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**FORMULATION OF NANOEMULSION PARIJOTO FRUIT EXTRACT (*Medinilla speciosa*) WITH VARIATION OF TWEENS STABILIZERS**

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Emerging Indigenous Food Processing in Solving Nutrition Problems

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# 1. Manuscript Submission and General History

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Original Research

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- ✓ 1. Initial Validation
- ✓ 2. Editorial Assignment
- ✓ 3. Independent Review
- ✓ 4. Interactive Review
- ✓ 5. Review Finalized
- ✓ 6. Final Validation
- ✓ 7. Final Decision

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<b>Date</b>	<b>Updates</b>				
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## 2. Revision from Reviewer 1

- Interactive Discussion Proof

### ▼ EVALUATION

**Q 1** Please list your revision requests for the authors and provide your detailed comments, including highlighting limitations and strengths of the study and evaluating the validity of the methods, results, and data interpretation. If you have additional comments based on Q2 and Q3 you can add them as well.

 Reviewer 1 | 29 Mar 2024 | 16:39

#1

The manuscript seems to be engaging in describing the research of the nanoemulsion formulation of Parjoto extract. However, several concerns need to be addressed, i.e.:

1. Please improve and revise the English language of the manuscript thoroughly.
2. What are the specifications of the Parjoto plants used in the study, and where was it cultivated and harvested? Is there any determination test performed to confirm the plant as *Medinilla speciosa*?
3. Was there any software used to perform the RSM-CCD approach? Why were 81 runs conducted for the CCD study, which seems too many to optimize three factors? Was the run-order randomized? What does "MLD" mean?
4. Is the statement "In Table 1, it is observed that the particle size range of the nanoemulsion is between  $14,603 \pm 16.73$  nm and  $118,053 \pm 4.5825$  nm" correct? Why are the particle sizes so big?

### 3. Submission of Revised Manuscript 1

- Interactive Discussion Proof

 Corresponding Author: Victoria Kristina Ananingsih | 19 Apr 2024 | 08:32

1. Please improve and revise the English language of the manuscript thoroughly.

Thank you for your suggestions in the new manuscript that we sent, we have corrected the grammar and improved the English language.

2. What are the specifications of the Parijoto plants used in the study, and where was it cultivated and harvested?

The research samples used in this study are fruits from the parijoto plant (*Medinilla speciosa*) cultivated and harvested on the slopes of Mount Maris, Kudus. The fruits used are ripe fruits harvested when the parijoto plant reaches full maturity, typically around 90-100 days after flowering. (We have added in the manuscript Line 100-102).

The Parijoto plants are carefully cultivated in the natural environment of the slopes of Mount Maris, Kudus, know for its conditions that support the growth of these plants. The planting and maintenance processes are carried out according to good cultivation practices to ensure the health and quality of the plants.

Is there any determination test performed to confirm the plant is *Medinilla speciosa*?

Although there has not been any definitive determination test conducted to confirm that the plants are *Medinilla speciosa*, this research still utilizes plants that morphologically and characteristic-wise match the description of *Medinilla speciosa*. Therefore, the Parijoto fruit samples used in this study are expected to represent the *Medinilla speciosa* species well, even though formal determination tests have not been conducted yet.

3. Was there any software used to perform the RSM-CCD approach? Why were 81 runs conducted for the CCD study which seems too many to optimize three factors? Was the run-order randomized? What does "MLD" mean?

Statistica 12.5 by StatSoft is a software commonly used for conducting the response surface methodology (RSM) combined with central composite design (CCD) approach. This software facilitates the design, analysis, and optimization of experiments, particularly in the context of studying multiple variables and their effects on a response.

As for conducting 81 experiments in the CCD study, this might seem excessive for optimizing three factors. However, the number of runs in a CCD is determined by the level of precision desired and the complexity of the response surface being studied. With 3 factors, a CCD typically involves a full factorial design with additional center points and possibly axial points. The number of runs is often a power of 2 plus additional center points, which can result in a seemingly high number of experiments. The purpose is to ensure thorough exploration of the design space and accurate estimation of the response surface. The sequence of experiments in a CCD is usually randomized to minimize the effects of potential confounding variables or systematic errors. Randomization helps to ensure that the results are not biased by the order in which experiments are conducted.

We apologize because there was a typewriting mistake, where MLD should be written as CCD.

4. Is the statement "In Table 1, it is observed that the particle size range of the nanoemulsion is between 14,603-16,73 nm and 118,053-4,5825 nm" correct? Why are the particle sizes so big?

The statement regarding the particle size range in Table 1, indicating sizes between 14,603-16,73 nm and 118,053-4,5825 nm, appears to be accurate based on the provided data. The particle size that we obtained in this research is in accordance with our target, namely a particle size below 300 nm which is suitable for food and beverage products. However, the particle sizes being relatively large could raise concerns and prompt further investigation into the factors influencing them. Various factors can impact particle size, including process conditions, surfactant type, mixing timing, mixing speed, filling extract, and surfactant concentration. In this analysis, the effects of surfactant type, filling extract, and surfactant concentration, particularly with various types of surfactants like Tween, were examined.

Different surfactants can interact differently with the components of the nanoemulsion, potentially affecting the resulting particle size distribution. Some surfactants may lead to larger particle sizes due to their molecular structure or interactions with the emulsion components. The nature of the filling extract used in the formulation can influence the size and stability of the nanoemulsion. Components of the filling extract may affect the emulsion droplet size during the formulation process. The concentration of surfactant in the formulation plays a critical role in stabilizing the nanoemulsion and controlling particle size. Higher concentrations of surfactant may lead to smaller particle sizes by reducing interfacial tension and preventing coalescence of droplets. Parameters such as mixing speed, mixing time, and temperature during emulsification can significantly impact particle size distribution. Suboptimal process conditions may result in larger particle sizes due to inadequate dispersion or coalescence of droplets.

In the analysis, the effects of surfactant type, filling extract, and surfactant concentration, especially with various types of surfactants such as Tween, were examined. This suggests that the study aimed to understand how different formulation parameters influence particle size in nanoemulsions. To address the issue of unusually large particle sizes, further investigation and optimization of the formulation and process parameters may be necessary.

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- Supporting file

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- Revision Manuscript



## FORMULATION OF NANOEMULSION PARIJOTO FRUIT EXTRACT (*Medinilla speciosa*) WITH VARIATION OF TWEENS STABILIZERS

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8 **Keywords:** Nanoemulsion<sub>1</sub>, Stabilizers<sub>2</sub>, Tweens<sub>3</sub>, Parijoto<sub>4</sub>, RSM<sub>5</sub>.

9 **Abstract**

10 Nanotechnology was deemed to possess substantial potential for development owing to its ability to  
11 modify surface characteristics and particle size, facilitating enhanced absorption of functional food  
12 compounds and controlled release of active substances to mitigate adverse effects. Nanoemulsion, a  
13 stable colloidal system formed by blending oil, emulsifier, and water, was identified as  
14 nanotechnology with promising applications. However, investigations into the impact of surfactants  
15 on characteristic nanoemulsions, needed to be more varied. This research gap necessitated further  
16 exploration in the advancement of nanotechnology-based foods. The parijoto fruit (*Medinilla*  
17 *speciosa*), an indigenous plant species in Indonesia, has yet to undergo extensive scrutiny for its  
18 potential use as a functional and nutraceutical food. Anthocyanins, a principal compound in the  
19 parijoto fruit, had exhibited efficacy in reducing the risk of cardiovascular diseases, diabetes, and  
20 inflammation, and demonstrated anti-inflammatory and antioxidant properties. This study aimed to  
21 investigate the characteristics of nanoemulsion formulations derived from parijoto fruit extract and to  
22 evaluate an optimum condition with various tween surfactant. The findings from this investigation  
23 could furnish valuable insights for the further advancement of anthocyanin nanoemulsions from  
24 parijoto fruit extract. The results comprised the characterization of nanoemulsion particle size,  
25 polydispersity index, zeta potential, conductivity, pH, and viscosity. RSM is used to optimize  
26 nanoemulsion by examining the relationships and interactions between independent variables and  
27 response variables through mathematical modeling and statistical methods. Furthermore, the  
28 characterization of nanoemulsion encompassed zeta potential, polydispersity, particle size,  
29 conductivity, pH, and viscosity. Elevated surfactant concentrations resulted in diminished particle  
30 sizes and more uniform size distribution, albeit reaching a plateau where surfactant aggregation and  
31 micelle formation ensued. Increased concentrations of surfactant type, concentration, and parijoto  
32 extract impacted the physical characteristics of nanoparticle size and polydispersity. The optimal  
33 process conditions for nanoemulsion comprised a 12% concentration of Tween 80 solvent, 12%  
34 Tween concentration, and 7.5% parijoto fruit extract concentration, yielding a desirability value of  
35 0.74, categorizing it as moderate.

36 **1 Introduction**

37 Nanotechnology underwent progressive evolution, characterized by measurements on the nanometer  
38 scale, approximately  $10^{-9}$  meters (Ariningsih, 2016). Acknowledgment from the World Health  
39 Organization (WHO) and the Food and Agriculture Organization (FAO) (2009) underscored  
40 nanotechnology's significant potential in enhancing food products, attributed to its capacity to modify  
41 surface characteristics and particle size. Such modifications facilitate targeted delivery of food  
42 compounds to specific organs and the controlled release of active compounds to mitigate adverse  
43 effects. The attributes of nanoscale food materials are pivotal in propelling diverse industries,  
44 including food, pharmaceuticals, and extensive nutraceutical applications (Rahman et al., 2020).  
45 Nanoemulsions denote a nanotechnological rendition of a stable colloidal system, achieving kinetic  
46 stability through the amalgamation of oil, emulsifier, and water (Mclements, 2016). Chang et al.  
47 (2022) conducted research utilizing surfactants as stabilizers in synthesizing nanoemulsions,  
48 showcasing the stability of nanoemulsion particle size in curcumin extract. Surfactants can diminish  
49 interfacial tension and form a substantially influential steric elastic film on the emulsion results (Xiao  
50 J & Huang, 2016).

51 Renowned for its tropical climate and vast biodiversity, Indonesia harbours at least 30,000 plant  
52 species, with 7,000 being herbal plants with documented health benefits (Widyowati & Agil, 2018;  
53 Jumiami & Komalasari, 2017). Parijoto (*Medinilla speciosa*), an endemic plant species in Indonesia,  
54 remains relatively understudied for its scientific potential in pharmacy, functional foods, and  
55 nutraceuticals. Analysis has confirmed that the parijoto fruit comprises phytochemical components  
56 such as anthocyanins, flavonoids, saponins, tannins, alkaloids, cardenolides, and glycosides  
57 (Balamurugan, 2014). Anthocyanins, a predominant compound in parijoto fruit, demonstrate efficacy  
58 in reducing the risk of cardiovascular diseases, diabetes, and inflammation while possessing notable  
59 anti-inflammatory and antioxidant properties. Extraction techniques yield varying anthocyanin  
60 contents, with the peel extract and whole fruit extract registering 208.75 and 173.7 mg/L,  
61 respectively (Sa'adah et al., 2020). Various factors influence anthocyanins' stability, including  
62 chemical structure, concentration, solvent, pH, storage temperature, light, oxygen, metal ions,  
63 proteins, and flavonoids. Weak stability under high pH, high temperature, and light exposure has  
64 been observed (Ito et al., 2021), with lower pH contributing to enhanced stability (Moldova et al.,  
65 2020). Heating at elevated temperatures accelerates anthocyanin degradation (Khoo et al., 2019).

66 In recent years, Response Surface Methodology (RSM) has emerged as a prominent multivariate  
67 statistical technique for optimizing various processes. Initially introduced by Box and colleagues in  
68 the 1950s, RSM facilitates the examination of the relationship and interactions among independent  
69 variables and response variables through mathematical modeling and statistical methods (Izayidan et  
70 al., 2019). RSM has been successfully employed in enhancing and optimizing therapeutic extract and  
71 drug nanoemulsion (Samim et al., 2020). In this study, Central Composite Design (CCD) Response  
72 Surface Methodology (RSM) was employed to optimize the quality parameters of the nanoemulsion.

73 Appropriate nano-encapsulation techniques, such as nanoemulsion, have shown the potential to  
74 enhance the stability, bioavailability, and solubility of lipophilic bioactive compounds while also  
75 preventing hydrolysis and oxidation (Rosso et al., 2020). Catechin nanoemulsion showed a  
76 remarkable improvement of stability and bioavailability in simulated gastrointestinal (Rafanar et al.,  
77 2016). Research conducted by Chang et al. (2022) used Tween as surfactant in the stable  
78 nanoemulsion synthesis loaded curcumin extract. This underscores the potential for developing  
79 nanoemulsion formulations for anthocyanins in parijoto fruit. Thus far, research on nanoemulsion  
80 formulation in parijoto fruit, incorporating various concentrations and stabilizers, still needs to be



122 involves observing the extract as the solvent evaporates, noting its increasing concentration  
123 evidenced by a thicker and more viscous appearance. Periodic weighing of the container or flask  
124 containing the extract allows for the tracking of weight loss as the solvent evaporates. Once the  
125 weight stabilizes or reaches a predetermined target, it signifies that the desired solvent removal rate  
126 has been attained, ensuring the production of a concentrated anthocyanin extract suitable for further  
127 analysis. Anthocyanin nanoemulsion was prepared using a combination of surfactants that have low,  
128 medium, and high hydrophile lipophile balance (HLB), namely Tween 20, Tween 60, and Tween 80.  
129 Then, surfactant (0.24 g) was added, and the mixture was homogenized entirely. This was followed  
130 by adding ( 2.76 g) deionized water and mixing again for complete dispersion of surfactant in water.  
131 The solution was then sonicated in a sonicator with a temperature of 35°C, frequency of 45 Hz, and  
132 100% power for 60 minutes. To produce a good nanoemulsion, homogenization was carried out  
133 using high shear homogenization at 15,000 rpm with a temperature of 4 C for 15 minutes.

### 134 **2.5 Characterization of Particle Size and Polydispersity Index of Nanoemulsion Parijoto** 135 **Fruit Extract**

136 The particle size analysis tool used in this study was the Zetasizer (Zetasizer Pro; Malvern et al.),  
137 which operates based on the general principle of dynamic light scattering (DLS). This tool has a  
138 detector placed at an angle of 173° from the transmitted light beam and detects size using a patented  
139 technology known as noninvasive backscattering. This technique is used for various purposes. One is  
140 to reduce the effect known as multiple scattering, making it easier to measure samples with high  
141 concentrations. Modifying McClements (2016), the particle size distribution and average particle size  
142 of nanoemulsions were determined by dynamic light scattering (DLS) at a wavelength of 633 nm and  
143 a temperature of 25 °C.

### 144 **2.6 Characterization of Zeta Potential Nanoemulsion Parijoto Fruit Extract**

145 The  $\zeta$ -potential of Parijoto Fruit Extract Nanoemulsion was evaluated using  $\zeta$ -potential analysis  
146 (Zetasizer Pro; Malvern Instruments, Ltd., Malvern) following the method described by Khalid et al.  
147 (2017). The  $\zeta$ -potential of the samples was evaluated automatically using 10 to 100 analytical runs  
148 after equilibration for 120 s at 25 °C. The zeta potential of the particles was measured by phase-  
149 analysis light scattering (PLS) using a Zeta dip cell.

### 150 **2.7 Characterization of the Conductivity of Nanoemulsion Parijoto Fruit Extract**

151 The conductivity of nanoemulsion particles was measured by phase-analysis light scattering (PLS)  
152 using a Zeta dip cell with a cuvet electrode. Samples were evaluated automatically using 10 to 100  
153 analytical runs after equilibration for 120 seconds at 25 °C. The detector is placed at an angle of 173°  
154 from the transmitted light beam.

### 155 **2.8 pH Measurement of Nanoemulsion Parijoto Fruit Extract**

156 The pH was determined using a Schott pH meter at room temperature ( $27 \pm 2$  °C), calibrated with a  
157 standard buffer of pH 7. The pH analysis of the Parijoto fruit extract nanoemulsion sample was  
158 carried out using a pH meter with a particular electrode. First, the pH meter is set and calibrated with  
159 a standard buffer solution at a known pH, generally at pH 4.0, 7.0, and 10.0. Samples were diluted  
160 with ten mM phosphate buffer pH seven before analysis to avoid multiple scattering effects during  
161 testing. The pH meter electrode is then carefully inserted into the sample to ensure good contact.  
162 Once the electrode is stable, a pH reading is taken and recorded. This step is repeated as necessary to  
163 obtain consistent results. This pH analysis provides essential information regarding the acidity or

164 alkalinity level of nanoemulsion and nanocitosan Parijoto fruit extract, which can affect the stability  
165 and quality of products using the nanoemulsion.

## 166 **2.9 Viscosity Measurement of Nanoemulsion Parijoto Fruit Extract**

167 Viscosity measurements are carried out using a viscometer instrument. 14 mL of sample was put into  
168 the cup and attached to the solvent trap provided. The viscometer was set at 200 rpm, three rotations,  
169 for 30 seconds. The measurement process begins by activating the viscometer, and this tool  
170 automatically measures the time required for a liquid to flow through the viscometer tube at a  
171 specific temperature and rpm. This time, a predetermined formula converts the reading into a  
172 viscosity value. Repeated measurements can be made to ensure consistent results.

## 173 **2.10 Statistical analysis uses Response Surface Methodology.**

174 In this study, primary data in 3 repetitions of extraction and three repetitions of testing were averaged  
175 and given a standard deviation value for each treatment combination using Statistica 12.5 by StatSoft.  
176 The data is then entered into a statistical application, arranged in a combination of factorial points,  
177 axial points, and central points with three repetitions. After that, the data was analyzed, and several  
178 test stages were carried out. The basis for testing is studentification from primary data.  
179 Studentification means that the scale of the variable is adjusted by dividing it by the estimated  
180 population standard deviation. Variability in sample standard deviation values contributes to  
181 additional uncertainty in the calculated value. This will cause problems in finding the probability  
182 distribution of each statistic studied.

### 183 **2.10.1 Effect Summary**

184 This test can summarise the effects of the combination of treatments used. The Longworth value in  
185 the results of this test is defined as  $-\log(p\text{-value})$  and is a transformation of the p-value based on the  
186 Pearson Chi-Squared test. The Pearson Chi-Squared test evaluates the possibility of the split being  
187 caused by chance. The higher the Pearson Chi-Squared value, the higher the probability of the split  
188 being caused by dependency. In general, if the worth is greater than 2, then the statistical model  
189 considers the variable necessary.

### 190 **2.10.2 Lack Of Fit**

191 Model suitability testing (lack of fit) is carried out to review whether the model equation is  
192 acceptable or not in predicting responses. In the lack of fit test, the following hypothesis is used:

193  $H_0$  = no lack of fit (suitable model)

194  $H_1$  = there is a lack of fit (the model is not suitable)

195 The hypothesis is concluded by comparing the calculated F value with the F table. The calculated F is  
196 obtained from the statistical test results and displayed in the ANOVA table. The F table value is  
197 obtained from the F Distribution Table. The criteria for the lack of fit test are:

198  $F_{\text{count}} < F_{\text{table}}$ , then  $H_0$  is accepted.  $F_{\text{count}} > F_{\text{table}}$ , then  $H_0$  is rejected.

199 Another parameter that can prove the suitability of the model obtained is by comparing the p-value  
200 with the  $\alpha$  value. If the p-value of lack of fit is smaller than the  $\alpha$  value, then there is a significant  
201 lack of fit, so the model obtained is not appropriate.

202 **2.10.3 Summary Of Fit**

203 The R square and Root Mean square error values are obtained in this test. Measures the difference in  
204 values from a model's predictions as estimates of the observed values. R square is also known as the  
205 coefficient of determination, which explains how far independent data can explain dependent data. R  
206 square has a value between 0 – 1 with the condition that the closer it is to one, the better it is. If the r  
207 square is 0.6, the independent variable can explain 60% of the distribution of the dependent variable.  
208 The independent variable cannot explain the remaining 40% or can be explained by variables outside  
209 the independent variable (error component).

210 **2.10.4 Parameter Estimates**

211 The parameter estimates are the coefficients of the linear predictor. This value represents the change  
212 in response if you have a certain level of a categorical predictor or a change of 1 unit for a continuous  
213 predictor, which means the same thing as in a multiple regression analysis with continuous response.

214 **2.10.5 Analysis Of Variance**

215 The ANOVA test (Analysis of Variance) has the following test criteria:

216 H0 is accepted if  $F_{\text{count}} < F_{\text{table}}$ , which means the model cannot be accepted statistically because  
217 no independent variables have a real influence on the response.

218 H1 is accepted if  $F_{\text{count}} > F_{\text{table}}$ , which means the model is statistically acceptable and at least one  
219 independent variable has a real influence on the response.

220 **2.10.6 Fitted Surfaces**

221 The depiction of the fitted surface is carried out using the Central Composite Design model. The  
222 experimental design is factorial, specifically Central Composite Design (CCD). CCD was chosen  
223 over Box-Behnken Design because CCD provides more design points in terms of axial points.  
224 Additionally, CCDs can run experiments at extreme values, providing better quadratic equations for  
225 analysis. CCD contains a factorial or fractional factorial design with a central point augmented by a  
226 group of 'axial points' that allow estimation of curvature. If the distance from the center of the design  
227 space to the factorial point is  $\pm 1$  unit for each factor, the distance from the center of the design space  
228 to the axial point is  $|\alpha| > 1$ . The exact value of  $\alpha$  depends on the properties desired for the design and  
229 the number of factors involved. The CCD has twice as many star points due to a factor in the design.

### 230 3 Result & Discussion

#### 231 3.1 Phytochemical Profiles of Dried Parijoto Fruit

232 Drying Parijoto Fruit is carried out using a cabinet dryer at a temperature of 70°C for 6 hours.  
233 The results of drying parijoto fruit were obtained through the preparation process, the  
234 antioxidant and anthocyanin activity profiles were expressed respectively in units of %  
235 inhibition and ppm. The results of the antioxidant activity of dried and extracted parijoto fruit  
236 were 79.14334.82%. % The total anthocyanin content in the dry samples and extracts was  
237 538.47 ± ppm. The dried Parijoto exhibited significant antioxidant activity, with a % inhibition  
238 value of 79.14 ± 34.82. This indicates a substantial capacity to neutralize free radicals, which  
239 are implicated in various chronic diseases and aging processes. The high antioxidant activity  
240 suggests that the drying process did not significantly diminish the antioxidant potential of  
241 Parijoto. The total anthocyanin content of the dried Parijoto was found to be 538.47 ± 4.67  
242 ppm. Anthocyanins are a group of pigmented compounds known for their antioxidant  
243 properties and potential health benefits. The retention of anthocyanins after the drying process  
244 indicates that cabinet drying effectively preserved these bioactive compounds in the dried  
245 Parijoto

246 The parijoto fruit extract was obtained through an extraction process using the Ultra-assisted  
247 extraction method. The antioxidant and anthocyanin activity profiles of parijoto fruit extract.  
248 The characterization of Parijoto extract as a filler in nanoemulsion involved various analyses  
249 to assess its antioxidant properties and phytochemical composition. The extraction method  
250 employed was ultra-assisted extraction, which is known for its efficiency in extracting  
251 bioactive compounds from plant materials. The antioxidant activity of the Parijoto extract was  
252 evaluated, yielding a % inhibition value of 50.776±6.18. This indicates a significant level of  
253 antioxidant capacity, which is crucial for combating oxidative stress and preventing cellular  
254 damage caused by free radicals. Furthermore, the total anthocyanin content of the extract was  
255 determined to be 94.43±4.14 ppm. Anthocyanins are well-known antioxidants found in many  
256 fruits and vegetables, known for their potential health benefits, including anti-inflammatory  
257 and anti-cancer properties. The flavonoid content of the Parijoto extract was measured to be  
258 126.85±1.15 g/L. Flavonoids are a class of polyphenolic compounds known for their  
259 antioxidant and anti-inflammatory effects. Additionally, the phenolic content of the extract was  
260 quantified as 8.43±0.70 GAE/g. Phenolic compounds are another group of bioactive  
261 compounds found in plants, known for their antioxidant and anti-inflammatory activities, as  
262 well as their potential role in reducing the risk of chronic diseases.

81 conducted. This study is dedicated to investigating the characteristics of nanoemulsion formulations  
82 derived from parijoto fruit extract and evaluating an optimum condition with various tween  
83 surfactant.

## 84 **2 Materials and Method**

### 85 **2.1 Materials**

86 Grinder (Binder), Erlenmeyer (Pyrex), beaker glass (Pyrex), volume pipette, test tube (Pyrex), test  
87 tube rack, funnel (Pyrex), measuring flask (Pyrex), vacuum n filter 0.22  $\mu\text{m}$  (Sartorius Stedim 11694-  
88 2-50-06), vial, micropipette (Socorex), blue tip (Biologix 1 mL pipette tips), hotplate (Cimarec et al.  
89 SP142025Q), vortex (Thermolyne et al.), Ultrasonic Cleaner (Biobase UC-10SD) modified, UV-VIS  
90 spectrophotometer (Shimadzu, UV-1280), aluminium foil, filter paper, 0.22  $\mu\text{m}$  filter membrane  
91 (Wattman), Cabinet dryer (HetoPowerDry LL1500), rotary evaporator (Biobase RE-2000E), syringe,  
92 analytical balance. Fresh parijoto, ethanol pro analysis (Merck, Germany), methanol pro analysis  
93 (Merck, Germany), distilled water, aqua bikes, folding ciocaltcu 10% (Merck, Germany),  $\text{Na}_2\text{CO}_3$   
94 7.5% (Merck, Germany), DPPH solution (Merck, Germany), Quarcetin (Merck, Germany),  $\text{AlCl}_3$   
95 (Merck, Germany), ammonium acetate 1 M (Merck, Germany), acetone (Merck, Germany),  
96 acetonitrile (Merck, Germany), standard cyanide (Zigma), delphinidin glu standard (Zigma), Tween  
97 20 (Merck, Germany), Tween 60 (Merck, Germany), Tween 80 (Merck, Germany), and Span 20  
98 (Merck, Germany).

### 99 **2.2 Preparation of Dry Samples of Parijoto Fruit Extract**

100 Samples used in this study are fruits from the Parijoto plant (*Medinilla speciosa*) cultivated and  
101 harvested on the slopes of Mount Muria, Kudus. The fruits used are ripe fruits harvested when the  
102 Parijoto plant reaches full maturity, typically around 90-100 days after flowering. Parijoto, which had  
103 been cleaned and sorted, was weighed 200 grams for each treatment. The fruit that has been weighed  
104 is then steam-blanching for 3 minutes. Prepare a citric acid solution with a concentration of 1% for  
105 pre-treatment of fruit before drying. After that, soak the parijoto fruit in the citric acid solution for 5  
106 minutes and drain. The Cabinet Dryer is cleaned before use to maintain hygiene and avoid cross-  
107 contamination. The drying temperature used was 70°C for 6 hours. The dried Parijoto fruit is then  
108 ground into powder using a herbal grinder for 2 minutes. After that, the sample will be extracted for  
109 further testing. The dried Parijoto will be chemically analyzed using UV-Vis spectroscopy.

### 110 **2.3 Making Parijoto Extract using Ultrasonic Assisted Extraction (UAE)**

111 Five grams of dry sample powder and 50 mL of 99.5% ethanol were mixed thoroughly for  
112 homogeneity in four 250 mL centrifuge bottles. Then, all vials were sonicated (40 KHz, 100 W) for  
113 30 min, followed by shaking for one hour, centrifuged at 4,000 rpm (4°C) for 10 min, collected the  
114 supernatant, and evaporated to dryness under vacuum. The residue was dissolved in 99.5% ethanol  
115 and diluted to 20 mL. After filtering through a 0.22  $\mu\text{m}$  membrane filter, parijoto fruit extract was  
116 obtained and stored at -20°C for analysis using UV-Vis.

### 117 **2.4 Preparation of Anthocyanin Nanoemulsion from Parijoto Extract**

118 Approximately 3 mL of anthocyanin nanoemulsion with concentrations of 2 mg/mL, 4 mg/mL, and 6  
119 mg/mL, respectively, were prepared by collecting a portion of parijoto extract, and the solvent was  
120 removed with nitrogen. The solvent removal process during anthocyanin extraction can be monitored  
121 using a combination of visual inspection and periodic weight measurements. Visual inspection

11	23	10	5	51.967	±	0.007	-24.427	±	1.251	0.009	±	0.010	3.50	±	0.068	4.881	±	0.021	0.296	±	0.013
12	29	10	5	39.237	±	2.857	-24.275	±	1.493	0.009	±	0.001	0.91	±	0.042	4.810	±	0.013	0.308	±	0.005
13	29	10	6	32.54	±	4.231	-22.470	±	1.207	0.005	±	0.028	3.39	±	0.04	4.897	±	0.003	0.308	±	0.023
14	33	10	6	54.087	±	0.472	-22.787	±	1.465	0.075	±	0.017	0.52	±	0.09	4.887	±	0.015	0.275	±	0.017
15	34	10	6	54.037	±	0.585	-25.150	±	1.802	0.007	±	0.010	0.59	±	0.04	4.700	±	0.013	0.307	±	0.013
16	39	10	9	77.963	±	0.708	-26.172	±	1.718	0.011	±	0.001	0.51	±	0.13	4.813	±	0.011	0.275	±	0.002
17	39	10	9	75.743	±	1.105	-24.927	±	0.811	0.005	±	0.019	0.71	±	0.04	4.830	±	0.013	0.305	±	0.014
18	39	10	9	77.369	±	0.672	-26.285	±	1.538	0.016	±	0.011	0.69	±	0.04	4.760	±	0.013	0.318	±	0.011
19	39	12	5	33.88	±	11.323	-26.007	±	1.311	0.002	±	0.011	1.57	±	0.13	4.890	±	0.013	0.302	±	0.013
20	39	12	1	30.4	±	14.628	-26.025	±	1.865	0.000	±	0.017	0.66	±	0.17	4.773	±	0.012	0.308	±	0.017
21	39	17	8	45.967	±	1.007	-26.051	±	1.508	0.005	±	0.016	0.61	±	0.13	4.771	±	0.012	0.305	±	0.015
22	39	12	0	31.28	±	1.239	-27.477	±	0.805	0.001	±	0.010	0.73	±	0.13	4.810	±	0.013	0.305	±	0.019
23	39	12	0	110.88	±	13.52	-27.072	±	1.708	0.001	±	0.017	0.57	±	0.17	4.870	±	0.013	0.305	±	0.017
24	39	17	6	116.23	±	7.064	-27.120	±	0.305	0.077	±	0.011	0.72	±	0.20	4.890	±	0.017	0.377	±	0.012
25	39	12	6	114.880	±	17.138	-26.725	±	1.908	0.010	±	0.011	0.61	±	0.19	4.813	±	0.011	0.318	±	0.015
26	39	12	9	118.33	±	4.185	-26.025	±	0.135	0.015	±	0.014	0.55	±	0.19	4.857	±	0.009	0.373	±	0.014
27	39	17	9	124.187	±	4.182	-24.670	±	0.014	0.009	±	0.010	0.75	±	0.21	4.867	±	0.015	0.308	±	0.013

263 **3.2 Fitting Model for RSM (Response Surface Methodology) in Parijoto Fruit Extract Nanoemulsion**

264 Data recorded for each run included nanoemulsion particle size, polydispersity index, zeta potential, conductivity, pH, and viscosity. Each  
 265 variable was measured with three repetitions and the measurements three times to get consistent results. This data will be used to analyze the  
 266 influence of various factors on the characteristics of nanoemulsions using the Response Surface Methodology method, which can be seen in the  
 267 table.

