

Nanoemulsion of clove essential oil (*Syzygium aromaticum*) as a natural preservative for broiler chicken meat

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Abstract

This study aims to determine the nanoemulsion formula of clove essential oil (*Syzygium aromaticum*) and the effectiveness of nanoemulsion on the physical quality of chicken meat during storage. The nanoemulsion was formulated using a self-nanoemulsifying drug delivery system (SNEDDS). SNEDDS formulation and optimization were carried out using D-optimal mixture design. Characterization of SNEDDS was performed to determine particle size, polydispersity index (PDI), zeta potential, and antibacterial activity. Data from this study, including characteristics, morphology, antioxidant and antibacterial activities, were analyzed descriptively to describe the pattern and main properties of the nanoemulsion. The formulation of clove essential oil (*Syzygium aromaticum*) nanoemulsion with a composition of 2.5% clove essential oil, VCO 2.5%, Tween 80 surfactant 63.36%, and PEG 400 co-surfactant 16.66% had particle size characteristics of 12.77 nm and PDI 0.372. Antibacterial tests against *Salmonella sp*, *Escherichia coli*, and *Staphylococcus aureus* showed moderate antibacterial activity. Antioxidant activity tests showed that clove essential oil nanoemulsion had an IC₅₀ value of 2.884 µg/ml. The application of clove essential oil nanoemulsion at a concentration of 25,000 ppm on broiler chicken meat effectively suppressed bacterial growth (TPC), maintained the physical quality of the meat, and improved the sensory quality in terms of color, aroma, and acceptability during storage.

Introduction

Broiler chicken meat, which contains water, protein, fat, vitamins, and minerals, is highly susceptible to spoilage. Meat spoilage can be caused by bacteria, mold, and yeast, as well as by chemical reactions, particularly fat and protein oxidation. This spoilage not only reduces sensory quality (such as color, flavor, and aroma) but can also lead to decreased nutritional value and even pose health risks if contaminated with pathogenic microbes (Yu et al., 2021).

The challenge of maintaining the quality of broiler chicken meat makes preservation a crucial aspect for maintaining product quality, safety, and shelf life. Various preservation methods, both traditional and modern, have been developed to extend shelf life without compromising sensory quality or nutritional value. Currently, the use of synthetic preservatives in meat is still common. The addition of these preservatives aims to slow the rate of meat spoilage. However, most synthetic preservatives raise concerns because they are considered unsafe for health.

One form of plant extract that has significant potential for meat preservation is essential oils. Essential oils are the result of the extraction of volatile fractions formed from the secondary metabolism of aromatic plants (Santana de Oliveira et al., 2023). Essential oils are known to possess potent bioactive compounds and are considered safe for use, making them ideal candidates as natural preservatives in food products, including broiler chicken meat.

One essential oil with potential as a meat preservative is clove essential oil. Clove essential oil (*Syzygium aromaticum*) is known to possess bioactive compounds, making it widely used in the cosmetics, food, and pharmaceutical industries due to its bioactivity. Clove essential oil has been tested against

pathogenic microbes and demonstrated antibacterial properties (Haro-González et al., 2021). Based on research conducted by Tahir et al. (2020), the antibacterial properties of clove essential oil are derived from the eugenol content in cloves.

The use of clove essential oil as a natural preservative in broiler meat has significant potential to extend the product's shelf life. However, its effectiveness is often hampered by its hydrophobic nature, which limits its distribution and interaction with meat, making its application less efficient. One approach to address these limitations is the application of nanotechnology, particularly in the form of nanoemulsions.

The use of nanotechnology in the meat industry not only plays a role in controlling microbial contamination but also makes a significant contribution to maintaining the sensory quality and chemical stability of products during storage. This innovation promises a more effective and sustainable solution in the development of natural preservation systems based on bioactive compounds. This is the basis for research on the use of nanotechnology, namely clove essential oil (*Syzygium aromaticum*) nanoemulsions as a natural preservative for broiler meat during storage.

This study aims to determine the formula for clove essential oil (*Syzygium aromaticum*) nanoemulsions and determine their effectiveness as a natural preservative on broiler meat quality during storage. This research provides information regarding the use of natural clove essential oil (*Syzygium aromaticum*) in maintaining broiler meat quality during storage. Furthermore, this research also contributes to scientific knowledge regarding the application of natural ingredients such as clove essential oil formulated in nanoemulsion form as an alternative natural preservative in the food industry

Materials and Methods

Nanoemulsion preparation

The preparation of the nanoemulsion began with the development of a self-nanoemulsifying drug delivery system (SNEDDS). Virgin coconut oil (VCO) was used as the oil phase, Tween 80 as the surfactant, and polyethylene glycol 400 (PEG 400) as the co-surfactant. Initially, Tween 80 and PEG 400 were mixed at various concentration ratios, after which VCO was gradually added to the surfactant-co-surfactant mixture until a SNEDDS was formed. Homogenization was carried out using a magnetic stirrer at moderate speed, followed by sonication using an ultrasonicator for 45 min. The resulting SNEDDS formulations were subsequently diluted 50-fold with distilled water as an initial step in formulation screening. Clarity evaluation (turbidity test) was performed as a preliminary assessment of formulation success. Solution clarity was determined based on transmittance values measured at a wavelength of 650 nm using distilled water as a blank (Ujilestari et al., 2018).

Optimization of Clove Essential Oil Nanoemulsion Formulation

The SNEDDS formulations obtained from the screening process were used to define the upper and lower limits for formulation optimization using a D-optimal mixture design. Optimization was conducted by establishing 16 formulation combinations based on variations in the composition of three main components: the oil phase (a mixture of clove essential oil and VCO), the surfactant (Tween 80), and the co-surfactant (PEG 400) (Baskara, 2020). The mixtures were homogenized using a magnetic stirrer at moderate speed, followed by sonication with an ultrasonicator for 45 min. Samples were prepared by mixing 0.1 mL of each formulation with 5 mL of distilled water and homogenized using a stirrer for 30 min. The percentage of transmittance was then measured using a UV spectrophotometer at a wavelength of 650 nm.

In this study, the relationship between three independent variables and one dependent variable was analyzed to optimize the formulation. The independent variables consisted of X_1 (oil phase, a mixture of clove essential oil and VCO), X_2 (surfactant, Tween 80), and X_3 (co-surfactant, PEG 400). The dependent variable was Y , defined as percent transmittance (%T), which served as the primary parameter for evaluating formulation clarity. The formulation data were analyzed using a D-optimal mixture design in Design-Expert software version 7.1.5 to assess the influence of each variable on the response and to determine the optimal formulation.

Nanoemulsion Characterization

Particle size, polydispersity index (PDI), and zeta potential (ζ) were measured using a particle size analyzer (PSA) (Microtrac MRB, USA) (Farshi et al., 2019). SNEDDS samples were diluted with sterile water at a ratio of 1:3, transferred into a cuvette, and analyzed using the PSA.

Morphology analysis of nanoemulsion

Morphology was analyzed using Transmission Electron Microscopy (TEM). The sample was dripped on a chopper grid and then carbon coated with an auto carbon coated tool for 5 seconds (JEM-JEOL, 1400, Shimadzu, Japan). The results obtained were then dried at room temperature, then inserted into the holder and analyzed with a voltage of 120 kV with a magnification of 10,000 to 80,000. Morphological testing was carried out by taking pictures of each of the smallest to largest magnifications.

Antioxidant activity

The test method used was the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Measurement of antioxidant activity of clove essential oil nanoemulsion was made in concentration variations of 100, 50, 25, 12.5, and 6.25 ppm then quercetin solution was made in concentration variations of 10, 8, 6, 4, and 2 ppm. Each concentration was pipetted as much as 1 mL put in a test tube, added 3 mL of DPPH mixture solution (50 ppm) in methanol. Homogenized, then incubated at 37°C for 30 minutes, measured the absorbance at a wavelength of 517 nm. The absorbance value obtained was calculated as the percent inhibition and then the equation was obtained to determine the IC_{50} value.

Antibacterial activity

The test method used was Kirby Bauer's pitting diffusion method. The test was carried out by making bacterial suspensions by planting *Salmonella* and *Escherichia coli* bacteria on Nutrient Broth (NB) media. Preparation of Nutrient Agar (NA) media, then poured into a petri dish until it is mushrooming, then made wells according to the number of samples to be tested. Samples were inserted into the wells that had been made, then incubated at 37°C for 24 hours. Observations were made after incubation was complete by measuring the clear zone that showed bacterial inhibition.

Application of clove essential oil nanoemulsion

SNEEDS clove essential oil was diluted with distilled water at a ratio of 1:4 to obtain a concentration of 25,000 ppm (P1), then diluted again to several concentrations, namely 12,500 ppm (P2), 6,250 ppm (P3), and 3,125 ppm (P4) to form a nanoemulsion. Broiler chicken meat was cut into uniform pieces. Each piece of meat was soaked in the nanoemulsion solution for 1 minute, until the entire surface of the meat was evenly coated with the nanoemulsion. The control sample (P0) was not treated. After soaking, all samples were placed in a closed container and stored at 4°C. During storage, observations were made on days 0, 4, 8, and 12 on the physical, chemical, microbiological characteristics, and sensory evaluation of the broiler chicken meat.

