted to assess the mechanical stability of the nanoemulsion. The BSF prepupae oil nanoemulsion was centrifuged at 3500 rpm for 30 min. Stable nanoemulsions can be identified by the absence of phase separation [14].

**Anti-hyaluronidase activity test of BSF prepupae oil nanoemulsion**

The sample was put into a 96-well microplate in five microliters. The mixture was then incubated for 10 min at 37 °C after 100 µl of hyaluronidase enzyme (1.5 U/ml in 20 mmol phosphate buffer, pH 7, containing 77 mmol NaCl and 0.01% bovine serum albumin (BSA)) was added. After that, 45 min at 37 °C was used for incubating 100 µl of hyaluronic acid substrate (0.03% in 300 mmol phosphate buffer, pH 5.35). After incubation, 1 ml of albumin acid solution (0.1% BSA in acetate buffer, pH 3.75) was added to stop the interaction between the substrate and the enzyme. After keeping the solution at room temperature for ten min, the absorbance at 600 nm was calculated. As a positive control, linoleic acid was employed [15].

$$\% \text{ inhibition} = \frac{A - B}{A} x100\%$$

A = Absorbance of the solution without the sample

B = absorbance of the solution with the sample added

The $IC_{50}$ value represents the concentration that provides 50% inhibition of hyaluronidase activity and is determined by interpolating the concentration-response curve. It can be established through linear regression analysis between the sample concentration and the percentage of enzyme activity inhibition using the equation y = a+bx [15].

**RESULTS AND DISCUSSION**

**Purification of BSF prepupae oil**

BSF prepupae oil is purified through degumming, dewaxing, and bleaching [11]. The degumming process helps remove phosphates in the form of phospholipids, reducing the risk of sediment formation cloudiness and enhancing the thermal stability of the oil without

reducing its fatty acid content. During degumming, phosphoric acid and sodium hydroxide are added. Phosphoric acid separates gum and other impurities from the sample by breaking the ester bonds of phospholipids, allowing the phospholipids to dissolve in water and be removed from the oil. Sodium hydroxide neutralizes the previously added phosphoric acid [16].

The dewaxing stage aims to remove waxes that cause cloudiness in the oil. After degumming, the BSF prepupae oil is centrifuged to separate the wax. In this process, the denser wax is pushed to the edge of the container while the lighter oil remains in the center, allowing for wax separation from the oil [11].

The bleaching stage removes pigments, contaminants, and residual colored and odorous compounds, enhancing the oil color and aroma's brightness, clarity, and stability. Activated carbon and bentonite are used as adsorbents due to their strong adsorption capabilities, effectively removing lipid peroxide compounds that can degrade the oil quality. This process improves the oil for cosmetics, food, and pharmaceutical use [16]. Based on the results of the organoleptic testing of BSF prepupae oil, it can be concluded that the purification process led to significant changes in several aspects of its physical properties and aroma. After purification, the oil became slightly less dense than its initial state, indicating an improvement in clarity, as reflected by the change in color from dark brown to clear yellow. Additionally, the pungent odor of the oil was reduced post-purification, suggesting that the process effectively decreased or eliminated the components responsible for the undesirable scent. Therefore, the purification process enhances the quality of BSF prepupae oil in terms of appearance and aroma.

**Formulation of BSF prepupae oil nanoemulsion**

The optimization of the nanoemulsion formula consists of a mixture of BSF prepupae oil as the oil phase, Tween 80 as the surfactant, PEG 400 as the co-surfactant, and distilled water as the carrier. The surfactant-to-co-surfactant ratios used were 1:7, 2:6, 3:5, 4:4, 5:3, 6:2, 7:1, 1:6, 2:5, 3:4, 4:3, 5:2, and 6:1. Based on fig. 1, the ratios of surfactant to co-surfactant that produced a visually clear nanoemulsion were 4:3, 5:2, and 6:1. These three ratios were established as the upper and lower limits of the surfactant and co-surfactant components entered into the Design Expert application. The recommended formula for further optimization can be found in table 2.