268 Table 3. Design of Experiment RSM Particle Size, Poly Dispersity Index, Zeta Potential, Conductivity, pH, Viscosity in Nanoemulsion

No. Run Test	Dependent Variables			Independent Variables					
	Types of Lyophobic Detergent	Dween Concentration (%)	Parijoto Fruit Extract Concentration (%)	Nanoparticle Size (nm)	Zeta Potential	Conductivity	Poly Dispersity Index	pH	Viscosity (cP)
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	Y <sub>1</sub>	Y <sub>2</sub>	Y <sub>3</sub>	Y <sub>4</sub>	Y <sub>5</sub>	Y <sub>6</sub>
1	20	3	3	13,511 ± 0,329	-21,14 ± 1,514	0,071 ± 0,005	2,41 ± 0,05	7,517 ± 0,085	0,17 ± 0,005
2	20	3	10	10,2 ± 0,337	-21,473 ± 1,857	0,057 ± 0,005	2,51 ± 0,05	6,870 ± 0,093	0,07 ± 0,003
3	20	3	15	11,069 ± 0,378	-21,740 ± 1,973	0,065 ± 0,005	2,57 ± 0,04	6,590 ± 0,092	0,08 ± 0,003
4	20	3	0	12,061 ± 0,389	-21,515 ± 1,150	0,067 ± 0,005	2,35 ± 0,05	6,890 ± 0,093	0,07 ± 0,005
5	20	3	6	10,017 ± 0,595	-21,127 ± 2,542	0,069 ± 0,005	2,45 ± 0,07	7,112 ± 0,023	0,09 ± 0,003
6	20	3	9	11,181 ± 0,719	-21,181 ± 2,719	0,070 ± 0,005	2,51 ± 0,10	7,011 ± 0,097	0,11 ± 0,006
7	20	3	9	12,547 ± 0,685	-20,645 ± 2,787	0,074 ± 0,005	2,52 ± 0,15	6,897 ± 0,088	0,11 ± 0,003
8	20	3	9	14,343 ± 1,737	-20,477 ± 3,578	0,074 ± 0,017	2,59 ± 0,05	7,110 ± 0,035	0,14 ± 0,017
9	20	3	9	15,307 ± 0,312	-20,417 ± 2,699	0,080 ± 0,005	2,61 ± 0,04	6,817 ± 0,035	0,09 ± 0,003
10	20	10	3	15,671 ± 0,888	-20,487 ± 1,204	0,085 ± 0,013	2,61 ± 0,04	6,790 ± 0,029	0,08 ± 0,003

270 Table 3 shows that the particle size range of the nanoemulsion is between  $14,603 \pm 16.73$  nm  
271 and  $118,053 \pm 4,5825$  nm. The largest and smallest nanoparticle sizes found are 126.47 nm  
272 and 13.72 nm, respectively, with most nanoparticle sizes falling within the 50-100 nm range.  
273 Similar results were confirmed by Noor El-Din et al. (2017), who reported nanoemulsion  
274 sizes ranging from 31.58 to 220.5 nm. Studies conducted by Delmas et al., Liu et al., and Mei  
275 et al. using ultrasonication and high emulsification methods also confirmed comparable  
276 results of 45–170 nm, 222.4–166.4 nm, and 170–280 nm, respectively (Delmas et al., 2016;  
277 Liu et al., 2017; Mei et al., 2019). Conversely, Peng et al. (2010) reported a nanoparticle size  
278 range of 21–530 nm. Zeta potential reflects the surface charge of particles and affects  
279 colloidal stability. High zeta potential can prevent particle aggregation due to electrostatic  
280 repulsion. The research includes the evaluation and characterization of zeta potential under  
281 various treatments. The study obtained zeta potential results for nanoemulsion ranging from -  
282  $22.197 \pm 0.738$  mV to  $-28.207 \pm 1.598$  mV, respectively. Similar results were confirmed by  
283 Wessam et al. (2023), obtaining results of +21.5 mV. Particles with high ZP values, between  
284 20 and 40 mV, provide system stability and are less likely to aggregate or increase particle  
285 size. However, it should be noted that ZP values are not an absolute measure of nanoparticle  
286 stability. Furthermore, emulsions with ZP variations  $>10$  mV are suggested to have better  
287 stability (Kadu et al., 2011). The ideal potential range for nanoparticle stability is (-30 to 20  
288 mV or +20 to +30 mV) (Liu et al., 2018). The produced values tend to be harmful due to the  
289 influence of acetic acid, resulting in a negative charge. This charge causes electrostatic  
290 repulsion forces between formed nanoparticles to prevent aggregation into larger sizes  
291 (Luthifayana et al., 2022). Higher zeta potential values increase nanoparticle stability due to  
292 higher electrostatic repulsion forces between nanoparticles.

293 Conductivity provides information about the ability of nanoemulsions to conduct electricity.  
294 Changes in conductivity can occur with changes in surface particle charge. Table 18 shows  
295 that the nanoemulsion conductivity of Parijoto fruit extract ranges from 0.03458 to 0.09987  
296 mS/cm. Good nanoemulsion conductivity measurements have higher electrical conductivity  
297 values (10–100  $\mu$ S/cm) (Akilu et al., 2019; Guo et al., 2016; Khader et al., 2016). Electrical  
298 conductivity values tend to decrease with decreasing water content in the emulsion. O/W type  
299 (Oil-in-Water) nanoemulsions have higher conductivity than W/O type (Water-in-Oil)  
300 nanoemulsions. This is because the more extensive water phase provides more pathways for  
301 ion conduction.

302 The type and concentration of surfactant in nanoemulsion can influence conductivity.  
303 Surfactants can provide ionic charge or facilitate ion conduction in the system. Viscosity is an  
304 essential parameter in evaluating the flow properties of nanoemulsion. Viscosity is one of the  
305 parameters used to determine the stability of polymers in a solution because it undergoes  
306 reduction during polymer storage due to polymer degradation (Aranaz et al., 2021). In this  
307 study, as shown in Table 1, the viscosity of nanoparticles ranges from 3,810 cP to 4,433 cP.  
308 Alemu et al. (2023) stated that viscosity can depend on particle size and storage time.  
309 Appropriate viscosity can affect the applicability and spread of the system. The viscosity of a  
310 preparation is related to the consistency and spreadability of the preparation, which will affect  
311 ease of use (Imanto et al., 2019). Viscosity values are influenced by several factors, such as  
312 temperature, pH, manufacturing conditions, and the quality and concentration of raw  
313 materials (Naini & Yusuf, 2018). The results of viscosity tests are shown in centipoise (cP).  
314 The higher the viscosity value of a preparation, the better the stability of the product, but the  
315 preparation will be difficult to apply to the skin, and the resistance of the preparation to flow  
316 will increase, making it difficult to remove from the container (Thakre, 2017). Meanwhile,  
317 low viscosity values will increase the flowability of the skin and make it easier to apply to the  
318 skin (Naini & Yusuf, 2018)



319 This ANOVA table is essential to evaluate the statistical significance of each model component and determine whether the quadratic model used  
 320 is good enough to explain the characteristics of the nanoemulsion or not. The p-value is used to determine statistical significance, and the  
 321 analysis results will help select an appropriate model and interpret the significance of factors that influence the characteristics of nanoemulsions,  
 322 which can be seen in the table.

323 Table 4. ANOVA (Analysis of Variance) for the RSM Quadratic Model Particle Size, Poly Dispersity Index, Zeta Potential, Conductivity, pH,  
 324 Viscosity in Nanoemulsion

Quadratic Model Equation	Sources of Variation	p-Value
<b>Particle Size</b> ( $R^2$ : 0,558 $R^2_1$ : 0,50156) $Y_1 = -0,000008 - 0,000069X_1 + 0,000040X_2 + 0,000032X_3 + 0,000056X_1^2 +$ $0,000064X_2^2 - 0,000003X_3^2 - 0,000056X_1X_2 - 0,000044X_2X_3 + 0,000065X_1X_3$	Model  <i>Lack of fit</i>	0,294 *  0,185
<b>Poly Dispersity Index</b> ( $R^2$ : 0,3643 $R^2_1$ : 0,2471) $Y_2 = 6,23086 + 0,58801 X_1 - 0,75655 X_2 + 84,3654 X_3 + 24,65 X_1^2 + 18,7663 X_2^2$ $20,744 X_3^2 + 23,0025 X_1X_2 + 26,3043 X_2X_3 + 9,5269 X_1X_3$	Model  <i>Lack of fit</i>	0,041*  0,692
<b>Zeta Potential</b> ( $R^2$ : 0,54003 $R^2_1$ : 0,56905) $Y_3 = 0,000062 - 0,000023 X_1 - 0,000010 X_2 + 0,000008 X_3 + -0,000007 X_1^2 +$ $0,000003 X_2^2 + 0,000008 X_3^2 + -0,000006 X_1X_2 - 0,000008 X_2X_3 + -0,000005 X_1X_3$	Model  <i>Lack of fit</i>	0,000*  0,980
<b>Conductivity</b> ( $R^2$ : 0,2444 $R^2_1$ : 0,3464) $Y_4 = 4035,80 - 1198,06X_1 + 833,22X_2 - 1083,49X_3 - 2597,39X_1^2 - 709,42X_2^2$ $+ 881,10X_3^2 + 305,68X_1X_2 - 700,69X_1X_3 - 943,96X_2X_3$	Model  <i>Lack of fit</i>	0,0004*  0,928
<b>pH</b> ( $R^2$ : 0,832 $R^2_1$ : 0,797) $Y_5 = 0,003122 - 0,000040X_1 - 0,000060X_2 + 0,000039X_3 - 0,000034X_1^2 +$ $0,000047X_2^2 + 0,000031X_3^2 - 0,000006X_1X_2 - 0,000015X_1X_3 + 0,000031 X_2X_3$	Model  <i>Lack of fit</i>	0,000*  0,067
<b>Viskositas</b> ( $R^2$ : 0,95976 $R^2_1$ : 0,95466) $Y_6 = 0,015177 - 0,009573X_1 - 0,003288X_2 - 0,000624X_3 - 0,008334X_1^2 -$ $0,000266X_2^2 - 20,744 X_3^2 + 23,0025 X_1X_2 + 26,3043 X_2X_3 + 9,5269 X_1X_3$	Model  <i>Lack of fit</i>	0,000*  0,103

325 Notes:  
 326 - \*: The model has a statistically significant effect (p<0.05)  
 327 - \*\*: Model mismatch or lack of fit occurs (p<0.05)

328 Based on the ANOVA RSM analysis of three factors, namely the type of Tween in  
329 nanoemulsion, Tween concentration, and Parijoto extract concentration, all ANOVA values  
330 show probabilities  $<0.0001$  ( $p<0.05$ ). This indicates that the quadratic response surface model  
331 used for both responses (dependent variables) is significant and can be used to optimize  
332 extraction factors (Wang et al., 2014). The coefficient of determination, or R square, depicts  
333 how independent data can explain dependent data. The range of R square values is between 0  
334 and 1, where values closer to 1 indicate better explanatory power.

335  
336 In the Central Composite Design analysis, the p-value indicates the significance of each  
337 coefficient in the built polynomial regression model. The lower the p-value, the more  
338 significant the contribution of the coefficient to the overall regression model (Zhong &  
339 Wang, 2010). It is important to note that using experimental data within the allowed range of  
340 variables in this study to create mathematical equations, which may have broader general  
341 applications, can provide the ability to predict system behavior when different factors are  
342 combined. From the perspective of optimizing the formation of emulsion nanoparticles, there  
343 is potential to develop more significant results, possibly based on the variables investigated in  
344 this study. Additionally, this optimization may be performed using the techniques outlined in  
345 this research to further test the effects of time and temperature or other conditions, as needed.

346 Table 4 shows details of the RSM approach used to assess particle size (nm), Poly Dispersity  
347 Index, Zeta Potential (mv), Conductivity, pH, and viscosity (Cp) in nanoemulsion of Parijoto  
348 fruit extract involved in a series of 81 experiments based on factorial design. The coefficients  
349 for the second-degree polynomial Equation are determined through experimental results,  
350 along with the regression coefficients for Particle Size (Y1), Poly Dispersity Index (Y2), Zeta  
351 Potential (Y3), Conductivity (Y4), pH (Y5), and viscosity (Y6). The Equation presented as  
352 Equation (2) shows the full quadratic model, while Table X shows the models predicting the  
353 response of the independent variables (Y1–Y6).

354 To assess the extent to which the equation model in RSM fits the data and how strong the  
355 influence of the variables is, the coefficient of determination or ( $R^2$ ) is used. Chin (1998) has  
356 categorized that for model suitability, the R-Square value is substantial if it is more than 0.67,  
357 moderate if it is more than 0.33 but lower than 0.67, and weak if it is more than 0.19 but  
358 lower than 0.33. pH and viscosity indicate strong model adequacy on these response  
359 variables. In contrast, the responses of Particle Size, Poly Dispersity Index, Zeta Potential,  
360 and Conductivity indicate a moderate model for these response variables. A lack of fit test  
361 was then performed to assess model fit for each response. With a p-value exceeding 0.05, it  
362 was confirmed that the model adequately fit the experimental data, as seen in Table 4.

### 363 **3.3 Contour plot on Particle Size, poly-dispersity index, Zeta Potential, Conductivity,** 364 **pH, and Viscosity as a function of Nanoemulsion Parijoto Fruit Extract.**

365 In this research, the model is created as a Contour plot, which can show the response: Particle  
366 Size, Poly Dispersity Index, Zeta Potential, Conductivity, pH, and Viscosity. Continued  
367 research shows a significant relationship between particle size and tween concentration and  
368 the type of lipophilic tween in nanoemulsions, as shown in Figures 1-6 the presented data  
369 offers valuable insights into the influence of lipophilic tween type and tween concentration  
370 on various properties of the nanoemulsion derived from parijoto fruit extract. Each figure  
371 depicts the contour plots illustrating the interaction effects between these two factors on  
372 different characteristics of the nanoemulsion.

373 In Figure 1, the contour plot demonstrates the interaction between the lipophilic tween type  
374 and tween concentration in controlling nanoparticle size. It reveals that as the lipophilic  
375 tween type increases from 20 to 80, and the tween concentration rises from 8% to 10%, there  
376 is a general trend of increasing particle size, albeit with a slight decreasing trend observed to  
377 some extent. This suggests that both factors play a role in determining the nanoparticle size,  
378 with higher concentrations leading to larger particle sizes. Moving to Figure 2, which  
379 illustrates the Zeta Potential of the nanoemulsion, an increase in the lipophilic Tween type  
380 from 60 to 80 and an increase in tween concentration from 8% to 10% correspond to an  
381 increase in Zeta Potential. Interestingly, no further changes are observed beyond this point.  
382 This indicates that these specific conditions result in optimal Zeta Potential, possibly  
383 indicating enhanced stability of the nanoemulsion.

384 Figure 3 showcases the influence of lipophilic tween type and tween concentration on the  
385 conductivity of the nanoemulsion. As the lipophilic tween type increases from 20 to 80 and  
386 the tween concentration rises from 8% to 12%, there is a consistent increase in conductivity  
387 without any further changes. This suggests a direct relationship between these factors and the  
388 conductivity of the nanoemulsion. The Contour plot presented in Figure 4 demonstrates the  
389 effect of lipophilic tween type and tween concentration on the Poly Dispersity Index (PDI) of  
390 the nanoemulsion. Interestingly, an increase in lipophilic tween type from 60 to 80 and a  
391 decrease in tween concentration from 12% to 8% lead to an increase in PDI value without  
392 further changes. This indicates a complex interaction between these factors in determining the  
393 homogeneity of particle size distribution within the nanoemulsion.

394 Figure 5 depicts the pH contour plot of the parijoto fruit extract nanoemulsion. An increase in  
395 lipophilic Tween type from 20 to 80 and an increase in tween concentration from 8% to 12%  
396 result in a consistent increase in pH without any further changes. This observation suggests  
397 that these specific conditions contribute to the alkalinity of the nanoemulsion, which may  
398 have implications for its stability and functionality. Finally, Figure 6 illustrates the viscosity  
399 contour plot of the nanoemulsion. An increase in lipophilic tween type from 35 to 80 and an  
400 increase in tween concentration from 8% to 12% lead to an increase in viscosity without  
401 further changes. This indicates that higher concentrations of lipophilic tween and tween result  
402 in a thicker consistency of the nanoemulsion, which affects its flow properties and  
403 application. The presented data highlights the intricate relationship between lipophilic tween  
404 type and tween concentration in influencing various physicochemical properties of the  
405 nanoemulsion derived from parijoto fruit extract. These findings provide valuable insights for  
406 optimizing the formulation and manufacturing process of the nanoemulsion for potential  
407 applications in various industries.

408 Research on the influence of surfactant type and concentration on nanoemulsion indicates  
409 that the selection of surfactant significantly affects the characteristics of nanoemulsion.  
410 Various surfactant types, such as Tween 20, Tween 60, and Tween 80, play different roles in  
411 forming nanoemulsions. The research results show that the particle size of Tween 80  
412 surfactant is the highest, with an average particle size of 107.196 nm. Similar results were  
413 reported by Chang et al. (2013), who obtained the smallest droplets in carvacrol-based  
414 nanoemulsion made with a mixture of food-grade non-ionic surfactants (Tween 20, 40, 60,  
415 80, and 85). Tween, a non-ionic surfactant derived from sorbitan ester, is soluble or  
416 dispersible in water and is commonly used as an oil-in-water emulsifier in the  
417 pharmaceutical, cosmetic, and cleaning industries. Among these surfactants, Tween 80 is one  
418 of the most commonly used. Research by Douglas et al. (2013) confirms that the type of non-  
419 ionic surfactant significantly influences the average particle diameter of the formed colloid  
420 dispersion. The smallest droplets were observed in systems prepared using Tween 80, while

421 the largest droplets formed in systems using Tween 85. The surfactant's Hydrophilic-  
422 Lipophilic Balance (HLB) plays a role in forming small particles. Surfactants with either too  
423 high (Tween 20) or too low (Tween 85) HLB values cannot form optimal nanoemulsions.  
424 Tween types with intermediate HLB values (Tween 40, 60, and 80) can form nanoemulsions  
425 with small particle sizes. However, there is no strong correlation between HLB values and  
426 particle sizes produced by these surfactants (Kumar et al., 2008). Small-molecule surfactants  
427 have higher surface activity and form smaller emulsion droplets than large ones (Qian &  
428 McClements, 2011; Teo, Goh & Lee, 2014).

429 Another critical factor for minimal droplet emulsion formation is the Hydrophilic-Lipophilic  
430 Balance (HLB) value of the surfactant (Sagitani, 1981), defined by Griffin as the ratio of  
431 surfactant hydrophilicity to lipophilicity (Griffin, 1949). A high HLB value indicates strong  
432 hydrophilicity, and the HLB values of non-ionic surfactants generally range from 0 to 20  
433 (Gad & Khairon, 2008), such as Tween 20 (HLB 16.7) and Tween 80 (HLB 15) (Dinarvand,  
434 Moghadam, Sheikhi & Atyabi, 2005). Emulsion stability is influenced by two polymer and  
435 particle surface tension mechanisms: steric stability caused by macromolecules adsorbed on  
436 particle surfaces and electrostatic stability due to repulsion between surface-charged droplets.  
437 In nanoemulsions made with Tween 80 surfactant, the surfactant may not have a charge on  
438 the hydrophobic group, causing the covered droplet surface to be non-charged and resulting  
439 in low zeta potential values, which can lead to increased particle size and PDI (Lian et al.,  
440 2016).

441 However, a different study proposed by Alam et al. (2023) suggests that Tween 20 helps  
442 improve PDI and allows for minimum polydispersity. Compared to other nanoparticles, the  
443 ability to maintain particle integrity using Tween 20 is significant. Increasing the Surfactant  
444 content in the formulation increases the polydispersity indices for natural extracts in the 3D  
445 response surface graph. This indicates that the use of Tween types with low and high HLB  
446 values can be applicable when combined with an optimal concentration of co-surfactant.

447 Surfactant concentration is also a critical factor in nanoemulsion formation. Research  
448 indicates that increasing surfactant concentration can result in smaller and more homogenous  
449 size distribution. However, there is a specific limit where surfactant concentration reaches a  
450 plateau level, leading to unadsorbed surfactant aggregation and micelle formation. The results  
451 show that the higher the Tween concentration, the higher the size and PDI. This is confirmed  
452 by Liat et al. (2016), stating that nanoemulsions prepared with higher surfactant  
453 concentrations significantly increase short-term stability. Systems with 15 or 20% weight of  
454 Tween 80 are highly unstable to increasing dilution, indicating that a medium surfactant  
455 concentration level may be more suitable for stable nanoemulsion preparation. Although the  
456 initial droplet size is small, higher surfactant concentrations can increase raw material costs  
457 and cause undesirable sensory (taste) issues in commercial applications. Therefore, this study  
458 uses a 10% weight of Tween 80 in further experiments.

459 Increasing surfactant concentration increases the number of surfactant molecules migrating  
460 from the oil phase to the emulsion water phase, and nanodroplets form. Frictional forces  
461 applied to the oil-water interface, coated with emulsifier, cause some emulsifiers to sink  
462 parallel to the surface layer, while others detach from the surface layer. Hasani et al. (2015)  
463 reported that droplet size increases by increasing surfactant concentration to 20%, and  
464 particles have a broad and non-uniform size distribution. The instability of nanoemulsion at  
465 high surfactant concentrations may be related to the depletion-flocculation mechanism of  
466 adsorbed surfactant. With increased surfactant concentration, additional surfactant molecules  
467 form micelles in the continuous phase rather than orienting on the particle surface. This leads  
468 to an increase in local osmotic pressure, causing the continuous phase between moving

469 droplets to decrease, reducing the continuous phase between those droplets. As a result,  
470 aggregation occurs, causing an increase in particle size. According to Oh et al. (2011) and  
471 Tadros et al. (2004), the average droplet size becomes smaller, and the size distribution  
472 becomes narrower with increasing emulsifier concentration, ultimately reaching a plateau  
473 level. Beyond the plateau level, free or unadsorbed emulsifiers may accumulate to form  
474 micelles. Nanoemulsions are known to be thermodynamically unstable, tending to minimize  
475 interfacial area through coalescence.

476 An increase in the concentration of the filler extract can lead to the tendency of nanoparticles  
477 to aggregate or form agglomerates also pH nanoemulsion. This phenomenon may occur due  
478 to physical or chemical interactions between nanoparticles and compounds in the filler  
479 extract. Findings by Alab et al. (2021) suggest that an increase in extract concentration results  
480 in an increase in particle size, particularly at the highest concentration of 347.2 nm. On the  
481 other hand, the smallest concentration has the lowest particle size at 86.98 nm. These results  
482 indicate that higher concentrations may increase the likelihood of particle agglomeration.

483 Furthermore, increasing the concentration of parijoto fruit extract can increase the total mass  
484 in the solution, which, in turn, can increase overall viscosity. Additional particles or  
485 molecules from the filler extract can contribute to the increase in viscosity. A study by Olan  
486 et al. (2021) shows that particles with the highest concentration have the highest viscosity and  
487 vice versa. This increase in viscosity may be caused by excess extract loaded into particles.  
488 The physicochemical characteristics of the filler extract may influence the viscosity  
489 properties of nanoparticles, and factors such as changes in pH, temperature, or chemical  
490 composition may also play a role in viscosity increase. Parijoto fruit is rich in active  
491 compounds, such as anthocyanins, which can affect the surface charge of nanoemulsion  
492 particles. At a certain pH, anthocyanins or other components may have specific charges that  
493 can influence the electrostatic stability of particles (Liu et al., 2016). Anthocyanins may  
494 undergo solubility changes at specific pH values, affecting the distribution and stability of the  
495 nanoemulsion's oil or water phase. The same occurs with surfactants, where variations in  
496 charge of the filler extract from parijoto fruit can affect the interaction between nanoparticles,  
497 anthocyanins, and other components in the system. The loading capacity of the extract in the  
498 nanoemulsion likely depends on its solubility in the system used (Costa et al., 2012).  
499 Anthocyanins tend to undergo color changes with pH (pH-dependent color shift).  
500 Additionally, the antioxidant activity of anthocyanins can be influenced by pH. This  
501 complexity can modulate the overall physicochemical properties of the nanoemulsion system.  
502

503 **3.4 Optimal Point Prediction from RSM in Nanoemulsion Parijoto Fruit Extract**

504 Optimal point predictions from the Response Surface Methodology are obtained by  
 505 combining optimal conditions based on interactions between independent variables. Profiler  
 506 predictions are obtained if the fitted surface graph is in minimum, maximum, or saddle form.  
 507 3D graphics on image x. shows a complex interaction between the variable factors of  
 508 lipophilic tween type and tween concentration on the response. Increasing the lipophilic  
 509 tween type value increases the response somewhat, but the tween concentration value can  
 510 modify the effect. There is an optimal region where the response reaches its peak. The  
 511 implication for practice is that by setting the variable factors at levels that are estimated to be  
 512 optimum, the research results can achieve the highest optimization in the desired response,  
 513 which can be seen in Figure 7.

514 Table 3. Prediction of Optimum Conditions for Parijoto Fruit Extract Nanoemulsion

Types of Analysis	Types of Lyphophilic Tweens	Tween Concentration (%)	Parijoto Fruit Extract Concentration (%)	Nanoparticle Size (nm)	Zeta Potential(mV)	Conductivity (mS/cm)	Poly Dispersity Index
<b>Optimum Condition Prediction</b>	80	12	7.5	61.97	-28.48	0.082	0.691
<b>Maximum Value at Optimum Conditions</b>	80	12	7.5	39.94	-32.48	0.048	0.371
<b>Minimum Value at Optimum Conditions</b>	80	12	7.5	163.88	-26.37	0.115	1,011

515

516 It can be seen in Table 9 that to achieve the maximum desired concentration of nanoparticle  
 517 size, zeta potential, Conductivity, Poly Dispersity Index, degree of acidity, and Viscosity, it is  
 518 necessary to set the Tween solvent concentration to 80, Tween concentration to 12% and  
 519 Parijoto fruit extract concentration to 7.5%. This set of conditions has a desirability value of  
 520 0.74. Because the value is almost close to 1 and falls into the moderate category, this set of  
 521 conditions is quite optimal for the aim of this research, namely to maximize the response.

522 The optimization of nanoemulsion formation from Parijoto fruit extract using Response  
 523 Surface Methodology (RSM) has been conducted in this study. RSM is a statistical method  
 524 used to design experiments and analyze the impact of multiple independent variables on a  
 525 measured response. As an output of this research, the synthesis process conditions of  
 526 nanoemulsion from Parijoto fruit extract can be optimized to achieve particle size,  
 527 polydispersity index (PDI), zeta potential, conductivity, pH, and viscosity levels. RSM  
 528 determines the optimal extraction time and temperature to maximize the response variable  
 529 outcomes (Granato et al., 2014). In line with this, predictions and observations are within a  
 530 narrow range and do not show significant differences at a 5% significance level, indicating  
 531 the model's suitability for optimization and process efficiency purposes.

532

533 The optimal point prediction from the Response Surface Methodology is obtained by  
534 integrating optimal conditions and depends on the interaction between independent variables,  
535 as Ratnawati et al. (2018) explained. The prediction profile is formed when the adjusted  
536 surface graphs show a minimum, maximum, or saddle shape. The optimization process can  
537 achieve optimal responses by analyzing each response beforehand, ultimately reducing effort  
538 and operational costs, as Nurmiah et al. (2013) stated. Desirability, with a range of values  
539 from 0 to 1, is used as the optimization target value, with low (0-0.49), moderate (0.5-0.79),  
540 and high (0.8-1) categories. The closer the value of 1 is, the greater the desirability, which  
541 indicates the suitability of the combination of process parameters to achieve optimal response  
542 variables.

543

544 It can be seen in Table 3 that to achieve the desired concentrations of nanoparticle size, zeta  
545 potential, conductivity, polydispersity index, acidity level, and viscosity, it is necessary to set  
546 the concentration of Tween 80, Tween concentration at 12%, and Parijoto fruit extract  
547 concentration at 7.5%. This set of conditions has a desirability value of 0.740349. Since its  
548 value is close to 1 and falls into the moderate category, this set of conditions is quite optimal  
549 for this research, which is to maximize the response

#### 550 **4 Conclusion**

551 In this series of experiments, nanoemulsion from parijoto fruit has been characterized, considering  
552 various physicochemical parameters such as particle size, polydispersity index, zeta potential,  
553 conductivity, pH, and viscosity. The research results indicate significant variations in the physical  
554 characteristics of both nanomaterials in terms of changes in surfactant and parijoto extract  
555 concentrations. Increased surfactant concentration tends to produce smaller particle sizes and a more  
556 homogeneous distribution, although certain limitations were found that lead to surfactant aggregation  
557 and micelle formation. The nanoemulsion characteristics, including zeta potential, polydispersity,  
558 particle size, conductivity, pH, and viscosity. The type and concentration of surfactants played a  
559 crucial role in determining the properties of the nanoemulsions. Variations in surfactant parameters  
560 resulted in observable differences in emulsion characteristics, highlighting the importance of  
561 surfactant selection and optimization. To achieve optimal nanoemulsion process conditions, it is  
562 recommended to use 12% Tween 80 solvent concentration, 12% Tween concentration, and 7.5%  
563 parijoto fruit extract concentration, resulting in a desirability value of 0.74, falling into the moderate  
564 category.

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## 4. Revision from Reviewer 2

- Interactive Discussion Proof

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 Reviewer 2 | 07 Apr 2024 | 03:48

#1

Overall, this research encourages exploring potential natural resources as producers of natural antioxidants from Indonesian plants.

Some revisions are needed to strengthen the discussion of this research. In detail, the revisions are as follows:

1. A discussion of linkages between all observed parameters needs to be explained. So, it is easy for readers to understand the direction and results of the research obtained quickly.
2. The main ingredient, parijoto, should be mentioned in detail, along with the fruit's origin specifications and maturity level. It has been known that the fruit's maturity will affect the anthocyanin content and other antioxidant components.
3. In the anthocyanin extraction part, the solvent removal step is done using nitrogen. When will this step be stopped? Please elaborate in detail so that readers can repeat this method if needed. (line 109)
4. Previous research related to nanoencapsulation can be added in the Introduction to strengthen the importance of this research.
5. Why is RSM used? The background of the RSM and its advantages should be mentioned in the abstract and introduction.
6. HPLC test results for phenol compounds have yet to be shown in the manuscript.
7. Three-dimensional images of RSM test results showing interactions between factors are commonly shown in the discussion to clarify the discussion.
8. In the Material section, an HPLC tool is used, but there is no explanation of the HPLC method, so it needs to be added to the method
9. The discussion section also needs to show the results and discussion for the HPLC test results. We suggest adding an HPLC test results table along with the discussion in the section discussion.
10. Table 1 of the RSM Design of Experiment, can be presented more concisely.
11. This study aimed to explore the surfactant's capability in forming anthocyanin nano emulsions from Parijoto fruit extract and assess the emulsion's characteristics, including pH, light, temperature, turbidity, emulsion type, and stability. However, the results, discussion, conclusion, and abstract do not explicitly describe this point. Optimal process conditions were not the objective of this research, but this was the conclusion of the results.

## 5. Submission of Revised Manuscript 2

- Interactive Discussion Proof

Corresponding Author: Victoria Kristine Ananingsih | 19 Apr 2024 | 09:01 #2

1. A discussion of linkages between all observed parameters needs to be explained. So, it is easy for readers to understand the direction and results of the research obtained quickly.

We have explained the relationships between the observed parameters in the discussion (Line 471 - 475) (Line 482-488) (Line 499-504)

Surfactant concentration is also a critical factor in nanoemulsion formation. Research indicates that increasing surfactant concentration can result in smaller and more homogeneous size distribution. However, there is a specific limit where surfactant concentration reaches a plateau level, leading to unadsorbed surfactant aggregation and micelle formation. The results show that the higher the Tween concentration, the higher the size and PDI. This is confirmed by Liat et al. (2016), stating that nanoemulsions prepared with higher surfactant concentrations significantly increase short-term stability. Systems with 15 or 20% weight of Tween 80 are highly unstable to increasing dilution, indicating that a medium surfactant concentration level may be more suitable for stable nanoemulsion preparation. Although the initial droplet size is small, higher surfactant concentrations can increase raw material costs and cause undesirable sensory (taste) issues in commercial applications. Therefore, this study uses a 10% weight of Tween 80 in further experiments.

With increased surfactant concentration, additional surfactant molecules form micelles in the continuous phase rather than orienting on the particle surface. This leads to an increase in local osmotic pressure, causing the continuous phase between moving droplets to decrease, reducing the continuous phase between these droplets. As a result, aggregation occurs, causing an increase in particle size.

An increase in the concentration of the filler extract can lead to the tendency of nanoparticles to aggregate or form agglomerates also pH nanoemulsion. This phenomenon may occur due to physical or chemical interactions between nanoparticles and compounds in the filler extract. Findings by Alab et al. (2021) suggest that an increase in extract concentration results in an increase in particle size, particularly at the highest concentration of 340.2 mg. On the other hand, the smallest concentration has the lowest particle size at 86.98 nm. These results indicate that higher concentrations may increase the likelihood of particle agglomeration.