Microbiological testing of meat

Microbiological testing was conducted on each treatment of broiler chicken meat on days 0, 4, 8, and 12 of storage. The parameters analyzed included Total Plate Count (TPC).

Analysis of the physical quality of broiler chicken meat

pH measurement. The pH of broiler chicken meat was measured using a Benchtop pH meter (Mettler-Toledo, SevenCompact™ S220 Basic, UK). pH measurements were taken for each treatment group on days 0, 4, 8, and 12 of storage (Feng et al., 2020).

Color parameters. Color measurements were performed using a Minolta® CR-400 Chromameter (Konica Minolta Sensing Inc., Japan). The color system used refers to the CIE (Commission Internationale de l'Éclairage) scheme with three main parameters: L* (lightness), a* (redness-greenness), and b* (yellowness-blueness) (Chouaibi et al., 2019).

Cooking shrinkage test. The cooking shrinkage test was performed on each treatment group on days 0, 4, 8, and 12 of storage. The chicken meat was weighed, placed in plastic bags, vacuum-sealed, and then placed in a water bath at 80°C for one hour. The meat samples were removed and cooled, then weighed after heating (Soeparno, 2015).

Meat tenderness. Chicken meat tenderness was measured using a Texture Profile Analyzer (TPA) and the results were expressed in Newtons. Chicken meat samples measuring 3x3x3 cm³ were pressed twice with a probe (6 mm diameter). The probe speed was set at 5 mm/second and the samples were pressed to 30% of their original height. The parameter observed was hardness.

Chemical content analysis of broiler chicken meat

Chemical analysis of broiler chicken meat includes determining the water, protein, and fat content using the Near Infrared Spectroscopy (NIRS) method. A 25-gram sample of meat was minced using a chopper, then placed in a sample dish and inserted into the FoodScan device. Measurements were taken using infrared light in the wavelength range of 780–2500 nm. The analysis data displayed by the instrument was then recorded for further evaluation.

Sensory evaluation

Sensory evaluation was conducted through hedonic testing in 4 sessions according to the storage duration treatments, namely 0, 4, 8, and 12 days, which aimed to assess the effect of the treatment on the samples based on consumer preferences compared to the control. Several attributes were assessed, including color, aroma, texture, and acceptability, using a 9-point hedonic scale, where 9 = very much liked and 1 = very much disliked, by a group of 30 untrained panelists on nanoencapsulated broiler chicken meat samples using clove essential oil.

Data Analysis

The experimental design used a completely randomized design with a one-way pattern. Data were analyzed using Statistical Program for Social Science or SPSS 29.0. If there were differences between the mean values for each treatment, a further test was conducted using Tukey HSD.

Results

Nanoemulsion characteristics

The preparation stage is the stage of determining the initial ratio between oil, surfactant, and co-surfactant, which is evaluated based on the transmittance percentage value. A high transmittance percentage value will be selected as the basis for the nanoemulsion formulation. The effect of changes in surfactant proportion on the transmittance value can be seen in Table 1.

The SNEDDS formulation has an oil phase composition consisting of a combination of two oils (50% clove essential oil and 50% VCO) (v/v) used as the basis for characterizing the optimal formulation. The upper and lower limits of the independent variable were determined from the transmittance results that had a value of more than 90% and lower transmittance results in the initial SNEDDS formulation, as well as the dependent variable objective used in the D-Optimal mixture design. The composition of ingredients in the SNEDDS formulation was determined using D-Optimal mixture design. The oil

component, consisting of 50% clove essential oil and 50% VCO had lower and upper limits of 16.67% and 20.00%. Tween 80 as a surfactant has lower and upper limits of 60.00% and 66.67%, and PEG 400 as a cosurfactant has lower and upper limits of 16.67% and 20.00%. The determination of these upper and lower limits aims to obtain the optimal composition combination in producing the highest transmittance value, as an indicator of nanoemulsion clarity.

The final formula for clove essential oil nanoemulsion (*Syzygium aromaticum*) is 2.5% clove essential oil, 2.5% VCO, 15.84% Tween 80, 4.15% PEG 400 as co-surfactant, and 75% distilled water.

Antibacterial test results

Antibacterial activity testing in Table 3 shows that clove essential oil has very strong inhibitory activity against the three types of test bacteria, namely *Salmonella sp* (31.6 ± 3.8 mm), *E. coli* (30.3 ± 3.1 mm), and *S. aureus* (29.15 ± 5.0 mm). These values are close to the antibacterial activity of the control (antibiotic), which had inhibition zones of 39.1 ± 1.5 mm for *Salmonella sp*, 31.65 ± 0.3 mm for *E. coli*, and 29.85 ± 1.6 mm for *S. aureus*. Tween 80 and PEG 400 did not show any inhibition zones against all test bacteria. VCO only showed an inhibition zone against *E. coli* of 8.15 mm, but not against *Salmonella sp* and *S. aureus*.

The SNEDDS formulation of clove essential oil showed lower antibacterial activity than pure clove essential oil, namely 17.7 ± 0.7 mm against *Salmonella sp*, 10.55 ± 0.6 mm against *E. coli*, and 13.2 ± 1.6 mm against *S. aureus*. The results of antibacterial activity testing showed that the 25,000 ppm nanoemulsion was able to form an inhibition zone against the three test bacteria, namely *Salmonella sp.*, *Escherichia coli*, and *Staphylococcus aureus*, with diameters of 8.5 ± 0.2 mm, 9.9 ± 0.3 mm, and 8.5 ± 0.3 mm, respectively. while the 12,500 ppm nanoemulsion showed antibacterial activity against *Salmonella sp.* and *Escherichia coli* with inhibition zone diameters of 8.3 ± 0.3 mm and 9.0 ± 0.3 mm, respectively, but did not show inhibitory activity against *Staphylococcus aureus*. In the 6250 ppm nanoemulsion, antibacterial activity was only detected against *Escherichia coli* with an inhibition zone diameter of 8.9 ± 0.3 mm, while the nanoemulsion with the lowest essential oil concentration, 3125 ppm, did not show any inhibition zone formation against all test bacteria.

The active compounds in cloves can interact directly with bacteria and inhibit bacterial growth. According to Morales et al. (2003), antibacterial inhibition zone activity is classified into four categories, namely: weak activity (< 5 mm), moderate (5–10 mm), strong (10–20 mm), and very strong (> 20–30 mm). The results of the antibacterial activity zone inhibition test against *Salmonella sp* bacteria can be seen in Fig. 2, *E. coli* in Fig. 3, and *S. aureus* in Fig. 4.

Antioxidant activity testing

The results of antioxidant activity testing on clove essential oil nanoemulsions using the DPPH method are presented in Table 4. Based on the inhibition percentage values obtained, clove essential oil nanoemulsions produced an IC₅₀ value of 2.884 µg/ml.

Microbiological Testing of Broiler Chicken Meat

Microbiological quality testing of broiler chicken meat using the Total Plate Count (TPC) method was conducted on days 0, 4, 8, and 12 with various treatments. The test results are shown in Table 9.

The results of TPC testing on broiler chicken meat that was given additional clove essential oil nanoemulsion during storage showed changes in the number of microbes. In general, TPC values in all treatments tended to increase with longer storage times. On day 0, treatments P1 and P3 were not significantly different from the control/P0, while treatments P2 and P4 were different from the control. On day 4, treatments P1 and P2 had significantly different bacterial counts compared to the control, while on day 8 only P1 was significantly different from the control. On day 8, samples supplemented with nanoemulsion at an essential oil concentration of 25,000 ppm (P1) had the lowest bacterial count compared to other samples. On day 12, all treatments were not significantly different from the control except for treatment P1, which was found to have a lower TPC value than the other treatments.

Analysis of the Physical Quality of Broiler Chicken Meat

pH Measurement. The pH value is one indicator used to determine meat quality during storage. Based on Table 6, the pH values of broiler chicken meat in all treatments showed a downward trend during 12 days of storage at 4°C. On day 0, there were no significant differences in pH values between treatments (P0–P4) ($p > 0.05$). On day 4, the pH in treatment P1 did not differ significantly from the control, while the other treatments showed slightly lower pH values. On day 8, treatments P1 and P2 showed pH values that were not significantly different from the control, while P3 and P4 had lower pH values. On day 12, almost all treatments were not significantly different from the control, except for P1, which had a lower pH value. The results of the pH testing of broiler chicken meat during storage can be seen in Table 10.

Color. Meat color is determined by the parameters L^* (brightness), a^* (redness), and b^* (yellowness). The results of the L^* parameter testing can be seen in Table 5. On day 0 and day 4, the L^* values between treatments did not show significant differences ($p > 0.05$), indicating that the application of nanoemulsion treatment did not have a significant effect on the brightness level of the meat at the initial stage of storage. During storage, on day 8, there was a significant difference in L^* values in treatment P3, while on day 12, there was a significant difference in P1 compared to the control.