**Fig. 1: Results of visual observation on the effect of tween 80 and PEG 400 ratios on nanoemulsion formation**

**Table 2: The recommended formula from design expert**

| Std | ID | Run | Space type | A: Tween 80 (ml) | B: PEG 400 (ml) | Transmittance (%) | pH |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | Vertex | 6 | 1 | 95.1 | 6.92 |
| 2 | 0 | 2 | Center | 5 | 2 | 98.0 | 7.19 |
| 6 | 0 | 3 | Center | 5 | 2 | 98.4 | 7.7 |
| 7 | 0 | 4 | Center | 5 | 2 | 98.5 | 7.4 |
| 3 | 3 | 5 | AxialCB | 5.5 | 1.5 | 97.4 | 7.3 |
| 5 | 2 | 6 | Vertex | 4 | 3 | 97.0 | 6.47 |
| 4 | 1 | 7 | Vertex | 6 | 1 | 95.4 | 6.6 |

After evaluating the responses for all the recommended formulas, data was analyzed to determine the optimal formula based on the best % transmittance and pH value by pH value. Two mixture components, Tween 80 (A) and PEG 400 (B), had varying volumes. Tween 80 (A) varied from 4 ml to 6 ml, while PEG 400 (B) varied from 1 ml to 3 ml (table 3).

**Table 3: The parameters analyzed with a simplex lattice design varied**

| Factors | Range (ml) | |
|---|---|---|
| | Low | High |
| Tween 80 (A) | 4 | 6 |
| PEG 400 (B) | 1 | 3 |

Fig. 2 shows that the highest transmittance percentage, 98.5%, is achieved with a combination of approximately 5 ml of Tween 80 and 2 ml of PEG 400. The transmittance value decreases if the Tween 80 or PEG 400 volume exceeds or falls short of this value. Fig. 3 demonstrates that the combination of around 5 ml of Tween 80 and 2 ml of PEG 400 remains within the permissible pH range. The pH value decreases when the Tween 80 or PEG 400 volume exceeds or falls below this combination. This optimal combination maintains both transmittance and pH within the desired range.

Both (fig. 2 and fig. 3) indicate an optimal combination of Tween 80 and PEG 400, yielding the highest transmittance and appropriate pH value. Variation in the volume of both components within certain limits still produces good results, but combinations too far from the optimal point reduce both transmittance and pH values. High transmittance indicates a highly transparent nanoemulsion, while appropriate pH values indicate good stability. This combination is suitable for applications requiring high transparency and stable pH value.

Fig. 4(a) and fig. 5(a) are graphs that examine whether the residuals (the difference between the model's predicted and observed values) are typically distributed. The normality of residuals is essential because many statistical methods assume that residuals follow a normal distribution. In the Normal Plot of Residuals, the x-axis typically represents the ordered residuals, while the y-axis shows

the expected residual values from the normal distribution (theoretical normal distribution). In both plots for percentage of Transmittance (%T) and pH, the points are close to a straight line, indicating that the residuals from the regression model approximate a normal distribution. It suggests that the regression model used is valid based on the assumption of residual normality.