At a certain pH, anthocyanins or other components may have specific charges that can influence the electrostatic stability of particles (Liu et al., 2016). Anthocyanins may undergo solubility changes at specific pH values, affecting the distribution and stability of the nanoemulsion's oil or water phase. The same occurs with surfactants, where variations in charge of the filler extract from parijoto fruit can affect the interaction between nanoparticles, anthocyanins, and other components in the system. The

2. The main ingredient, parijoto, should be mentioned in detail, along with the fruit's origin specifications and maturity level. It has been known that the fruit's maturity will affect the anthocyanin content and other antioxidant components.

We already added the information about parijoto fruit in line 100-102.

Samples used in this study are fruits from the Parijoto plant (*Medinilla speciosa*) cultivated and harvested on the slopes of Mount Meru, Kuala. The fruits used are ripe fruits harvested when the Parijoto plant reaches full maturity, typically around 90-100 days after flowering.

3. In the anthocyanin extraction part, the solvent removal step is done using nitrogen. When will this step be stopped? Please elaborate in detail so that readers can repeat this method if needed. (line 109)

We have added the method in more detail as written in line 120 - 127.

The solvent removal process during anthocyanin extraction can be monitored using a combination of visual inspection and periodic weight measurements. Visual inspection involves observing the extract as the solvent

- Supporting File

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We have added the method in more detail as written in line 120 - 127.

The solvent removal process during anthocyanin extraction can be monitored using a combination of visual inspection and periodic weight measurements. Visual inspection involves observing the extract as the solvent evaporates, noting its increasing concentration evidenced by a thicker and more viscous appearance. Periodic weighing of the container or flask containing the extract allows for the tracking of weight loss as the solvent evaporates. Once the weight stabilizes or reaches a predetermined target, it signifies that the desired solvent removal rate has been attained, ensuring the production of a concentrated anthocyanin extract suitable for further analysis.

4. Previous research related to nanoencapsulation can be added in the Introduction to strengthen the importance of this research.

We have added in the manuscript (Line 73-78)

Appropriate nano-encapsulation techniques, such as nanoemulsion, have shown the potential to enhance the stability, bioavailability, and solubility of lipophilic bioactive compounds while also preventing hydrolysis and oxidation (Rosso et al., 2020). Catechin nanoemulsion showed a remarkable improvement of stability and bioavailability in simulated gastrointestinal (Rafanar et al., 2016). Research conducted by Chang et al. (2022) used Tween as surfactant in the stable nanoemulsion synthesis loaded curcumin extract.

5. Why is RSM used? The background of the RSM and its advantages should be mentioned in the abstract and Introduction.

We have added in the abstract (Line 25-27)

RSM is used to optimize nanoemulsion by examining the relationships and interactions between independent variables and response variables through mathematical modeling and statistical methods.

We have added in the introduction (Line 66-72 )

This study aimed to investigate the characteristics of nanoemulsion formulations derived from parijoto fruit extract and evaluate an optimum condition with various tween surfactant.

Thus far, research on nanoemulsion formulation in parijoto fruit, incorporating various concentrations and stabilizers, still needs to be conducted. This study is dedicated to investigating the characteristics of nanoemulsion formulations derived from parijoto fruit extract and evaluating an optimum condition with various tween surfactant.

6. HPLC test results for phenol compounds have yet to be shown in the manuscript.

We did not do the phenol analysis using HPLC in this research. Therefore, we did not mention it. However, we have mentioned Phytochemical Profiles of Dried Parijoto Fruit in section 3.1. (Line 232-262).

Drying Parijoto Fruit is carried out using a cabinet dryer at a temperature of 70°C for 6 hours. The results of drying parijoto fruit were obtained through the preparation process, the antioxidant and anthocyanin activity profiles were expressed respectively in units of % inhibition and ppm. The results of the antioxidant activity of dried and extracted parijoto fruit were 79.14334.82%. % The total anthocyanin content in the dry samples and extracts was 538.47 ± ppm. The dried Parijoto exhibited significant antioxidant activity, with a % inhibition value of 79.14 ± 34.82. This indicates a substantial capacity to neutralize free radicals, which are implicated in various chronic diseases and aging processes. The high antioxidant activity suggests that the drying process did not significantly diminish the antioxidant potential of Parijoto. The total anthocyanin content of the dried Parijoto was found to be 538.47 ± 4.67 ppm. Anthocyanins are a group of pigmented compounds known for their antioxidant properties and potential health benefits. The retention of anthocyanins after the drying process indicates that cabinet drying effectively preserved these bioactive compounds in the dried Parijoto

The parijoto fruit extract was obtained through an extraction process using the Ultra-assisted extraction method. The antioxidant and anthocyanin activity profiles of parijoto fruit extract. The characterization of Parijoto extract as a filler in nanoemulsion involved various analyses to assess its antioxidant properties and phytochemical composition. The extraction method employed was ultra-assisted extraction, which is known for its efficiency in extracting bioactive compounds from plant materials. The antioxidant activity of the Parijoto extract was evaluated, yielding a % inhibition value of 50.776±6.18. This indicates a significant level of antioxidant capacity, which is crucial for combating oxidative stress and preventing cellular damage caused by free radicals. Furthermore, the total anthocyanin content of the extract was determined to be 94.43±4.14 ppm. Anthocyanins are well-known antioxidants found in many fruits and vegetables, known for their potential health benefits, including anti-inflammatory and anti-cancer properties. The flavonoid content of the Parijoto extract was measured to be 126.85±1.15 g/L. Flavonoids are a class of polyphenolic compounds known for their antioxidant and anti-inflammatory effects. Additionally, the phenolic content of the extract was quantified as 8.43±0.70 GAE/g. Phenolic compounds are another group of bioactive compounds found in plants, known for their antioxidant and anti-inflammatory activities, as well as their potential role in reducing the risk of chronic diseases.

7. Three-dimensional images of RSM test results showing interactions between factors are commonly shown in the discussion to clarify the discussion.

A three-dimensional image of the RSM test results showing the interactions between common factors has been displayed and explained in the discussion. (Line 373-416)

In this research, the model is created as a Contour plot, which can show the response: Particle Size, Poly Dispersity Index, Zeta Potential, Conductivity, pH, and Viscosity. Continued research shows a significant relationship between particle size and tween concentration and the type of lipophilic tween in nanoemulsions, as shown in Figures 1-6 the presented data offers valuable insights into the influence of lipophilic tween type and tween concentration on various properties of the nanoemulsion derived from parijoto fruit extract. Each figure depicts the contour plots illustrating the interaction effects between these two factors on different characteristics of the nanoemulsion.

In Figure 1, the contour plot demonstrates the interaction between the lipophilic tween type and tween concentration in controlling nanoparticle size. It reveals that as the lipophilic tween type increases from 20 to 80, and the tween concentration rises from 8% to 10%, there is a general trend of increasing particle size, albeit with a slight decreasing trend observed to some extent. This suggests that both factors play a role in determining the nanoparticle size, with higher concentrations leading to larger particle sizes. Moving to Figure 2, which illustrates the Zeta Potential of the nanoemulsion, an increase in the lipophilic Tween type from 60 to 80 and an increase in tween concentration from 8% to 10% correspond to an increase in Zeta Potential. Interestingly, no further changes are observed beyond this point. This indicates that these specific conditions result in optimal Zeta Potential, possibly indicating enhanced stability of the nanoemulsion.

Figure 3 showcases the influence of lipophilic tween type and tween concentration on the conductivity of the nanoemulsion. As the lipophilic tween type increases from 20 to 80 and the tween concentration rises from 8% to 12%, there is a consistent increase in conductivity without any further changes. This suggests a direct relationship between these factors and the conductivity of the nanoemulsion. The Contour plot presented in Figure 4 demonstrates the effect of lipophilic tween type and tween concentration on the Poly Dispersity Index (PDI) of the nanoemulsion. Interestingly, an increase in lipophilic tween type from 60 to 80 and a decrease in tween concentration from 12% to 8% lead to an increase in PDI value without further changes. This indicates a complex interaction between these factors in determining the homogeneity of particle size distribution within the nanoemulsion.

Figure 5 depicts the pH contour plot of the parijoto fruit extract nanoemulsion. An increase in lipophilic Tween type from 20 to 80 and an increase in tween concentration from 8% to 12% result in a consistent increase in pH without any further changes. This observation suggests that these specific conditions contribute to the alkalinity of the nanoemulsion, which may have implications for its stability

and functionality. Finally, Figure 6 illustrates the viscosity contour plot of the nanoemulsion. An increase in lipophilic tween type from 35 to 80 and an increase in tween concentration from 8% to 12% lead to an increase in viscosity without further changes. This indicates that higher concentrations of lipophilic tween and tween result in a thicker consistency of the nanoemulsion, which affects its flow properties and application. The presented data highlights the intricate relationship between lipophilic tween type and tween concentration in influencing various physicochemical properties of the nanoemulsion derived from parijoto fruit extract. These findings provide valuable insights for optimizing the formulation and manufacturing process of the nanoemulsion for potential applications in various industries.

8. In the Material section, an HPLC tool is used, but there is no explanation of the HPLC method, so it needs to be added to the method

We did not use HPLC tool in this research. We already deleted the use of HPLC tool in the method.

9. The discussion section also needs to show the results and discussion for the HPLC test results. We suggest adding an HPLC test results table along with the discussion in the section discussion.

We did not do the analysis using HPLC in this research. Therefore, we did not make the discussion about it.

10. Table 1 of the RSM Design of Experiment, can be presented more concisely.

We have revised Table 1.

11. This study aimed to explore the surfactant's capability in forming anthocyanin nano emulsions from Parijoto fruit extract and assess the emulsion's characteristics, including pH, light, temperature, turbidity, emulsion type, and stability. However, the results, discussion, conclusion, and abstract do not explicitly describe this point. Optimal process conditions were not the objective of this research, but this was the conclusion of the results.

We will add optimization to the goal of this research beside investigate nanoemulsion characteristic such as particle size, PDI, zeta potential, conductivity, and viscosity of the nanoemulsion. It is important to note that although optimal process conditions were not the main focus of this study, the results indirectly indicate that certain conditions provide better results in terms of emulsion characteristics. In the future, it would be useful to provide a more detailed analysis of these characteristics to better understand the implications and potential applications of anthocyanin nanoemulsions derived from Parijoto fruit extract.

(Line 10-35) an (Line73-85)

In this series of experiments, nanoemulsion from parijoto fruit has been characterized, considering various physicochemical parameters such as particle size, polydispersity index, zeta potential, conductivity, pH, and viscosity. The research results indicate significant variations in the physical characteristics of both nanomaterials in terms of changes in surfactant and parijoto extract concentrations. Increased surfactant concentration tends to produce smaller particle sizes and a more homogeneous distribution, although certain limitations were found that lead to surfactant aggregation and micelle formation. The nanoemulsion characteristics, including zeta potential, polydispersity, particle size, conductivity, pH, and viscosity. The type and concentration of surfactants played a crucial role in determining the properties of the nanoemulsions. Variations in surfactant parameters resulted in observable differences in emulsion characteristics, highlighting the importance of surfactant selection and optimization. To achieve optimal nanoemulsion process conditions, it is recommended to use 12% Tween 80 solvent concentration, 12% Tween concentration, and 7.5% parijoto fruit extract concentration, resulting in a desirability value of 0.74, falling into the moderate category.

- Revised Manuscript 2



## FORMULATION OF NANOEMULSION PARIJOTO FRUIT EXTRACT (*Medinilla speciosa*) WITH VARIATION OF TWEENS STABILIZERS

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2 Sarsita Putra<sup>1</sup>

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8 **Keywords:** Nanoemulsion<sup>1</sup>, Stabilizers<sup>2</sup>, Tweens<sup>3</sup>, Parijoto<sup>4</sup>, RSM<sup>5</sup>.

9 **Abstract**

10 Nanotechnology was deemed to possess substantial potential for development owing to its ability to  
11 modify surface characteristics and particle size, facilitating enhanced absorption of functional food  
12 compounds and controlled release of active substances to mitigate adverse effects. Nanoemulsion, a  
13 stable colloidal system formed by blending oil, emulsifier, and water, was identified as  
14 nanotechnology with promising applications. However, investigations into the impact of surfactants  
15 on characteristic nanoemulsions, needed to be more varied. This research gap necessitated further  
16 exploration in the advancement of nanotechnology-based foods. The parijoto fruit (*Medinilla*  
17 *speciosa*), an indigenous plant species in Indonesia, has yet to undergo extensive scrutiny for its  
18 potential use as a functional and nutraceutical food. Anthocyanins, a principal compound in the  
19 parijoto fruit, had exhibited efficacy in reducing the risk of cardiovascular diseases, diabetes, and  
20 inflammation, and demonstrated anti-inflammatory and antioxidant properties. This study aimed to  
21 investigate the characteristics of nanoemulsion formulations derived from parijoto fruit extract and to  
22 evaluate an optimum condition with various tween surfactant. The findings from this investigation  
23 could furnish valuable insights for the further advancement of anthocyanin nanoemulsions from  
24 parijoto fruit extract. The results comprised the characterization of nanoemulsion particle size,  
25 polydispersity index, zeta potential, conductivity, pH, and viscosity. RSM is used to optimize  
26 nanoemulsion by examining the relationships and interactions between independent variables and  
27 response variables through mathematical modeling and statistical methods. Furthermore, the  
28 characterization of nanoemulsion encompassed zeta potential, polydispersity, particle size,  
29 conductivity, pH, and viscosity. Elevated surfactant concentrations resulted in diminished particle  
30 sizes and more uniform size distribution, albeit reaching a plateau where surfactant aggregation and  
31 micelle formation ensued. Increased concentrations of surfactant type, concentration, and parijoto  
32 extract impacted the physical characteristics of nanoparticle size and polydispersity. The optimal  
33 process conditions for nanoemulsion comprised a 12% concentration of Tween 80 solvent, 12%  
34 Tween concentration, and 7.5% parijoto fruit extract concentration, yielding a desirability value of  
35 0.74, categorizing it as moderate.

36 **1 Introduction**

37 Nanotechnology underwent progressive evolution, characterized by measurements on the nanometer  
38 scale, approximately  $10^{-9}$  meters (Ariningsih, 2016). Acknowledgment from the World Health  
39 Organization (WHO) and the Food and Agriculture Organization (FAO) (2009) underscored  
40 nanotechnology's significant potential in enhancing food products, attributed to its capacity to modify  
41 surface characteristics and particle size. Such modifications facilitate targeted delivery of food  
42 compounds to specific organs and the controlled release of active compounds to mitigate adverse  
43 effects. The attributes of nanoscale food materials are pivotal in propelling diverse industries,  
44 including food, pharmaceuticals, and extensive nutraceutical applications (Rahman et al., 2020).  
45 Nanoemulsions denote a nanotechnological rendition of a stable colloidal system, achieving kinetic  
46 stability through the amalgamation of oil, emulsifier, and water (McLements, 2016). Chang et al.  
47 (2022) conducted research utilizing surfactants as stabilizers in synthesizing nanoemulsions,  
48 showcasing the stability of nanoemulsion particle size in curcumin extract. Surfactants can diminish  
49 interfacial tension and form a substantially influential steric elastic film on the emulsion results (Xiao  
50 J & Hwang, 2016).

51 Renowned for its tropical climate and vast biodiversity, Indonesia harbours at least 30,000 plant  
52 species, with 7,000 being herbal plants with documented health benefits (Widyowati & Agil, 2018;  
53 Jumiami & Komalasari, 2017). Parijoto (*Medinilla speciosa*), an endemic plant species in Indonesia,  
54 remains relatively understudied for its scientific potential in pharmacy, functional foods, and  
55 nutraceuticals. Analysis has confirmed that the parijoto fruit comprises phytochemical components  
56 such as anthocyanins, flavonoids, saponins, tannins, alkaloids, cardenolides, and glycosides  
57 (Balamurugan, 2014). Anthocyanins, a predominant compound in parijoto fruit, demonstrate efficacy  
58 in reducing the risk of cardiovascular diseases, diabetes, and inflammation while possessing notable  
59 anti-inflammatory and antioxidant properties. Extraction techniques yield varying anthocyanin  
60 contents, with the peel extract and whole fruit extract registering 208.75 and 173.7 mg/L,  
61 respectively (Sa'adah et al., 2020). Various factors influence anthocyanins' stability, including  
62 chemical structure, concentration, solvent, pH, storage temperature, light, oxygen, metal ions,  
63 proteins, and flavonoids. Weak stability under high pH, high temperature, and light exposure has  
64 been observed (Ito et al., 2021), with lower pH contributing to enhanced stability (Moldova et al.,  
65 2020). Heating at elevated temperatures accelerates anthocyanin degradation (Khoo et al., 2019).

66 In recent years, Response Surface Methodology (RSM) has emerged as a prominent multivariate  
67 statistical technique for optimizing various processes. Initially introduced by Box and colleagues in  
68 the 1950s, RSM facilitates the examination of the relationship and interactions among independent  
69 variables and response variables through mathematical modeling and statistical methods (Izayidan et  
70 al., 2019). RSM has been successfully employed in culancing and optimizing therapeutic extract and  
71 drug nanoemulsion (Samim et al., 2020). In this study, Central Composite Design (CCD) Response  
72 Surface Methodology (RSM) was employed to optimize the quality parameters of the nanoemulsion.

73 Appropriate nano-encapsulation techniques, such as nanoemulsion, have shown the potential to  
74 enhance the stability, bioavailability, and solubility of lipophilic bioactive compounds while also  
75 preventing hydrolysis and oxidation (Rosso et al., 2020). Catechin nanoemulsion showed a  
76 remarkable improvement of stability and bioavailability in simulated gastrointestinal (Rafanar et al.,  
77 2016). Research conducted by Chang et al. (2022) used Tween as surfactant in the stable  
78 nanoemulsion synthesis loaded curcumin extract. This underscores the potential for developing  
79 nanoemulsion formulations for anthocyanins in parijoto fruit. Thus far, research on nanoemulsion  
80 formulation in parijoto fruit, incorporating various concentrations and stabilizers, still needs to be

81 conducted. This study is dedicated to investigating the characteristics of nanoemulsion formulations  
82 derived from parijoto fruit extract and evaluating an optimum condition with various tween  
83 surfactant.

## 84 **2 Materials and Method**

### 85 **2.1 Materials**

86 Grinder (Binder), Erlenmeyer (Pyrex), beaker glass (Pyrex), volume pipette, test tube (Pyrex), test  
87 tube rack, funnel (Pyrex), measuring flask (Pyrex), vacuum n filter 0.22  $\mu$ m (Sartorius Stedim 11694-  
88 2-50-06), vial, micropipette (Socorex), blue tip (Biologix 1 nmL pipette tips), hotplate (Cimarec et al.  
89 SP142025Q), vortex (Thermolyne et al.), Ultrasonic Cleaner (Biobase UC-10SD) modified, UV-VIS  
90 spectrophotometer (Shimadzu, UV-1280), aluminium foil, filter paper, 0.22  $\mu$ m filter membrane  
91 (Wattman), Cabinet dryer (HetoPowerDry LL1500), rotary evaporator (Biobase RE-2000E), syringe,  
92 analytical balance. Fresh parijoto, ethanol pro analysis (Merck, Germany), methanol pro analysis  
93 (Merck, Germany), distilled water, aqua bikes, folding ciocaltcu 10% (Merck, Germany), Na<sub>2</sub>CO<sub>3</sub>  
94 7.5% (Merck, Germany), DPPH solution (Merck, Germany), Quarcetin (Merck, Germany), AlCl<sub>3</sub>  
95 (Merck, Germany), ammonium acetate 1 M (Merck, Germany), acetone (Merck, Germany),  
96 acetonitrile (Merck, Germany), standard cyanide (Zigma), delphinidin glu standard (Zigma), Tween  
97 20 (Merck, Germany), Tween 60 (Merck, Germany), Tween 80 (Merck, Germany), and Span 20  
98 (Merck, Germany).

### 99 **2.2 Preparation of Dry Samples of Parijoto Fruit Extract**

100 Samples used in this study are fruits from the Parijoto plant (*Medinilla speciosa*) cultivated and  
101 harvested on the slopes of Mount Muria, Kudus. The fruits used are ripe fruits harvested when the  
102 Parijoto plant reaches full maturity, typically around 90-100 days after flowering. Parijoto, which had  
103 been cleaned and sorted, was weighed 200 grams for each treatment. The fruit that has been weighed  
104 is then steam-blanching for 3 minutes. Prepare a citric acid solution with a concentration of 1% for  
105 pre-treatment of fruit before drying. After that, soak the parijoto fruit in the citric acid solution for 5  
106 minutes and drain. The Cabinet Dryer is cleaned before use to maintain hygiene and avoid cross-  
107 contamination. The drying temperature used was 70°C for 6 hours. The dried Parijoto fruit is then  
108 ground into powder using a herbal grinder for 2 minutes. After that, the sample will be extracted for  
109 further testing. The dried Parijoto will be chemically analyzed using UV-Vis spectroscopy.

### 110 **2.3 Making Parijoto Extract using Ultrasonic Assisted Extraction (UAE)**

111 Five grams of dry sample powder and 50 mL of 99.5% ethanol were mixed thoroughly for  
112 homogeneity in four 250 mL centrifuge bottles. Then, all vials were sonicated (40 KHz, 100 W) for  
113 30 min, followed by shaking for one hour, centrifuged at 4,000 rpm (4°C) for 10 min, collected the  
114 supernatant, and evaporated to dryness under vacuum. The residue was dissolved in 99.5% ethanol  
115 and diluted to 20 mL. After filtering through a 0.22  $\mu$ m membrane filter, parijoto fruit extract was  
116 obtained and stored at -20°C for analysis using UV-Vis.

### 117 **2.4 Preparation of Anthocyanin Nanoemulsion from Parijoto Extract**

118 Approximately 3 mL of anthocyanin nanoemulsion with concentrations of 2 mg/mL, 4 mg/mL, and 6  
119 mg/mL, respectively, were prepared by collecting a portion of parijoto extract, and the solvent was  
120 removed with nitrogen. The solvent removal process during anthocyanin extraction can be monitored  
121 using a combination of visual inspection and periodic weight measurements. Visual inspection



122 involves observing the extract as the solvent evaporates, noting its increasing concentration  
123 evidenced by a thicker and more viscous appearance. Periodic weighing of the container or flask  
124 containing the extract allows for the tracking of weight loss as the solvent evaporates. Once the  
125 weight stabilizes or reaches a predetermined target, it signifies that the desired solvent removal rate  
126 has been attained, ensuring the production of a concentrated anthocyanin extract suitable for further  
127 analysis. Anthocyanin nanoemulsion was prepared using a combination of surfactants that have low,  
128 medium, and high hydrophile lipophile balance (H.L.B), namely Tween 20, Tween 60, and Tween 80.  
129 Then, surfactant (0.24 g) was added, and the mixture was homogenized entirely. This was followed  
130 by adding ( 2.76 g) deionized water and mixing again for complete dispersion of surfactant in water.  
131 The solution was then sonicated in a sonicator with a temperature of 35°C, frequency of 45 Hz, and  
132 100% power for 60 minutes. To produce a good nanoemulsion, homogenization was carried out  
133 using high shear homogenization at 15,000 rpm with a temperature of 4 C for 15 minutes.

### 134 **2.5 Characterization of Particle Size and Polydispersity Index of Nanoemulsion Parijoto** 135 **Fruit Extract**

136 The particle size analysis tool used in this study was the Zetasizer (Zetasizer Pro; Malvern et al.),  
137 which operates based on the general principle of dynamic light scattering (DLS). This tool has a  
138 detector placed at an angle of 173° from the transmitted light beam and detects size using a patented  
139 technology known as noninvasive backscattering. This technique is used for various purposes. One is  
140 to reduce the effect known as multiple scattering, making it easier to measure samples with high  
141 concentrations. Modifying McClements (2016), the particle size distribution and average particle size  
142 of nanoemulsions were determined by dynamic light scattering (DLS) at a wavelength of 633 nm and  
143 a temperature of 25 °C.

### 144 **2.6 Characterization of Zeta Potential Nanoemulsion Parijoto Fruit Extract**

145 The  $\zeta$ -potential of Parijoto Fruit Extract Nanoemulsion was evaluated using  $\zeta$ -potential analysis  
146 (Zetasizer Pro; Malvern Instruments, Ltd., Malvern) following the method described by Khalid et al.  
147 (2017). The  $\zeta$ -potential of the samples was evaluated automatically using 10 to 100 analytical runs  
148 after equilibration for 120 s at 25 °C. The zeta potential of the particles was measured by phase-  
149 analysis light scattering (PLS) using a Zeta dip cell.

### 150 **2.7 Characterization of the Conductivity of Nanoemulsion Parijoto Fruit Extract**

151 The conductivity of nanoemulsion particles was measured by phase-analysis light scattering (PLS)  
152 using a Zeta dip cell with a cuvet electrode. Samples were evaluated automatically using 10 to 100  
153 analytical runs after equilibration for 120 seconds at 25 °C. The detector is placed at an angle of 173°  
154 from the transmitted light beam.

### 155 **2.8 pH Measurement of Nanoemulsion Parijoto Fruit Extract**

156 The pH was determined using a Schott pH meter at room temperature ( $27 \pm 2$  °C), calibrated with a  
157 standard buffer of pH 7. The pH analysis of the Parijoto fruit extract nanoemulsion sample was  
158 carried out using a pH meter with a particular electrode. First, the pH meter is set and calibrated with  
159 a standard buffer solution at a known pH, generally at pH 4.0, 7.0, and 10.0. Samples were diluted  
160 with ten mM phosphate buffer pH seven before analysis to avoid multiple scattering effects during  
161 testing. The pH meter electrode is then carefully inserted into the sample to ensure good contact.  
162 Once the electrode is stable, a pH reading is taken and recorded. This step is repeated as necessary to  
163 obtain consistent results. This pH analysis provides essential information regarding the acidity or

164 alkalinity level of nanoemulsion and nanocitosan Parijoto fruit extract, which can affect the stability  
165 and quality of products using the nanoemulsion.

## 166 **2.9 Viscosity Measurement of Nanoemulsion Parijoto Fruit Extract**

167 Viscosity measurements are carried out using a viscometer instrument. 14 mL of sample was put into  
168 the cup and attached to the solvent trap provided. The viscometer was set at 200 rpm, three rotations,  
169 for 30 seconds. The measurement process begins by activating the viscometer, and this tool  
170 automatically measures the time required for a liquid to flow through the viscometer tube at a  
171 specific temperature and rpm. This time, a predetermined formula converts the reading into a  
172 viscosity value. Repeated measurements can be made to ensure consistent results.

## 173 **2.10 Statistical analysis uses Response Surface Methodology.**

174 In this study, primary data in 3 repetitions of extraction and three repetitions of testing were averaged  
175 and given a standard deviation value for each treatment combination using Statistica 12.5 by StatSoft.  
176 The data is then entered into a statistical application, arranged in a combination of factorial points,  
177 axial points, and central points with three repetitions. After that, the data was analyzed, and several  
178 test stages were carried out. The basis for testing is studentification from primary data.  
179 Studentification means that the scale of the variable is adjusted by dividing it by the estimated  
180 population standard deviation. Variability in sample standard deviation values contributes to  
181 additional uncertainty in the calculated value. This will cause problems in finding the probability  
182 distribution of each statistic studied.

### 183 **2.10.1 Effect Summary**

184 This test can summarise the effects of the combination of treatments used. The Longworth value in  
185 the results of this test is defined as  $-\log(p\text{-value})$  and is a transformation of the p-value based on the  
186 Pearson Chi-Squared test. The Pearson Chi-Squared test evaluates the possibility of the split being  
187 caused by chance. The higher the Pearson Chi-Squared value, the higher the probability of the split  
188 being caused by dependency. In general, if the worth is greater than 2, then the statistical model  
189 considers the variable necessary.

### 190 **2.10.2 Lack Of Fit**

191 Model suitability testing (lack of fit) is carried out to review whether the model equation is  
192 acceptable or not in predicting responses. In the lack of fit test, the following hypothesis is used:

193  $H_0$  = no lack of fit (suitable model)

194  $H_1$  = there is a lack of fit (the model is not suitable)

195 The hypothesis is concluded by comparing the calculated F value with the F table. The calculated F is  
196 obtained from the statistical test results and displayed in the ANOVA table. The F table value is  
197 obtained from the F Distribution Table. The criteria for the lack of fit test are:

198  $F_{\text{count}} < F_{\text{table}}$ , then  $H_0$  is accepted.  $F_{\text{count}} > F_{\text{table}}$ , then  $H_0$  is rejected.

199 Another parameter that can prove the suitability of the model obtained is by comparing the p-value  
200 with the  $\alpha$  value. If the p-value of lack of fit is smaller than the  $\alpha$  value, then there is a significant  
201 lack of fit, so the model obtained is not appropriate.

202 **2.10.3 Summary Of Fit**

203 The R square and Root Mean square error values are obtained in this test. Measures the difference in  
204 values from a model's predictions as estimates of the observed values. R square is also known as the  
205 coefficient of determination, which explains how far independent data can explain dependent data. R  
206 square has a value between 0 – 1 with the condition that the closer it is to one, the better it is. If the r  
207 square is 0.6, the independent variable can explain 60% of the distribution of the dependent variable.  
208 The independent variable cannot explain the remaining 40% or can be explained by variables outside  
209 the independent variable (error component).

210 **2.10.4 Parameter Estimates**

211 The parameter estimates are the coefficients of the linear predictor. This value represents the change  
212 in response if you have a certain level of a categorical predictor or a change of 1 unit for a continuous  
213 predictor, which means the same thing as in a multiple regression analysis with continuous response.

214 **2.10.5 Analysis Of Variance**

215 The ANOVA test (Analysis of Variance) has the following test criteria:

216 H0 is accepted if  $F_{\text{count}} < F_{\text{table}}$ , which means the model cannot be accepted statistically because  
217 no independent variables have a real influence on the response.

218 H1 is accepted if  $F_{\text{count}} > F_{\text{table}}$ , which means the model is statistically acceptable and at least one  
219 independent variable has a real influence on the response.

220 **2.10.6 Fitted Surfaces**

221 The depiction of the fitted surface is carried out using the Central Composite Design model. The  
222 experimental design is factorial, specifically Central Composite Design (CCD). CCD was chosen  
223 over Box-Behnken Design because CCD provides more design points in terms of axial points.  
224 Additionally, CCDs can run experiments at extreme values, providing better quadratic equations for  
225 analysis. CCD contains a factorial or fractional factorial design with a central point augmented by a  
226 group of 'axial points' that allow estimation of curvature. If the distance from the center of the design  
227 space to the factorial point is  $\pm 1$  unit for each factor, the distance from the center of the design space  
228 to the axial point is  $|\alpha| > 1$ . The exact value of  $\alpha$  depends on the properties desired for the design and  
229 the number of factors involved. The CCD has twice as many star points due to a factor in the design.

230 **3 Result & Discussion**

231 **3.1 Phytochemical Profiles of Dried Parijoto Fruit**

232 Drying Parijoto Fruit is carried out using a cabinet dryer at a temperature of 70°C for 6 hours.  
233 The results of drying parijoto fruit were obtained through the preparation process, the  
234 antioxidant and anthocyanin activity profiles were expressed respectively in units of %  
235 inhibition and ppm. The results of the antioxidant activity of dried and extracted parijoto fruit  
236 were 79.14334.82%. % The total anthocyanin content in the dry samples and extracts was  
237 538.47 ± ppm. The dried Parijoto exhibited significant antioxidant activity, with a % inhibition  
238 value of 79.14 ± 34.82. This indicates a substantial capacity to neutralize free radicals, which  
239 are implicated in various chronic diseases and aging processes. The high antioxidant activity  
240 suggests that the drying process did not significantly diminish the antioxidant potential of  
241 Parijoto. The total anthocyanin content of the dried Parijoto was found to be 538.47 ± 4.67  
242 ppm. Anthocyanins are a group of pigmented compounds known for their antioxidant  
243 properties and potential health benefits. The retention of anthocyanins after the drying process  
244 indicates that cabinet drying effectively preserved these bioactive compounds in the dried  
245 Parijoto

246 The parijoto fruit extract was obtained through an extraction process using the Ultra-assisted  
247 extraction method. The antioxidant and anthocyanin activity profiles of parijoto fruit extract.  
248 The characterization of Parijoto extract as a filler in nanoemulsion involved various analyses  
249 to assess its antioxidant properties and phytochemical composition. The extraction method  
250 employed was ultra-assisted extraction, which is known for its efficiency in extracting  
251 bioactive compounds from plant materials. The antioxidant activity of the Parijoto extract was  
252 evaluated, yielding a % inhibition value of 50.776±6.18. This indicates a significant level of  
253 antioxidant capacity, which is crucial for combating oxidative stress and preventing cellular  
254 damage caused by free radicals. Furthermore, the total anthocyanin content of the extract was  
255 determined to be 94.43±4.14 ppm. Anthocyanins are well-known antioxidants found in many  
256 fruits and vegetables, known for their potential health benefits, including anti-inflammatory  
257 and anti-cancer properties. The flavonoid content of the Parijoto extract was measured to be  
258 126.85±1.15 g/L. Flavonoids are a class of polyphenolic compounds known for their  
259 antioxidant and anti-inflammatory effects. Additionally, the phenolic content of the extract was  
260 quantified as 8.43±0.70 GAE/g. Phenolic compounds are another group of bioactive  
261 compounds found in plants, known for their antioxidant and anti-inflammatory activities, as  
262 well as their potential role in reducing the risk of chronic diseases.