The a^* parameter represents the red-green color dimension, where positive values indicate a more reddish color and negative values indicate a more greenish color. The results of the a^* color test with various treatments and storage periods are presented in Table 6. The test results show that on day 0 and day 8, there was no difference in a^* values between samples treated with clove essential oil nanoemulsion and the control. On day 4, only sample P5 differed from the control, while on day 12, the a^* value in treatment P1 differed from the control, indicating a difference in the intensity of redness in the sample.

The b^* parameter represents the yellow-blue color dimension, where positive values indicate a more yellow color and negative values indicate a more blue color. The color test data (b^*) with various treatments and storage periods are presented in Table 13. The test results show that on day 0 there was

no significant difference in b^* values between samples treated with clove essential oil nanoemulsion and the control, while testing on day 4 showed that all treatments were significantly different from the control. On days 8 to 12, the b^* values in the control samples were not significantly different from those in the samples given additional nanoemulsion. Overall, the b^* values in the samples increased compared to day 0, although there were no significant differences between treatments.

Cooking shrinkage. Cooking shrinkage testing on samples given clove essential oil nanoemulsion showed no significant difference from the control. The cooking shrinkage values of broiler chicken meat from day 0 to day 12 during storage ranged from $31.76 \pm 1.61\%$ to $37.30 \pm 4.48\%$ and showed no significant differences between the clove essential oil nanoemulsion treatment and the control. This indicates that the addition of nanoemulsion did not have a negative effect on the cooking shrinkage of broiler chicken meat during storage for up to 12 days. Cooking shrinkage test data are presented in Table 14.

Water-holding capacity. Water-holding capacity (WHC) describes the ability of meat to retain water during storage. The results showed that the DIA of broiler chicken meat supplemented with clove essential oil nanoemulsion was not significantly different from the control on days 0, 4, and 12, while on day 8, P3 and P4 were significantly different from the control. The DIA of broiler chickens obtained from the research results ranged from $42.49 \pm 4.67\%$ to $58.60 \pm 5.00\%$ during 12 days of storage. The WHC test results can be seen in Table 15.

Tenderness. The results showed that meat tenderness during storage from day 0 to day 12 fluctuated. On day 0, all treatments were not significantly different from the control, while on day 4 only treatment P2 was significantly different from the control. On days 8 and 12, the results showed that P1 and P2 were significantly different from the control, with higher hardness values than the control. Higher hardness values indicate that the meat texture is harder, as more force is required to compress it. There is a negative relationship between water content and hardness, i.e., when meat muscles lose water content, the hardness level increases. The results of tenderness testing of broiler chicken meat with added broiler chicken essential oil nanoemulsion are presented in Table 16.

Chemical Quality Analysis of Broiler Chicken Meat

The chemical tests conducted in this study included tests for moisture, fat, and protein content. The results of testing the moisture content of chicken meat during storage showed changes in moisture content, but the moisture content was still within the normal range of 72–75%. On day 0, there was a significant difference ($P < 0.05$) between treatment P4 and the control in terms of moisture content. On day 4, treatment P3 was significantly different from the control, while on day 8, all treatments were different from the control. Testing on day 12 showed that the moisture content of treatment P3 was significantly different from the control.

Protein plays an important role in the formation of muscle tissue structure and affects meat tenderness. The results of testing the protein content of broiler chicken meat showed that the protein content of

broiler chicken meat ranged from $20.50 \pm 0.35\%$ to $22.66 \pm 0.41\%$. On day 0, treatment P2 was significantly different from the control. From day 4 to day 8, the results of the broiler chicken meat protein analysis were not significantly different, while on day 12, treatment P3 was not significantly different from the control. The results of testing the fat content of broiler chicken meat during storage showed that on day 0, treatments P2 and P3 were significantly different from the control. On day 4, treatments P2 and P3 also showed significant differences from the control. Furthermore, on day 8, treatment P1 was significantly different from the control, while on day 12, treatments P1, P2, and P4 showed significant differences compared to the control.

Sensory Evaluation of Broiler Chicken Meat

The sensory evaluation assessed included appearance, aroma, texture, and acceptability on a hedonic scale of 1 to 9 points by a group of 30 untrained panelists on the samples. Sensory test results for broiler chicken meat color parameters during 12 days of storage ranged from 6.33 to 3.73. From day 0 to day 8 of storage, there were no significant differences between all treatments and the control. However, on day 12 of storage, treatments P1 and P3 showed a significant difference compared to the control, with higher liking scores. Sensory test results for broiler chicken meat aroma parameters during 12 days of storage showed scores ranging from 6.23 to 3.53. The highest value indicated a relatively fresh aroma, while the lowest value indicated a decline in aroma quality over the course of storage. Based on the observations, on days 0, 4, and 12, the control samples consistently had the lowest sensory aroma scores compared to the samples treated with nanoemulsion. The results of sensory tests on the color and texture parameters of broiler chicken meat during 12 days of storage showed a score range of 6.33 to 3.73. Sensory tests on the texture parameters of broiler chicken meat showed that there was no significant difference between the control samples and the treated samples during storage. On day 0, the sensory score ranged from 5.8 to 6.6, then on day 4 it was 5.47 to 6.07, and on day 8 it ranged from 5.23 to 6.0. During the period from day 0 to day 8, statistical test results showed no significant differences between treatments. However, on the 12th day of storage, the sensory score decreased further to a range of 4.13–5.9 and showed a significant difference between treatments compared to the control. The results of the sensory evaluation of broiler chicken meat can be seen in Table 20.

Discussion

Nanoemulsion characteristics

The transmittance values in the preparation of SNEDDS, which are independent and dependent variables, as well as the responses from 16 runs of the formulation mixture recommended by the D-Optimal mixture design, can be seen in Table 2. Based on the test results from 16 runs of the formulation mixture, mathematical equations were obtained from the analysis of the response of independent variables to dependent variables. The transmittance percentage is one of the parameters used in formulation optimization. A transmittance value close to 100% indicates that the entire optimized formulation is

clear and transparent (Kotta et al., 2015). A transmittance value above 90% indicates that the self-nanoemulsification process in the SNEDDS system is proceeding efficiently (Gupta et al., 2011).

The final formula for clove essential oil nanoemulsion (*Syzygium aromaticum*) is 2.5% clove essential oil, 2.5% VCO, 15.84% Tween 80, 4.15% PEG 400 as co-surfactant, and 75% distilled water.

According to Khan et al. (2012), a formulation that appears cloudy will scatter and refract light more, resulting in a lower transmittance value. Thus, the visual clarity of a formulation is directly proportional to its transmittance value. Nanoemulsions with transmittance close to 100% indicate that the particle size is in the nanometer range (Yadav et al., 2014).

The particle size of the formulation was found to be 12.77 nm with a particle distribution expressed in polydispersity index (PDI) of 0.372 and a zeta potential of 29.6 mV. These results indicate that the formulation is nano-sized and has a uniform particle distribution. According to Solans et al. (2005) SNEDDS can produce nanoemulsions with particle sizes less than 200 nm after dilution. Particle size is an important factor that determines the dispersion rate of compounds with low water solubility (Wang et al., 2009) and determines the rate and release and absorption of bioactive compounds (Parmar et al., 2011). A polydispersity index value of less than 0.5 indicates a uniform particle size distribution (Kassem et al., 2016).

The zeta potential value describes the amount of electrical charge on the surface of particles that plays an important role in the stability of nanoemulsion dispersions. According to Yang and Benita (2000), a nanoemulsion system is categorized as stable if it has a zeta potential value of less than – 30 mV or greater than + 30 mV, because the electrostatic repulsive force between particles is strong enough to prevent aggregation. In this study, the zeta potential value of 29.6 mV is close to the threshold of electrostatic stability. This positive zeta potential value indicates that the nanoemulsion droplets are positively charged, which is likely due to the adsorption of H⁺ ions on the oil–water interface

The morphology of oil particles in SNEDDS formulations was observed using Transmission Electron Microscopy (TEM). TEM is a widely used characterization technique for analyzing very small materials and has a resolution capability of up to 0.2 nm (Silva et al., 2012). The process of SNEDDS formation and deposition during the addition of the aqueous phase can be clearly visualized through TEM analysis (Kotta et al., 2015).

Antibacterial test

The active compounds in cloves can interact directly with bacteria and inhibit bacterial growth. According to Morales et al. (2003), antibacterial inhibition zone activity is classified into four categories, namely: weak activity (< 5 mm), moderate (5–10 mm), strong (10–20 mm), and very strong (> 20–30 mm). The results of the antibacterial activity zone inhibition test against *Salmonella sp* bacteria can be seen in Fig. 2, *E. coli* in Fig. 3, and *S. aureus* in Fig. 4.

Antioxidant activity testing

The results of antioxidant activity testing on clove essential oil nanoemulsions using the DPPH method are presented in Table 4. Based on the inhibition percentage values obtained, clove essential oil nanoemulsions produced an IC₅₀ value of 2.884 µg/ml. An increase in the concentration of clove essential oil nanoemulsion also increased the percentage of inhibition, which is in line with Damanis et al. (2020), who reported that the level of inhibition tended to increase with the increasing concentration of antioxidant compounds contained in the sample.