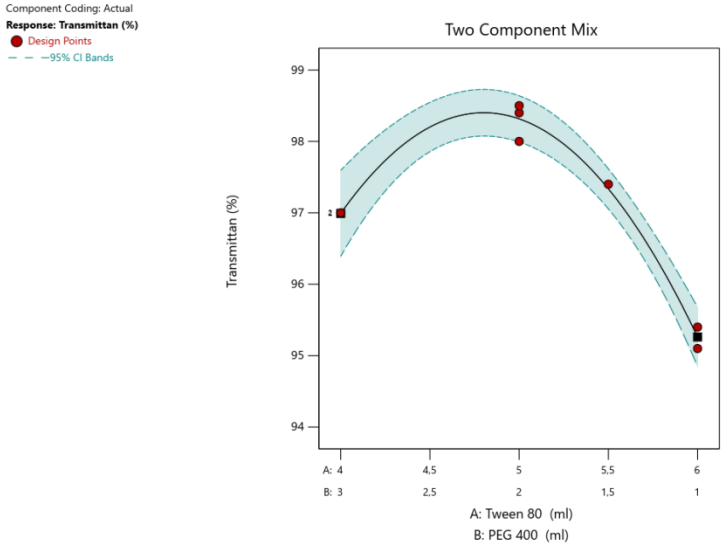



**Fig. 2: Graph showing the relationship between the mixture components (Tween 80 and PEG 400) and % transmittance**

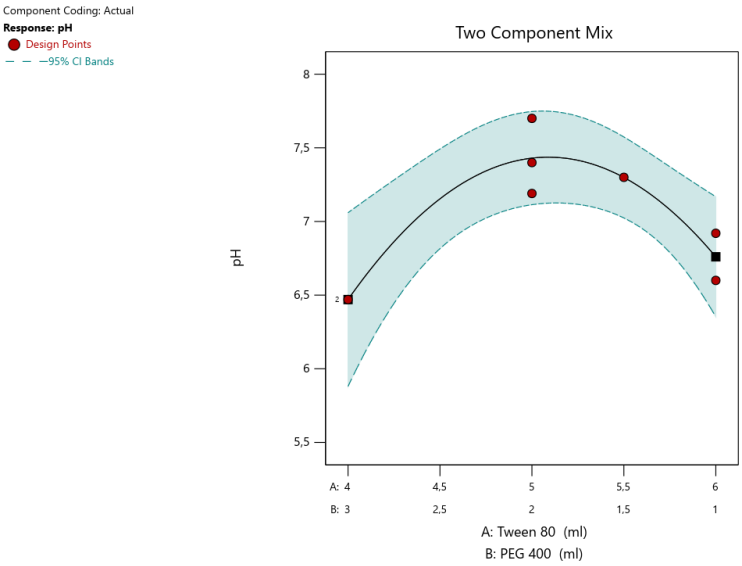
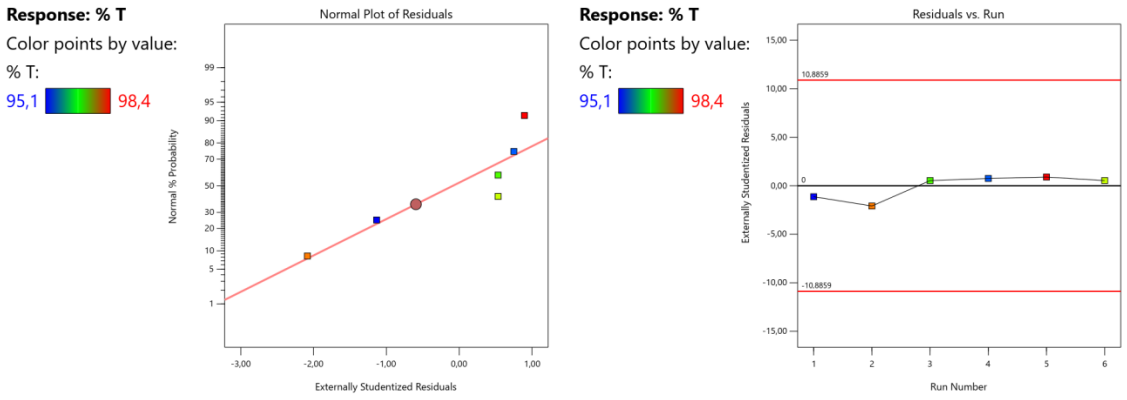
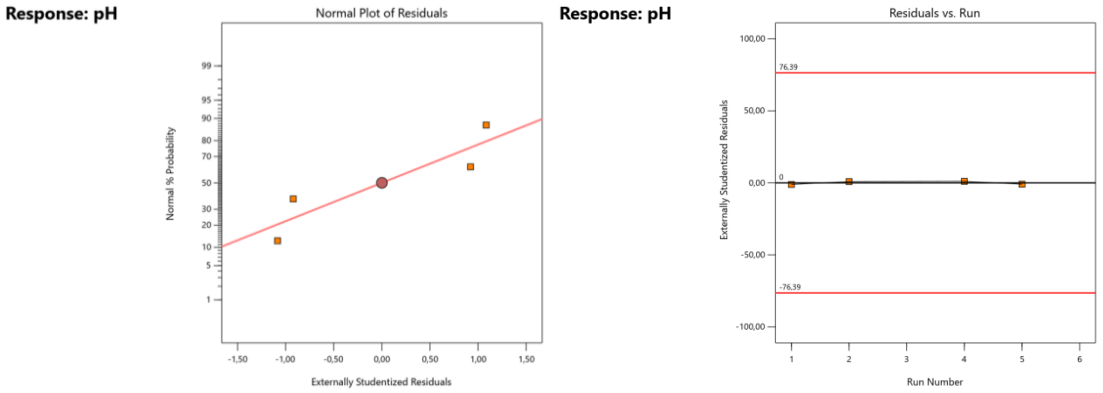