263 **3.2 Fitting Model for RSM (Response Surface Methodology) in Parijoto Fruit Extract Nanoemulsion**

264 Data recorded for each run included nanoemulsion particle size, polydispersity index, zeta potential, conductivity, pH, and viscosity. Each  
 265 variable was measured with three repetitions and the measurements three times to get consistent results. This data will be used to analyze the  
 266 influence of various factors on the characteristics of nanoemulsions using the Response Surface Methodology method, which can be seen in the  
 267 table.

268 Table 3. Design of Experiment RSM Particle Size, Poly Dispersity Index, Zeta Potential, Conductivity, pH, Viscosity in Nanoemulsion

No. Run Test	Dependent Variables			Independent Variables					
	Types of Lyophobic Detergent	Detergent Concentration (%)	Parijoto Fruit Extract Concentration (%)	Nanoparticle Size (nm)	Zeta Potential	Conductivity	Poly Dispersity Index	pH	Viscosity (cP)
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	Y <sub>1</sub>	Y <sub>2</sub>	Y <sub>3</sub>	Y <sub>4</sub>	Y <sub>5</sub>	Y <sub>6</sub>
1	20	3	3	15.511 ± 0.228	-22.12 ± 1.514	0.071 ± 0.005	0.43 ± 0.025	7.517 ± 0.005	0.07 ± 0.000
2	20	3	10	10.2 ± 0.337	-25.473 ± 2.857	0.057 ± 0.005	0.51 ± 0.065	6.870 ± 0.003	0.07 ± 0.003
3	20	3	5	15.069 ± 0.278	-22.740 ± 1.513	0.060 ± 0.001	0.57 ± 0.034	6.960 ± 0.002	0.06 ± 0.000
4	20	5	0	22.061 ± 0.300	-22.535 ± 1.510	0.067 ± 0.001	0.35 ± 0.064	6.890 ± 0.003	0.07 ± 0.000
5	20	3	0	20.017 ± 0.090	-22.127 ± 2.542	0.069 ± 0.000	0.48 ± 0.007	7.712 ± 0.002	0.09 ± 0.003
6	20	3	0	41.981 ± 0.159	-17.181 ± 4.719	0.070 ± 0.000	0.45 ± 0.010	6.811 ± 0.007	0.07 ± 0.000
7	20	3	0	42.547 ± 0.085	-20.645 ± 2.707	0.074 ± 0.000	0.52 ± 0.15	6.897 ± 0.008	0.07 ± 0.003
8	20	3	0	44.743 ± 1.707	-24.477 ± 3.578	0.074 ± 0.007	0.50 ± 0.005	6.810 ± 0.005	0.07 ± 0.007
9	20	3	0	49.307 ± 0.317	-24.617 ± 4.699	0.080 ± 0.001	0.40 ± 0.004	6.817 ± 0.005	0.06 ± 0.003
10	20	10	3	35.071 ± 0.300	-24.607 ± 1.204	0.085 ± 0.003	0.61 ± 0.001	6.790 ± 0.009	0.06 ± 0.003

11	28	10	5	51.967	-	0.007	-24.437	-	1.251	0.000	-	1.000	1.50	-	0.60	1.801	-	0.021	0.296	-	0.013
12	29	10	5	39.231	+	2.851	-24.270	+	1.493	0.009	+	1.001	1.91	+	0.62	1.810	+	0.013	0.308	+	0.005
13	29	10	6	32.54	+	4.231	-23.70	+	1.207	0.005	+	1.020	1.99	+	0.64	1.871	+	0.003	0.308	+	0.023
14	30	10	6	64.087	-	0.470	-23.787	-	1.445	0.075	-	1.017	1.52	-	0.69	1.807	-	0.015	0.275	-	0.017
15	30	10	6	84.037	-	0.585	-25.190	-	1.800	0.087	-	1.010	1.59	-	0.64	1.700	-	0.013	0.307	-	0.013
16	30	10	9	77.961	-	17.908	-26.172	-	1.718	0.091	+	1.000	1.51	-	0.71	1.811	-	0.011	0.271	-	0.002
17	30	10	9	75.943	-	1.101	-26.927	-	1.851	0.081	+	1.019	1.51	-	0.65	1.830	-	0.013	0.305	-	0.014
18	30	10	9	77.369	+	16.972	-26.281	+	1.530	0.070	+	1.021	1.60	+	0.61	1.760	+	0.003	0.278	+	0.011
19	30	12	5	83.88	-	11.920	-26.007	+	1.311	0.082	-	1.011	1.57	+	0.73	1.890	+	0.013	0.302	+	0.013
20	30	12	1	80.4	-	14.828	-26.025	-	1.800	0.080	-	1.017	1.66	-	0.77	1.771	-	0.012	0.308	-	0.017
21	30	12	8	85.967	-	7.087	-26.051	+	1.500	0.081	+	1.016	1.61	+	0.75	1.771	-	0.002	0.305	-	0.015
22	30	12	0	91.49	+	1.209	-27.471	+	1.801	0.081	+	1.010	1.73	+	0.71	1.810	+	0.013	0.301	+	0.019
23	30	12	0	110.88	+	19.52	-27.071	+	1.708	0.081	-	1.017	1.57	+	0.77	1.870	-	0.003	0.301	-	0.011
24	30	12	6	110.23	-	7.064	-27.120	-	1.300	0.077	-	1.011	1.52	-	0.70	1.800	-	0.017	0.277	-	0.012
25	30	12	6	111.880	+	17.178	-26.720	+	1.600	0.080	+	1.010	1.61	+	0.79	1.811	+	0.011	0.278	+	0.005
26	30	12	9	118.33	+	4.181	-26.025	+	1.139	0.075	+	1.014	1.55	+	0.73	1.857	+	0.003	0.273	+	0.014
27	30	12	9	124.187	-	4.182	-26.670	-	1.014	0.089	-	1.010	1.75	-	0.71	1.857	-	0.015	0.308	-	0.013

270 Table 3 shows that the particle size range of the nanoemulsion is between  $14,603 \pm 16.73$  nm  
271 and  $118,053 \pm 4,5825$  nm. The largest and smallest nanoparticle sizes found are 126.47 nm  
272 and 13.72 nm, respectively, with most nanoparticle sizes falling within the 50-100 nm range.  
273 Similar results were confirmed by Noor El-Din et al. (2017), who reported nanoemulsion  
274 sizes ranging from 31.58 to 220.5 nm. Studies conducted by Delmas et al., Liu et al., and Mei  
275 et al. using ultrasonication and high emulsification methods also confirmed comparable  
276 results of 45–170 nm, 222.4–166.4 nm, and 170–280 nm, respectively (Delmas et al., 2016;  
277 Liu et al., 2017; Mei et al., 2019). Conversely, Peng et al. (2010) reported a nanoparticle size  
278 range of 21–530 nm. Zeta potential reflects the surface charge of particles and affects  
279 colloidal stability. High zeta potential can prevent particle aggregation due to electrostatic  
280 repulsion. The research includes the evaluation and characterization of zeta potential under  
281 various treatments. The study obtained zeta potential results for nanoemulsion ranging from  
282  $-22.197 \pm 0.738$  mV to  $-28.207 \pm 1.598$  mV, respectively. Similar results were confirmed by  
283 Wessam et al. (2023), obtaining results of +21.5 mV. Particles with high ZP values, between  
284 20 and 40 mV, provide system stability and are less likely to aggregate or increase particle  
285 size. However, it should be noted that ZP values are not an absolute measure of nanoparticle  
286 stability. Furthermore, emulsions with ZP variations  $>10$  mV are suggested to have better  
287 stability (Kadu et al., 2011). The ideal potential range for nanoparticle stability is (-30 to 20  
288 mV or +20 to +30 mV) (Liu et al., 2018). The produced values tend to be harmful due to the  
289 influence of acetic acid, resulting in a negative charge. This charge causes electrostatic  
290 repulsion forces between formed nanoparticles to prevent aggregation into larger sizes  
291 (Luthifayana et al., 2022). Higher zeta potential values increase nanoparticle stability due to  
292 higher electrostatic repulsion forces between nanoparticles.

293 Conductivity provides information about the ability of nanoemulsions to conduct electricity.  
294 Changes in conductivity can occur with changes in surface particle charge. Table 18 shows  
295 that the nanoemulsion conductivity of Parijoto fruit extract ranges from 0.03458 to 0.09987  
296 mS/cm. Good nanoemulsion conductivity measurements have higher electrical conductivity  
297 values (10–100  $\mu$ S/cm) (Akilu et al., 2019; Guo et al., 2016; Khader et al., 2016). Electrical  
298 conductivity values tend to decrease with decreasing water content in the emulsion. O/W type  
299 (Oil-in-Water) nanoemulsions have higher conductivity than W/O type (Water-in-Oil)  
300 nanoemulsions. This is because the more extensive water phase provides more pathways for  
301 ion conduction.

302 The type and concentration of surfactant in nanoemulsion can influence conductivity.  
303 Surfactants can provide ionic charge or facilitate ion conduction in the system. Viscosity is an  
304 essential parameter in evaluating the flow properties of nanoemulsion. Viscosity is one of the  
305 parameters used to determine the stability of polymers in a solution because it undergoes  
306 reduction during polymer storage due to polymer degradation (Aranaz et al., 2021). In this  
307 study, as shown in Table 1, the viscosity of nanoparticles ranges from 3,810 cP to 4,433 cP.  
308 Alemu et al. (2023) stated that viscosity can depend on particle size and storage time.  
309 Appropriate viscosity can affect the applicability and spread of the system. The viscosity of a  
310 preparation is related to the consistency and spreadability of the preparation, which will affect  
311 ease of use (Imanto et al., 2019). Viscosity values are influenced by several factors, such as  
312 temperature, pH, manufacturing conditions, and the quality and concentration of raw  
313 materials (Naini & Yusuf, 2018). The results of viscosity tests are shown in centipoise (cP).  
314 The higher the viscosity value of a preparation, the better the stability of the product, but the  
315 preparation will be difficult to apply to the skin, and the resistance of the preparation to flow  
316 will increase, making it difficult to remove from the container (Thakre, 2017). Meanwhile,  
317 low viscosity values will increase the flowability of the skin and make it easier to apply to the  
318 skin (Naini & Yusuf, 2018)





319 This ANOVA table is essential to evaluate the statistical significance of each model component and determine whether the quadratic model used  
 320 is good enough to explain the characteristics of the nanoemulsion or not. The p-value is used to determine statistical significance, and the  
 321 analysis results will help select an appropriate model and interpret the significance of factors that influence the characteristics of nanoemulsions,  
 322 which can be seen in the table.

323 Table 4. ANOVA (Analysis of Variance) for the RSM Quadratic Model Particle Size, Poly Dispersity Index, Zeta Potential, Conductivity, pH,  
 324 Viscosity in Nanoemulsion

Quadratic Model Equation	Sources of Variation	p-Value
<b>Particle Size</b> ( $R^2$ : 0,558 $R^2_1$ : 0,50156) $Y_1 = -0,000008 - 0,000069X_1 + 0,000040X_2 + 0,000032X_3 + 0,000056X_1^2 +$ $0,000064X_2^2 - 0,000003X_3^2 - 0,000056X_1X_2 - 0,000044X_2X_3 + 0,000065X_1X_3$	Model  <i>Lack of fit</i>	0,294 *  0,185
<b>Poly Dispersity Index</b> ( $R^2$ : 0,3643 $R^2_1$ : 0,2471) $Y_2 = 6,23086 + 0,58801 X_1 - 0,75655 X_2 + 84,3654 X_3 + 24,65 X_1^2 + 18,7663 X_2^2$ $20,744 X_3^2 + 23,0025 X_1X_2 + 26,3043 X_2X_3 + 9,5269 X_1X_3$	Model  <i>Lack of fit</i>	0,041*  0,692
<b>Zeta Potential</b> ( $R^2$ : 0,54003 $R^2_1$ : 0,56905) $Y_3 = 0,000062 - 0,000023 X_1 - 0,000010 X_2 + 0,000008 X_3 + -0,000007 X_1^2 +$ $0,000003 X_2^2 + 0,000008 X_3^2 + -0,000006 X_1X_2 - 0,000008 X_2X_3 + -0,000005 X_1X_3$	Model  <i>Lack of fit</i>	0,000*  0,980
<b>Conductivity</b> ( $R^2$ : 0,2444 $R^2_1$ : 0,3464) $Y_4 = 4035,80 - 1198,06X_1 + 833,22X_2 - 1083,49X_3 - 2597,39X_1^2 - 709,42X_2^2$ $+ 881,10X_3^2 + 305,68X_1X_2 - 700,69X_1X_3 - 943,96X_2X_3$	Model  <i>Lack of fit</i>	0,0004*  0,928
<b>pH</b> ( $R^2$ : 0,832 $R^2_1$ : 0,797) $Y_5 = 0,003122 - 0,000040X_1 - 0,000060X_2 + 0,000039X_3 - 0,000034X_1^2 +$ $0,000047X_2^2 + 0,000031X_3^2 - 0,000006X_1X_2 - 0,000015X_1X_3 + 0,000031 X_2X_3$	Model  <i>Lack of fit</i>	0,000*  0,067
<b>Viskositas</b> ( $R^2$ : 0,95976 $R^2_1$ : 0,95466) $Y_6 = 0,015177 - 0,009573X_1 - 0,003288X_2 - 0,000624X_3 - 0,008334X_1^2 -$ $0,000266X_2^2 - 20,744 X_3^2 + 23,0925 X_1X_2 + 26,3043 X_2X_3 + 9,5269 X_1X_3$	Model  <i>Lack of fit</i>	0,000*  0,103

325 Notes:  
 326 - \*: The model has a statistically significant effect (p<0.05)  
 327 - \*\*: Model mismatch or lack of fit occurs (p<0.05)

328 Based on the ANOVA RSM analysis of three factors, namely the type of Tween in  
329 nanoemulsion, Tween concentration, and Parijoto extract concentration, all ANOVA values  
330 show probabilities  $<0.0001$  ( $p<0.05$ ). This indicates that the quadratic response surface model  
331 used for both responses (dependent variables) is significant and can be used to optimize  
332 extraction factors (Wang et al., 2014). The coefficient of determination, or R square, depicts  
333 how independent data can explain dependent data. The range of R square values is between 0  
334 and 1, where values closer to 1 indicate better explanatory power.

335  
336 In the Central Composite Design analysis, the p-value indicates the significance of each  
337 coefficient in the built polynomial regression model. The lower the p-value, the more  
338 significant the contribution of the coefficient to the overall regression model (Zhong &  
339 Wang, 2010). It is important to note that using experimental data within the allowed range of  
340 variables in this study to create mathematical equations, which may have broader general  
341 applications, can provide the ability to predict system behavior when different factors are  
342 combined. From the perspective of optimizing the formation of emulsion nanoparticles, there  
343 is potential to develop more significant results, possibly based on the variables investigated in  
344 this study. Additionally, this optimization may be performed using the techniques outlined in  
345 this research to further test the effects of time and temperature or other conditions, as needed.

346 Table 4 shows details of the RSM approach used to assess particle size (nm), Poly Dispersity  
347 Index, Zeta Potential (mv), Conductivity, pII, and viscosity (Cp) in nanoemulsion of Parijoto  
348 fruit extract involved in a series of 81 experiments based on factorial design. The coefficients  
349 for the second-degree polynomial Equation are determined through experimental results,  
350 along with the regression coefficients for Particle Size (Y1), Poly Dispersity Index (Y2), Zeta  
351 Potential (Y3), Conductivity (Y4), pH (Y5), and viscosity (Y6). The Equation presented as  
352 Equation (2) shows the full quadratic model, while Table X shows the models predicting the  
353 response of the independent variables (Y1–Y6).

354 To assess the extent to which the equation model in RSM fits the data and how strong the  
355 influence of the variables is, the coefficient of determination or ( $R^2$ ) is used. Chin (1998) has  
356 categorized that for model suitability, the R-Square value is substantial if it is more than 0.67,  
357 moderate if it is more than 0.33 but lower than 0.67, and weak if it is more than 0.19 but  
358 lower than 0.33. pII and viscosity indicate strong model adequacy on these response  
359 variables. In contrast, the responses of Particle Size, Poly Dispersity Index, Zeta Potential,  
360 and Conductivity indicate a moderate model for these response variables. A lack of fit test  
361 was then performed to assess model fit for each response. With a p-value exceeding 0.05, it  
362 was confirmed that the model adequately fit the experimental data, as seen in Table 4.

### 363 **3.3 Contour plot on Particle Size, poly-dispersity index, Zeta Potential, Conductivity,** 364 **pII, and Viscosity as a function of Nanoemulsion Parijoto Fruit Extract.**

365 In this research, the model is created as a Contour plot, which can show the response: Particle  
366 Size, Poly Dispersity Index, Zeta Potential, Conductivity, pH, and Viscosity. Continued  
367 research shows a significant relationship between particle size and tween concentration and  
368 the type of lipophilic tween in nanoemulsions, as shown in Figures 1-6 the presented data  
369 offers valuable insights into the influence of lipophilic tween type and tween concentration  
370 on various properties of the nanoemulsion derived from parijoto fruit extract. Each figure  
371 depicts the contour plots illustrating the interaction effects between these two factors on  
372 different characteristics of the nanoemulsion.



373 In Figure 1, the contour plot demonstrates the interaction between the lipophilic tween type  
374 and tween concentration in controlling nanoparticle size. It reveals that as the lipophilic  
375 tween type increases from 20 to 80, and the tween concentration rises from 8% to 10%, there  
376 is a general trend of increasing particle size, albeit with a slight decreasing trend observed to  
377 some extent. This suggests that both factors play a role in determining the nanoparticle size,  
378 with higher concentrations leading to larger particle sizes. Moving to Figure 2, which  
379 illustrates the Zeta Potential of the nanoemulsion, an increase in the lipophilic Tween type  
380 from 60 to 80 and an increase in tween concentration from 8% to 10% correspond to an  
381 increase in Zeta Potential. Interestingly, no further changes are observed beyond this point.  
382 This indicates that these specific conditions result in optimal Zeta Potential, possibly  
383 indicating enhanced stability of the nanoemulsion.

384 Figure 3 showcases the influence of lipophilic tween type and tween concentration on the  
385 conductivity of the nanoemulsion. As the lipophilic tween type increases from 20 to 80 and  
386 the tween concentration rises from 8% to 12%, there is a consistent increase in conductivity  
387 without any further changes. This suggests a direct relationship between these factors and the  
388 conductivity of the nanoemulsion. The Contour plot presented in Figure 4 demonstrates the  
389 effect of lipophilic tween type and tween concentration on the Poly Dispersity Index (PDI) of  
390 the nanoemulsion. Interestingly, an increase in lipophilic tween type from 60 to 80 and a  
391 decrease in tween concentration from 12% to 8% lead to an increase in PDI value without  
392 further changes. This indicates a complex interaction between these factors in determining the  
393 homogeneity of particle size distribution within the nanoemulsion.

394 Figure 5 depicts the pH contour plot of the parijoto fruit extract nanoemulsion. An increase in  
395 lipophilic Tween type from 20 to 80 and an increase in tween concentration from 8% to 12%  
396 result in a consistent increase in pH without any further changes. This observation suggests  
397 that these specific conditions contribute to the alkalinity of the nanoemulsion, which may  
398 have implications for its stability and functionality. Finally, Figure 6 illustrates the viscosity  
399 contour plot of the nanoemulsion. An increase in lipophilic tween type from 35 to 80 and an  
400 increase in tween concentration from 8% to 12% lead to an increase in viscosity without  
401 further changes. This indicates that higher concentrations of lipophilic tween and tween result  
402 in a thicker consistency of the nanoemulsion, which affects its flow properties and  
403 application. The presented data highlights the intricate relationship between lipophilic tween  
404 type and tween concentration in influencing various physicochemical properties of the  
405 nanoemulsion derived from parijoto fruit extract. These findings provide valuable insights for  
406 optimizing the formulation and manufacturing process of the nanoemulsion for potential  
407 applications in various industries.

408 Research on the influence of surfactant type and concentration on nanoemulsion indicates  
409 that the selection of surfactant significantly affects the characteristics of nanoemulsion.  
410 Various surfactant types, such as Tween 20, Tween 60, and Tween 80, play different roles in  
411 forming nanoemulsions. The research results show that the particle size of Tween 80  
412 surfactant is the highest, with an average particle size of 107.196 nm. Similar results were  
413 reported by Chang et al. (2013), who obtained the smallest droplets in carvacrol-based  
414 nanoemulsion made with a mixture of food-grade non-ionic surfactants (Tween 20, 40, 60,  
415 80, and 85). Tween, a non-ionic surfactant derived from sorbitan ester, is soluble or  
416 dispersible in water and is commonly used as an oil-in-water emulsifier in the  
417 pharmaceutical, cosmetic, and cleaning industries. Among these surfactants, Tween 80 is one  
418 of the most commonly used. Research by Douglas et al. (2013) confirms that the type of non-  
419 ionic surfactant significantly influences the average particle diameter of the formed colloid  
420 dispersion. The smallest droplets were observed in systems prepared using Tween 80, while



421 the largest droplets formed in systems using Tween 85. The surfactant's Hydrophilic-  
422 Lipophilic Balance (HLB) plays a role in forming small particles. Surfactants with either too  
423 high (Tween 20) or too low (Tween 85) HLB values cannot form optimal nanoemulsions.  
424 Tween types with intermediate HLB values (Tween 40, 60, and 80) can form nanoemulsions  
425 with small particle sizes. However, there is no strong correlation between HLB values and  
426 particle sizes produced by these surfactants (Kumar et al., 2008). Small-molecule surfactants  
427 have higher surface activity and form smaller emulsion droplets than large ones (Qian &  
428 McClements, 2011; Teo, Goh & Lee, 2014).

429 Another critical factor for minimal droplet emulsion formation is the Hydrophilic-Lipophilic  
430 Balance (HLB) value of the surfactant (Sagitani, 1981), defined by Griffin as the ratio of  
431 surfactant hydrophilicity to lipophilicity (Griffin, 1949). A high HLB value indicates strong  
432 hydrophilicity, and the HLB values of non-ionic surfactants generally range from 0 to 20  
433 (Gad & Khairou, 2008), such as Tween 20 (HLB 16.7) and Tween 80 (HLB 15) (Dinarvand,  
434 Moghadam, Sheikhi & Atyabi, 2005). Emulsion stability is influenced by two polymer and  
435 particle surface tension mechanisms: steric stability caused by macromolecules adsorbed on  
436 particle surfaces and electrostatic stability due to repulsion between surface-charged droplets.  
437 In nanoemulsions made with Tween 80 surfactant, the surfactant may not have a charge on  
438 the hydrophobic group, causing the covered droplet surface to be non-charged and resulting  
439 in low zeta potential values, which can lead to increased particle size and PDI (Lian et al.,  
440 2016).

441 However, a different study proposed by Alam et al. (2023) suggests that Tween 20 helps  
442 improve PDI and allows for minimum polydispersity. Compared to other nanoparticles, the  
443 ability to maintain particle integrity using Tween 20 is significant. Increasing the Surfactant  
444 content in the formulation increases the polydispersity indices for natural extracts in the 3D  
445 response surface graph. This indicates that the use of Tween types with low and high HLB  
446 values can be applicable when combined with an optimal concentration of co-surfactant.

447 Surfactant concentration is also a critical factor in nanoemulsion formation. Research  
448 indicates that increasing surfactant concentration can result in smaller and more homogenous  
449 size distribution. However, there is a specific limit where surfactant concentration reaches a  
450 plateau level, leading to unadsorbed surfactant aggregation and micelle formation. The results  
451 show that the higher the Tween concentration, the higher the size and PDI. This is confirmed  
452 by Liat et al. (2016), stating that nanoemulsions prepared with higher surfactant  
453 concentrations significantly increase short-term stability. Systems with 15 or 20% weight of  
454 Tween 80 are highly unstable to increasing dilution, indicating that a medium surfactant  
455 concentration level may be more suitable for stable nanoemulsion preparation. Although the  
456 initial droplet size is small, higher surfactant concentrations can increase raw material costs  
457 and cause undesirable sensory (taste) issues in commercial applications. Therefore, this study  
458 uses a 10% weight of Tween 80 in further experiments.

459 Increasing surfactant concentration increases the number of surfactant molecules migrating  
460 from the oil phase to the emulsion water phase, and nanodroplets form. Frictional forces  
461 applied to the oil-water interface, coated with emulsifier, cause some emulsifiers to sink  
462 parallel to the surface layer, while others detach from the surface layer. Hasani et al. (2015)  
463 reported that droplet size increases by increasing surfactant concentration to 20%, and  
464 particles have a broad and non-uniform size distribution. The instability of nanoemulsion at  
465 high surfactant concentrations may be related to the depletion-flocculation mechanism of  
466 adsorbed surfactant. With increased surfactant concentration, additional surfactant molecules  
467 form micelles in the continuous phase rather than orienting on the particle surface. This leads  
468 to an increase in local osmotic pressure, causing the continuous phase between moving



469 droplets to decrease, reducing the continuous phase between those droplets. As a result,  
470 aggregation occurs, causing an increase in particle size. According to Ohi et al. (2011) and  
471 Tadros et al. (2004), the average droplet size becomes smaller, and the size distribution  
472 becomes narrower with increasing emulsifier concentration, ultimately reaching a plateau  
473 level. Beyond the plateau level, free or unadsorbed emulsifiers may accumulate to form  
474 micelles. Nanoemulsions are known to be thermodynamically unstable, tending to minimize  
475 interfacial area through coalescence.

476 An increase in the concentration of the filler extract can lead to the tendency of nanoparticles  
477 to aggregate or form agglomerates also pH nanoemulsion. This phenomenon may occur due  
478 to physical or chemical interactions between nanoparticles and compounds in the filler  
479 extract. Findings by Alab et al. (2021) suggest that an increase in extract concentration results  
480 in an increase in particle size, particularly at the highest concentration of 347.2 nm. On the  
481 other hand, the smallest concentration has the lowest particle size at 86.98 nm. These results  
482 indicate that higher concentrations may increase the likelihood of particle agglomeration.

483 Furthermore, increasing the concentration of parijoto fruit extract can increase the total mass  
484 in the solution, which, in turn, can increase overall viscosity. Additional particles or  
485 molecules from the filler extract can contribute to the increase in viscosity. A study by Olan  
486 et al. (2021) shows that particles with the highest concentration have the highest viscosity and  
487 vice versa. This increase in viscosity may be caused by excess extract loaded into particles.  
488 The physicochemical characteristics of the filler extract may influence the viscosity  
489 properties of nanoparticles, and factors such as changes in pH, temperature, or chemical  
490 composition may also play a role in viscosity increase. Parijoto fruit is rich in active  
491 compounds, such as anthocyanins, which can affect the surface charge of nanoemulsion  
492 particles. At a certain pH, anthocyanins or other components may have specific charges that  
493 can influence the electrostatic stability of particles (Liu et al., 2016). Anthocyanins may  
494 undergo solubility changes at specific pH values, affecting the distribution and stability of the  
495 nanoemulsion's oil or water phase. The same occurs with surfactants, where variations in  
496 charge of the filler extract from parijoto fruit can affect the interaction between nanoparticles,  
497 anthocyanins, and other components in the system. The loading capacity of the extract in the  
498 nanoemulsion likely depends on its solubility in the system used (Costa et al., 2012).  
499 Anthocyanins tend to undergo color changes with pH (pH-dependent color shift).  
500 Additionally, the antioxidant activity of anthocyanins can be influenced by pH. This  
501 complexity can modulate the overall physicochemical properties of the nanoemulsion system.  
502





503 **3.4 Optimal Point Prediction from RSM in Nanoemulsion Parijoto Fruit Extract**

504 Optimal point predictions from the Response Surface Methodology are obtained by  
 505 combining optimal conditions based on interactions between independent variables. Profiler  
 506 predictions are obtained if the fitted surface graph is in minimum, maximum, or saddle form.  
 507 3D graphics on image x. shows a complex interaction between the variable factors of  
 508 lipophilic tween type and tween concentration on the response. Increasing the lipophilic  
 509 tween type value increases the response somewhat, but the tween concentration value can  
 510 modify the effect. There is an optimal region where the response reaches its peak. The  
 511 implication for practice is that by setting the variable factors at levels that are estimated to be  
 512 optimum, the research results can achieve the highest optimization in the desired response,  
 513 which can be seen in Figure 7.

514 Table 3. Prediction of Optimum Conditions for Parijoto Fruit Extract Nanoemulsion

Types of Analysis	Types of Lyphophilic Tweens	Tween Concentration (%)	Parijoto Fruit Extract Concentration (%)	Nanoparticle Size (nm)	Zeta Potential(mV)	Conductivity (mS/cm)	Poly Dispersity Index
<b>Optimum Condition Prediction</b>	80	12	7.5	61.97	-28.48	0.082	0.691
<b>Maximum Value at Optimum Conditions</b>	80	12	7.5	39.94	-32.48	0.048	0.371
<b>Minimum Value at Optimum Conditions</b>	80	12	7.5	163.88	-26.37	0.115	1,011

515

516 It can be seen in Table 9 that to achieve the maximum desired concentration of nanoparticle  
 517 size, zeta potential, Conductivity, Poly Dispersity Index, degree of acidity, and Viscosity, it is  
 518 necessary to set the Tween solvent concentration to 80, Tween concentration to 12% and  
 519 Parijoto fruit extract concentration to 7.5%. This set of conditions has a desirability value of  
 520 0.74. Because the value is almost close to 1 and falls into the moderate category, this set of  
 521 conditions is quite optimal for the aim of this research, namely to maximize the response.

522 The optimization of nanoemulsion formation from Parijoto fruit extract using Response  
 523 Surface Methodology (RSM) has been conducted in this study. RSM is a statistical method  
 524 used to design experiments and analyze the impact of multiple independent variables on a  
 525 measured response. As an output of this research, the synthesis process conditions of  
 526 nanoemulsion from Parijoto fruit extract can be optimized to achieve particle size,  
 527 polydispersity index (PDI), zeta potential, conductivity, pH, and viscosity levels. RSM  
 528 determines the optimal extraction time and temperature to maximize the response variable  
 529 outcomes (Granato et al., 2014). In line with this, predictions and observations are within a  
 530 narrow range and do not show significant differences at a 5% significance level, indicating  
 531 the model's suitability for optimization and process efficiency purposes.



532

533 The optimal point prediction from the Response Surface Methodology is obtained by  
534 integrating optimal conditions and depends on the interaction between independent variables,  
535 as Ratnawati et al. (2018) explained. The prediction profile is formed when the adjusted  
536 surface graphs show a minimum, maximum, or saddle shape. The optimization process can  
537 achieve optimal responses by analyzing each response beforehand, ultimately reducing effort  
538 and operational costs, as Nurmiah et al. (2013) stated. Desirability, with a range of values  
539 from 0 to 1, is used as the optimization target value, with low (0-0.49), moderate (0.5-0.79),  
540 and high (0.8-1) categories. The closer the value of 1 is, the greater the desirability, which  
541 indicates the suitability of the combination of process parameters to achieve optimal response  
542 variables.

