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Characteristics of *Nigella sativa* Emulsion Using Tween 80 Surfactant Variation

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ABSTRACT

Background: *Nigella sativa* (*NS*) is a renowned natural antioxidant with anti-inflammatory, anticancer, and antidiabetic properties. However, its active components are highly susceptible to degradation upon exposure to air and light, significantly reducing their stability and therapeutic potential.

Objective: This study aimed to improve the stability of *NS* oil by formulating emulsions using Tween 80 surfactant.

Research Methods: Emulsions were prepared with Tween 80 concentrations of 10%, 15%, and 20%, and their physical properties were evaluated, including organoleptic characteristics, pH, homogeneity, and viscosity.

Results: The emulsions demonstrated homogeneous, milky-white appearances with the characteristic aroma of *NS* and pH values ranging from 5.65 to 5.85. Viscosity increased proportionally with higher Tween 80 concentrations, measuring 104 cP, 180 cP, and 384 cP, respectively. These findings highlight the potential of Tween 80-based emulsions to stabilize *NS* oil, offering favorable physical characteristics that support further pharmaceutical and nutraceutical applications.

Conclusion: The best performing Nigella sativa (black cumin oil) emulsion formula identified in this study was Formula 3 (F3) with good viscosity stability. The important role of surfactant concentration and storage conditions in maintaining the stability and quality of the emulsion.

Keywords: *Emulsion; Physical characteristics; Nigella sativa; Tween 80*

INTRODUCTION

Black cumin oil, derived from Nigella sativa (NS), has long been recognized for its significance in traditional medicine, as well as its use as a spice and food preservative. This plant is commonly cultivated in the Middle East, Eastern and Western Europe, and Central Asia (Rahman, et al., 2021). The seeds of NS are black and have a slightly bitter taste. Nutritional studies suggest that NS is a rich source of various minerals, including sodium, zinc, calcium, iron, magnesium, copper, potassium, and copper (Hossain, et al., 2021). Previous research has demonstrated that NS is particularly abundant in phytochemicals, especially antioxidants such as thymoquinone, thymohydroquinone, and dithymoquinone, which are known for their anti-inflammatory, anticancer, and antidiabetic properties (Gholamnezhad, and Keyhanmanesh, 2015). These compounds help protect cells and tissues from damage caused by free radicals (Anjastika, 2020). Further studies have shown that NS can enhance the immune system and effectively treat asthma and dry cough (Saleh et al., 2023).

Moreover, NS has exhibited antimicrobial activity against various pathogens, including *Escherichia coli*, *Bacillus subtilis*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* (Shafodino, and Lusilao, 2022). Consequently, NS has become an attractive candidate for use as an additive in food product development, potentially extending shelf life by inhibiting fungal growth (Ramadan, 2016). However, one of the challenges of using NS oil is its low antioxidant stability, particularly when exposed to oxygen and light (Wafa, and Betha, 2023). Therefore, strategies to improve the stability of antioxidants in NS oil are essential. A promising approach involves the preparation of NS oil emulsions (Husni, et al., 2019). Emulsions are stable dispersion systems composed of two immiscible phases: the aqueous phase and the oil phase, with one phase dispersed as small droplets within the continuous phase. The preparation of NS oil emulsions can enhance antioxidant stability by protecting the active compounds in NS oil from adverse interactions with other materials and the environment.

In the formulation of NS emulsions, Tween 80 is commonly used as a surfactant due to its favorable properties, including a low critical micelle concentration and a high hydrophilic-lipophilic balance value (Pratiwi, et al, 2024). Although Tween 80 has been shown to be effective in forming stable oil-in-water emulsions, there is limited research on its use specifically for stabilizing NS emulsions. Therefore, developing an optimal emulsion formulation with the appropriate concentration of NS oil and Tween 80 surfactant is critical for enhancing stability, bioavailability, and antioxidant efficacy. The formulation of NS oil emulsions with Tween 80 surfactant holds promising potential for the development of health and beauty products, combining antioxidant effectiveness with the ideal emulsion formulation. This study, therefore, aims to utilize Tween 80 as a surfactant to stabilize NS emulsions and develop a stable and effective emulsion system.

RESEARCH METHODS

1. Equipments and Materials

Equipments:

The equipment utilized in this study included a 50 mL measuring cup, dropper, 50 mL and 100 mL beakers, glass bottles, thermometer, stirring rod, spatula, 10 mL and 5 mL measuring flasks, pH meter, conical tubes, test tubes, test tube rack, analytical balance, magnetic stirrer, hotplate, Brookfield viscometer, homogenizer, oven, and centrifuge.

Materials:

The materials used in the study were *Nigella sativa* seeds, Tween 80, distilled water (aquadest), glycerin, propylene glycol, and Virgin Coconut Oil (VCO).

TABLE 1. Formulation of Emulsion NS

Materials	Function	Formula (%)		
		F1	F2	F3
Nigella sativa	Active ingredients	15	15	15
VCO	Oil phase	15	15	15
Glycerin	Cosurfactan	20	20	20
Propylene glycol	Cosurfactan	20	20	20
Tween 80	Surfactan	10	15	20
Aquadest	Water phase	Ad 100	Ad 100	Ad 100

2. Preparation of Nigella sativa Emulsion

The emulsion was prepared by mixing Tween 80 with distilled water using a magnetic stirrer set at 1000 rpm, followed by heating the mixture to 70°C and homogenizing it for 15 minutes. Concurrently, the oil phase, comprising *Nigella sativa* oil, Virgin Coconut Oil (VCO), glycerin, and propylene glycol, was heated and homogenized under identical conditions. The oil phase was then gradually added to the aqueous phase while stirring at

1000 rpm, followed by additional homogenization using a homogenizer to ensure consistency and stability.