The IC₅₀ value in clove essential oil nanoemulsion indicates strong antioxidant activity. Antioxidant activity testing was also conducted on quercetin as a comparison, which produced an IC₅₀ value of 1.148 µg/ml. This is confirmed by Souhoka et al. (2019), who stated that the lower the IC₅₀ value produced, the higher the antioxidant activity of a substance, with an IC₅₀ value below 50 ppm categorized as strong antioxidant activity. The results of testing the antioxidant activity of clove essential oil nanoemulsion are also in line with research conducted by Haro-González et al. (2023), who reported that the antioxidant activity of pure clove essential oil, as indicated by an IC₅₀ value of 0.78 µg/mL, decreased after being formulated into a nanoemulsion, with the IC₅₀ value increasing to 2.43 µg/mL. This increase in the IC₅₀ value indicates that the antioxidant capacity of nanoemulsions is lower than that of pure essential oils, which is thought to be related to changes in the interaction and distribution of active compounds during the nanoemulsion formulation process. However, other studies have shown that clove essential oil nanoemulsions still have high antioxidant activity. Taher et al. (2024) reported that clove essential oil nanoemulsion was able to produce a maximum inhibition percentage of 65% at a concentration of 100 µg/mL, which is higher than the clove nano-gel sample, which only reached 59.2% at the same concentration, with a clove nanoemulsion IC₅₀ value of 3.25 µg/mL compared to 6.58 µg/mL for clove nano-gel.

Microbiological Testing of Broiler Chicken Meat

Microbiological quality testing of broiler chicken meat using the Total Plate Count (TPC) method was conducted on days 0, 4, 8, and 12 with various treatments. The test results are shown in Table 9. Test results from days 0 to 12 showed that the number of microbial contaminants exceeded the maximum limit set in the Indonesian National Standard (SNI), which is a maximum of 1×10^6 CFU/mL. Microbial counts exceeding this limit can occur due to contamination during transportation, marketing, and slaughtering processes (Edi and Rahmah, 2018). Research shows that the addition of clove essential oil nanoemulsion treatment can inhibit bacterial growth compared to the control. The treatment that showed its effectiveness in inhibiting bacterial growth in this study was treatment P1 because it used the highest concentration of clove essential oil, which indicates the strongest antibacterial ability. This is in line with the research conducted by Sun et al. (2020), which states that clove oil nanoemulsion is effective in inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus*. Furthermore, Anwer et al. (2016) explained that clove essential oil nanoemulsion can enhance the antibacterial activity of natural bioactive compounds, which is influenced by the very small size of the nanoparticles, the stability of the system, and the optimal composition of the surfactant, thus having the potential to be developed as a natural antibacterial agent.

Analysis of the Physical Quality of Broiler Chicken Meat

pH Measurement. The pH value is one indicator used to determine meat quality during storage. This pH reduction mechanism is related to the lipid oxidation process, in which fatty acids undergo degradation and produce weak acids that cause a decrease in pH (Yun et al., 2007). In addition, triglyceride hydrolysis in meat tissue can produce free fatty acids that increase hydrogen ion concentration. According to Kristott (2000), oxidative and hydrolytic damage to fat components in food materials can trigger lipid oxidation, which leads to a decrease in pH. Furthermore, microbial activity that produces acidic metabolites, autolytic enzymes that release amino acids from proteins, and protein denaturation due to prolonged storage can also contribute to a decrease in pH, as they increase the accumulation of acidic compounds during storage (Warenska et al., 2023).

Cooking shrinkage. Cooking shrinkage testing on samples given clove essential oil nanoemulsion showed no significant difference from the control. Soeparno (2015) states that cooking loss is closely related to meat DIA, and that meat cooking loss varies between 1.5–54.5% with a range of 15–40%. Based on this opinion, we can conclude that the broiler chicken meat in this study is still within normal limits. Cooking loss is caused by the denaturation of meat proteins during heat treatment, which reduces the binding capacity between water molecules and proteins (Jeong and Han, 2019).

Water-holding capacity. Water-holding capacity (WHC) describes the ability of meat to retain water during storage. Soeparno (2015) states that DIA is the ability of meat to bind added water when there is external force, and the normal range of DIA is between 20% and 60%. The DIA value in this study is still within the normal range of broiler chicken meat DIA. The DIA value tends to decrease with longer storage times. According to Nurjaya et al. (2023), differences in meat water-binding capacity are influenced by the protein and carbohydrate content of the meat; low meat protein content is followed by lower water-binding capacity. Furthermore, low water-binding capacity is influenced by nanoemulsions that dissolve water in the meat, indicating that the coating on the meat has been successfully applied.

Tenderness. Broiler chicken meat showed changes in tenderness as storage time increased. Soeparno (2015) stated that meat tenderness is influenced by the amount of connective tissue and water content in muscle fibers. The tenderness of broiler chicken meat was measured using TPA and observed in terms of hardness in Newtons (N). According to Indiarito et al. (2012), hardness is the maximum peak at the first pressure or at the first bite. Until the end of storage (day 12), all samples showed an overall decrease in tenderness compared to day 0. This finding is consistent with the results reported by Sujiwo et al. (2018), which stated that the shear force of chicken breast meat decreases with storage time. This is also in line with the opinion of Risnawati et al. (2025) that the texture of chicken meat, which includes hardness, elasticity, cohesiveness, tenderness, chewiness, and resistance, will decrease over time when stored at low temperatures (4°C) (Risnawati et al., 2025). The tenderness of meat is determined by the level of proteolysis of myofibrillar proteins, which serve to maintain the structural integrity of muscle fibers (Marcinkowska-Lesiak et al., 2016). Kruk et al. (2011) also reported that protein degradation in meat can be caused by bacterial or enzymatic processes. This is supported by Soeparno (2015), who

stated that the decrease in meat tenderness is the result of metabolism in meat, either naturally (autolysis) or due to energy performance in meat cells.

Chemical Quality Analysis of Broiler Chicken Meat

Moisture content is one of the important parameters in testing the chemical quality of meat. The moisture content of chicken meat during storage showed changes in moisture content, but the moisture content was still within the normal range of 72–75%. This is in accordance with the opinion of Li et al. (2025) that fresh chicken breast meat contains about 70–75% water. According to Wala et al. (2016), the longer meat is stored, the higher its moisture content, which can affect its quality and shelf life. Higher moisture content can contribute to a softer texture, but it can also increase the risk of reduced shelf life if not handled properly, making it prone to spoilage.

Protein plays an important role in the formation of muscle tissue structure and affects meat tenderness. The results of testing the protein content of broiler chicken meat showed that the protein content of broiler chicken meat ranged from $20.50 \pm 0.35\%$ to $22.66 \pm 0.41\%$. On day 0, treatment P2 was significantly different from the control. From day 4 to day 8, the results of the broiler chicken meat protein analysis were not significantly different, while on day 12, treatment P3 was not significantly different from the control. According to Soeparno (2015), the protein content of meat ranges from 19% to 22%. The protein content in this study shows values that are still within that range.

The research results show that the fat content of broiler chicken meat decreased from day 0 to day 12 of storage. The length of storage can affect the fat content of meat. This statement is also supported by Nadia et al. (2023), who state that the lower the fat content in food products, such as broiler chicken meat, the better and more beneficial it is, and it does not cause adverse effects on health.

Sensory Evaluation of Broiler Chicken Meat

Sensory qualities such as appearance, color, taste, and texture determine consumer assessment of a product (Barboza and Kazal, 2018). Sensory quality is one of the important assessments that can be used to predict consumer acceptance of a product (Alcantra and Freitas Sa, 2018), especially food products. The result indicates that the treatments did not have a significant effect on meat color perception at the beginning to midway through the storage period. However, on day 12 of storage, treatments P1 and P3 showed a significant difference compared to the control, with higher liking scores. This increase indicates that the meat color in treatments P1 and P3 was increasingly preferred by the panelists.

The color of processed meat products plays an important role in consumer acceptance. This is in line with Sujiwo et al. (2018), who stated that sensory evaluation of chicken breast meat stored at low temperatures showed a decrease in sensory quality as storage time increased. The meat color score was reported to have decreased significantly during storage, reflecting visual changes in the meat. These results are in line with the study by Vaithiyanathan et al. (2011), which reported that the sensory color score of chicken breast meat decreased significantly during storage at 4°C.

Based on the observations, on days 0, 4, and 12, the control samples consistently had the lowest sensory aroma scores compared to the samples treated with nanoemulsion. This shows that the addition of nanoemulsion plays a role in maintaining the aroma quality of the meat during storage. The ability of nanoemulsion to inhibit microbial activity and oxidation processes is thought to be the main factor contributing to aroma stability, so that the treated samples were preferred by the panelists over the control, especially during longer storage. When chicken meat is stored at cold temperatures, in addition to color changes, there is also an increase in unpleasant odor scores, which are closely related to lipid oxidation and protein degradation. Lipid oxidation produces volatile compounds that contribute to the emergence of off-odors and flavors, while protein degradation due to microbial activity also accelerates the formation of off-odors in meat (Duan et al., 2017). This is in line with the statement by Ghasemi et al. (2022) that the addition of clove oil to meat products can reduce lipid oxidation.