543

544 It can be seen in Table 3 that to achieve the desired concentrations of nanoparticle size, zeta  
545 potential, conductivity, polydispersity index, acidity level, and viscosity, it is necessary to set  
546 the concentration of Tween 80, Tween concentration at 12%, and Parijoto fruit extract  
547 concentration at 7.5%. This set of conditions has a desirability value of 0.740349. Since its  
548 value is close to 1 and falls into the moderate category, this set of conditions is quite optimal  
549 for this research, which is to maximize the response

#### 550 **4 Conclusion**

551 In this series of experiments, nanoemulsion from parijoto fruit has been characterized, considering  
552 various physicochemical parameters such as particle size, polydispersity index, zeta potential,  
553 conductivity, pH, and viscosity. The research results indicate significant variations in the physical  
554 characteristics of both nanomaterials in terms of changes in surfactant and parijoto extract  
555 concentrations. Increased surfactant concentration tends to produce smaller particle sizes and a more  
556 homogeneous distribution, although certain limitations were found that lead to surfactant aggregation  
557 and micelle formation. The nanoemulsion characteristics, including zeta potential, polydispersity,  
558 particle size, conductivity, pH, and viscosity. The type and concentration of surfactants played a  
559 crucial role in determining the properties of the nanoemulsions. Variations in surfactant parameters  
560 resulted in observable differences in emulsion characteristics, highlighting the importance of  
561 surfactant selection and optimization. To achieve optimal nanoemulsion process conditions, it is  
562 recommended to use 12% Tween 80 solvent concentration, 12% Tween concentration, and 7.5%  
563 parijoto fruit extract concentration, resulting in a desirability value of 0.74, falling into the moderate  
564 category.

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## 6. Revision from Reviewer 3

- Interactive Discussion Proof

**Q1** Please list your revision requests for the authors and provide your detailed comments, including highlighting limitations and strengths of the study and evaluating the validity of the methods, results, and data interpretation. If you have additional comments based on Q2 and Q3 you can add them as well.

Reviewer 3 | 27 Apr 2024 | 16:00

#1

Reviewers' comments:

Manuscript ID: 1398809

FORMULATION OF NANOEMULSION PARIJOTO FRUIT EXTRACT (*Medinilla speciosa*) WITH VARIATION OF TWEEN'S STABILIZERS

In the manuscript, under review, the current manuscript aims to This study aimed to investigate the characteristics of nanoemulsion formulations derived from parijoto fruit extract and to evaluate an optimum condition with various tween surfactant. In general, the manuscript needs considerable improvement. However, there are some insufficient flaws, which are listed below. Therefore, major revisions are required before it can be published.

Abstract:

Line No. 29-30: Particle sizes or droplet sizes? I would suggest droplet sizes would be more appropriate.

Line No. 32-38: The author should clear the composition discussion: 12% concentration of Tween 80 solvent, 12% tween concentration...?

What is yielding a desirability value of 0.74? kindly rephrase the sentence with clarity.

Line No. 49: The reference (Xiao S J & Huang, 2016) has to corrected as journal format.

Introduction:

Line No. 64-68: The sentence formation is poorly described, the author should rephrase it.

1. Elaborate the Introduction: The manuscript's introduction is very short and needs to be expanded to provide a clear context and background for the research. This will help the readers understand the significance of the research and its potential impact.

2. Include recent research: The manuscript needs to include the last 10 years of research and review articles from Nanoemulsion. The absence of recent research is a significant flaw that needs to be addressed. Additionally, the manuscript should present the most recent findings related to the bioactivity of essential oil nanoemulsions.

General: Please check your spelling and grammar

1. Do proper alignment

2. Include the list of abbreviations

3. References should follow as mentioned in the journal author guidelines.

Line No. 76: Include the "Condition" after the "simulated gastrointestinal"

Line No. 79: "So far" not "This far"

Line No. 79-82: The sentence formation is poorly described, rephrase it

Line No. 101: The fruits used are ripe fruits...? Fruits means ripened, do not use ripened fruit.

Line No. 107, 113 & 116: Need to give space between number and symbol "70°C". The same has to be followed throughout the manuscript

Line No. 113: include make and instrument details for "centrifuge"

Line No. 131: include make and instrument details for "sonicator"

Line No. 136: Malvern et al.?

Line No. 167: include make and instrument details for the "viscometer instrument"

Line No. 215: ANOVA test, Which software was used?

Line No. 236: Check the number "79.14334.628"...? ?

Line No. 246: What is the Ultra-assisted extraction method?

Challenges associated with the Stability of nanoemulsion need to be included in the brief.

Also, industrial application of parijoto fruit or extract must be included.

Revise the conclusion: The conclusion part should be written with improvement. Instead, the collective research information should be presented in the conclusion part. This will help to summarize the research's key findings and their implications effectively.

I strongly recommend that the author needs to re-write the sentence formation in many places and keep the units and symbols appropriate in the manuscript. Also, English language and typographical errors need to be avoided in many places. Overall, this manuscript required more improvement to meet the standard in the field of nanoemulsion.

## 7. Submission of Revised Manuscript 3

- Interactive Discussion Proof

Corresponding Author: Victoria Kristina Ananingsih | 01 May 2024 | 07:57

41

We would like to extend my heartfelt gratitude for taking the time to review our manuscript. Your insightful feedback and constructive criticism have immensely contributed to the refinement of our research.

Your meticulous attention to detail and suggestions for improvement have been invaluable in enhancing the clarity and quality of our work. Your expertise and thoughtful comments have guided us towards addressing several crucial aspects, ensuring a more robust and comprehensive manuscript.

Warm regards,

- Line No. 29-30: Particle sizes or droplet sizes? I would suggest droplet sizes would be more appropriate.

Thank you for your input. We appreciate the suggestion to use the term "droplet size" in the abstract, but we would like to maintain consistency with the terminology used in our research, which is "particle size." We believe that this term is more appropriate for the characteristics we are studying. We hope that our decision to use the term "particle size" can be accepted.

- Line No. 32-35: The author should clear the composition discussion: 12% concentration of Tween 80 solvent, 12% Tween concentration. What is yielding a desirability value of 0.74? Kindly rephrase the sentence with clarity. Line No. 46: The reference (Xiao S J & Huang, 2016) has to corrected as journal format.

We apologize for any confusion regarding the composition discussion mentioned in lines 32-35. The statement clarifies the use of Tween 80 as an optimal type of stabilizer, along with a separate 12% concentration of Tween utilized in the formulation. Additionally, the desirability value of 0.74 was established through an evaluation process, and we will amend the sentence to provide clearer context.

We already revised it (Line 32-35)

"The optimal process conditions for nanoemulsion consisting of the type of Tween used are Tween 80, Tween concentration of 12 %, and perilla fruit extract concentration of 7.5 %, yielding a desirability value of 0.74, categorizing it as moderate."

Regarding the reference to Xiao and Huang (2016) cited in line 46, we acknowledge the oversight and already corrected the reference to adhere to journal formatting standards (Line 53-54)

"Surfactants can diminish interfacial tension and form a substantially influential steric-elastic film on the emulsion results (Xiao S Huang, 2016)."

- Introduction:

Line No. 64-65: The sentence formation is poorly described, the author should rephrase it.

1. Elaborate the introduction: The manuscript's introduction is very short and needs to be expanded to provide a clear context and background for the research. This will help the readers understand the significance of the research and its potential impact.

2. Include recent research: The manuscript needs to include the last 10 years of research and review articles from Nanoemulsion. The absence of recent research is a significant flaw that needs to be addressed. Additionally, the manuscript should present the most recent findings related to the bioactivity of essential oil nanoemulsions.

We have revised it (line 37-54 and line 77-98).

Line 37-54

Nanotechnology underwent progressive evolution, characterized by measurements on the nanometer scale, approximately 10<sup>-9</sup> meters (Ariningsih, 2016). Acknowledgment from the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) (2009) underscored nanotechnology's significant potential in enhancing food products, attributed to its capacity to modify surface characteristics and particle size. Such modifications facilitate targeted delivery of food compounds to specific organs and the controlled release of active compounds to mitigate adverse effects. The attributes of nanoscale food materials are pivotal in propelling diverse industries, including food, pharmaceuticals, and extensive nutraceutical applications (Rahman et al., 2020). Due to their substantial surface area-to-volume ratio, nanoemulsions exhibit enhanced stability against gravitational separation and aggregation, owing to their distinct physicochemical and biological characteristics compared to conventional emulsions. The droplets or globules inherent in nanoemulsions mitigate gravitational forces and Brownian motion, thereby averting creaming or sedimentation during storage. Nanoemulsions denote a nanotechnological rendition of a stable colloidal system, achieving kinetic stability through the amalgamation of oil, emulsifier, and water (McLemerts, 2016). Chang et al. (2022) conducted research utilizing surfactants as stabilizers in synthesizing nanoemulsions, showcasing the stability of nanoemulsion particle size in curcumin extract. Surfactants can diminish interfacial tension and form a substantially influential steric elastic film on the emulsion results (Xiao & Huang, 2016).

Line 77 - 99

Appropriate nano-encapsulation techniques, such as nanoemulsion, have shown the potential to enhance the stability, bioavailability, and solubility of lipophilic bioactive compounds while also preventing hydrolysis and oxidation (Russo et al., 2020). Nanoemulsions are widely utilized nanoformulations in food-related industries through active or passive targeting mechanisms. Gunasekaran et al. (2014) introduced nanotechnology as an effective tool for enhancing the bioavailability and bioactivity of phytochemicals. Nanoemulsion has emerged as a novel technology, providing opportunities to address challenges associated with delivering micronutrients in functional food (Joyce et al., 2014). Shin et al. (2015) explored recent advancements in nanoformulation of lipophilic functional foods. Moreover, nanotechnology-based strategies have been explored to associate complex matrices derived from plant extracts, offering promising prospects for developing novel therapeutic formulations (Zorzi et al., 2015). Synthesis of nanoemulsion using mangosteen peel extract rich in anthocyanins as the main ingredient of the formulation can increase the dominant penetration of  $\alpha$ -mangosin through the stratum corneum (Pratiwi et al., 2017). Catechin nanoemulsion showed a remarkable improvement in stability and bioavailability in simulated gastrointestinal conditions (Rafanar et al., 2016). Mulia et al. (2017) showed the optimum results using a high-speed homogenization and Tween surfactant to prepare nanoemulsions with nanoemulsion. Research conducted by Chang et al. (2022) used Tween as the surfactant in the stable nanoemulsion synthesis loaded curcumin extract. This highlights the opportunity to develop nanoemulsion formulations for anthocyanins found in perijato fruit. So far, research on nanoemulsion formulation in perijato fruit involving various concentrations and stabilizers still needs to be conducted. This study is conducted to investigate the characteristics of nanoemulsion formulations derived from perijato fruit extract and to evaluate an optimum condition with various tween surfactant.

- Line No. 76: Include the "Condition" after the "simulated gastrointestinal"

We have revised the sentence (line 89 - 91).

Catechin nanoemulsion showed a remarkable improvement in stability and bioavailability in simulated gastrointestinal conditions (Rafanar et al., 2016).

- Line No. 79: "So far" not "Thus far"

We have revised the sentence (line 95 - 98).

So far, research on nanoemulsion formulation in perijato fruit involving various concentrations and stabilizers still needs to be conducted. This study is conducted to investigate the characteristics of nanoemulsion formulations derived from perijato fruit extract and to evaluate an optimum condition with various tween surfactant.

- Line No. 79-B: The sentence formation is poorly described, rephrase it

We already rephrased it (line 95 - 98).

So far, research on nanoemulsion formulation in perijato fruit involving various concentrations and stabilizers still needs to be conducted. This study is conducted to investigate the characteristics of nanoemulsion formulations derived from perijato fruit extract and to evaluate an optimum condition with various tween surfactant.

- Line No. 101: The fruits used are ripe fruits...? Fruits means ripened, do not use ripened fruit.

We use the term "ripe fruit" because perijoto fruit can be consumed locally when it is "half-ripe". Meanwhile, what we use is "fully ripe" perijoto fruit with a slightly soft texture.

- Line No. 107, 113 & 116: Need to give space between number and symbol "°C". The same has to be followed throughout the manuscript.

We have revised it.

The drying temperature used was 70 °C for 6 hours. The dried Perijoto fruit is then ground into powder using a herbal grinder for 2 minutes.

- Line No. 113: Include make and instrument details for "centrifuge"

We have written it in more details (line 125-132).

Five grams of dry sample powder and 50 mL of 99.5% ethanol were mixed thoroughly for homogeneity in four 250 mL centrifuge bottles. Then, all vials were sonicated using a Bio-Sized Ultrasonic Waterbath with a 40 kHz frequency and 100 W power for 30 minutes. Subsequently, the samples were subjected to shaking for one hour. The centrifugation step was performed at 4,000 rpm at 4 °C for 10 minutes (Ohaus, USA). The supernatant was then carefully collected, and the remaining solution was evaporated to dryness under vacuum conditions. The residue was dissolved in 99.5% ethanol and diluted to 20 mL. After filtering through a 0.22 µm membrane filter, perijoto fruit extract was obtained and stored at -20 °C for UV-Vis analysis.

- Line No. 131: Include make and instrument details for "sonicator".

We have written it in more details (line 125-132).

Five grams of dry sample powder and 50 mL of 99.5% ethanol were mixed thoroughly for homogeneity in four 250 mL centrifuge bottles. Then, all vials were sonicated using a Bio-Sized Ultrasonic Waterbath with a 40 kHz frequency and 100 W power for 30 minutes. Subsequently, the samples were subjected to shaking for one hour. The centrifugation step was performed at 4,000 rpm at 4 °C for 10 minutes (Ohaus, USA). The supernatant was then carefully collected, and the remaining solution was evaporated to dryness under vacuum conditions. The residue was dissolved in 99.5% ethanol and diluted to 20 mL. After filtering through a 0.22 µm membrane filter, perijoto fruit extract was obtained and stored at -20 °C for UV-Vis analysis.

- Line No. 136: Malvern et al.?

We have revised it (line 153)

(Zetasizer Pro; Malvern Instruments, Ltd., Malvern.)

- Line No. 167: Include make and instrument details for the "viscometer instrument"

We have written it in more details (line 183-188).

Viscosity measurements are carried out using a viscometer Brookfield. 14 mL of sample was put into the cup and attached to the solvent trap provided. The viscometer was set at 200 rpm, three rotations, for 30 seconds.

The total anthocyanin content in the dry samples and extracts was 538.47 ± ppm. The dried Parijoto exhibited significant antioxidant activity, with a % inhibition value of 79.14 ± 34.82.

- Line No. 246: What is the Ultra-assisted extraction method?

We have revised and mentioned it (line 261-267).

The parijoto fruit extract was obtained through an extraction process using the Ultra-assisted extraction method. The Ultra-assisted extraction method involves the utilization of a modified ultrasonic water bath for the extraction of parity fruit. This method harnesses ultrasonic energy to enhance the extraction process by facilitating cell wall breakdown and increasing target compounds' solubility. During extraction, the parijoto fruit is immersed in a solvent within the ultrasonic waterbath, where ultrasonic waves are applied to the sample.

We have revised and justified typowriting based on the suggestions given by reviewers which can be seen in the lines below.

- Challenges associated with the Stability of nanoemulsion need to be included in the brief. Also, Industrial application of parijoto fruit or extract must be included.

We have revised it (Line 520-531).

The challenge is the propensity for Ostwald ripening, wherein larger droplets grow at the expense of smaller ones, leading to phase separation and reduced shelf-life. Additionally, factors such as temperature fluctuations, pH changes, and exposure to light can exacerbate instability, causing particle aggregation and creaming. Surfactant degradation over time is another concern, as it can compromise the emulsion's ability to maintain a stable dispersion. However, the industrial application of parijoto fruit or extract holds significant potential. Parijoto fruit, known for its rich content of bioactive compounds, including anthocyanins, flavonoids, and phenolic acids, offers various health benefits such as antioxidant and anti-inflammatory properties. Incorporating parijoto extract into nanoemulsions can enhance its bioavailability and efficacy, making it suitable for a range of industrial applications especially food functional and nutraceutical.

- Revise the conclusion: The conclusion part should be written with improvement. Instead, the collective research information should be presented in the conclusion part. This will help to summarize the research's key findings and their implications effectively.

We have revised the conclusion (line 562-576).

In this series of experiments, nanoemulsion from parijoto fruit has been characterized, considering various physicochemical parameters such as particle size, polydispersity index,  $\zeta$ -potential, conductivity, pH, and viscosity respectively ranged from 14,603±16.73 nm to 118,053±4,5825 nm, 0.402±0.038 to 0.874±0.100, -22.197±0.738 mV to -28.207±1.598 mV, 0.064±0.013 to 0.090±0.010 mS/cm, and 6,747±0.035 to 6.897±0.006, and 3.827±0.021 to 5.633±0.058. The research results indicate significant variations in the physical characteristics of both nanomaterials regarding changes in surfactant and parijoto extract concentrations. Increased surfactant concentration tends to produce smaller particle sizes and a more homogeneous distribution, although certain limitations were found that lead to surfactant aggregation and micelle formation. The nanoemulsion characteristics include  $\zeta$ -potential, polydispersity, particle size, conductivity, pH, and viscosity. The type and concentration of surfactants played a crucial role in determining the properties of the nanoemulsions. Variations in surfactant parameters resulted in observable differences in emulsion characteristics, highlighting the importance of surfactant selection and optimization. To achieve optimal nanoemulsion process conditions, it is recommended to use Tween 80 with 12% Tween concentration and 7.5% parijoto fruit extract concentration, resulting in a desirability value of 0.74, into the moderate category.

- Supporting File

We would like to extend my heartfelt gratitude for taking the time to review our manuscript. Your insightful feedback and constructive criticism have immensely contributed to the refinement of our research.

Your meticulous attention to detail and suggestions for improvement have been invaluable in enhancing the clarity and quality of our work. Your expertise and thoughtful comments have guided us towards addressing several crucial aspects, ensuring a more robust and comprehensive manuscript.

Warm regards,



- Line No. 29-30: Particle sizes or droplet sizes? I would suggest droplet sizes would be more appropriate.

Thank you for your input. We appreciate the suggestion to use the term "droplet size" in the abstract, but we would like to maintain consistency with the terminology used in our research, which is "particle size." We believe that this term is more appropriate for the characteristics we are studying. We hope that our decision to use the term "particle size" can be accepted.

- Line No. 32-35: The author should clear the composition discussion: 12% concentration of Tween 80 solvent, 12% Tween concentration...? What is yielding a desirability value of 0.74? Kindly rephrase the sentence with clarity. Line No. 49: The reference (Xiao 50 J & Huang, 2016) has to corrected as journal format.

We apologize for any confusion regarding the composition discussion mentioned in lines 32-35. The statement clarifies the use of Tween 80 as an optimal type of stabilizer, along with a separate 12% concentration of Tween utilized in the formulation. Additionally, the desirability value of 0.74 was established through an evaluation process, and we will amend the sentence to provide clearer context.

We already revised it (Line 32-35)

"The optimal process conditions for nanoemulsion consisting of the type of Tween used are Tween 80, Tween concentration of 12 %, and parijoto fruit extract concentration of 7.5 %, yielding a desirability value of 0.74, categorizing it as moderate."

Regarding the reference to Xiao and Huang (2016) cited in line 49, we acknowledge the oversight and already corrected the reference to adhere to journal formatting standards (Line 53 -54)

"Surfactants can diminish interfacial tension and form a substantially influential steric elastic film on the emulsion results (Xiao & Huang, 2016)."

- Introduction:

Line No. 64-65: The sentence formation is poorly described, the author should rephrase it.

1. Elaborate the introduction: The manuscript's introduction is very short and needs to be expanded to provide a clear context and background for the research. This will help the readers understand the significance of the research and its potential impact.

2. Include recent research: The manuscript needs to include the last 10 years of research and review articles from Nanoemulsion. The absence of recent research is a significant flaw that needs to be addressed. Additionally, the manuscript should present the most recent findings related to the bioactivity of essential oil nanoemulsions.

We have revised it (line 37-54 and line 77-98).

Line 37-54

Nanotechnology underwent progressive evolution, characterized by measurements on the nanometer scale, approximately  $10^{-9}$  meters (Ariningsih, 2016). Acknowledgment from the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) (2009) underscored nanotechnology's significant potential in enhancing food products, attributed to its capacity to modify surface characteristics and particle size. Such modifications facilitate targeted delivery of food compounds to specific organs and the controlled release of active compounds to mitigate adverse effects. The attributes of nanoscale food materials are pivotal in propelling diverse industries, including food, pharmaceuticals, and extensive nutraceutical applications (Rahman et al., 2020). Due to their substantial surface area-to-volume ratio, nanoemulsions exhibit enhanced stability against gravitational separation and aggregation, owing to their distinct physicochemical and biological characteristics compared to conventional emulsions. The droplets or globules inherent in nanoemulsions mitigate gravitational forces and Brownian motion, thereby averting creaming or sedimentation during storage. Nanoemulsions denote a nanotechnological rendition of a stable colloidal system, achieving kinetic stability through the amalgamation of oil, emulsifier, and water (McLements, 2016). Chang et al. (2022) conducted research utilizing surfactants as stabilizers in synthesizing nanoemulsions, showcasing the stability of nanoemulsion particle size in curcumin extract. Surfactants can diminish interfacial tension and form a substantially influential steric elastic film on the emulsion results (Xiao & Huang, 2016).

Line 77 - 99

Appropriate nano-encapsulation techniques, such as nanoemulsion, have shown the potential to enhance the stability, bioavailability, and solubility of lipophilic bioactive

compounds while also preventing hydrolysis and oxidation (Rosso et al., 2020). Nanoemulsions are widely utilized nanoformulations in food-related industries through active or passive targeting mechanisms: Gunasekaran et al. (2014) introduced nanotechnology as an effective tool for enhancing the bioavailability and bioactivity of phytomedicine. Nanoemulsion has emerged as a novel technology, providing opportunities to address challenges associated with delivering micronutrients in functional food (Joyce et al., 2014). Shin et al. (2015) explored recent advancements in nanoformulation of lipophilic functional foods. Moreover, nanotechnology-based strategies have been explored to associate complex matrices derived from plant extracts, offering promising prospects for developing novel therapeutic formulations (Zorzi et al., 2015). Synthesis of nanoemulsion using mangosteen peel extract rich in anthocyanins as the main ingredient of the formulation can increase the dominant penetration of  $\alpha$ -mangostin through the stratum corneum (Pratiwi et al., 2017). Catechin nanoemulsion showed a remarkable improvement in stability and bioavailability in simulated gastrointestinal conditions (Rafanar et al., 2016). Mulia et al. (2017) showed the optimum results using a high-speed homogenization and Tween surfactant to prepare nanoemulsions with nanoemulsion. Research conducted by Chang et al. (2022) used Tween as the surfactant in the stable nanoemulsion synthesis loaded curcumin extract. This highlights the opportunity to develop nanoemulsion formulations for anthocyanins found in parijoto fruit. So far, research on nanoemulsion formulation in parijoto fruit involving various concentrations and stabilizers still needs to be conducted. This study is conducted to investigate the characteristics of nanoemulsion formulations derived from parijoto fruit extract and to evaluate an optimum condition with various tween surfactant.

- Line No. 76: Include the "Condition" after the "simulated gastrointestinal"

We have revised the sentence (line 89 - 91).

Catechin nanoemulsion showed a remarkable improvement in stability and bioavailability in simulated gastrointestinal conditions (Rafanar et al., 2016).

- Line No. 79: "So far" not "Thus far"

We have revised the sentence (line 95 - 98).

So far, research on nanoemulsion formulation in parijoto fruit involving various concentrations and stabilizers still needs to be conducted. This study is conducted to

investigate the characteristics of nanoemulsion formulations derived from parijoto fruit extract and to evaluate an optimum condition with various tween surfactant.

- Line No. 79-83: The sentence formation is poorly described, rephrase it

We already rephrased it (line 95 - 98).

So far, research on nanoemulsion formulation in parijoto fruit involving various concentrations and stabilizers still needs to be conducted. This study is conducted to investigate the characteristics of nanoemulsion formulations derived from parijoto fruit extract and to evaluate an optimum condition with various tween surfactant.

- Line No. 101: The fruits used are ripe fruits...? Fruits means ripened, do not use ripened fruit.

We use the term "ripe fruit" because parijoto fruit can be consumed locally when it is "half-ripe". Meanwhile, what we use is "fully ripe" parijoto fruit with a slightly soft texture.

- Line No. 107, 113 & 116: Need to give space between number and symbol "70°C". The same has to be followed throughout the manuscript.

We have revised it.

The drying temperature used was 70 °C for 6 hours. The dried Parijoto fruit is then ground into powder using a herbal grinder for 2 minutes.

- Line No. 113: Include make and instrument details for "centrifuge"

We have written it in more details (line 125-132).

Five grams of dry sample powder and 50 mL of 99.5% ethanol were mixed thoroughly for homogeneity in four 250 mL centrifuge bottles. Then, all vials were sonicated using a Bio-Based Ultrasonic Waterbath with a 40 KHz frequency and 100 W power for 30 minutes. Subsequently, the samples were subjected to shaking for one hour. The centrifugation step was performed at 4,000 rpm at 4°C for 10 minutes (Ohaus, USA). The supernatant was then carefully collected, and the remaining solution was evaporated to dryness under vacuum conditions. The residue was dissolved in 99.5%

ethanol and diluted to 20 mL. After filtering through a 0.22 µm membrane filter, parijoto fruit extract was obtained and stored at -20 °C for UV-Vis analysis.

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(Zetasizer Pro; Malvern Instruments, Ltd., Malvern.)

- Line No. 167: Include make and instrument details for the “viscometer instrument”

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Viscosity measurements are carried out using a viscometer brookfield. 14 mL of sample was put into the cup and attached to the solvent trap provided. The viscometer was set at 200 rpm, three rotations, for 30 seconds.

- Line No. 215: ANOVA test, Which software was used?

All statistical analyzes were carried out using the statistics 12.5 application presented at the beginning of the sub-chapter (line 190-194).

In this study, primary data in 3 repetitions of extraction and three repetitions of testing were averaged and given a standard deviation value for each treatment combination using Statistica 12.5 by StatSoft. The data is then entered into a statistical application, arranged in a combination of factorial points, axial points, and central points with three repetitions. After that, the data was analyzed, and several test stages were carried out.

- Line No. 236: Check the number "79.14334.82%"... ?

We have revised it (line 251-252).

The total anthocyanin content in the dry samples and extracts was  $538.47 \pm$  ppm. The dried Parijoto exhibited significant antioxidant activity, with a % inhibition value of  $79.14 \pm 34.82$ .

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The challenge is the propensity for Ostwald ripening, wherein larger droplets grow at the expense of smaller ones, leading to phase separation and reduced shelf-life. Additionally, factors such as temperature fluctuations, pH changes, and exposure to

light can exacerbate instability, causing particle aggregation and creaming. Surfactant degradation over time is another concern, as it can compromise the emulsion's ability to maintain a stable dispersion. However, the industrial application of parijoto fruit or extract holds significant potential. Parijoto fruit, known for its rich content of bioactive compounds, including anthocyanins, flavonoids, and phenolic acids, offers various health benefits such as antioxidant and anti-inflammatory properties. Incorporating parijoto extract into nanoemulsions can enhance its bioavailability and efficacy, making it suitable for a range of industrial applications especially food functional and nutraceutical.

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In this series of experiments, nanoemulsion from parijoto fruit has been characterized, considering various physicochemical parameters such as particle size, polydispersity index,  $\zeta$ -potential, conductivity, pH, and viscosity respectively ranged from  $14,603 \pm 16.73$  nm to  $118,053 \pm 4.5825$  nm,  $0.402 \pm 0.038$  to  $0.874 \pm 0.100$ ,  $-22.197 \pm 0.738$  mV to  $-28.207 \pm 1.598$  mV,  $0.064 \pm 0.013$  to  $0.090 \pm 0.010$  mS/cm, and  $6.747 \pm 0.035$  to  $6.897 \pm 0.006$ , and  $3.827 \pm 0.021$  to  $5.633 \pm 0.058$ . The research results indicate significant variations in the physical characteristics of both nanomaterials regarding changes in surfactant and parijoto extract concentrations. Increased surfactant concentration tends to produce smaller particle sizes and a more homogeneous distribution, although certain limitations were found that lead to surfactant aggregation and micelle formation. The nanoemulsion characteristics include  $\zeta$ -potential, polydispersity, particle size, conductivity, pH, and viscosity. The type and concentration of surfactants played a crucial role in determining the properties of the nanoemulsions. Variations in surfactant parameters resulted in observable differences in emulsion characteristics, highlighting the importance of surfactant selection and optimization. To achieve optimal nanoemulsion process conditions, it is recommended to use Tween 80 with 12% Tween concentration and 7.5% parijoto fruit extract concentration, resulting in a desirability value of 0.74, into the moderate category.

- Revised Manuscript 3



## FORMULATION OF NANOEMULSION PARIJOTO FRUIT EXTRACT (*Medinilla speciosa*) WITH VARIATION OF TWEENS STABILIZERS

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8 **Keywords:** Nanoemulsion<sub>1</sub>, Stabilizers<sub>2</sub>, Tween<sub>3</sub>, Parijoto<sub>4</sub>, RSM<sub>5</sub>.

### 9 Abstract

10 Nanotechnology was deemed to possess substantial potential for development owing to its ability to  
11 modify surface characteristics and particle size, facilitating enhanced absorption of functional food  
12 compounds and controlled release of active substances to mitigate adverse effects. Nanoemulsion, a  
13 stable colloidal system formed by blending oil, emulsifier, and water, was identified as  
14 nanotechnology with promising applications. However, investigations into the impact of surfactants  
15 on characteristic nanoemulsions, needed to be more varied. This research gap necessitated further  
16 exploration in the advancement of nanotechnology-based foods. The parijoto fruit (*Medinilla*  
17 *speciosa*), an indigenous plant species in Indonesia, has yet to undergo extensive scrutiny for its  
18 potential use as a functional and nutraceutical food. Anthocyanins, a principal compound in the  
19 parijoto fruit, had exhibited efficacy in reducing the risk of cardiovascular diseases, diabetes, and  
20 inflammation, and demonstrated anti-inflammatory and antioxidant properties. This study aimed to  
21 investigate the characteristics of nanoemulsion formulations derived from parijoto fruit extract and to  
22 evaluate an optimum condition with various tween surfactant. The findings from this investigation  
23 could furnish valuable insights for the further advancement of anthocyanin nanoemulsions from  
24 parijoto fruit extract. The results comprised the characterization of nanoemulsion particle size,  
25 polydispersity index, zeta potential, conductivity, pH, and viscosity. RSM is used to optimize  
26 nanoemulsion by examining the relationships and interactions between independent variables and  
27 response variables through mathematical modeling and statistical methods. Furthermore, the  
28 characterization of nanoemulsion encompassed zeta potential, polydispersity, particle size,  
29 conductivity, pH, and viscosity. Elevated surfactant concentrations resulted in diminished particle  
30 sizes and more uniform size distribution, albeit reaching a plateau where surfactant aggregation and  
31 micelle formation ensued. Increased concentrations of surfactant type, concentration, and parijoto  
32 extract impacted the physical characteristics of nanoparticle size and polydispersity. The optimal  
33 process conditions for nanoemulsion comprised a 12% concentration of Tween 80 solvent, 12%  
34 Tween concentration, and 7.5% parijoto fruit extract concentration, yielding a desirability value of  
35 0.74, categorizing it as moderate.

36 **1 Introduction**

37 Nanotechnology underwent progressive evolution, characterized by measurements on the nanometer  
38 scale, approximately  $10^{-9}$  meters (Ariningsih, 2016). Acknowledgment from the World Health  
39 Organization (WHO) and the Food and Agriculture Organization (FAO) (2009) underscored  
40 nanotechnology's significant potential in enhancing food products, attributed to its capacity to modify  
41 surface characteristics and particle size. Such modifications facilitate targeted delivery of food  
42 compounds to specific organs and the controlled release of active compounds to mitigate adverse  
43 effects. The attributes of nanoscale food materials are pivotal in propelling diverse industries,  
44 including food, pharmaceuticals, and extensive nutraceutical applications (Rahman et al., 2020).  
45 Nanoemulsions denote a nanotechnological rendition of a stable colloidal system, achieving kinetic  
46 stability through the amalgamation of oil, emulsifier, and water (McLements, 2016). Chang et al.  
47 (2022) conducted research utilizing surfactants as stabilizers in synthesizing nanoemulsions,  
48 showcasing the stability of nanoemulsion particle size in curcumin extract. Surfactants can diminish  
49 interfacial tension and form a substantially influential steric elastic film on the emulsion results (Xiao  
50 J & Hwang, 2016).