3. Evaluation of Emulsion

Organoleptic Test

The emulsion was subjected to organoleptic evaluation by observing its physical appearance, color, and odor.

Homogeneity Test

Homogeneity was evaluated to ensure uniform distribution of components within the emulsion. A small sample of the emulsion was placed on a glass slide and examined under a microscope to detect the presence of particles or uneven dispersion. Achieving homogeneity is essential for maintaining emulsion stability and preventing phase separation.

pH Test

The pH value of the emulsion was determined using a pH meter, following the method described by Husni et al. (2019).

Viscosity Test

The viscosity of the emulsion was measured using a Brookfield Viscometer at 25°C with spindle number 4, following the method detailed by Husfianingsi, and Sinala (2023).

4. Data Analysis

The results of the physical property evaluations were analyzed descriptively to interpret the emulsion's performance characteristics.

RESULTS AND DISCUSSION

Organoleptic Test

The organoleptic evaluation of *Nigella sativa* (black cumin oil) emulsion preparations was conducted through visual assessments of color, form, and odor. This evaluation aimed to detect any physical changes in the emulsion after storage, as described by Husni et al. (2019). The results indicated that all three *Nigella sativa* emulsion formulations remained stable without any signs of phase separation. For Formula 1, the emulsion exhibited a milky white color, liquid form, and a characteristic Virgin Coconut Oil (VCO) odor. Formula 2 displayed a more yellow hue compared to Formula 1, retained its liquid form, and had a stronger VCO-like scent. Similarly, Formula 3 exhibited a yellowish-white color, liquid form, and a more intense VCO odor compared to Formula 2.

FIGURE 1. Organoleptic Test

TABLE 2. Result of Test

Formulation	Color	Form	Odor	Homogenity	Mean pH	Viscosity (cP)
F1	Milky white	Liquid	Characteristic VCO (+)	Homogeneous	5,85	104
F2	Yellowish white	Liquid	Characteristic VCO (++)	Homogeneous	5,65	180
F3	Yellowish white	Liquid	Characteristic VCO (+++)	Homogeneous	5,75	384

Homogenity Test

The homogeneity test confirmed that all three formulations met the required standards, with no visible solid particles detected. This result is in accordance with the findings of Husni et al. (2019), who emphasized the significance of proper mixing to achieve uniformity in emulsion preparations. Homogeneous emulsions are crucial for ensuring consistent dosing and therapeutic efficacy, as reported in previous studies.

pH Test

The pH test was conducted to evaluate the compatibility between the pH of the emulsion and the digestive tract, ensuring optimal absorption in the stomach. The digestive tract typically exhibits a pH range of 5–7. The pH value is a critical parameter influencing the efficacy and stability of the active substances within the emulsion. Changes in pH during storage may indicate chemical reactions or degradation of components, potentially impacting the desired therapeutic effects (Pratiwi, et al., 2023). A lower pH value in the emulsion can accelerate lipid oxidation, leading to reduced emulsion stability, whereas a higher pH can slow the rate of lipid oxidation. Additionally, the rate of lipid oxidation is influenced by storage temperature (Husni et al., 2019). The pH measurements of the *Nigella sativa* (black cumin oil) emulsion formulations are presented in Table 2. The results indicate minimal variation in pH values among the formulations. Formula 1 exhibited a pH of 5.85, Formula 2 had a pH of 5.65, and Formula 3 recorded a pH of 5.75. All three formulations fall within the typical pH range of the digestive tract (5–7), suggesting that the emulsions meet the required specifications for compatibility and stability.

Viscosity Test

The viscosity test was performed to evaluate the thickness of the emulsion preparations, as higher viscosity indicates a thicker formulation (Husni et al., 2019). The viscosity measurements, expressed in centipoise (cP), for *Nigella sativa* (black cumin oil) emulsions at two storage temperatures, including 25°C, are presented in Figure 2. Figure 2 demonstrates that the viscosity values for the F1, F2, and F3 emulsions were 104 cP, 180 cP, and 384 cP, respectively. Formula F1 exhibited the lowest viscosity, while F3 displayed the highest viscosity and stability, likely due to its higher Tween 80 concentration (20%) compared to F1 (10%) and F2 (15%). The increased viscosity in F3 may enhance its stability by reducing the risk of phase separation.Furthermore, a decline in viscosity during storage was observed, which could be attributed to temperature fluctuations. Higher temperatures reduce the viscosity of Tween 80 and other emulsifiers, affecting the overall stability of the emulsion. This highlights the importance of optimizing both surfactant concentration and storage conditions to maintain emulsion stability.

CONCLUSION

The best-performing *Nigella sativa* (black cumin oil) emulsion formula identified in this study was Formula 3 (F3), despite Formula 1 (F1) showing better results in the organoleptic evaluation. F1 contained 10% Tween 80, whereas F3 had a higher concentration of 20%. F3 demonstrated superior stability compared to F1 and F2 at a storage temperature of 25°C. Further physical stability testing revealed that F3 exhibited better viscosity stability than F1 and F2. These results suggest that the physical stability of the emulsion is influenced not only by the concentration of Tween 80 but also by the storage temperature. This finding underscores the critical role of both surfactant concentration and storage conditions in maintaining the stability and quality of the emulsion.

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