Sensory tests on the texture parameters of broiler chicken meat showed that there was no significant difference between the control samples and the treated samples during storage. This indicates that the treatment, including the application of nanoemulsion, did not have a significant effect on the panelists' perception of meat texture. In general, sensory texture attributes are influenced by factors such as muscle tissue structure, water content, protein and fat composition, as well as storage conditions and treatments applied. Other factors that influence sensory texture assessment are handling and storage methods, which can trigger physical and chemical changes in meat.

During the period from day 0 to day 8, statistical test results showed no significant differences between treatments. The sensory evaluation results showed that the acceptability scores of broiler chicken meat during storage decreased as the storage time increased. The control sample consistently had the lowest sensory scores compared to the other treatments. Although for most of the storage period the differences between treatments were not statistically significant, the trend values showed that the control treatment always had a lower level of acceptability. Various studies have reported that the overall acceptability score of chicken breast meat decreases during cold storage, reaching a level that is not sensorially acceptable after a certain storage period. This decrease in acceptability reflects the accumulation of physical, chemical, and microbiological changes that occur during storage, making the meat unfit for consumption after too long a storage period (Sujiwo et al., 2018).

Declarations

Conflict of Interest

The authors declare that there is no conflict of interest.

Institutional Review Board

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Author Contribution

DAD carried out the experiments with support from NR, APK, and NIK. DAD wrote the manuscript with support from PIS, ET, Z, UAK, and SZK. ET and Z supervised the project. ET conceived

References

1. Anwer, M. K., S. Jamil, E. O. Ibnouf, dan F. Shakeel. 2014. Enhanced antibacterial effects of clove essential oil by nanoemulsion. *J. Oleo Sci.* 63(4):347–354.
2. Baskara, A. P. 2020. Efek Antimikrobia dan Imunomodulator Nano-Partikel Minyak Atsiri Minyak Atsiri Kayu Manis (*Cinnamomum burmannii*) yang Diberikan pada Ayam Broiler Melalui Air Minum. Disertasi Fakultas Peternakan Universitas Gadjah Mada.
3. Beya, M.M., M.E. Netzel, Y. Sultanbawa, H. Smyth, dan L.C. Hoffman. 2021. Plant-based phenolic molecules as natural preservatives in comminuted meats: A review. *Antioxidants*. 10(2):263.
4. Chouaibi, M., L. Rezig, A. Boussaid, dan S. Hamdi. 2019. Insoluble Tomato-Fiber Effect on Wheat Dough Rheology and Cookies' Quality. *Journal Food Science*. 31:1–18.
5. Damanis, F. V. M., D. S. Wewengkang, dan I. Antasionasti. 2020. Uji aktivitas antioksidan ekstrak etanol Ascidian *Herdmania monus* dengan metode DPPH. *Pharmacon* 9(3):464–469.
6. Edi, S., dan R. S. N. Rahmah. 2018. Pengaruh lama penyimpanan daging ayam pada suhu ruang dan refrigerator terhadap angka lempeng total bakteri dan keberadaan *Salmonella* sp. *J. Biosci.* 4(1):23–31.
7. Falleh, H., M.B. Jemaa, M.A. Neves, H. Isoda, M. Nakajima, dan R. Ksouri. 2021. Peppermint and Myrtle Nanoemulsions: Formulations, Stability, and Antimicrobial Activity. *Food Sci. Technol.* 152(112377):1–7.
8. Farshi, P., M. Tabibiazar, M. Ghorbani, M. Mohammadifar, M.B. Amirkhiz, dan H. Hamishehkar. 2019. Whey protein isolate-guar gum stabilized cumin seed oil nanoemulsion. *Food Biosci.* 28:49–56. doi:10.1016/j.fbio.2019.01.011.
9. Feng, X., J. Y. Y. Tjia, Y. Zhou, Q. Liu, C. Fu, dan H. Yang. 2020. Effect of Tocopherol Nanoemulsion Addition on Fish Sausage Properties and Fatty Acid Oxidation. *LWT - Food Science and Technology* 118(108737):1–8
10. Ghasemi, B., M. J. Varidi, M. Varidi, Z. Kazemi-Taskooh, dan S. A. Emami. 2022. The effect of plant essential oils on physicochemical properties of chicken nuggets. *J. Food Meas. Charact.* 16(1):772–783.

11. Gupta, S., S. Chavhana, dan K.K. Sawant. 2011. Self-nanoemulsifying drug delivery system for adefovir dipivoxil: design, characterization, in vitro and ex vivo evaluation. *Colloids Surf. A: Physicochem. Eng. Aspects*. 392:145–155.
12. Haro-González, J.N., G.A. Castillo-Herrera, M. Martínez-Velázquez, dan H. Espinosa-Andrews. 2021. Clove essential oil (*Syzygium aromaticum* L. Myrtaceae): Extraction, chemical composition, food applications, and essential bioactivity for human health. *Molecules*. 26(21):6387.
13. Indiarto, R., B. Nurhadi, dan E. Subroto. 2012. Kajian karakteristik tekstur dan organoleptik daging ayam asap berbasis asap cair tempurung kelapa. *Jurnal Teknologi Hasil Pertanian*. 5(2):106–116.
14. Jabbar, M., I. Baboo, H. Majeed, Z. Farooq, V. Palangi, dan M. Lackner. 2024. Preparation and characterization of cumin essential oil nanoemulsion (CEONE) as an antibacterial agent and growth promoter in broilers: A study on efficacy, safety, and health impact. *Animals*. 14(19):2860.
15. Kalembe, D.A.A.K. dan A. Kunicka. 2003. Antibacterial and antifungal properties of essential oils. *Curr. Med. Chem*. 10(10):813–829.
16. Kassem, A. A., A. M. Mohsen, R. S. Ahmed, dan T. M. Essam. 2016. Self-nanoemulsifying drug delivery system with enhanced solubilization of nystatin. *J. Mol. Liq*. 218:219–232.
17. Khan, F., M.S. Islam, M.A. Roni, dan R. Jalil. 2012. Systematic development of self-emulsifying drug delivery systems of atorvastatin with improved bioavailability potential. *Scientia Pharm*. 80:1027–1043.
18. Kotta, S., A.W. Khan, S.H. Ansari, R.K. Sharma, dan J. Ali. 2015. Formulation of nanoemulsion: a comparison between phase inversion composition method and high-pressure homogenization method. *Drug Deliv*. 22:455–466.
19. Kruk, Z. A., H. Yun, D. L. Rutley, E. J. Lee, Y. J. Kim, dan C. Jo. 2011. Effect of high pressure on microbial population and quality of chicken breast fillet. *Food Control*. 22:6–12.
20. Lee, S. K., J. W. Chon, Y. K. Yun, J. C. Lee, C. Jo, K. Y. Song, D. H. Kim, D. Bae, H. Kim, J. S. Moon dan K. H. Seo. 2022. Properties of broiler breast meat with pale color and a new approach for evaluating meat freshness in poultry processing plants. *Poultry Science*. 101(3), 101627
21. Li, R., L. Liu, D. Zhou, M. Li, X. Kou, dan S. Wang. 2025. Influence of additives on moisture variation and edible quality of minced chicken breast. *Innov. Food Sci. Emerg. Technol*. 102:104009.
22. Marcinkowska-Lesiak, M., Z. Zdanowska-Sasiadek, A. Stelmasiak, K. Damaziak, M. Michalczyk, E. Polawska, J. Wyrwicz, dan A. Wierzbicka. 2016. Effect of packaging method and cold-storage time on chicken meat quality. *CyTA–J. Food* 14:41–46.
23. Risnawati, H., R. Triani, P. B. Purwandoko, N. K. I. Mayasti, N. D. Susanti, F. Novianti, dan E. D. Kurniawan. 2025. Effect of low temperature on physical characteristics of chicken meat during storage. *IOP Conf. Ser. Earth Environ. Sci*. 1446:012011.
24. Santana de Oliveira, M., O. Vostinaru, D. Rigano, dan E.H. de Aguiar Andrade. 2023. Bioactive compounds present in essential oils: Advances and pharmacological applications. *Front. Pharmacol*. 14:1130097.
25. Sharma, O.P. dan T.K. Bhat. 2009. DPPH antioxidant assay revisited. *Food Chem*. 113(4):1202–1205.