51 Renowned for its tropical climate and vast biodiversity, Indonesia harbours at least 30,000 plant  
52 species, with 7,000 being herbal plants with documented health benefits (Widyowati & Agil, 2018;  
53 Jumiami & Komalasari, 2017). Parijoto (*Medinilla speciosa*), an endemic plant species in Indonesia,  
54 remains relatively understudied for its scientific potential in pharmacy, functional foods, and  
55 nutraceuticals. Analysis has confirmed that the parijoto fruit comprises phytochemical components  
56 such as anthocyanins, flavonoids, saponins, tannins, alkaloids, cardenolides, and glycosides  
57 (Balamurugan, 2014). Anthocyanins, a predominant compound in parijoto fruit, demonstrate efficacy  
58 in reducing the risk of cardiovascular diseases, diabetes, and inflammation while possessing notable  
59 anti-inflammatory and antioxidant properties. Extraction techniques yield varying anthocyanin  
60 contents, with the peel extract and whole fruit extract registering 208.75 and 173.7 mg/L,  
61 respectively (Sa'adah et al., 2020). Various factors influence anthocyanins' stability, including  
62 chemical structure, concentration, solvent, pH, storage temperature, light, oxygen, metal ions,  
63 proteins, and flavonoids. Weak stability under high pH, high temperature, and light exposure has  
64 been observed (Ito et al., 2021), with lower pH contributing to enhanced stability (Moldova et al.,  
65 2020). Heating at elevated temperatures accelerates anthocyanin degradation (Khoo et al., 2019).

66 In recent years, Response Surface Methodology (RSM) has emerged as a prominent multivariate  
67 statistical technique for optimizing various processes. Initially introduced by Box and colleagues in  
68 the 1950s, RSM facilitates the examination of the relationship and interactions among independent  
69 variables and response variables through mathematical modeling and statistical methods (Izayidan et  
70 al., 2019). RSM has been successfully employed in culancing and optimizing therapeutic extract and  
71 drug nanoemulsion (Samim et al., 2020). In this study, Central Composite Design (CCD) Response  
72 Surface Methodology (RSM) was employed to optimize the quality parameters of the nanoemulsion.

73 Appropriate nano-encapsulation techniques, such as nanoemulsion, have shown the potential to  
74 enhance the stability, bioavailability, and solubility of lipophilic bioactive compounds while also  
75 preventing hydrolysis and oxidation (Rosso et al., 2020). Catechin nanoemulsion showed a  
76 remarkable improvement of stability and bioavailability in simulated gastrointestinal (Rafanar et al.,  
77 2016). Research conducted by Chang et al. (2022) used Tween as surfactant in the stable  
78 nanoemulsion synthesis loaded curcumin extract. This underscores the potential for developing  
79 nanoemulsion formulations for anthocyanins in parijoto fruit. Thus far, research on nanoemulsion  
80 formulation in parijoto fruit, incorporating various concentrations and stabilizers, still needs to be

81 conducted. This study is dedicated to investigating the characteristics of nanoemulsion formulations  
82 derived from parijoto fruit extract and evaluating an optimum condition with various tween  
83 surfactant.

## 84 **2 Materials and Method**

### 85 **2.1 Materials**

86 Grinder (Binder), Erlenmeyer (Pyrex), beaker glass (Pyrex), volume pipette, test tube (Pyrex), test  
87 tube rack, funnel (Pyrex), measuring flask (Pyrex), vacuum n filter 0.22  $\mu$ m (Sartorius Stedim 11694-  
88 2-50-06), vial, micropipette (Socorex), blue tip (Biologix 1 nmL pipette tips), hotplate (Cimarec et al.  
89 SP142025Q), vortex (Thermolyne et al.), Ultrasonic Cleaner (Biobase UC-10SD) modified, UV-VIS  
90 spectrophotometer (Shimadzu, UV-1280), aluminium foil, filter paper, 0.22  $\mu$ m filter membrane  
91 (Wattman), Cabinet dryer (HetoPowerDry LL1500), rotary evaporator (Biobase RE-2000E), syringe,  
92 analytical balance. Fresh parijoto, ethanol pro analysis (Merck, Germany), methanol pro analysis  
93 (Merck, Germany), distilled water, aqua bikes, folding ciocalteu 10% (Merck, Germany), Na<sub>2</sub>CO<sub>3</sub>  
94 7.5% (Merck, Germany), DPPH solution (Merck, Germany), Quarcetin (Merck, Germany), AlCl<sub>3</sub>  
95 (Merck, Germany), ammonium acetate 1 M (Merck, Germany), acetone (Merck, Germany),  
96 acetonitrile (Merck, Germany), standard cyanide (Zigma), delphinidin glu standard (Zigma), Tween  
97 20 (Merck, Germany), Tween 60 (Merck, Germany), Tween 80 (Merck, Germany), and Span 20  
98 (Merck, Germany).

### 99 **2.2 Preparation of Dry Samples of Parijoto Fruit Extract**

100 Samples used in this study are fruits from the Parijoto plant (*Medinilla speciosa*) cultivated and  
101 harvested on the slopes of Mount Muria, Kudus. The fruits used are ripe fruits harvested when the  
102 Parijoto plant reaches full maturity, typically around 90-100 days after flowering. Parijoto, which had  
103 been cleaned and sorted, was weighed 200 grams for each treatment. The fruit that has been weighed  
104 is then steam-blanching for 3 minutes. Prepare a citric acid solution with a concentration of 1% for  
105 pre-treatment of fruit before drying. After that, soak the parijoto fruit in the citric acid solution for 5  
106 minutes and drain. The Cabinet Dryer is cleaned before use to maintain hygiene and avoid cross-  
107 contamination. The drying temperature used was 70°C for 6 hours. The dried Parijoto fruit is then  
108 ground into powder using a herbal grinder for 2 minutes. After that, the sample will be extracted for  
109 further testing. The dried Parijoto will be chemically analyzed using UV-Vis spectroscopy.

### 110 **2.3 Making Parijoto Extract using Ultrasonic Assisted Extraction (UAE)**

111 Five grams of dry sample powder and 50 mL of 99.5% ethanol were mixed thoroughly for  
112 homogeneity in four 250 mL centrifuge bottles. Then, all vials were sonicated (40 KHz, 100 W) for  
113 30 min, followed by shaking for one hour, centrifuged at 4,000 rpm (4°C) for 10 min, collected the  
114 supernatant, and evaporated to dryness under vacuum. The residue was dissolved in 99.5% ethanol  
115 and diluted to 20 mL. After filtering through a 0.22  $\mu$ m membrane filter, parijoto fruit extract was  
116 obtained and stored at -20°C for analysis using UV-Vis.

### 117 **2.4 Preparation of Anthocyanin Nanoemulsion from Parijoto Extract**

118 Approximately 3 mL of anthocyanin nanoemulsion with concentrations of 2 mg/mL, 4 mg/mL, and 6  
119 mg/mL, respectively, were prepared by collecting a portion of parijoto extract, and the solvent was  
120 removed with nitrogen. The solvent removal process during anthocyanin extraction can be monitored  
121 using a combination of visual inspection and periodic weight measurements. Visual inspection

122 involves observing the extract as the solvent evaporates, noting its increasing concentration  
123 evidenced by a thicker and more viscous appearance. Periodic weighing of the container or flask  
124 containing the extract allows for the tracking of weight loss as the solvent evaporates. Once the  
125 weight stabilizes or reaches a predetermined target, it signifies that the desired solvent removal rate  
126 has been attained, ensuring the production of a concentrated anthocyanin extract suitable for further  
127 analysis. Anthocyanin nanoemulsion was prepared using a combination of surfactants that have low,  
128 medium, and high hydrophile lipophile balance (H.L.B), namely Twen 20, Tween 60, and Tween 80.  
129 Then, surfactant (0.24 g) was added, and the mixture was homogenized entirely. This was followed  
130 by adding ( 2.76 g) deionized water and mixing again for complete dispersion of surfactant in water.  
131 The solution was then sonicated in a sonicator with a temperature of 35°C, frequency of 45 Hz, and  
132 100% power for 60 minutes. To produce a good nanoemulsion, homogenization was carried out  
133 using high shear homogenization at 15,000 rpm with a temperature of 4 C for 15 minutes.

### 134 **2.5 Characterization of Particle Size and Polydispersity Index of Nanoemulsion Parijoto** 135 **Fruit Extract**

136 The particle size analysis tool used in this study was the Zetasizer (Zetasizer Pro; Malvern et al.),  
137 which operates based on the general principle of dynamic light scattering (DLS). This tool has a  
138 detector placed at an angle of 173° from the transmitted light beam and detects size using a patented  
139 technology known as noninvasive backscattering. This technique is used for various purposes. One is  
140 to reduce the effect known as multiple scattering, making it easier to measure samples with high  
141 concentrations. Modifying McClements (2016), the particle size distribution and average particle size  
142 of nanoemulsions were determined by dynamic light scattering (DLS) at a wavelength of 633 nm and  
143 a temperature of 25 °C.

### 144 **2.6 Characterization of Zeta Potential Nanoemulsion Parijoto Fruit Extract**

145 The  $\zeta$ -potential of Parijoto Fruit Extract Nanoemulsion was evaluated using  $\zeta$ -potential analysis  
146 (Zetasizer Pro; Malvern Instruments, Ltd., Malvern) following the method described by Khalid et al.  
147 (2017). The  $\zeta$ -potential of the samples was evaluated automatically using 10 to 100 analytical runs  
148 after equilibration for 120 s at 25 °C. The zeta potential of the particles was measured by phase-  
149 analysis light scattering (PLS) using a Zeta dip cell.

### 150 **2.7 Characterization of the Conductivity of Nanoemulsion Parijoto Fruit Extract**

151 The conductivity of nanoemulsion particles was measured by phase-analysis light scattering (PLS)  
152 using a Zeta dip cell with a cuvet electrode. Samples were evaluated automatically using 10 to 100  
153 analytical runs after equilibration for 120 seconds at 25 °C. The detector is placed at an angle of 173°  
154 from the transmitted light beam.

### 155 **2.8 pH Measurement of Nanoemulsion Parijoto Fruit Extract**

156 The pH was determined using a Schott pH meter at room temperature ( $27 \pm 2$  °C), calibrated with a  
157 standard buffer of pH 7. The pH analysis of the Parijoto fruit extract nanoemulsion sample was  
158 carried out using a pH meter with a particular electrode. First, the pH meter is set and calibrated with  
159 a standard buffer solution at a known pH, generally at pH 4.0, 7.0, and 10.0. Samples were diluted  
160 with ten mM phosphate buffer pH seven before analysis to avoid multiple scattering effects during  
161 testing. The pH meter electrode is then carefully inserted into the sample to ensure good contact.  
162 Once the electrode is stable, a pH reading is taken and recorded. This step is repeated as necessary to  
163 obtain consistent results. This pH analysis provides essential information regarding the acidity or

164 alkalinity level of nanoemulsion and nanocitosan Parijoto fruit extract, which can affect the stability  
165 and quality of products using the nanoemulsion.

## 166 **2.9 Viscosity Measurement of Nanoemulsion Parijoto Fruit Extract**

167 Viscosity measurements are carried out using a viscometer instrument. 14 mL of sample was put into  
168 the cup and attached to the solvent trap provided. The viscometer was set at 200 rpm, three rotations,  
169 for 30 seconds. The measurement process begins by activating the viscometer, and this tool  
170 automatically measures the time required for a liquid to flow through the viscometer tube at a  
171 specific temperature and rpm. This time, a predetermined formula converts the reading into a  
172 viscosity value. Repeated measurements can be made to ensure consistent results.

## 173 **2.10 Statistical analysis uses Response Surface Methodology.**

174 In this study, primary data in 3 repetitions of extraction and three repetitions of testing were averaged  
175 and given a standard deviation value for each treatment combination using Statistica 12.5 by StatSoft.  
176 The data is then entered into a statistical application, arranged in a combination of factorial points,  
177 axial points, and central points with three repetitions. After that, the data was analyzed, and several  
178 test stages were carried out. The basis for testing is studentification from primary data.  
179 Studentification means that the scale of the variable is adjusted by dividing it by the estimated  
180 population standard deviation. Variability in sample standard deviation values contributes to  
181 additional uncertainty in the calculated value. This will cause problems in finding the probability  
182 distribution of each statistic studied.

### 183 **2.10.1 Effect Summary**

184 This test can summarise the effects of the combination of treatments used. The Longworth value in  
185 the results of this test is defined as  $-\log(p\text{-value})$  and is a transformation of the p-value based on the  
186 Pearson Chi-Squared test. The Pearson Chi-Squared test evaluates the possibility of the split being  
187 caused by chance. The higher the Pearson Chi-Squared value, the higher the probability of the split  
188 being caused by dependency. In general, if the worth is greater than 2, then the statistical model  
189 considers the variable necessary.

### 190 **2.10.2 Lack Of Fit**

191 Model suitability testing (lack of fit) is carried out to review whether the model equation is  
192 acceptable or not in predicting responses. In the lack of fit test, the following hypothesis is used:

193  $H_0$  = no lack of fit (suitable model)

194  $H_1$  = there is a lack of fit (the model is not suitable)

195 The hypothesis is concluded by comparing the calculated F value with the F table. The calculated F is  
196 obtained from the statistical test results and displayed in the ANOVA table. The F table value is  
197 obtained from the F Distribution Table. The criteria for the lack of fit test are:

198  $F_{\text{count}} < F_{\text{table}}$ , then  $H_0$  is accepted.  $F_{\text{count}} > F_{\text{table}}$ , then  $H_0$  is rejected.

199 Another parameter that can prove the suitability of the model obtained is by comparing the p-value  
200 with the  $\alpha$  value. If the p-value of lack of fit is smaller than the  $\alpha$  value, then there is a significant  
201 lack of fit, so the model obtained is not appropriate.

202 **2.10.3 Summary Of Fit**

203 The R square and Root Mean square error values are obtained in this test. Measures the difference in  
204 values from a model's predictions as estimates of the observed values. R square is also known as the  
205 coefficient of determination, which explains how far independent data can explain dependent data. R  
206 square has a value between 0 – 1 with the condition that the closer it is to one, the better it is. If the r  
207 square is 0.6, the independent variable can explain 60% of the distribution of the dependent variable.  
208 The independent variable cannot explain the remaining 40% or can be explained by variables outside  
209 the independent variable (error component).

210 **2.10.4 Parameter Estimates**

211 The parameter estimates are the coefficients of the linear predictor. This value represents the change  
212 in response if you have a certain level of a categorical predictor or a change of 1 unit for a continuous  
213 predictor, which means the same thing as in a multiple regression analysis with continuous response.

214 **2.10.5 Analysis Of Variance**

215 The ANOVA test (Analysis of Variance) has the following test criteria:

216 H0 is accepted if  $F_{\text{count}} < F_{\text{table}}$ , which means the model cannot be accepted statistically because  
217 no independent variables have a real influence on the response.

218 H1 is accepted if  $F_{\text{count}} > F_{\text{table}}$ , which means the model is statistically acceptable and at least one  
219 independent variable has a real influence on the response.

220 **2.10.6 Fitted Surfaces**

221 The depiction of the fitted surface is carried out using the Central Composite Design model. The  
222 experimental design is factorial, specifically Central Composite Design (CCD). CCD was chosen  
223 over Box-Behnken Design because CCD provides more design points in terms of axial points.  
224 Additionally, CCDs can run experiments at extreme values, providing better quadratic equations for  
225 analysis. CCD contains a factorial or fractional factorial design with a central point augmented by a  
226 group of 'axial points' that allow estimation of curvature. If the distance from the center of the design  
227 space to the factorial point is  $\pm 1$  unit for each factor, the distance from the center of the design space  
228 to the axial point is  $|\alpha| > 1$ . The exact value of  $\alpha$  depends on the properties desired for the design and  
229 the number of factors involved. The CCD has twice as many star points due to a factor in the design.

230 **3 Result & Discussion**

231 **3.1 Phytochemical Profiles of Dried Parijoto Fruit**

232 Drying Parijoto Fruit is carried out using a cabinet dryer at a temperature of 70°C for 6 hours.  
233 The results of drying parijoto fruit were obtained through the preparation process, the  
234 antioxidant and anthocyanin activity profiles were expressed respectively in units of %  
235 inhibition and ppm. The results of the antioxidant activity of dried and extracted parijoto fruit  
236 were 79.14334.82%. % The total anthocyanin content in the dry samples and extracts was  
237 538.47 ± ppm. The dried Parijoto exhibited significant antioxidant activity, with a % inhibition  
238 value of 79.14 ± 34.82. This indicates a substantial capacity to neutralize free radicals, which  
239 are implicated in various chronic diseases and aging processes. The high antioxidant activity  
240 suggests that the drying process did not significantly diminish the antioxidant potential of  
241 Parijoto. The total anthocyanin content of the dried Parijoto was found to be 538.47 ± 4.67  
242 ppm. Anthocyanins are a group of pigmented compounds known for their antioxidant  
243 properties and potential health benefits. The retention of anthocyanins after the drying process  
244 indicates that cabinet drying effectively preserved these bioactive compounds in the dried  
245 Parijoto

246 The parijoto fruit extract was obtained through an extraction process using the Ultra-assisted  
247 extraction method. The antioxidant and anthocyanin activity profiles of parijoto fruit extract.  
248 The characterization of Parijoto extract as a filler in nanoemulsion involved various analyses  
249 to assess its antioxidant properties and phytochemical composition. The extraction method  
250 employed was ultra-assisted extraction, which is known for its efficiency in extracting  
251 bioactive compounds from plant materials. The antioxidant activity of the Parijoto extract was  
252 evaluated, yielding a % inhibition value of 50.776±6.18. This indicates a significant level of  
253 antioxidant capacity, which is crucial for combating oxidative stress and preventing cellular  
254 damage caused by free radicals. Furthermore, the total anthocyanin content of the extract was  
255 determined to be 94.43±4.14 ppm. Anthocyanins are well-known antioxidants found in many  
256 fruits and vegetables, known for their potential health benefits, including anti-inflammatory  
257 and anti-cancer properties. The flavonoid content of the Parijoto extract was measured to be  
258 126.85±1.15 g/L. Flavonoids are a class of polyphenolic compounds known for their  
259 antioxidant and anti-inflammatory effects. Additionally, the phenolic content of the extract was  
260 quantified as 8.43±0.70 GAE/g. Phenolic compounds are another group of bioactive  
261 compounds found in plants, known for their antioxidant and anti-inflammatory activities, as  
262 well as their potential role in reducing the risk of chronic diseases.

263 **3.2 Fitting Model for RSM (Response Surface Methodology) in Parijoto Fruit Extract Nanoemulsion**

264 Data recorded for each run included nanoemulsion particle size, polydispersity index, zeta potential, conductivity, pH, and viscosity. Each  
 265 variable was measured with three repetitions and the measurements three times to get consistent results. This data will be used to analyze the  
 266 influence of various factors on the characteristics of nanoemulsions using the Response Surface Methodology method, which can be seen in the  
 267 table.

268 Table 3. Design of Experiment RSM Particle Size, Poly Dispersity Index, Zeta Potential, Conductivity, pH, Viscosity in Nanoemulsion

No. Run Test	Dependent Variables			Independent Variables					
	Types of Lyophobic Detergent	Detergent Concentration (%)	Parijoto Fruit Extract Concentration (%)	Nanoparticle Size (nm)	Zeta Potential	Conductivity	Poly Dispersity Index	pH	Viscosity (cP)
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	Y <sub>1</sub>	Y <sub>2</sub>	Y <sub>3</sub>	Y <sub>4</sub>	Y <sub>5</sub>	Y <sub>6</sub>
1	20	3	3	15.511 ± 0.228	-21.12 ± 1.514	0.071 ± 0.005	0.41 ± 0.05	7.517 ± 0.005	0.07 ± 0.000
2	20	3	10	10.2 ± 0.337	-20.433 ± 2.857	0.057 ± 0.005	0.51 ± 0.05	6.870 ± 0.003	0.07 ± 0.003
3	20	3	5	15.069 ± 0.278	-20.740 ± 1.973	0.060 ± 0.001	0.57 ± 0.04	6.960 ± 0.002	0.06 ± 0.000
4	20	5	0	17.061 ± 0.300	-20.535 ± 1.150	0.067 ± 0.001	0.35 ± 0.05	6.890 ± 0.003	0.07 ± 0.000
5	20	3	0	20.017 ± 0.090	-20.107 ± 2.542	0.069 ± 0.000	0.48 ± 0.07	6.702 ± 0.002	0.06 ± 0.003
6	20	3	0	41.981 ± 0.159	-19.181 ± 4.719	0.070 ± 0.000	0.44 ± 0.02	6.811 ± 0.007	0.07 ± 0.000
7	20	3	0	42.547 ± 0.085	-20.640 ± 2.707	0.074 ± 0.000	0.52 ± 0.15	6.897 ± 0.008	0.07 ± 0.003
8	20	3	0	44.343 ± 1.707	-20.477 ± 3.578	0.074 ± 0.007	0.50 ± 0.05	6.810 ± 0.005	0.07 ± 0.007
9	20	3	0	49.307 ± 0.307	-20.417 ± 4.699	0.080 ± 0.001	0.40 ± 0.04	6.817 ± 0.005	0.06 ± 0.003
10	20	10	3	35.071 ± 0.300	-20.407 ± 1.204	0.085 ± 0.003	0.81 ± 0.04	6.790 ± 0.009	0.06 ± 0.003



11	28	10	5	51.967	-	0.007	-24.437	-	1.251	0.000	-	1.000	1.50	-	0.60	1.801	-	0.021	0.296	-	0.013
12	29	10	5	39.231	+	1.881	-24.170	+	1.873	0.009	+	1.001	1.91	+	0.62	1.810	+	0.013	0.308	+	0.005
13	29	10	6	32.54	+	4.231	-23.70	+	1.207	0.005	+	1.020	1.99	+	0.64	1.871	+	0.003	0.308	+	0.023
14	30	10	6	64.087	-	0.470	-23.787	-	1.465	0.075	-	1.017	1.52	-	0.69	1.807	-	0.015	0.275	-	0.017
15	30	10	6	84.037	-	0.585	-25.190	-	1.800	0.087	-	1.010	1.59	-	0.64	1.700	-	0.013	0.307	-	0.013
16	30	10	9	77.961	+	17.908	-26.172	+	1.718	0.011	+	1.000	1.51	+	0.71	1.811	+	0.011	0.271	+	0.002
17	30	10	9	75.943	+	1.101	-26.927	+	1.851	0.001	+	1.019	1.51	+	0.65	1.800	+	0.013	0.305	+	0.014
18	30	10	9	77.969	+	16.972	-26.281	+	1.510	0.010	+	1.021	1.60	+	0.61	1.700	+	0.003	0.278	+	0.011
19	30	12	5	81.88	-	11.923	-26.007	+	1.311	0.001	-	1.011	1.57	+	0.73	1.800	+	0.013	0.302	+	0.013
20	30	12	1	80.4	-	14.828	-26.025	-	1.800	0.000	-	1.017	1.66	-	0.77	1.771	-	0.012	0.308	-	0.017
21	30	12	8	85.967	+	7.007	-26.051	+	1.500	0.001	+	1.010	1.61	+	0.75	1.771	+	0.002	0.305	+	0.015
22	30	12	0	91.49	+	1.209	-27.471	+	1.801	0.001	+	1.010	1.73	+	0.71	1.810	+	0.013	0.301	+	0.019
23	30	12	0	110.88	+	19.51	-27.071	+	1.700	0.001	+	1.017	1.57	+	0.77	1.870	+	0.003	0.301	+	0.011
24	30	12	6	110.23	-	7.004	-27.120	-	1.300	0.077	-	1.011	1.52	-	0.70	1.800	-	0.017	0.277	-	0.012
25	30	12	6	111.880	+	17.178	-26.720	+	1.600	0.010	+	1.010	1.61	+	0.79	1.811	+	0.011	0.278	+	0.003
26	30	12	9	118.33	+	4.181	-26.025	+	1.119	0.015	+	1.014	1.55	+	0.73	1.857	+	0.003	0.271	+	0.014
27	30	12	9	124.187	+	4.182	-26.670	+	1.014	0.009	+	1.010	1.75	+	0.71	1.857	+	0.015	0.308	+	0.013

270 Table 3 shows that the particle size range of the nanoemulsion is between  $14,603 \pm 16.73$  nm  
271 and  $118,053 \pm 4,5825$  nm. The largest and smallest nanoparticle sizes found are 126.47 nm  
272 and 13.72 nm, respectively, with most nanoparticle sizes falling within the 50-100 nm range.  
273 Similar results were confirmed by Noor El-Din et al. (2017), who reported nanoemulsion  
274 sizes ranging from 31.58 to 220.5 nm. Studies conducted by Delmas et al., Liu et al., and Mei  
275 et al. using ultrasonication and high emulsification methods also confirmed comparable  
276 results of 45–170 nm, 222.4–166.4 nm, and 170–280 nm, respectively (Delmas et al., 2016;  
277 Liu et al., 2017; Mei et al., 2019). Conversely, Peng et al. (2010) reported a nanoparticle size  
278 range of 21–530 nm. Zeta potential reflects the surface charge of particles and affects  
279 colloidal stability. High zeta potential can prevent particle aggregation due to electrostatic  
280 repulsion. The research includes the evaluation and characterization of zeta potential under  
281 various treatments. The study obtained zeta potential results for nanoemulsion ranging from  
282  $-22.197 \pm 0.738$  mV to  $-28.207 \pm 1.598$  mV, respectively. Similar results were confirmed by  
283 Wessam et al. (2023), obtaining results of +21.5 mV. Particles with high ZP values, between  
284 20 and 40 mV, provide system stability and are less likely to aggregate or increase particle  
285 size. However, it should be noted that ZP values are not an absolute measure of nanoparticle  
286 stability. Furthermore, emulsions with ZP variations  $>10$  mV are suggested to have better  
287 stability (Kadu et al., 2011). The ideal potential range for nanoparticle stability is (-30 to 20  
288 mV or +20 to +30 mV) (Liu et al., 2018). The produced values tend to be harmful due to the  
289 influence of acetic acid, resulting in a negative charge. This charge causes electrostatic  
290 repulsion forces between formed nanoparticles to prevent aggregation into larger sizes  
291 (Luthifayana et al., 2022). Higher zeta potential values increase nanoparticle stability due to  
292 higher electrostatic repulsion forces between nanoparticles.

293 Conductivity provides information about the ability of nanoemulsions to conduct electricity.  
294 Changes in conductivity can occur with changes in surface particle charge. Table 18 shows  
295 that the nanoemulsion conductivity of Parijoto fruit extract ranges from 0.03458 to 0.09987  
296 mS/cm. Good nanoemulsion conductivity measurements have higher electrical conductivity  
297 values (10–100  $\mu$ S/cm) (Akilu et al., 2019; Guo et al., 2016; Khader et al., 2016). Electrical  
298 conductivity values tend to decrease with decreasing water content in the emulsion. O/W type  
299 (Oil-in-Water) nanoemulsions have higher conductivity than W/O type (Water-in-Oil)  
300 nanoemulsions. This is because the more extensive water phase provides more pathways for  
301 ion conduction.

302 The type and concentration of surfactant in nanoemulsion can influence conductivity.  
303 Surfactants can provide ionic charge or facilitate ion conduction in the system. Viscosity is an  
304 essential parameter in evaluating the flow properties of nanoemulsion. Viscosity is one of the  
305 parameters used to determine the stability of polymers in a solution because it undergoes  
306 reduction during polymer storage due to polymer degradation (Aranaz et al., 2021). In this  
307 study, as shown in Table 1, the viscosity of nanoparticles ranges from 3,810 cP to 4,433 cP.  
308 Alemu et al. (2023) stated that viscosity can depend on particle size and storage time.  
309 Appropriate viscosity can affect the applicability and spread of the system. The viscosity of a  
310 preparation is related to the consistency and spreadability of the preparation, which will affect  
311 ease of use (Imanto et al., 2019). Viscosity values are influenced by several factors, such as  
312 temperature, pH, manufacturing conditions, and the quality and concentration of raw  
313 materials (Naini & Yusuf, 2018). The results of viscosity tests are shown in centipoise (cP).  
314 The higher the viscosity value of a preparation, the better the stability of the product, but the  
315 preparation will be difficult to apply to the skin, and the resistance of the preparation to flow  
316 will increase, making it difficult to remove from the container (Thakre, 2017). Meanwhile,  
317 low viscosity values will increase the flowability of the skin and make it easier to apply to the  
318 skin (Naini & Yusuf, 2018)



319 This ANOVA table is essential to evaluate the statistical significance of each model component and determine whether the quadratic model used  
 320 is good enough to explain the characteristics of the nanoemulsion or not. The p-value is used to determine statistical significance, and the  
 321 analysis results will help select an appropriate model and interpret the significance of factors that influence the characteristics of nanoemulsions,  
 322 which can be seen in the table.

323 Table 4. ANOVA (Analysis of Variance) for the RSM Quadratic Model Particle Size, Poly Dispersity Index, Zeta Potential, Conductivity, pH,  
 324 Viscosity in Nanoemulsion

Quadratic Model Equation	Sources of Variation	p-Value
<b>Particle Size</b> ( $R^2$ : 0,558 $R^2_1$ : 0,50156) $Y_1 = -0,000008 - 0,000069X_1 + 0,000040X_2 + 0,000032X_3 + 0,000056X_1^2 +$ $0,000064X_2^2 - 0,000003X_3^2 - 0,000056X_1X_2 - 0,000044X_2X_3 + 0,000065X_1X_3$	Model  <i>Lack of fit</i>	0,294 *  0,185
<b>Poly Dispersity Index</b> ( $R^2$ : 0,3643 $R^2_1$ : 0,2471) $Y_2 = 6,23086 + 0,58801 X_1 - 0,75655 X_2 + 84,3654 X_3 + 24,65 X_1^2 + 18,7663 X_2^2$ $20,744 X_3^2 + 23,0025 X_1X_2 + 26,3043 X_2X_3 + 9,5269 X_1X_3$	Model  <i>Lack of fit</i>	0,041*  0,692
<b>Zeta Potential</b> ( $R^2$ : 0,54003 $R^2_1$ : 0,56905) $Y_3 = 0,000062 - 0,000023 X_1 - 0,000010 X_2 + 0,000008 X_3 + -0,000007 X_1^2 +$ $0,000003 X_2^2 + 0,000008 X_3^2 + -0,000006 X_1X_2 - 0,000008 X_2X_3 + -0,000005 X_1X_3$	Model  <i>Lack of fit</i>	0,000*  0,980
<b>Conductivity</b> ( $R^2$ : 0,2444 $R^2_1$ : 0,3464) $Y_4 = 4035,80 - 1198,06X_1 + 833,22X_2 - 1083,49X_3 - 2597,59X_1^2 - 709,42X_2^2$ $+ 881,10X_3^2 + 305,68X_1X_2 - 700,69X_1X_3 - 943,96X_2X_3$	Model  <i>Lack of fit</i>	0,0004*  0,928
<b>pH</b> ( $R^2$ : 0,832 $R^2_1$ : 0,797) $Y_5 = 0,003122 - 0,000040X_1 - 0,000060X_2 + 0,000039X_3 - 0,000034X_1^2 +$ $0,000047X_2^2 + 0,000031X_3^2 - 0,000006X_1X_2 - 0,000015X_1X_3 + 0,000031 X_2X_3$	Model  <i>Lack of fit</i>	0,000*  0,067
<b>Viskositas</b> ( $R^2$ : 0,95976 $R^2_1$ : 0,95466) $Y_6 = 0,015177 - 0,009573X_1 - 0,003288X_2 - 0,000624X_3 - 0,008334X_1^2 -$ $0,000266X_2^2 - 20,744 X_3^2 + 23,0925 X_1X_2 + 26,3043 X_2X_3 + 9,5269 X_1X_3$	Model  <i>Lack of fit</i>	0,000*  0,103

325 Notes:  
 326 - \*: The model has a statistically significant effect (p<0.05)  
 327 - \*\*: Model mismatch or lack of fit occurs (p<0.05)

328 Based on the ANOVA RSM analysis of three factors, namely the type of Tween in  
329 nanoemulsion, Tween concentration, and Parijoto extract concentration, all ANOVA values  
330 show probabilities  $<0.0001$  ( $p<0.05$ ). This indicates that the quadratic response surface model  
331 used for both responses (dependent variables) is significant and can be used to optimize  
332 extraction factors (Wang et al., 2014). The coefficient of determination, or R square, depicts  
333 how independent data can explain dependent data. The range of R square values is between 0  
334 and 1, where values closer to 1 indicate better explanatory power.