**Fig. 3: Graph showing the relationship between the two mixture components (Tween 80 and PEG 400) and pH value**




(a) (b)

**Fig. 4: A residual parameter graph for the transmittance**




(a) (b)

**Fig. 5: A residual parameter graph for the pH value**

Fig. 4(b) and fig. 5(b) illustrate the Residuals vs. Run plots, which are employed to evaluate whether there are any patterns in the residuals relative to the run number (sequence of experiments). Ideally, residuals should be randomly distributed around the zero axis, indicating that no systematic patterns arise with the sequence of trials. The presence of patterns, such as an upward or downward trend, may suggest potential issues with the model (e. g., other factors not accounted for in the model that could influence the outcomes). The points should be randomly scattered around the zero line in these plots. Random residual patterns suggest that the model does not exhibit systematic errors related to the sequence of trials. The residuals in the graphs for %T and pH are randomly distributed with no discernible patterns, indicating that the model does not exhibit systematic errors. Suppose the points form a pattern (e. g., an increasing or decreasing trend). In that case, this may suggest potential issues such as changes in experimental conditions or errors in the predictive model.

The design expert's optimal formula contains 4.803 ml of Tween 80 and 2.197 ml of PEG 400. Additionally, variations in the concentration of BSF prepupae oil in the formulation were conducted at concentrations of 11% (1 ml) and 16% (1.5 ml), as shown in table 4.

**Table 4: Variations in BSF prepupae oil in the nanoemulsion formulation**

| Component | PP1 (ml) | PP2 (ml) |
|---|---|---|
| Tween 80 | 4.803 | 4.803 |
| PEG 400 | 2.197 | 2.197 |
| BSF prepupae oil | 1 | 1.5 |
| Distilled water | 0.5 | 0.5 |

**Optimization of the preparation method for BSF prepupae oil nanoemulsion**

The preparation method was optimized to enhance the percentage transmittance of the nanoemulsion. A higher transmittance value indicates smaller emulsion droplet sizes, approaching the nanometer scale. The size of the dispersed phase significantly impacts the appearance of the emulsion. When the globules in the emulsion are tiny, and light can pass through them, the solution appears transparent, resulting in a high transmittance value. Distilled water was used as a reference because it contains no particles that obstruct light transmission, thereby resulting in a transmittance value of 100% [17]. In this study, transmittance testing was performed on both formulations using different preparation methods: one involving mixing and the other involving mixing, followed by sonication.

**Table 5: Transmittance testing for the mechanical mixing method and mechanical mixing followed by sonication**

| Formula | Transmittance (%) | |
|---|---|---|
| | Mechanical mixing (250 rpm, 37 °C, 40 min) | Mechanical mixing (250 rpm, 37 °C, 40 min) followed by sonication (40 °C, 10 min) |
| PP1 | 98.6 | 99.8 |
| PP2 | 94.3 | 98.4 |

According to the results shown in table 5, transmittance testing with mechanical mixing (250 rpm, 37 °C, 40 min) followed by sonication (40 °C, 10 min) yielded higher results compared to the preparation method using only mechanical mixing (250 rpm, 37 °C, 40 min). Combining mechanical mixing and sonication improved the percentage transmittance in the nanoemulsion preparation by producing smaller, more uniform droplets approaching the nanometer scale. The small globule size allows light to pass through the solution, resulting in a more transparent nanoemulsion and a higher transmittance value. This method of mechanical mixing followed by sonication is more effective than mechanical mixing alone, as it maximizes homogenization and emulsion stability. Sonication effectively reduces droplet size, while mixing ensures uniform blending, improving nanoemulsion quality [18].