26. Solans, C., P. Izquierdo, J. Nolla, N. Azemar, dan M.J. Garcia-Celma. 2005. Nano emulsions. *Curr. Opin. Colloid Interface Sci.* 10:102–110.
27. Souhoka, F. A., N. Hattu, dan M. Huliselan. 2019. Uji aktivitas antioksidan ekstrak metanol biji kesumba keling. *Indones. J. Chem. Res.* 7(1):25–31.
28. Sujiwo, J., D. Kim, dan A. Jang. 2018. Relation among quality traits of chicken breast meat during cold storage: correlations between freshness traits and torrymeter values. *Poult. Sci.* 97(8), 2887–2894.
29. Sun, H., D. Luo, S. Zheng, Z. Li, dan W. Xu. 2022. Antimicrobial behavior and mechanism of clove oil nanoemulsion. *J. Food Sci. Technol.* 59(5):1939–1947.
30. Taher, M. K., I. Q. Falih, dan Y. J. Abdullah. 2024. Design of nanoemulsion delivery systems of clove essential oil. *J. Prev. Diagn. Treat. Strateg. Med.* 3(3):163–170.
31. Tahir, M., S. Chuzaemi, E. Widodo, dan H. Hafsah. 2020. Chemical compounds and antioxidant contents of clove leaves essential oil. *Agroland: J. Agric. Sci.* 7(1):37–44.
32. Ujilestari, T., Martien, R., Ariyadi, B., & Dono, N. D. 2018. Self-nanoemulsifying drug delivery system (SNEDDS) of *Amomum compactum* essential oil: design, formulation, and characterization. *Journal of Applied Pharmaceutical Science*, 8(6), 014–021.
33. Vélez, M.A., M.C. Perotti, L. Santiago, A.M. Gennaro, dan E. Hynes. 2017. Bioactive compounds delivery using nanotechnology: Design and applications in dairy food. Dalam: *Nutrient Delivery*. Academic Press, Cambridge, MA, USA. hlm. 221–250.
34. Wahyono, N.D. dan M.M.D. Utami. 2018. A review of the poultry meat production industry for food safety in Indonesia. *J. Phys.: Conf. Ser.* 953:012125.
35. Wala, J., T. Ransaleleh, I. Wahyuni, dan M. Rotinsulu. 2016. Kadar air, pH, dan total mikroba daging ayam dengan penambahan kunyit putih. *Zootec* 36(2):405–416.
36. Wang, L., J. Dong, J. Chen, J. Eastoe, dan X. Li. 2009. Design and optimization of a new self-nanoemulsifying drug delivery system. *J. Colloid Interface Sci.* 330(2):443–448.
37. Warenska, M., dan A. Okruszek. 2023. Impact of frozen storage on functional properties and sensory evaluation of goose meat. *Poult. Sci.* 102(10):102894.
38. Yu, H.H., Y.W. Chin, dan H.D. Paik. 2021. Application of natural preservatives for meat and meat products against food-borne pathogens and spoilage bacteria: a review. *Foods*. 10(10):2418.

Tables

Table 1. Transmittance value of initial SNEDDS formulation (v/v)

Oil (%)	Tween 80 (%)	PEG 400 (%)	Transmittance (%)
33,33	33,33	33,33	4,30 ± 0,02
25	50	25	14,32 ± 0,22
20	60	20	77,74 ± 0,18
16,67	66,67	16,67	90,71 ± 0,24
14,29	71,43	14,29	90,85 ± 0,12
12,5	75	12,5	91,06 ± 0,12

Table 2. Transmittance value of SNEDDS formulation (v/v)

Run	Component (v/v)			Transmittance (%)
	Oil (%)	Tween 80 (%)	PEG 400 (%)	
1	18,47	63,47	18,07	91,35 ± 0,12
2	20,00	60,00	20,00	91,08 ± 0,32
3	20,00	63,34	16,66	91,41 ± 0,12
4	19,87	61,16	18,97	90,66 ± 0,67
5	17,25	62,75	20,00	91,34 ± 0,12
6	20,00	60,00	20,00	90,99 ± 0,03
7	16,68	66,66	16,66	91,10 ± 0,36
8	16,68	66,66	16,66	90,87 ± 0,32
9	16,66	64,65	18,69	91,62 ± 0,24
10	17,25	62,75	20,00	91,13 ± 0,12
11	19,70	62,35	17,95	90,50 ± 0,02
12	20,00	63,34	16,66	91,27 ± 0,24
13	16,66	64,65	18,69	91,31 ± 0,12
14	17,37	65,34	17,29	91,48 ± 0,24
15	18,51	61,49	20,00	91,20 ± 0,01
16	18,75	64,59	16,66	91,69 ± 0,12

Table 3. Clear zone diameter results

Sample	Diameter (mm)		
	<i>Salmonella sp</i>	<i>E. coli</i> FNCC 0091	<i>S. aureus</i> ATCC 259
Clove essential oil	31,6 ± 3,8	30,3 ± 3,1	29,15 ± 5,0
Tween 80	-	-	-
PEG 400	-	-	-
VCO	-	8,15 ± 0,3	-
Snedds	17,7 ± 0,7	10,55 ± 0,6	13,2 ± 1,6
Nanoemulsion 25.000 ppm	8,5 ± 0,2	9,9 ± 0,3	8,5 ± 0,3
Nanoemulsion 12.500 ppm	8,3 ± 0,3	9,0 ± 0,3	-
Nanoemulsion 6.250 ppm	-	8,9 ± 0,3	-
Nanoemulsion 3.125 ppm	-	-	-
Tetracyclin 30 ppm	39,1 ± 1,7	31,65 ± 0,3	29,85 ± 1,6

Table 4. Antioxidant activity of clove essential oil

Sampel	Concentration (µg/ml)	IC ₅₀
Quercetin	10	1,148
	8	
	6	
	4	
	2	
clove essential oil nanoemulsion	100	2,884
	50	
	25	
	12.5	
	6.25	

Table 9. Broiler chicken meat TPC test results

Perlakuan	Lama Penyimpanan Mikro			
	H0	H4	H8	H12
	(×10 ⁶ CFU/mL)	(×10 ⁶ CFU/mL)	(×10 ⁶ CFU/mL)	(×10 ⁸ CFU/mL)
P0	1,13 ± 0,32 ^a	6,53 ± 0,29 ^b	75,00 ± 3,04 ^{bc}	2,30 ± 0,10 ^b
P1	1,15 ± 0,05 ^a	3.45 ± 0,18 ^a	3,46 ± 0,28 ^a	1,15 ± 0,19 ^a
P2	1,87 ± 0,15 ^c	4,30 ± 0,18 ^a	61,33 ± 4,04 ^b	2,30 ± 0,26 ^b
P3	1,47 ± 0,09 ^{ab}	6,81 ± 0,27 ^b	73,00 ± 9,53 ^{bc}	2,28 ± 0,14 ^b
P4	1,39 ± 0,13 ^b	8,50 ± 0,77 ^c	81,16 ± 9,82 ^c	2,39 ± 0,55 ^b

a,b,c Different superscripts in the same column indicate significant differences (P<0.05).

P0 = No treatment; P1 = Clove essential oil nanoemulsion 25,000 ppm; P2 = Clove essential oil nanoemulsion 12,500 ppm; P3 = Clove essential oil nanoemulsion 6,250 ppm; P4 = Clove essential oil nanoemulsion 3,125 ppm; H0 = Storage period 0 days; H4 = Storage period 4 days; H8 = Storage period 8 days; H12 = Storage period 12 days

Table 10. Results of broiler chicken meat pH testing

Treatment	Storage Period			
	H0	H4	H8	H12
P0	6,13 ± 0,03	5,92 ± 0,03 ^c	5,92 ± 0,09 ^c	5,91 ± 0,05 ^b
P1	6,13 ± 0,02	5,95 ± 0,04 ^c	5,91 ± 0,03 ^{bc}	5,84 ± 0,02 ^a
P2	6,15 ± 0,03	5,99 ± 0,04 ^d	5,91 ± 0,03 ^{bc}	5,93 ± 0,02 ^b
P3	6,15 ± 0,03	5,86 ± 0,03 ^b	5,85 ± 0,03 ^b	5,94 ± 0,06 ^b
P4	6,12 ± 0,02	5,79 ± 0,02 ^a	5,73 ± 0,04 ^a	5,93 ± 0,03 ^b

a,b,c Different superscripts in the same column indicate significant differences (P<0.05).

P0 = No treatment; P1 = Clove essential oil nanoemulsion 25,000 ppm; P2 = Clove essential oil nanoemulsion 12,500 ppm; P3 = Clove essential oil nanoemulsion 6,250 ppm; P4 = Clove essential oil nanoemulsion 3,125 ppm; H0 = Storage period 0 days; H4 = Storage period 4 days; H8 = Storage period 8 days; H12 = Storage period 12 days

Table 11. Color test results L* (brightness)

Treatment	Storage Period			
	H0	H4	H8	H12
P0	59,22 ± 2,76	57,30 ± 4,16	59,03 ± 3,75 ^{ab}	62,49 ± 3,73 ^b
P1	58,98 ± 4,47	61,68 ± 3,02	58,49 ± 1,31 ^a	56,66 ± 3,46 ^a
P2	58,96 ± 1,42	61,32 ± 4,39	63,37 ± 3,81 ^{bc}	64,03 ± 1,48 ^b
P3	59,90 ± 1,70	60,73 ± 3,39	66,07 ± 2,57 ^c	61,50 ± 2,03 ^b
P4	59,89 ± 3,34	58,96 ± 1,79	61,68 ± 4,77 ^{abc}	61,67 ± 2,38 ^b

a,b,c Different superscripts in the same column indicate significant differences (P<0.05).