335  
336 In the Central Composite Design analysis, the p-value indicates the significance of each  
337 coefficient in the built polynomial regression model. The lower the p-value, the more  
338 significant the contribution of the coefficient to the overall regression model (Zhong &  
339 Wang, 2010). It is important to note that using experimental data within the allowed range of  
340 variables in this study to create mathematical equations, which may have broader general  
341 applications, can provide the ability to predict system behavior when different factors are  
342 combined. From the perspective of optimizing the formation of emulsion nanoparticles, there  
343 is potential to develop more significant results, possibly based on the variables investigated in  
344 this study. Additionally, this optimization may be performed using the techniques outlined in  
345 this research to further test the effects of time and temperature or other conditions, as needed.

346 Table 4 shows details of the RSM approach used to assess particle size (nm), Poly Dispersity  
347 Index, Zeta Potential (mv), Conductivity, pII, and viscosity (Cp) in nanoemulsion of Parijoto  
348 fruit extract involved in a series of 81 experiments based on factorial design. The coefficients  
349 for the second-degree polynomial Equation are determined through experimental results,  
350 along with the regression coefficients for Particle Size (Y1), Poly Dispersity Index (Y2), Zeta  
351 Potential (Y3), Conductivity (Y4), pH (Y5), and viscosity (Y6). The Equation presented as  
352 Equation (2) shows the full quadratic model, while Table X shows the models predicting the  
353 response of the independent variables (Y1–Y6).

354 To assess the extent to which the equation model in RSM fits the data and how strong the  
355 influence of the variables is, the coefficient of determination or ( $R^2$ ) is used. Chin (1998) has  
356 categorized that for model suitability, the R-Square value is substantial if it is more than 0.67,  
357 moderate if it is more than 0.33 but lower than 0.67, and weak if it is more than 0.19 but  
358 lower than 0.33. pII and viscosity indicate strong model adequacy on these response  
359 variables. In contrast, the responses of Particle Size, Poly Dispersity Index, Zeta Potential,  
360 and Conductivity indicate a moderate model for these response variables. A lack of fit test  
361 was then performed to assess model fit for each response. With a p-value exceeding 0.05, it  
362 was confirmed that the model adequately fit the experimental data, as seen in Table 4.

### 363 **3.3 Contour plot on Particle Size, poly-dispersity index, Zeta Potential, Conductivity,** 364 **pII, and Viscosity as a function of Nanoemulsion Parijoto Fruit Extract.**

365 In this research, the model is created as a Contour plot, which can show the response: Particle  
366 Size, Poly Dispersity Index, Zeta Potential, Conductivity, pH, and Viscosity. Continued  
367 research shows a significant relationship between particle size and tween concentration and  
368 the type of lipophilic tween in nanoemulsions, as shown in Figures 1-6 the presented data  
369 offers valuable insights into the influence of lipophilic tween type and tween concentration  
370 on various properties of the nanoemulsion derived from parijoto fruit extract. Each figure  
371 depicts the contour plots illustrating the interaction effects between these two factors on  
372 different characteristics of the nanoemulsion.



373 In Figure 1, the contour plot demonstrates the interaction between the lipophilic tween type  
374 and tween concentration in controlling nanoparticle size. It reveals that as the lipophilic  
375 tween type increases from 20 to 80, and the tween concentration rises from 8% to 10%, there  
376 is a general trend of increasing particle size, albeit with a slight decreasing trend observed to  
377 some extent. This suggests that both factors play a role in determining the nanoparticle size,  
378 with higher concentrations leading to larger particle sizes. Moving to Figure 2, which  
379 illustrates the Zeta Potential of the nanoemulsion, an increase in the lipophilic Tween type  
380 from 60 to 80 and an increase in tween concentration from 8% to 10% correspond to an  
381 increase in Zeta Potential. Interestingly, no further changes are observed beyond this point.  
382 This indicates that these specific conditions result in optimal Zeta Potential, possibly  
383 indicating enhanced stability of the nanoemulsion.

384 Figure 3 showcases the influence of lipophilic tween type and tween concentration on the  
385 conductivity of the nanoemulsion. As the lipophilic tween type increases from 20 to 80 and  
386 the tween concentration rises from 8% to 12%, there is a consistent increase in conductivity  
387 without any further changes. This suggests a direct relationship between these factors and the  
388 conductivity of the nanoemulsion. The Contour plot presented in Figure 4 demonstrates the  
389 effect of lipophilic tween type and tween concentration on the Poly Dispersity Index (PDI) of  
390 the nanoemulsion. Interestingly, an increase in lipophilic tween type from 60 to 80 and a  
391 decrease in tween concentration from 12% to 8% lead to an increase in PDI value without  
392 further changes. This indicates a complex interaction between these factors in determining the  
393 homogeneity of particle size distribution within the nanoemulsion.

394 Figure 5 depicts the pH contour plot of the parijoto fruit extract nanoemulsion. An increase in  
395 lipophilic Tween type from 20 to 80 and an increase in tween concentration from 8% to 12%  
396 result in a consistent increase in pH without any further changes. This observation suggests  
397 that these specific conditions contribute to the alkalinity of the nanoemulsion, which may  
398 have implications for its stability and functionality. Finally, Figure 6 illustrates the viscosity  
399 contour plot of the nanoemulsion. An increase in lipophilic tween type from 35 to 80 and an  
400 increase in tween concentration from 8% to 12% lead to an increase in viscosity without  
401 further changes. This indicates that higher concentrations of lipophilic tween and tween result  
402 in a thicker consistency of the nanoemulsion, which affects its flow properties and  
403 application. The presented data highlights the intricate relationship between lipophilic tween  
404 type and tween concentration in influencing various physicochemical properties of the  
405 nanoemulsion derived from parijoto fruit extract. These findings provide valuable insights for  
406 optimizing the formulation and manufacturing process of the nanoemulsion for potential  
407 applications in various industries.

408 Research on the influence of surfactant type and concentration on nanoemulsion indicates  
409 that the selection of surfactant significantly affects the characteristics of nanoemulsion.  
410 Various surfactant types, such as Tween 20, Tween 60, and Tween 80, play different roles in  
411 forming nanoemulsions. The research results show that the particle size of Tween 80  
412 surfactant is the highest, with an average particle size of 107.196 nm. Similar results were  
413 reported by Chang et al. (2013), who obtained the smallest droplets in carvacrol-based  
414 nanoemulsion made with a mixture of food-grade non-ionic surfactants (Tween 20, 40, 60,  
415 80, and 85). Tween, a non-ionic surfactant derived from sorbitan ester, is soluble or  
416 dispersible in water and is commonly used as an oil-in-water emulsifier in the  
417 pharmaceutical, cosmetic, and cleaning industries. Among these surfactants, Tween 80 is one  
418 of the most commonly used. Research by Douglas et al. (2013) confirms that the type of non-  
419 ionic surfactant significantly influences the average particle diameter of the formed colloid  
420 dispersion. The smallest droplets were observed in systems prepared using Tween 80, while





421 the largest droplets formed in systems using Tween 85. The surfactant's Hydrophilic-  
422 Lipophilic Balance (HLB) plays a role in forming small particles. Surfactants with either too  
423 high (Tween 20) or too low (Tween 85) HLB values cannot form optimal nanoemulsions.  
424 Tween types with intermediate HLB values (Tween 40, 60, and 80) can form nanoemulsions  
425 with small particle sizes. However, there is no strong correlation between HLB values and  
426 particle sizes produced by these surfactants (Kumar et al., 2008). Small-molecule surfactants  
427 have higher surface activity and form smaller emulsion droplets than large ones (Qian &  
428 McClements, 2011; Teo, Goh & Lee, 2014).

429 Another critical factor for minimal droplet emulsion formation is the Hydrophilic-Lipophilic  
430 Balance (HLB) value of the surfactant (Sagitani, 1981), defined by Griffin as the ratio of  
431 surfactant hydrophilicity to lipophilicity (Griffin, 1949). A high HLB value indicates strong  
432 hydrophilicity, and the HLB values of non-ionic surfactants generally range from 0 to 20  
433 (Gad & Khairou, 2008), such as Tween 20 (HLB 16.7) and Tween 80 (HLB 15) (Dinarvand,  
434 Moghadam, Sheikhi & Atyabi, 2005). Emulsion stability is influenced by two polymer and  
435 particle surface tension mechanisms: steric stability caused by macromolecules adsorbed on  
436 particle surfaces and electrostatic stability due to repulsion between surface-charged droplets.  
437 In nanoemulsions made with Tween 80 surfactant, the surfactant may not have a charge on  
438 the hydrophobic group, causing the covered droplet surface to be non-charged and resulting  
439 in low zeta potential values, which can lead to increased particle size and PDI (Lian et al.,  
440 2016).

441 However, a different study proposed by Alam et al. (2023) suggests that Tween 20 helps  
442 improve PDI and allows for minimum polydispersity. Compared to other nanoparticles, the  
443 ability to maintain particle integrity using Tween 20 is significant. Increasing the Surfactant  
444 content in the formulation increases the polydispersity indices for natural extracts in the 3D  
445 response surface graph. This indicates that the use of Tween types with low and high HLB  
446 values can be applicable when combined with an optimal concentration of co-surfactant.

447 Surfactant concentration is also a critical factor in nanoemulsion formation. Research  
448 indicates that increasing surfactant concentration can result in smaller and more homogenous  
449 size distribution. However, there is a specific limit where surfactant concentration reaches a  
450 plateau level, leading to unadsorbed surfactant aggregation and micelle formation. The results  
451 show that the higher the Tween concentration, the higher the size and PDI. This is confirmed  
452 by Liat et al. (2016), stating that nanoemulsions prepared with higher surfactant  
453 concentrations significantly increase short-term stability. Systems with 15 or 20% weight of  
454 Tween 80 are highly unstable to increasing dilution, indicating that a medium surfactant  
455 concentration level may be more suitable for stable nanoemulsion preparation. Although the  
456 initial droplet size is small, higher surfactant concentrations can increase raw material costs  
457 and cause undesirable sensory (taste) issues in commercial applications. Therefore, this study  
458 uses a 10% weight of Tween 80 in further experiments.

459 Increasing surfactant concentration increases the number of surfactant molecules migrating  
460 from the oil phase to the emulsion water phase, and nanodroplets form. Frictional forces  
461 applied to the oil-water interface, coated with emulsifier, cause some emulsifiers to sink  
462 parallel to the surface layer, while others detach from the surface layer. Hasani et al. (2015)  
463 reported that droplet size increases by increasing surfactant concentration to 20%, and  
464 particles have a broad and non-uniform size distribution. The instability of nanoemulsion at  
465 high surfactant concentrations may be related to the depletion-flocculation mechanism of  
466 adsorbed surfactant. With increased surfactant concentration, additional surfactant molecules  
467 form micelles in the continuous phase rather than orienting on the particle surface. This leads  
468 to an increase in local osmotic pressure, causing the continuous phase between moving



469 droplets to decrease, reducing the continuous phase between those droplets. As a result,  
470 aggregation occurs, causing an increase in particle size. According to Ohi et al. (2011) and  
471 Tadros et al. (2004), the average droplet size becomes smaller, and the size distribution  
472 becomes narrower with increasing emulsifier concentration, ultimately reaching a plateau  
473 level. Beyond the plateau level, free or unadsorbed emulsifiers may accumulate to form  
474 micelles. Nanoemulsions are known to be thermodynamically unstable, tending to minimize  
475 interfacial area through coalescence.

476 An increase in the concentration of the filler extract can lead to the tendency of nanoparticles  
477 to aggregate or form agglomerates also pH nanoemulsion. This phenomenon may occur due  
478 to physical or chemical interactions between nanoparticles and compounds in the filler  
479 extract. Findings by Alab et al. (2021) suggest that an increase in extract concentration results  
480 in an increase in particle size, particularly at the highest concentration of 347.2 nm. On the  
481 other hand, the smallest concentration has the lowest particle size at 86.98 nm. These results  
482 indicate that higher concentrations may increase the likelihood of particle agglomeration.

483 Furthermore, increasing the concentration of parijoto fruit extract can increase the total mass  
484 in the solution, which, in turn, can increase overall viscosity. Additional particles or  
485 molecules from the filler extract can contribute to the increase in viscosity. A study by Olan  
486 et al. (2021) shows that particles with the highest concentration have the highest viscosity and  
487 vice versa. This increase in viscosity may be caused by excess extract loaded into particles.  
488 The physicochemical characteristics of the filler extract may influence the viscosity  
489 properties of nanoparticles, and factors such as changes in pH, temperature, or chemical  
490 composition may also play a role in viscosity increase. Parijoto fruit is rich in active  
491 compounds, such as anthocyanins, which can affect the surface charge of nanoemulsion  
492 particles. At a certain pH, anthocyanins or other components may have specific charges that  
493 can influence the electrostatic stability of particles (Liu et al., 2016). Anthocyanins may  
494 undergo solubility changes at specific pH values, affecting the distribution and stability of the  
495 nanoemulsion's oil or water phase. The same occurs with surfactants, where variations in  
496 charge of the filler extract from parijoto fruit can affect the interaction between nanoparticles,  
497 anthocyanins, and other components in the system. The loading capacity of the extract in the  
498 nanoemulsion likely depends on its solubility in the system used (Costa et al., 2012).  
499 Anthocyanins tend to undergo color changes with pH (pH-dependent color shift).  
500 Additionally, the antioxidant activity of anthocyanins can be influenced by pH. This  
501 complexity can modulate the overall physicochemical properties of the nanoemulsion system.  
502



503 **3.4 Optimal Point Prediction from RSM in Nanoemulsion Parijoto Fruit Extract**

504 Optimal point predictions from the Response Surface Methodology are obtained by  
 505 combining optimal conditions based on interactions between independent variables. Profiler  
 506 predictions are obtained if the fitted surface graph is in minimum, maximum, or saddle form.  
 507 3D graphics on image x. shows a complex interaction between the variable factors of  
 508 lipophilic tween type and tween concentration on the response. Increasing the lipophilic  
 509 tween type value increases the response somewhat, but the tween concentration value can  
 510 modify the effect. There is an optimal region where the response reaches its peak. The  
 511 implication for practice is that by setting the variable factors at levels that are estimated to be  
 512 optimum, the research results can achieve the highest optimization in the desired response,  
 513 which can be seen in Figure 7.

514 Table 3. Prediction of Optimum Conditions for Parijoto Fruit Extract Nanoemulsion

Types of Analysis	Types of Lyphophilic Tweens	Tween Concentration (%)	Parijoto Fruit Extract Concentration (%)	Nanoparticle Size (nm)	Zeta Potential(mV)	Conductivity (mS/cm)	Poly Dispersity Index
<b>Optimum Condition Prediction</b>	80	12	7.5	61.97	-28.48	0.082	0.691
<b>Maximum Value at Optimum Conditions</b>	80	12	7.5	39.94	-32.48	0.048	0.371
<b>Minimum Value at Optimum Conditions</b>	80	12	7.5	163.88	-26.37	0.115	1,011

515

516 It can be seen in Table 9 that to achieve the maximum desired concentration of nanoparticle  
 517 size, zeta potential, Conductivity, Poly Dispersity Index, degree of acidity, and Viscosity, it is  
 518 necessary to set the Tween solvent concentration to 80, Tween concentration to 12% and  
 519 Parijoto fruit extract concentration to 7.5%. This set of conditions has a desirability value of  
 520 0.74. Because the value is almost close to 1 and falls into the moderate category, this set of  
 521 conditions is quite optimal for the aim of this research, namely to maximize the response.

522 The optimization of nanoemulsion formation from Parijoto fruit extract using Response  
 523 Surface Methodology (RSM) has been conducted in this study. RSM is a statistical method  
 524 used to design experiments and analyze the impact of multiple independent variables on a  
 525 measured response. As an output of this research, the synthesis process conditions of  
 526 nanoemulsion from Parijoto fruit extract can be optimized to achieve particle size,  
 527 polydispersity index (PDI), zeta potential, conductivity, pH, and viscosity levels. RSM  
 528 determines the optimal extraction time and temperature to maximize the response variable  
 529 outcomes (Granato et al., 2014). In line with this, predictions and observations are within a  
 530 narrow range and do not show significant differences at a 5% significance level, indicating  
 531 the model's suitability for optimization and process efficiency purposes.



532

533 The optimal point prediction from the Response Surface Methodology is obtained by  
534 integrating optimal conditions and depends on the interaction between independent variables,  
535 as Ratnawati et al. (2018) explained. The prediction profile is formed when the adjusted  
536 surface graphs show a minimum, maximum, or saddle shape. The optimization process can  
537 achieve optimal responses by analyzing each response beforehand, ultimately reducing effort  
538 and operational costs, as Nurmiah et al. (2013) stated. Desirability, with a range of values  
539 from 0 to 1, is used as the optimization target value, with low (0-0.49), moderate (0.5-0.79),  
540 and high (0.8-1) categories. The closer the value of 1 is, the greater the desirability, which  
541 indicates the suitability of the combination of process parameters to achieve optimal response  
542 variables.

543

544 It can be seen in Table 3 that to achieve the desired concentrations of nanoparticle size, zeta  
545 potential, conductivity, polydispersity index, acidity level, and viscosity, it is necessary to set  
546 the concentration of Tween 80, Tween concentration at 12%, and Parijoto fruit extract  
547 concentration at 7.5%. This set of conditions has a desirability value of 0.740349. Since its  
548 value is close to 1 and falls into the moderate category, this set of conditions is quite optimal  
549 for this research, which is to maximize the response





#### 550 **4 Conclusion**

551 In this series of experiments, nanoemulsion from parijoto fruit has been characterized, considering  
552 various physicochemical parameters such as particle size, polydispersity index, zeta potential,  
553 conductivity, pH, and viscosity. The research results indicate significant variations in the physical  
554 characteristics of both nanomaterials in terms of changes in surfactant and parijoto extract  
555 concentrations. Increased surfactant concentration tends to produce smaller particle sizes and a more  
556 homogeneous distribution, although certain limitations were found that lead to surfactant aggregation  
557 and micelle formation. The nanoemulsion characteristics, including zeta potential, polydispersity,  
558 particle size, conductivity, pH, and viscosity. The type and concentration of surfactants played a  
559 crucial role in determining the properties of the nanoemulsions. Variations in surfactant parameters  
560 resulted in observable differences in emulsion characteristics, highlighting the importance of  
561 surfactant selection and optimization. To achieve optimal nanoemulsion process conditions, it is  
562 recommended to use 12% Tween 80 solvent concentration, 12% Tween concentration, and 7.5%  
563 parijoto fruit extract concentration, resulting in a desirability value of 0.74, falling into the moderate  
564 category.

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## FORMULATION OF NANOEMULSION PARIJOTO FRUIT EXTRACT (*Medinilla speciosa*) WITH VARIATION OF TWEENS STABILIZERS

Victoria K. Ananingsih<sup>1\*</sup>, Yohanes A. Sarsita Putra<sup>1</sup>, Alberta R. Pratiwi<sup>1</sup>, Bernadeta Soedarini<sup>1</sup>

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### Scope Statement

The manuscript aligns seamlessly with the scope of Frontiers in Nutrition and its specialty in Nutrition and Food Technology. Our research significantly contributes to the current understanding of Nutrition and Food Technology, addressing key questions in the field. The innovative methodologies and novel findings presented in our study make it a perfect fit for publication in Frontiers in Nutrition, where it will undoubtedly advance knowledge in Nutrition and Food Technology and stimulate meaningful discussions among the journal's readership.

### Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

### CRedit Author Statement

**Bernadeta Soedarini:** Data curation, Formal Analysis, Methodology, Validation, Writing - original draft, Writing - review & editing. **Victoria Kristina Ananingsih:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing - original draft, Writing - review & editing. **Alberta Rika Pratiwi:** Data curation, Formal Analysis, Methodology, Project administration, Validation, Writing - original draft, Writing - review & editing. **Yohanes Alan Sarsita Putra:** Data curation, Formal Analysis, Investigation, Resources, Software, Validation, Visualization, Writing - original draft, Writing - review & editing.

### Keywords

Nanoemulsion1, Stabilizers2, Tween3, Parijoto4, RSM5

### Abstract

Word count: 308

Nanotechnology has substantial potential for development due to its ability to modify surface characteristics and particle size, facilitating enhanced absorption of functional food compounds and controlled release of active substances to mitigate adverse effects. Nanoemulsion, a stable colloidal system formed by blending oil, emulsifier, and water, was identified as nanotechnology with promising applications. However, investigations into the impact of surfactants on characteristic nanoemulsions need to be more varied. This research gap necessitated further exploration in the advancement of nanotechnology-based foods. The parijoto fruit (*Medinilla speciosa*), an indigenous plant species in Indonesia, has yet to undergo extensive scrutiny for its potential use as a functional and nutraceutical food. Anthocyanins, a principal compound in the parijoto fruit, had exhibited efficacy in reducing the risk of cardiovascular disease diabetes, demonstrating anti-inflammatory and antioxidant properties. This study aimed to investigate the characteristics of nanoemulsion formulations derived from parijoto fruit extract and to evaluate an optimum condition with various tween surfactants. The findings from this investigation could furnish valuable insights for the further advancement of anthocyanin nanoemulsions from parijoto fruit extract. The results comprised the characterization of nanoemulsion particle size, polydispersity index,  $\zeta$ -potential, conductivity, pH, and viscosity. Through mathematical modelling and statistical methods, RSM optimizes nanoemulsion by examining the relationships and interactions between independent and response variables. Furthermore, the characterization of nanoemulsion encompassed  $\zeta$ -potential, polydispersity, particle size, conductivity, pH, and viscosity. Elevated surfactant concentrations resulted in diminished particle sizes and more uniform size distribution, albeit reaching a plateau where surfactant aggregation and micelle formation ensued. Increased concentrations of surfactant type, concentration, and parijoto extract impacted the physical characteristics of nanoparticle size and polydispersity. The optimal process conditions for nanoemulsion consisting of the type of Tween used are Tween 80, Tween concentration of 12 %, and parijoto fruit extract concentration of 7.5 %, yielding a desirability value of 0.74, categorizing it as moderate.

### Funding statement

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In review

## FORMULATION OF NANOEMULSION PARIJOTO FRUIT EXTRACT (*Medinilla speciosa*) WITH VARIATION OF TWEENS STABILIZERS

1 Victoria Kristina Ananingsih<sup>1</sup>, Alberta Rika Pratiwi<sup>1</sup>, Bernadeta Soedarini<sup>1</sup>, Yohanes Alan  
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8 **Keywords:** Nanoemulsion<sup>1</sup>, Stabilizers<sup>2</sup>, Tween<sup>3</sup>, Parijoto<sup>4</sup>, RSM<sup>5</sup>.

9 **Abstract**

10 Nanotechnology has substantial potential for development due to its ability to modify surface  
11 characteristics and particle size, facilitating enhanced absorption of functional food compounds and  
12 controlled release of active substances to mitigate adverse effects. Nanoemulsion, a stable colloidal  
13 system formed by blending oil, emulsifier, and water, was identified as nanotechnology with  
14 promising applications. However, investigations into the impact of surfactants on characteristic  
15 nanoemulsions need to be more varied. This research gap necessitated further exploration in the  
16 advancement of nanotechnology-based foods. The parijoto fruit (*Medinilla speciosa*), an indigenous  
17 plant species in Indonesia, has yet to undergo extensive scrutiny for its potential use as a functional  
18 and nutraceutical food. Anthocyanins, a principal compound in the parijoto fruit, had exhibited  
19 efficacy in reducing the risk of cardiovascular disease diabetes, demonstrating anti-inflammatory and  
20 antioxidant properties. This study aimed to investigate the characteristics of nanoemulsion  
21 formulations derived from parijoto fruit extract and to evaluate an optimum condition with various  
22 tween surfactants. The findings from this investigation could furnish valuable insights for the further  
23 advancement of anthocyanin nanoemulsions from parijoto fruit extract. The results comprised the  
24 characterization of nanoemulsion particle size, polydispersity index,  $\zeta$ -potential, conductivity, pH,  
25 and viscosity. Through mathematical modelling and statistical methods, RSM optimizes  
26 nanoemulsion by examining the relationships and interactions between independent and response  
27 variables.

28 Furthermore, the characterization of nanoemulsion encompassed  $\zeta$ -potential, polydispersity, particle  
29 size, conductivity, pH, and viscosity. Elevated surfactant concentrations resulted in diminished  
30 particle sizes and more uniform size distribution, albeit reaching a plateau where surfactant  
31 aggregation and micelle formation ensued. Increased concentrations of surfactant type, concentration,  
32 and parijoto extract impacted the physical characteristics of nanoparticle size and polydispersity. The  
33 optimal process conditions for nanoemulsion consisting of the type of Tween used are Tween 80,

34 Tween concentration of 12 %, and parijoto fruit extract concentration of 7.5 %, yielding a desirability  
35 value of 0.74, categorizing it as moderate.

## 36 1 Introduction

37 Nanotechnology underwent progressive evolution, characterized by measurements on the nanometer  
38 scale, approximately  $10^{-9}$  meters (Ariningsih, 2016). Acknowledgment from the World Health  
39 Organization (WHO) and the Food and Agriculture Organization (FAO) (2009) underscored  
40 nanotechnology's significant potential in enhancing food products, attributed to its capacity to modify  
41 surface characteristics and particle size. Such modifications facilitate targeted delivery of food  
42 compounds to specific organs and the controlled release of active compounds to mitigate adverse  
43 effects. The attributes of nanoscale food materials are pivotal in propelling diverse industries,  
44 including food, pharmaceuticals, and extensive nutraceutical applications (Rahman et al., 2020). Due  
45 to their substantial surface area-to-volume ratio, nanoemulsions exhibit enhanced stability against  
46 gravitational separation and aggregation, owing to their distinct physicochemical and biological  
47 characteristics compared to conventional emulsions. The droplets or globules inherent in  
48 nanoemulsions mitigate gravitational forces and Brownian motion, thereby averting creaming or  
49 sedimentation during storage. Nanoemulsions denote a nanotechnological rendition of a stable  
50 colloidal system, achieving kinetic stability through the amalgamation of oil, emulsifier, and water  
51 (McLements, 2016). Chang et al. (2022) conducted research utilizing surfactants as stabilizers in  
52 synthesizing nanoemulsions, showcasing the stability of nanoemulsion particle size in curcumin  
53 extract. Surfactants can diminish interfacial tension and form a substantially influential steric elastic  
54 film on the emulsion results (Xiao & Huang, 2016).

55 Renowned for its tropical climate and vast biodiversity, Indonesia harbours at least 30,000 plant  
56 species, with 7,000 being herbal plants with documented health benefits (Widyowati & Agil, 2018;  
57 Juniarni & Komalasari, 2017). Parijoto (*Medinilla speciosa*), an endemic plant species in Indonesia,  
58 remains relatively understudied for its scientific potential in pharmacy, functional foods, and  
59 nutraceuticals. Analysis has confirmed that the parijoto fruit comprises phytochemical components  
60 such as anthocyanins, flavonoids, saponins, tannins, alkaloids, cardenolides, and glycosides  
61 (Balamurugan, 2014). Anthocyanins, a predominant compound in parijoto fruit, demonstrate efficacy  
62 in reducing the risk of cardiovascular diseases, diabetes, and inflammation while possessing notable  
63 anti-inflammatory and antioxidant properties. Extraction techniques yield varying anthocyanin  
64 contents, with the peel extract and whole fruit extract registering 208.75 and 173.7 mg/L,  
65 respectively (Sa'adah et al., 2020). Various factors influence anthocyanins' stability, including  
66 chemical structure, concentration, solvent, pH, storage temperature, light, oxygen, metal ions,  
67 proteins, and flavonoids. Weak stability under high pH, high temperature, and light exposure has  
68 been observed (Ito et al., 2021), with lower pH contributing to enhanced stability (Moldova et al.,  
69 2020). Heating at elevated temperatures accelerates anthocyanin degradation (Khoo et al., 2019).

70 Response Surface Methodology (RSM) has emerged as a prominent multivariate statistical technique  
71 for optimizing various processes in recent years. Initially introduced by Box and colleagues in the  
72 1950s, RSM facilitates examining the relationship and interactions among independent and response  
73 variables through mathematical modelling and statistical methods (Izayidan et al., 2019). RSM has  
74 successfully enhanced and optimized therapeutic extract and drug nanoemulsion (Samim et al.,  
75 2020). In this study, Central Composite Design (CCD) Response Surface Methodology (RSM) was  
76 employed to optimize the quality parameters of the nanoemulsion.



77 Appropriate nano-encapsulation techniques, such as nanoemulsion, have shown the potential to  
78 enhance the stability, bioavailability, and solubility of lipophilic bioactive compounds while also  
79 preventing hydrolysis and oxidation (Rosso et al., 2020). Nanoemulsions are widely utilized  
80 nanoformulations in food-related industries through active or passive targeting mechanisms.  
81 Gunasekaran et al. (2014) introduced nanotechnology as an effective tool for enhancing the  
82 bioavailability and bioactivity of phytomedicine. Nanoemulsion has emerged as a novel technology,  
83 providing opportunities to address challenges associated with delivering micronutrients in functional  
84 food (Joyce et al., 2014). Shin et al. (2015) explored recent advancements in nanoformulation of  
85 lipophilic functional foods. Moreover, nanotechnology-based strategies have been explored to  
86 associate complex matrices derived from plant extracts, offering promising prospects for developing  
87 novel therapeutic formulations (Zorzi et al., 2015). Synthesis of nanoemulsion using mangosteen peel  
88 extract rich in anthocyanins as the main ingredient of the formulation can increase the dominant  
89 penetration of  $\alpha$ -mangostin through the stratum corneum (Pratiwi et al., 2017). Catechin  
90 nanoemulsion showed a remarkable improvement in stability and bioavailability in simulated  
91 gastrointestinal conditions (Rafanar et al., 2016). Mulia et al. (2017) showed the optimum results  
92 using a high-speed homogenization and Tween surfactant to prepare nanoemulsions with  
93 nanoemulsion. Research conducted by Chang et al. (2022) used Tween as the surfactant in the stable  
94 nanoemulsion synthesis loaded curcumin extract. This highlights the opportunity to develop  
95 nanoemulsion formulations for anthocyanins found in parijoto fruit. So far, research on nanoemulsion  
96 formulation in parijoto fruit involving various concentrations and stabilizers still needs to be  
97 conducted. This current study investigates the characteristics of nanoemulsion formulations derived  
98 from parijoto fruit extract and evaluates an optimum condition with various tween surfactants.

## 99 2 Materials and Method

### 100 2.1 Materials

101 Grönder (Binder), Erlenmeyer (Pyrex), beaker glass (Pyrex), volume pipette, test tube (Pyrex), test  
102 tube rack, funnel (Pyrex), measuring flask (Pyrex), vacuum n filter 0.22  $\mu$ m (Sartorius Stedim 11694-  
103 2-50-06), vial, micropipette (Socorex), blue tip (Biologix 1 mL pipette tips), hotplate (Cinarc et al.  
104 SP142025Q), vortex (Thermolyne et al.), Ultrasonic Cleaner (Biobase UC-10SD) modified, UV-VIS  
105 spectrophotometer (Shimadzu, UV-1280), aluminium foil, filter paper, 0.22  $\mu$ m filter membrane  
106 (Wattman), Cabinet dryer (HetoPowerDry II.1500), rotary evaporator (Biobase RE-2000F), syringe,  
107 analytical balance. Fresh parijoto, ethanol pro analysis (Merck, Germany), methanol pro analysis  
108 (Merck, Germany), distilled water, F. ciocalten 10% (Merck, Germany), Na<sub>2</sub>CO<sub>3</sub> 7.5% (Merck,  
109 Germany), DPPH solution (Merck, Germany), Quercetin (Merck, Germany), AlCl<sub>3</sub> (Merck,  
110 Germany), ammonium acetate 1 M (Merck, Germany), acetone (Merck, Germany), acetonitrile  
111 (Merck, Germany), standard cyanide (Zigma), delphinidin glu standard (Zigma), Tween 20 (Merck,  
112 Germany), Tween 60 (Merck, Germany), and Tween 80 (Merck, Germany).