**Preparation of BSF prepupae oil nanoemulsion**

From the optimization of formulas and methods, an optimal formula was determined with two variations of BSF prepupae oil: 11% (1 ml) and 16% (1.5 ml). Sonication and magnetic stirring were used in tandem to prepare them. Both nanoemulsion formulas were evaluated for their physical properties and anti-aging activity *in vitro* (hyaluronidase enzyme inhibition test).

**Droplet size, zeta potential (ZP), and polydispersity index measurements of BSF prepupae oil nanoemulsion**

The Particle Size Analysis (PSA) of BSF prepupae oil nanoemulsion reveals significant differences in particle size, polydispersity index, and zeta potential between the two samples with varying oil

concentrations (table 6). The average particle size of the PP1 formulation (11% oil) is 15.3 nm. In contrast, the PP2 formulation (16% oil) exhibits a significantly larger average particle size of 307.8 nm. Although the particle size in PP2 is much larger than in PP1, it remains within the normal range for nanoemulsions (20–500 nm). More extensive oil phases tend to form larger droplets, resulting in a larger average particle size. Conversely, smaller particle sizes can enhance the stability and homogeneity of the emulsion system [19].

**Table 6: Test results for droplet size, polydispersity index, and zeta potential**

| Formula | Droplet size (nm) | Polydispersity index | Potential zeta (mV) |
|---|---|---|---|
| PP1 | 15.3 | 0.193 | -0.4 |
| PP2 | 307.8 | 0.656 | -40.0 |

The polydispersity index (PDI) measures the distribution of particle sizes in a colloidal system. It indicates the degree of non-uniformity in particle size distribution. A lower PDI value (typically less than 0.5) suggests a more homogeneous and stable system, while a higher PDI indicates a broader size distribution, which can affect stability [20]. The PDI for PP1 is 0.193, while PP2 has a PDI of 0.656. The lower PDI value in PP1 suggests a more consistent particle size distribution than PP2, indicating that the emulsification process was more effective in PP1. It may be attributed to the increased viscosity of the emulsion system in PP2, which affects the emulsification process and particle stability [21].

Zeta potential measures the electrical charge on the surface of particles in a colloidal system. It is a critical parameter that influences the stability of colloidal dispersions [22]. A high absolute value of zeta potential (either positive or negative) typically indicates good stability, as it suggests strong repulsive forces between particles, preventing aggregation. The zeta potential for PP1 is-0.4 mV, whereas PP2 has a zeta potential of-40 mV. The acceptable range for zeta potential indicating good colloidal stability is above+30 mV or below-30 mV [23]. The shallow zeta potential of PP1 suggests that particles in the PP1 nanoemulsion have low kinetic stability and are more prone to aggregation or sedimentation over time. In contrast, PP2 demonstrates good electrostatic stability. The higher zeta potential in PP2 suggests that particles in this formulation tend to repel each other, thereby reducing the likelihood of aggregation and enhancing the stability of the nanoemulsion system [24].

Comparison between the two samples indicates that increasing the oil concentration from 11% to 16% significantly affects the characteristics of the nanoemulsion. At the lower oil concentration (11%) in PP1, smaller particle sizes and a narrower distribution are achieved, but with low kinetic stability due to a nearly neutral zeta potential. Conversely, at the higher oil concentration (16%) in PP2, although particle size increases and the size distribution widens, system stability improves due to the higher zeta potential.

These results highlight a trade-off between particle size and stability in the BSF prepupae oil nanoemulsion formulation. A lower oil concentration may be preferred for applications requiring small and homogeneous particle sizes, although additional stabilizers might be needed to enhance stability. Conversely, a higher oil concentration could be considered for applications prioritizing emulsion stability, as the particle size remains within the nano range. Further research could focus on optimizing the formulation by adjusting the type and concentration of surfactants to achieve an optimal balance between particle size, homogeneity, and stability of the BSF prepupae oil nanoemulsion.

**Measurement of viscosity**

The viscosity test of BSF prepupae oil nanoemulsion was conducted to determine the thickness of the resulting formulation. The viscosity range for nanoemulsions can vary depending on the intended application and specific formulation, typically ranging from 10 to 2000 cPs [17]. The viscosity measurements for the PP1 formulation were 375±7.071 and 538.667±8.083 cPs. Based on these results, both formulations exhibit ideal viscosities as they fall within the typical nanoemulsion viscosity range.