P0 = No treatment; P1 = Clove essential oil nanoemulsion 25,000 ppm; P2 = Clove essential oil nanoemulsion 12,500 ppm; P3 = Clove essential oil nanoemulsion 6,250 ppm; P4 = Clove essential oil nanoemulsion 3,125 ppm; H0 = Storage period 0 days; H4 = Storage period 4 days; H8 = Storage period 8 days; H12 = Storage period 12 days

Table 14. Results of cooking shrinkage tests on broiler chicken meat

Treatment	Storage Period			
	H0 (%)	H4 (%)	H8 (%)	H12 (%)
P0	34,70 ± 2,29	35,47 ± 4,09	33,30 ± 0,88	33,62 ± 4,05
P1	35,19 ± 0,87	37,30 ± 4,48	32,90 ± 2,70	33,16 ± 2,87
P2	34,49 ± 1,11	35,46 ± 1,24	32,67 ± 3,04	34,23 ± 1,02
P3	34,31 ± 0,50	32,86 ± 1,38	33,01 ± 2,90	34,78 ± 0,32
P4	35,24 ± 2,23	31,76 ± 1,61	32,23 ± 1,72	32,59 ± 2,06

a,b,c Different superscripts in the same column indicate significant differences (P<0.05).

P0 = No treatment; P1 = Clove essential oil nanoemulsion 25,000 ppm; P2 = Clove essential oil nanoemulsion 12,500 ppm; P3 = Clove essential oil nanoemulsion 6,250 ppm; P4 = Clove essential oil nanoemulsion 3,125 ppm; H0 = Storage period 0 days; H4 = Storage period 4 days; H8 = Storage period 8 days; H12 = Storage period 12 days

Table 15. Results of water binding capacity testing of broiler chicken meat

Treatment	Storage Period			
	H0 (%)	H4 (%)	H8 (%)	H12 (%)
P0	48,78 ± 3,76	51,89 ± 4,41	58,60 ± 5,00 ^b	47,62 ± 3,33
P1	47,56 ± 1,39	52,86 ± 0,66	50,61 ± 1,99 ^{ab}	50,48 ± 0,42
P2	47,03 ± 0,32	48,85 ± 1,31	49,34 ± 2,76 ^{ab}	48,96 ± 4,19
P3	48,16 ± 1,31	45,33 ± 1,88	46,25 ± 5,35 ^a	48,31 ± 2,35
P4	48,62 ± 3,72	47,96 ± 4,14	42,49 ± 4,67 ^a	46,37 ± 1,96

a,b,c Different superscripts in the same column indicate significant differences (P<0.05).

P0 = No treatment; P1 = Clove essential oil nanoemulsion 25,000 ppm; P2 = Clove essential oil nanoemulsion 12,500 ppm; P3 = Clove essential oil nanoemulsion 6,250 ppm; P4 = Clove essential oil nanoemulsion 3,125 ppm; H0 = Storage period 0 days; H4 = Storage period 4 days; H8 = Storage period 8 days; H12 = Storage period 12 days

Table 16. Results of broiler chicken meat tenderness testing

Treatment	Storage Period			
	H0 (N)	H4 (N)	H8 (N)	H12 (N)
P0	48,32 ± 5,69	65,27 ± 0,79 ^b	39,57 ± 3,84 ^b	33,48 ± 2,72 ^a
P1	55,71 ± 2,89	61,02 ± 8,33 ^{ab}	54,31 ± 0,98 ^c	55,17 ± 3,77 ^b
P2	56,22 ± 5,69	49,58 ± 4,25 ^a	27,59 ± 4,14 ^a	54,53 ± 2,75 ^b
P3	61,34 ± 7,77	57,99 ± 1,34 ^{ab}	38,90 ± 4,56 ^b	33,18 ± 5,69 ^a
P4	56,64 ± 2,90	55,18 ± 1,69 ^{ab}	36,22 ± 2,13 ^{ab}	41,55 ± 3,56 ^a

a,b,c Different superscripts in the same column indicate significant differences (P<0.05).

P0 = No treatment; P1 = Clove essential oil nanoemulsion 25,000 ppm; P2 = Clove essential oil nanoemulsion 12,500 ppm; P3 = Clove essential oil nanoemulsion 6,250 ppm; P4 = Clove essential oil nanoemulsion 3,125 ppm; H0 = Storage period 0 days; H4 = Storage period 4 days; H8 = Storage period 8 days; H12 = Storage period 12 days

Table 17. Results of moisture content testing of broiler chicken meat

Treatment	Storage Period			
	H0 (%)	H4 (%)	H8 (%)	H12 (%)
P0	74,20 ± 0,21 ^{ab}	72,99 ± 0,15 ^a	75,12 ± 0,11 ^c	74,21 ± 0,21 ^a
P1	74,81 ± 0,18 ^b	72,93 ± 0,02 ^a	73,92 ± 0,07 ^{ab}	74,68 ± 0,00 ^a
P2	74,15 ± 0,15 ^a	73,22 ± 0,15 ^{ab}	74,05 ± 0,09 ^b	74,38 ± 0,17 ^a
P3	74,61 ± 0,15 ^{ab}	73,53 ± 0,11 ^b	73,37 ± 0,18 ^a	75,72 ± 0,00 ^b
P4	75,76 ± 0,06 ^c	73,19 ± 0,17 ^{ab}	73,97 ± 0,24 ^a	74,38 ± 0,47 ^a

a,b,c Different superscripts in the same column indicate significant differences (P<0.05).

P0 = No treatment; P1 = Clove essential oil nanoemulsion 25,000 ppm; P2 = Clove essential oil nanoemulsion 12,500 ppm; P3 = Clove essential oil nanoemulsion 6,250 ppm; P4 = Clove essential oil

nanoemulsion 3,125 ppm; H0 = Storage period 0 days; H4 = Storage period 4 days; H8 = Storage period 8 days; H12 = Storage period 12 days

Table 18. Broiler chicken meat protein test results

Treatment	Storage Period			
	H0 (%)	H4 (%)	H8 (%)	H12 (%)
P0	22,14 ± 0,08 ^b	22,66 ± 0,41	21,91 ± 0,16	22,14 ± 0,08 ^b
P1	21,07 ± 0,13 ^a	22,33 ± 0,89	22,19 ± 0,11	21,07 ± 0,13 ^a
P2	22,14 ± 0,28 ^b	22,43 ± 0,58	22,51 ± 0,50	22,14 ± 0,28 ^b
P3	20,50 ± 0,35 ^a	22,34 ± 0,52	21,84 ± 0,04	20,50 ± 0,35 ^a
P4	21,11 ± 0,22 ^a	22,52 ± 0,03	21,54 ± 0,04	20,85 ± 0,14 ^a

a,b,c Different superscripts in the same column indicate significant differences (P<0.05).

P0 = No treatment; P1 = Clove essential oil nanoemulsion 25,000 ppm; P2 = Clove essential oil nanoemulsion 12,500 ppm; P3 = Clove essential oil nanoemulsion 6,250 ppm; P4 = Clove essential oil nanoemulsion 3,125 ppm; H0 = Storage period 0 days; H4 = Storage period 4 days; H8 = Storage period 8 days; H12 = Storage period 12 days

Table 19. Broiler chicken meat fat test results

Treatment	Storage Period			
	H0 (%)	H4 (%)	H8 (%)	H12 (%)
P0	4,09 ± 0,03 ^a	4,51 ± 0,21 ^b	3,84 ± 0,22 ^b	4,08 ± 0,03 ^b
P1	2,36 ± 0,25 ^b	4,03 ± 0,13 ^{ab}	3,28 ± 0,12 ^a	2,35 ± 0,23 ^a
P2	2,91 ± 0,05 ^a	3,93 ± 0,04 ^a	3,78 ± 0,12 ^b	2,75 ± 0,34 ^a
P3	2,81 ± 0,25 ^a	3,92 ± 0,07 ^a	3,42 ± 0,01 ^{ab}	3,71 ± 0,12 ^b
P4	3,71 ± 0,12 ^b	3,70 ± 0,08 ^b	3,42 ± 0,01 ^{ab}	2,75 ± 0,17 ^a

a,b,c Different superscripts in the same column indicate significant differences (P<0.05).

P0 = No treatment; P1 = Clove essential oil nanoemulsion 25,000 ppm; P2 = Clove essential oil nanoemulsion 12,500 ppm; P3 = Clove essential oil nanoemulsion 6,250 ppm; P4 = Clove essential oil nanoemulsion 3,125 ppm; H0 = Storage period 0 days; H4 = Storage period 4 days; H8 = Storage period 8 days; H12 = Storage period 12 days