**Studies on the thermodynamic stability of BSF prepupae oil nanoemulsion**

This study employed centrifugation and freeze-thaw cycle techniques in thermodynamic stability tests to identify metastable formulations [13]. The tests successfully identified the ideal formulation, which exhibited no instability. For BSF prepupae oil nanoemulsion to form a properly correct digestive system, it must spontaneously emulsify and maintain stability to avoid creaming, cracking, or precipitation. The thermodynamic stability tests confirmed that the prepared BSF prepupae oil nanoemulsion was stable, even for the shortest duration tested.

**Anti-hyaluronidase activity test of BSF prepupae oil nanoemulsion**

IC50, or the half maximal inhibitory concentration, is a key metric used to evaluate the potency of a substance in inhibiting a specific enzyme or biological function [3]. In the context of this study, it measures the concentration needed to inhibit 50% of the hyaluronidase enzyme activity. A lower IC50 value indicates a more potent inhibitor, meaning a smaller substance is required to achieve significant enzyme inhibition. The study compared the IC50 values for different formulations of Black Soldier Fly (BSF) prepupae oil. The pure BSF prepupae oil had an IC50 of 0.173%, while the nanoemulsion formulations PP1 and PP2 had IC50 values of 0.053% and 0.066%, respectively (table 7). It demonstrates that the nanoemulsion formulations are more effective in inhibiting hyaluronidase activity than the pure oil. PP1 was more effective than PP2, likely due to its smaller particle size, which is 20 times smaller than that of PP2. The improved anti-hyaluronidase activity in nanoemulsion formulations is attributed to enhanced stability, better distribution, and increased skin penetration [25].

**Table 7: Anti-hyaluronidase activity test of BSF prepupae oil nanoemulsion and BSF prepupae oil**

| Sample | $IC_{50}$ (%) |
|---|---|
| PP1 | 0.053 |
| PP2 | 0.066 |
| BSF Prepupae Oil | 0.173 |

Although the anti-hyaluronidase activity between PP1 and PP2 was not significantly different, PP2 exhibited better overall performance due to its superior zeta potential and stability despite its larger particle size. Therefore, PP2 was selected as the optimal formulation, offering a balance of effectiveness, stability, and bioavailability. These findings suggest that BSF prepupae oil nanoemulsions could be a potential agent in skincare products, particularly inhibiting hyaluronidase activity.

Linoleic acid was used as a positive control in the anti-hyaluronidase activity test to ensure the method's accuracy and conditions. As a polyunsaturated fatty acid, linoleic acid is known for inhibiting hyaluronidase. The $IC_{50}$ of linoleic acid was 0.00013%, demonstrating its high efficacy in inhibiting 50% of hyaluronidase activity at deficient concentrations. The use of linoleic acid as a positive control confirmed the reliability of the test results, as it produced the expected outcomes. Positive controls are crucial for ensuring the accuracy of experimental data, and the successful results from the linoleic acid control validate the accuracy of the anti-hyaluronidase test conducted.

**CONCLUSION**

The selected nanoemulsion formulation, PP2, consists of Tween 80,

PEG 400, distilled water, and BSF prepupae Oil in 53.5%, 24.5%, 6%, and 16%, respectively. It has a droplet size of 307.8 nm, a polydispersity index of 0.656, and a zeta potential of-40 mV and remained stable in both freeze-thaw and centrifugation stability tests. The BSF prepupae oil in formulations PP1 (IC50: 0.052%) and PP2 (IC50: 0.066%) exhibited higher anti-hyaluronidase activity compared to pure BSF prepupae Oil (IC50: 0.173%).

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**AUTHORS CONTRIBUTIONS**

Concept: AJ, RA, RR; Design: RA, AJ; Data Collection or Processing: SA, AJ, RA, RR; Analysis or Interpretation: RA, AJ, RR, SA; Literature Search: SA, AJ, RA Writing: AJ, SA, RR, RA.

**CONFLICT OF INTERESTS**

The authors declare no conflict of interest with the data contained in the manuscript.

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