Table 20. Evaluasi sensori daging ayam broiler

Parameter	Perlakuan	Lama Penyimpanan			
		H0	H4	H8	H12
Warna	P0	6,20 ± 1,35	5,53 ± 1,63	5,33 ± 1,49	3,73 ± 1,41 ^a
	P1	6,33 ± 1,42	5,50 ± 1,46	5,07 ± 1,57	5,13 ± 1,69 ^b
	P2	5,93 ± 1,48	5,60 ± 1,57	5,30 ± 1,58	4,20 ± 1,69 ^{ab}
	P3	6,07 ± 1,44	5,30 ± 1,29	5,37 ± 1,49	5,30 ± 1,47 ^b
	P4	5,90 ± 1,45	5,83 ± 1,29	4,77 ± 1,57	4,83 ± 1,72 ^{ab}
Aroma	P0	5,20 ± 1,61 ^a	4,27 ± 1,44 ^a	5,33 ± 1,94 ^a	3,53 ± 1,68 ^a
	P1	5,73 ± 1,68 ^{ab}	5,50 ± 1,33 ^b	5,87 ± 1,36 ^b	5,63 ± 1,25 ^b
	P2	5,23 ± 1,65 ^a	6,00 ± 1,46 ^b	5,97 ± 1,09 ^b	5,10 ± 1,54 ^b
	P3	6,43 ± 1,16 ^b	5,33 ± 1,89 ^{ab}	5,53 ± 1,38 ^b	5,70 ± 1,32 ^b
	P4	6,23 ± 1,14 ^{ab}	5,70 ± 1,46 ^b	4,30 ± 1,32 ^a	4,90 ± 1,73 ^b
Tekstur	P0	6,13 ± 1,04	6,43 ± 1,19	5,73 ± 1,62	5,03 ± 1,52
	P1	6,33 ± 1,21	5,57 ± 1,28	6,07 ± 1,20	5,60 ± 1,28
	P2	6,33 ± 1,29	5,83 ± 1,51	5,60 ± 1,04	5,23 ± 1,50
	P3	6,53 ± 1,01	5,57 ± 1,61	5,60 ± 1,22	5,63 ± 1,40
	P4	6,40 ± 1,06	5,73 ± 1,26	5,53 ± 1,43	5,60 ± 1,16
Daya Terima	P0	5,80 ± 1,24	5,47 ± 1,61	5,23 ± 1,76	4,13 ± 1,46 ^a
	P1	6,13 ± 1,38	5,70 ± 1,15	6,03 ± 1,19	5,67 ± 1,29 ^b
	P2	5,90 ± 1,42	6,07 ± 1,20	5,90 ± 1,12	5,27 ± 1,36 ^b
	P3	6,60 ± 0,93	5,53 ± 1,69	6,00 ± 1,29	5,90 ± 1,16 ^b
	P4	6,37 ± 0,89	5,97 ± 1,09	5,17 ± 1,39	5,17 ± 1,51 ^b

a,b,c Different superscripts in the same column indicate significant differences ($P<0.05$).

P0 = No treatment; P1 = Clove essential oil nanoemulsion 25,000 ppm; P2 = Clove essential oil nanoemulsion 12,500 ppm; P3 = Clove essential oil nanoemulsion 6,250 ppm; P4 = Clove essential oil nanoemulsion 3,125 ppm; H0 = Storage period 0 days; H4 = Storage period 4 days; H8 = Storage period 8 days; H12 = Storage period 12 days

Tables 5 to 8, 12, 13 are not available with this version.

Figures

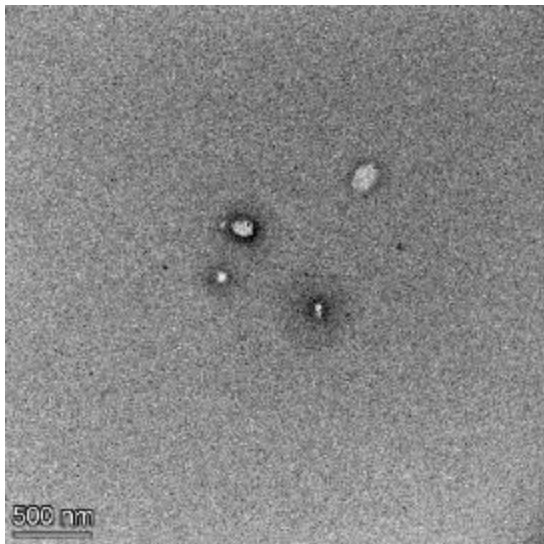


Figure 1

Morphology of clove essential oil nanoemulsion with 50,000x magnification visualized using TEM. The nanoemulsion particles are white, while the aqueous phase is dark.

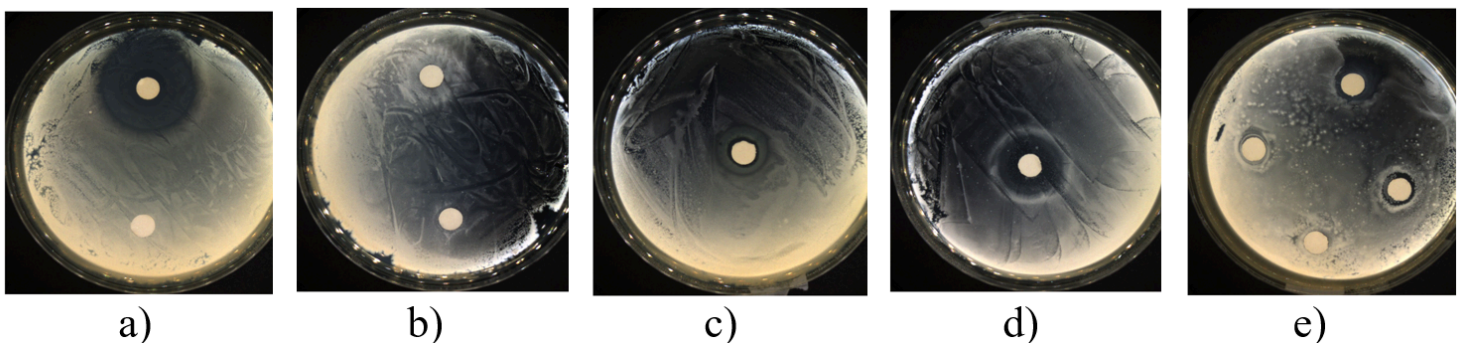


Figure 2

Results of antibacterial activity tests against *S. aureus* ATCC 259 bacteria. a) clove essential oil and VCO, b) Tween 80 and PEG 400, c) SNEDDS clove essential oil, d) antibiotics e) nanoemulsion

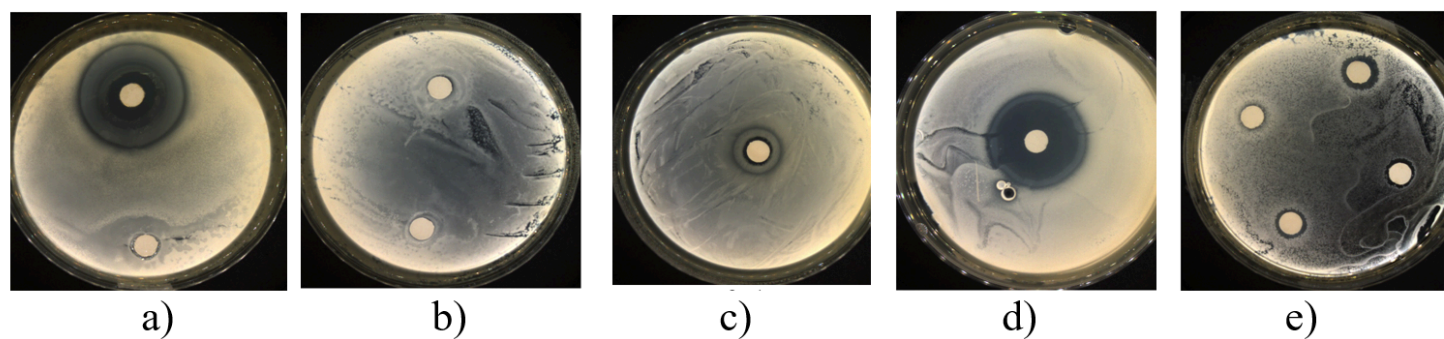


Figure 3

Results of antibacterial activity tests against *E. Coli* bacteria FNCC 0091. a) clove essential oil and VCO, b) Tween 80 and PEG 400, c) SNEDDS clove essential oil, d) antibiotics, e) nanoemulsion

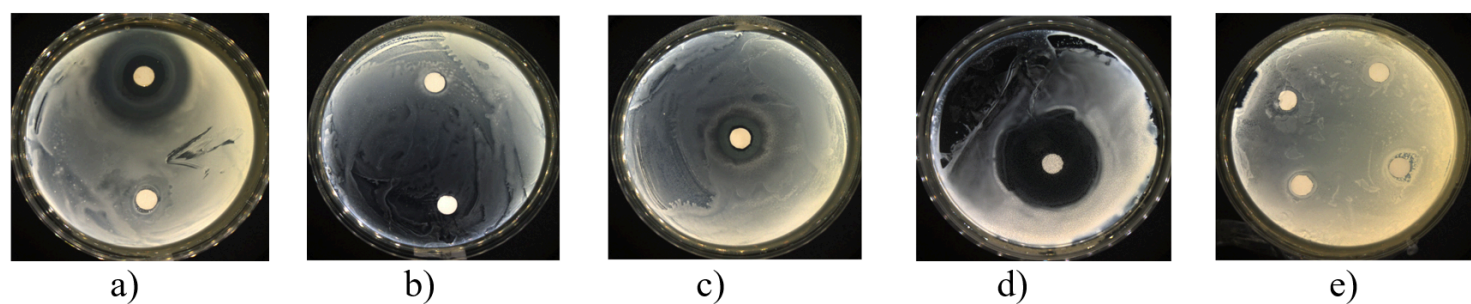


Figure 4

Results of antibacterial activity tests against *Salmonella* sp. bacteria a) clove essential oil and VCO, b) Tween 80 and PEG 400, c) SNEDDS clove essential oil, d) antibiotics, e) nanoemulsion