### 113 2.2 Preparation of Dry Samples of Parijoto Fruit Extract

114 Samples used in this study are fruits from the Parijoto plant (*Medinilla speciosa*) cultivated and  
115 harvested on the slopes of Mount Muria, Kudus. The fruits used are ripe fruits harvested when the  
116 Parijoto plant reaches full maturity, typically around 90-100 days after flowering. Parijoto, which had  
117 been cleaned and sorted, was weighed 200 grams for each treatment. The fruit that has been weighed  
118 is then steam-blanched for 3 minutes. Prepare a citric acid solution with a concentration of 1% for  
119 pre-treatment of fruit before drying. After that, soak the parijoto fruit in the citric acid solution for 5  
120 minutes and drain. The Cabinet Dryer is cleaned before use to maintain hygiene and avoid cross-  
121 contamination. The drying temperature used was 70 °C for 6 hours. The dried Parijoto fruit is then

122 ground into powder using a herbal grinder for 2 minutes. After that, the sample will be extracted for  
123 further testing. The dried Parijoto will be chemically analyzed using UV-Vis spectroscopy.

### 124 **2.3 Making Parijoto Extract using Ultrasonic Assisted Extraction (UAE)**

125 Five grams of dry sample powder and 50 mL of 99.5% ethanol were mixed thoroughly for  
126 homogeneity in four 250 mL centrifuge bottles. Then, all vials were sonicated using a Bio-Based  
127 Ultrasonic Waterbath with a 40 KHz frequency and 100 W power for 30 minutes. Subsequently, the  
128 samples were subjected to shaking for one hour. The centrifugation step was performed at 4,000 rpm  
129 at 4 °C for 10 minutes (Ohaus, USA). The supernatant was then carefully collected, and the  
130 remaining solution was evaporated to dryness under vacuum conditions. The residue was dissolved in  
131 99.5% ethanol and diluted to 20 mL. After filtering through a 0.22 µm membrane filter, parijoto fruit  
132 extract was obtained and stored at -20 °C for UV-Vis analysis.

### 133 **2.4 Preparation of Anthocyanin Nanoemulsion from Parijoto Extract**

134 Approximately 3 mL of anthocyanin nanoemulsion with concentrations of 2 mg/mL, 4 mg/mL, and 6  
135 mg/mL, respectively, were prepared by collecting a portion of parijoto extract, and the solvent was  
136 removed with nitrogen. The solvent removal process during anthocyanin extraction can be monitored  
137 using a combination of visual inspection and periodic weight measurements. Visual inspection  
138 involves observing the extract as the solvent evaporates, noting its increasing concentration,  
139 evidenced by a thicker and more viscous appearance. Periodic weighing of the container or flask  
140 containing the extract allows for weight loss tracking as the solvent evaporates. Once the weight  
141 stabilizes or reaches a predetermined target, the desired solvent removal rate has been attained,  
142 ensuring the production of a concentrated anthocyanin extract suitable for further analysis.  
143 Anthocyanin nanoemulsion was prepared using a combination of surfactants that have low, medium,  
144 and high hydrophilic lipophilic balance (HLB), namely Tween 20, Tween 60, and Tween 80. Then,  
145 surfactant (0.24 g) was added, and the mixture was homogenized entirely. This was followed by  
146 adding (2.76 g) deionized water and mixing again for complete dispersion of surfactant in water. The  
147 solution was then sonicated in a sonicator with a temperature of 35 °C, frequency of 45 Hz, and  
148 100% power for 60 minutes. To produce a good nanoemulsion, homogenization was carried out  
149 using high shear homogenization at 15,000 rpm with a temperature of 4 °C for 15 minutes.

### 150 **2.5 Characterization of Particle Size and Polydispersity Index of Nanoemulsion Parijoto** 151 **Fruit Extract**

152 The particle size analysis tool used in this study was the Zetasizer (Zetasizer Pro; Malvern  
153 Instruments, Ltd., Malvern), which operates based on the general principle of dynamic light  
154 scattering (DLS). This tool has a detector placed at an angle of 173 ° from the transmitted light beam  
155 and detects size using a patented technology known as noninvasive backscattering. This technique is  
156 used for various purposes. One is to reduce the effect known as multiple scattering, making it easier  
157 to measure samples with high concentrations. Modifying McClements (2016), the particle size  
158 distribution and average particle size of nanoemulsions were determined by dynamic light scattering  
159 (DLS) at a wavelength of 633 nm and a temperature of 25 °C.

### 160 **2.6 Characterization of Z-potential Nanoemulsion Parijoto Fruit Extract**

161 The ζ-potential of Parijoto Fruit Extract Nanoemulsion was evaluated using ζ-potential analysis  
162 (Zetasizer Pro; Malvern Instruments, Ltd., Malvern) following the method described by Khalid et al.  
163 (2017). The ζ-potential of the samples was evaluated automatically using 10 to 100 analytical runs

164 after equilibration for 120 s at 25 °C. The  $\zeta$ -potential of the particles was measured by phase-analysis  
165 light scattering (PLS) using a Zeta dip cell.

## 166 **2.7 Characterization of the Conductivity of Nanoemulsion Parijoto Fruit Extract**

167 The conductivity of nanoemulsion particles was measured by phase-analysis light scattering (PLS)  
168 using a Zeta dip cell with a cuvet electrode. Samples were evaluated automatically using 10 to 100  
169 analytical runs after equilibration for 120 seconds at 25 °C. The detector is placed at an angle of 173°  
170 from the transmitted light beam.

## 171 **2.8 pH Measurement of Nanoemulsion Parijoto Fruit Extract**

172 The pH was determined using a Schott pH meter at room temperature ( $27 \pm 2$  °C), calibrated with a  
173 standard buffer of pH 7. The pH analysis of the Parijoto fruit extract nanoemulsion sample was  
174 carried out using a pH meter with a particular electrode. First, the pH meter is set and calibrated with  
175 a standard buffer solution at a known pH, generally at pH 4.0, 7.0, and 10.0. Samples were diluted  
176 with ten mM phosphate buffer pH seven before analysis to avoid multiple scattering effects during  
177 testing. The pH meter electrode is then carefully inserted into the sample to ensure good contact.  
178 Once the electrode is stable, a pH reading is taken and recorded. This step is repeated as necessary to  
179 obtain consistent results. This pH analysis provides essential information regarding the acidity or  
180 alkalinity level of nanoemulsion and nanocitosan Parijoto fruit extract, which can affect the stability  
181 and quality of products using the nanoemulsion.

## 182 **2.9 Viscosity Measurement of Nanoemulsion Parijoto Fruit Extract**

183 Viscosity measurements are carried out using a viscometer brookfield. 14 mL of sample was put into  
184 the cup and attached to the solvent trap provided. The viscometer was set at 200 rpm, three rotations,  
185 for 30 seconds. The measurement process begins by activating the viscometer, and this tool  
186 automatically measures the time required for a liquid to flow through the viscometer tube at a  
187 specific temperature and rpm. This time, a predetermined formula converts the reading into a  
188 viscosity value. Repeated measurements can be made to ensure consistent results.

## 189 **2.10 Statistical analysis uses Response Surface Methodology.**

190 In this study, primary data in 3 repetitions of extraction and three repetitions of testing were averaged  
191 and given a standard deviation value for each treatment combination using Statistica 12.5 by StatSoft.  
192 The data is then entered into a statistical application, arranged in a combination of factorial points,  
193 axial points, and central points with three repetitions. After that, the data was analyzed, and several  
194 test stages were carried out. The basis for testing is studentification from primary data.  
195 Studentification means that the scale of the variable is adjusted by dividing it by the estimated  
196 population standard deviation. Variability in sample standard deviation values contributes to  
197 additional uncertainty in the calculated value. This will cause problems in finding the probability  
198 distribution of each statistic studied.

### 199 **2.10.1 Effect Summary**

200 This test can summarise the effects of the combination of treatments used. The Longworth value in  
201 the results of this test is defined as  $-\log(p\text{-value})$  and is a transformation of the p-value based on the  
202 Pearson Chi-Squared test. The Pearson Chi-Squared test evaluates the possibility of the split being  
203 caused by chance. The higher the Pearson Chi-Squared value, the higher the probability of the split

204 being caused by dependency. Generally, if the worth is greater than 2, the statistical model considers  
205 the variable necessary.

### 206 **2.10.2 Lack Of Fit**

207 Model suitability testing (lack of fit) is carried out to review whether the model equation is  
208 acceptable or not in predicting responses. In the lack of fit test, the following hypothesis is used:

209  $H_0$  = no lack of fit (suitable model)

210  $H_1$  = there is a lack of fit (the model is not suitable)

211 The hypothesis is concluded by comparing the calculated F value with the F table. The calculated F is  
212 obtained from the statistical test results and displayed in the ANOVA table. The F table value is  
213 obtained from the F Distribution Table. The criteria for the lack of fit test are:

214  $F_{count} < F_{table}$ , then  $H_0$  is accepted.  $F_{count} > F_{table}$ , then  $H_0$  is rejected.

215 Another parameter that can prove the suitability of the model obtained is by comparing the p-value  
216 with the  $\alpha$  value. If the p-value of lack of fit is smaller than the  $\alpha$  value, then there is a significant  
217 lack of fit, so the model obtained is inappropriate.

### 218 **2.10.3 Summary Of Fit**

219 The R square and Root Mean square error values are obtained in this test. Measures the difference in  
220 values from a model's predictions as estimates of the observed values. R square is also known as the  
221 coefficient of determination, which explains how far independent data can explain dependent data. R  
222 square has a value between 0 – 1 with the condition that the closer it is to one, the better it is. If the r  
223 square is 0.6, the independent variable can explain 60% of the distribution of the dependent variable.  
224 The independent variable cannot explain the remaining 40% or can be explained by variables outside  
225 the independent variable (error component).

### 226 **2.10.4 Parameter Estimates**

227 The parameter estimates are the coefficients of the linear predictor. This value represents the change  
228 in response if you have a certain level of a categorical predictor or a change of 1 unit for a continuous  
229 predictor, which means the same thing as in a multiple regression analysis with continuous response.

### 230 **2.10.5 Analysis Of Variance**

231 The ANOVA test (Analysis of Variance) has the following test criteria:

232  $H_0$  is accepted if  $F_{count} < F_{table}$ , which means the model cannot be accepted statistically because  
233 no independent variables directly influence the response.

234  $H_1$  is accepted if  $F_{count} > F_{table}$ , which means the model is statistically acceptable and at least one  
235 independent variable has a real influence on the response.

### 236 **2.10.6 Fitted Surfaces**

237 The depiction of the fitted surface is carried out using the Central Composite Design model. The  
238 experimental design is factorial, specifically Central Composite Design (CCD). CCD was chosen  
239 over Box-Behnken Design because CCD provides more design points in terms of axial points.

240 Additionally, CCDs can run experiments at extreme values, providing better quadratic equations for  
241 analysis. CCD contains a factorial or fractional factorial design with a central point augmented by a  
242 group of 'axial points' that allow estimation of curvature. If the distance from the centre of the design  
243 space to the factorial point is  $\pm 1$  unit for each factor, the distance from the centre of the design space  
244 to the axial point is  $|\alpha| > 1$ . The exact value of  $\alpha$  depends on the properties desired for the design and  
245 the number of factors involved. The CCD has twice as many star points due to a factor in the design.

In review

246 **3 Result & Discussion**

247 **3.1 Phytochemical Profiles of Dried Parijoto Fruit**

248 Drying Parijoto Fruit is carried out using a cabinet dryer at a temperature 70°C for 6 hours. The  
249 results of drying parijoto fruit were obtained through the preparation process; the antioxidant  
250 and anthocyanin activity profiles were expressed respectively in units of % inhibition and ppm.  
251 The total anthocyanin content in the dry samples and extracts was  $538.47 \pm 4.67$  ppm. The dried  
252 Parijoto exhibited significant antioxidant activity, with a % inhibition value of  $79.14 \pm 34.82$ .  
253 This indicates a substantial capacity to neutralize free radicals in various chronic diseases and  
254 ageing processes. The high antioxidant activity suggests that the drying process did not  
255 significantly diminish the antioxidant potential of Parijoto. The total anthocyanin content of  
256 the dried Parijoto was  $538.47 \pm 4.67$  ppm. Anthocyanins are a group of pigmented compounds  
257 known for their antioxidant properties and potential health benefits. The retention of  
258 anthocyanins after drying indicates that cabinet drying effectively preserved these bioactive  
259 compounds in the dried Parijoto.

260 The parijoto fruit extract was obtained through an extraction process using the Ultra-assisted  
261 extraction method. The Ultra-assisted extraction method involves the utilization of a modified  
262 ultrasonic water bath for the extraction of parijoto fruit. This method harnesses ultrasonic  
263 energy to enhance the extraction process by facilitating cell wall breakdown and increasing  
264 target compounds' solubility. During extraction, the parijoto fruit is immersed in a solvent  
265 within the ultrasonic waterbath, where ultrasonic waves are applied to the sample. This results  
266 in intensified agitation and cavitation within the solvent, leading to improved extraction  
267 efficiency and higher yields of bioactive compounds from the fruit. The characterization of  
268 Parijoto extract as a filler in nanoemulsion involved various analyses to assess its antioxidant  
269 properties and phytochemical composition. The extraction method was ultra-assisted  
270 extraction, known for its efficiency in extracting bioactive compounds from plant materials.  
271 The antioxidant activity of the Parijoto extract was evaluated, yielding a % inhibition value of  
272  $50.776 \pm 6.18$ . This indicates a significant antioxidant capacity, crucial for combating oxidative  
273 stress and preventing cellular damage caused by free radicals.

274 Furthermore, the total anthocyanin content of the extract was determined to be  $94.43 \pm 4.14$   
275 ppm. Anthocyanins are well-known antioxidants in many fruits and vegetables. They are  
276 known for their potential health benefits, including anti-inflammatory and anti-cancer  
277 properties. The flavonoid content of the Parijoto extract was measured to be  $126.85 \pm 1.15$  g/L.  
278 Flavonoids are a class of polyphenolic compounds known for their antioxidant and anti-  
279 inflammatory effects. Additionally, the phenolic content of the extract was quantified as  
280  $8.43 \pm 0.70$  GAE/g. Phenolic compounds are another group of bioactive compounds found in  
281 plants, known for their antioxidant and anti-inflammatory activities and their potential role in  
282 reducing the risk of chronic diseases.

283 **3.2 Fitting Model for RSM (Response et al.) in Parijoto Fruit Extract Nanoemulsion**

284 Data recorded for each run included nanoemulsion particle size, polydispersity index,  $\zeta$ -potential, conductivity, pH, and viscosity. Each variable  
 285 was measured with three repetitions and the measurements three times to get consistent results. This data will be used to analyze the influence of  
 286 various factors on the characteristics of nanoemulsions using the Response Surface Methodology method, which can be seen in the table.

287 Table 3. Design of Experiment RSM Particle Size, Poly Dispersity Index, Z-potential, Conductivity, pH, Viscosity in Nanoemulsion

No. Run Test	Dependent Variables			Independent Variables										
	Types of Hydrophilic Tween	Tween Concentration (%)	Parijoto Fruit Extract Concentration (%)	Nanoparticle Size (nm)		Z potential	Conductivity		Poly Dispersity Index		pH		Viscosity(Cp)	
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	Y <sub>1</sub>	Y <sub>2</sub>	Y <sub>3</sub>	Y <sub>4</sub>	Y <sub>5</sub>	Y <sub>6</sub>	Y <sub>7</sub>	Y <sub>8</sub>	Y <sub>9</sub>	Y <sub>10</sub>	
1	20	5	5	15.53 ± 0.129	-39.14 ± 1.574	0.051 ± 0.005	0.45 ± 0.05	6.767 ± 0.095	0.171 ± 0.005					
2	20	0	5	15.2 ± 0.137	-29.43 ± 2.807	0.057 ± 0.013	0.54 ± 0.08	6.870 ± 0.023	0.057 ± 0.015					
3	20	0	10	15.063 ± 0.1728	-37.50 ± 1.171	0.066 ± 0.007	0.57 ± 0.07	6.700 ± 0.093	0.066 ± 0.007					
4	20	5	0	22.061 ± 0.1830	-25.525 ± 1.128	0.067 ± 0.007	0.55 ± 0.05	6.891 ± 0.053	0.061 ± 0.005					
5	20	0	10	20.017 ± 0.0585	-22.177 ± 2.542	0.065 ± 0.010	0.48 ± 0.07	6.753 ± 0.023	0.068 ± 0.013					
6	20	0	0	41.593 ± 0.1759	-22.127 ± 0.731	0.074 ± 0.006	0.45 ± 0.07	6.823 ± 0.002	0.074 ± 0.006					
7	20	5	0	62.041 ± 0.0055	-24.645 ± 0.761	0.010 ± 0.010	0.57 ± 0.15	6.897 ± 0.089	0.014 ± 0.013					
8	20	0	0	84.549 ± 0.1707	-24.471 ± 0.379	0.074 ± 0.017	0.50 ± 0.05	6.810 ± 0.023	0.074 ± 0.017					
9	20	0	0	91.067 ± 0.0312	-24.627 ± 0.809	0.064 ± 0.013	0.40 ± 0.04	6.817 ± 0.023	0.064 ± 0.013					
10	20	10	5	51.877 ± 0.1598	-24.627 ± 1.206	0.065 ± 0.013	0.61 ± 0.04	6.750 ± 0.013	0.065 ± 0.013					
11	20	10	5	55.567 ± 0.007	-24.427 ± 1.213	0.069 ± 0.010	0.50 ± 0.09	6.881 ± 0.021	0.069 ± 0.010					

12	23	10	s	56.237	±	1.937	-24.275	±	1.973	0.049	±	1.001	1.51	±	0.02	1.10	±	0.03	0.308	±	0.001
12	23	10	o	61.54	±	4.231	-21.730	±	1.201	0.086	±	1.029	1.59	±	0.03	1.077	±	0.03	0.300	±	0.015
14	23	10	s	84.087	±	0.472	-25.797	±	1.442	0.075	±	1.017	1.32	±	0.03	1.009	±	0.025	0.373	±	0.017
15	23	10	o	84.027	±	0.566	-25.150	±	1.202	0.087	±	1.020	1.59	±	0.04	1.100	±	0.03	0.317	±	0.013
16	23	10	y	72.287	±	12.208	-26.127	±	1.716	0.071	±	1.040	1.55	±	0.11	1.041	±	0.01	0.375	±	0.002
17	23	10	y	75.143	±	1.101	-26.242	±	1.843	0.081	±	1.079	1.51	±	0.14	1.000	±	0.013	0.405	±	0.013
18	23	10	y	77.963	±	10.473	-26.343	±	1.518	0.076	±	1.057	1.59	±	0.01	1.100	±	0.013	0.375	±	0.013
19	23	12	s	81.69	±	11.525	-26.047	±	1.333	0.080	±	1.012	1.57	±	0.13	1.090	±	0.013	0.382	±	0.015
20	23	12	s	81.4	±	11.528	-26.025	±	1.260	0.089	±	1.017	1.55	±	0.17	1.172	±	0.012	0.395	±	0.017
21	23	12	s	83.067	±	7.467	-26.645	±	1.258	0.085	±	1.016	1.64	±	0.15	1.177	±	0.012	0.385	±	0.018
22	23	12	o	91.14	±	11.159	-27.172	±	1.403	0.081	±	1.016	1.70	±	0.14	1.110	±	0.013	0.385	±	0.015
23	23	12	o	110.88	±	10.51	-27.077	±	1.508	0.081	±	1.017	1.57	±	0.27	1.110	±	0.013	0.385	±	0.017
24	23	12	o	110.23	±	7.094	-27.230	±	1.326	0.077	±	1.011	1.72	±	0.13	1.090	±	0.017	0.377	±	0.015
25	23	12	y	117.647	±	17.119	-28.217	±	1.928	0.076	±	1.010	1.64	±	0.23	1.073	±	0.021	0.378	±	0.010
26	23	12	y	119.11	±	1.581	-28.015	±	1.718	0.076	±	1.011	1.61	±	0.19	1.067	±	0.018	0.379	±	0.011
27	23	12	o	124.707	±	4.182	-28.070	±	1.824	0.089	±	1.010	1.75	±	0.21	1.057	±	0.015	0.388	±	0.015



289 Table 3 shows that the particle size range of the nanoemulsion is between  $14,603 \pm 16.73$  nm  
290 and  $118,053 \pm 4,5825$  nm. The largest and smallest nanoparticle sizes found are 126.47 nm  
291 and 13.72 nm, respectively, with most nanoparticle sizes falling within the 50-100 nm range.  
292 Similar results were confirmed by Noor El-Din et al. (2017), who reported nanoemulsion  
293 sizes ranging from 31.58 to 220.5 nm. Studies conducted by Delmas et al., Liu et al., and Mei  
294 et al. using ultrasonication and high emulsification methods also confirmed comparable  
295 results of 45–170 nm, 222.4–166.4 nm, and 170–280 nm, respectively (Delmas et al., 2016;  
296 Liu et al., 2017; Mei et al., 2019). Conversely, Peng et al. (2010) reported a nanoparticle size  
297 range of 21–530 nm. Z-potential reflects the surface charge of particles and affects colloidal  
298 stability. High  $\zeta$ -potential can prevent particle aggregation due to electrostatic repulsion. The  
299 research includes the evaluation and characterization of  $\zeta$ -potential under various treatments.  
300 The study obtained  $\zeta$ -potential results for nanoemulsion ranging from  $-22.197 \pm 0.738$  mV to  
301  $-28.207 \pm 1.598$  mV, respectively. Similar results were confirmed by Wessam et al. (2023),  
302 obtaining results of +21.5 mV. Particles with high ZP values, between 20 and 40 mV, provide  
303 system stability and are less likely to aggregate or increase particle size. However, it should  
304 be noted that ZP values are not an absolute measure of nanoparticle stability. Furthermore,  
305 emulsions with ZP variations  $>10$  mV are suggested to have better stability (Kadı et al.,  
306 2011). The ideal potential range for nanoparticle stability is (-30 to 20 mV or +20 to +30 mV)  
307 (Lin et al., 2018). The produced values tend to be harmful due to the influence of acetic acid,  
308 resulting in a negative charge. This charge causes electrostatic repulsion forces between  
309 formed nanoparticles to prevent aggregation into larger sizes (Luthifayana et al., 2022).  
310 Higher  $\zeta$ -potential values increase nanoparticle stability due to higher electrostatic repulsion  
311 forces between nanoparticles.

312 Conductivity provides information about the ability of nanoemulsions to conduct electricity.  
313 Changes in conductivity can occur with changes in surface particle charge. Table 18 shows  
314 that the nanoemulsion conductivity of Parijoto fruit extract ranges from 0.03458 to 0.09987  
315 mS/cm. Good nanoemulsion conductivity measurements have higher electrical conductivity  
316 values (10–100  $\mu$ S/cm) (Akilu et al., 2019; Guo et al., 2016; Khader et al., 2016). Electrical  
317 conductivity values tend to decrease with decreasing water content in the emulsion. O/W type  
318 (Oil-in-Water) nanoemulsions have higher conductivity than W/O type (Water-in-Oil)  
319 nanoemulsions. This is because the more extensive water phase provides more pathways for  
320 ion conduction.

321 The type and concentration of surfactant in nanoemulsion can influence conductivity.  
322 Surfactants can provide ionic charge or facilitate ion conduction in the system. Viscosity is an  
323 essential parameter in evaluating the flow properties of nanoemulsion. Viscosity is one of the  
324 parameters used to determine the stability of polymers in a solution because it undergoes  
325 reduction during polymer storage due to polymer degradation (Aranaz et al., 2021). In this  
326 study, as shown in Table 1, the viscosity of nanoparticles ranges from 3,810 cP to 4,433 cP.  
327 Alemu et al. (2023) stated that viscosity can depend on particle size and storage time.  
328 Appropriate viscosity can affect the applicability and spread of the system. The viscosity of a  
329 preparation is related to the consistency and spreadability of the preparation, which will affect  
330 ease of use (Imanto et al., 2019). Viscosity values are influenced by several factors, such as  
331 temperature, pH, manufacturing conditions, and the quality and concentration of raw  
332 materials (Naini & Yusuf, 2018). The results of viscosity tests are shown in centipoise (cP).  
333 The higher the viscosity value of a preparation, the better the stability of the product, but the  
334 preparation will be difficult to apply to the skin, and the resistance of the preparation to flow  
335 will increase, making it difficult to remove from the container (Thakre, 2017). Meanwhile,  
336 low viscosity values will increase the flowability of the skin and make it easier to apply to the  
337 skin (Naini & Yusuf, 2018)

338 This ANOVA table is essential to evaluate the statistical significance of each model component and determine whether the quadratic model used  
 339 is good enough to explain the characteristics of the nanoemulsion or not. The p-value is used to determine statistical significance, and the  
 340 analysis results will help select an appropriate model and interpret the significance of factors that influence the characteristics of nanoemulsions,  
 341 which can be seen in the table.

342 Table 4. ANOVA (Analysis of Variance) for the RSM Quadratic Model Particle Size, Poly Dispersity Index, Z-potential, Conductivity, pH, and  
 343 Viscosity in Nanoemulsion

Quadratic Model Equation	Sources of Variation	p-Value
<b>Particle Size</b> ( $R^2$ : 0,558 $R^2_1$ : 0,50156) $Y_1 = -0,000008 - 0,000069X_1 + 0,000040X_2 + 0,000032X_3 + 0,000056X_1^2 +$ $0,000064X_2^2 - 0,000003X_3^2 - 0,000056X_1X_2 - 0,000044X_2X_3 + 0,000065X_1X_3$	Model	0,294 *
	Lack of fit	0,185
<b>Poly Dispersity Index</b> ( $R^2$ : 0,3643 $R^2_1$ : 0,2471) $Y_2 = 6,23086 + 0,58801 X_1 - 0,75655 X_2 + 84,3654 X_3 + 24,65 X_1^2 + 18,7663 X_2^2$ $20,744 X_3^2 + 23,0025 X_1X_2 + 26,3043 X_2X_3 + 9,5269 X_1X_3$	Model	0,041*
	Lack of fit	0,692
<b>Z-potential</b> ( $R^2$ : 0,54003 $R^2_1$ : 0,56905) $Y_3 = 0,000062 - 0,000023 X_1 - 0,000010 X_2 + 0,000008 X_3 + -0,000007 X_1^2 +$ $0,000003 X_2^2 + 0,000008 X_3^2 + -0,000006 X_1X_2 - 0,000008 X_2X_3 + -0,000005 X_1X_3$	Model	0,000*
	Lack of fit	0,980
<b>Conductivity</b> ( $R^2$ : 0,2444 $R^2_1$ : 0,3464) $Y_4 = 4035,80 - 1198,06X_1 + 833,22X_2 - 1083,49X_3 - 2597,59X_1^2 - 709,42X_2^2$ $+ 881,10X_3^2 + 305,68X_1X_2 - 700,69X_1X_3 - 943,96X_2X_3$	Model	0,0004*
	Lack of fit	0,928
<b>pH</b> ( $R^2$ : 0,832 $R^2_1$ : 0,797) $Y_5 = 0,003122 - 0,000040X_1 - 0,000060X_2 + 0,000039X_3 - 0,000034X_1^2 -$ $0,000047X_2^2 + 0,000031X_3^2 - 0,00006X_1X_2 - 0,000015X_1X_3 + 0,000031 X_2X_3$	Model	0,000*
	Lack of fit	0,067
<b>Viskositas</b> ( $R^2$ : 0,95976 $R^2_1$ : 0,95466) $Y_6 = 0,015177 - 0,009573X_1 - 0,003288X_2 - 0,000624X_3 - 0,008334X_1^2 -$ $0,000266X_2^2 - 20,744 X_3^2 + 23,0025 X_1X_2 + 26,3043 X_2X_3 + 9,5269 X_1X_3$	Model	0,000*
	Lack of fit	0,103

344 Notes :  
 345 - \*: The model has a statistically significant effect (p<0.05)  
 346 - \*\*: Model mismatch or lack of fit occurs (p<0.05)

347 Based on the ANOVA RSM analysis of three factors, namely the type of Tween in  
348 nanoemulsion, Tween concentration, and Parijoto extract concentration, all ANOVA values  
349 show probabilities  $<0.0001$  ( $p<0.05$ ). This indicates that the quadratic response surface model  
350 used for both responses (dependent variables) is significant and can be used to optimize  
351 extraction factors (Wang et al., 2014). The coefficient of determination, or R square, depicts  
352 how independent data can explain dependent data. The range of R square values is between 0  
353 and 1, where values closer to 1 indicate better explanatory power.

354  
355 In the Central Composite Design analysis, the p-value indicates the significance of each  
356 coefficient in the built polynomial regression model. The lower the p-value, the more  
357 significant the contribution of the coefficient to the overall regression model (Zhong &  
358 Wang, 2010). Using experimental data within the allowed range of variables in this study to  
359 create mathematical equations, which may have broader general applications, can provide the  
360 ability to predict system behaviour when different factors are combined. From the perspective  
361 of optimizing the formation of emulsion nanoparticles, there is potential to develop more  
362 significant results based on the variables investigated in this study. Additionally, this  
363 optimization may be performed using the techniques outlined in this research to test further  
364 the effects of time and temperature or other conditions, as needed.

365 Table 4 shows details of the RSM approach used to assess particle size (nm), Poly Dispersity  
366 Index, Z-potential (mv), Conductivity, pH, and viscosity (Cp) in nanoemulsion of Parijoto  
367 fruit extract involved in a series of 81 experiments based on factorial design. The coefficients  
368 for the second-degree polynomial Equation are determined through experimental results,  
369 along with the regression coefficients for Particle Size (Y1), Poly Dispersity Index (Y2), Z-  
370 potential (Y3), Conductivity (Y4), pH (Y5), and viscosity (Y6). The Equation presented as  
371 Equation (2) shows the full quadratic model, while Table X shows the models predicting the  
372 response of the independent variables (Y1–Y6).

373 To assess the extent to which the equation model in RSM fits the data and how strong the  
374 influence of the variables is, the coefficient of determination or ( $R^2$ ) is used. Chin (1998) has  
375 categorized that for model suitability, the R-Square value is substantial if it is more than 0.67,  
376 moderate if it is more than 0.33 but lower than 0.67, and weak if it is more than 0.19 but  
377 lower than 0.33. pH and viscosity indicate strong model adequacy on these response  
378 variables. In contrast, the responses of Particle Size, Poly Dispersity Index, Z-potential, and  
379 Conductivity indicate a moderate model for these response variables. A lack of fit test was  
380 then performed to assess model fit for each response. With a p-value exceeding 0.05, it was  
381 confirmed that the model adequately fit the experimental data, as seen in Table 4.

### 382 **3.3 Contour plot on Particle Size, poly-dispersity index, Z-potential, Conductivity, pH,** 383 **and Viscosity as a function of Nanoemulsion Parijoto Fruit Extract.**

384 In this research, the model is created as a Contour plot, showing the response: Particle Size,  
385 Poly Dispersity Index, Z-potential, Conductivity, pH, and Viscosity. Continued research  
386 shows a significant relationship between particle size and tween concentration and the type of  
387 lipophilic Tween in nanoemulsions, as shown in Figures 1-6. The presented data offers  
388 valuable insights into the influence of lipophilic tween type and tween concentration on  
389 various properties of the nanoemulsion derived from parijoto fruit extract. Each figure depicts  
390 the contour plots illustrating the interaction effects between these two factors on different  
391 characteristics of the nanoemulsion.

392 Figure 1, the contour plot demonstrates the interaction between the lipophilic tween type and  
393 tween concentration in controlling nanoparticle size. It reveals that as the lipophilic tween  
394 type increases from 20 to 80, and the tween concentration rises from 8% to 10%, there is a  
395 general trend of increasing particle size, albeit with a slight decreasing trend observed to  
396 some extent. This suggests that both factors play a role in determining the nanoparticle size,  
397 with higher concentrations leading to larger particle sizes. Moving to Figure 2, which  
398 illustrates the Z-potential of the nanoemulsion, an increase in the lipophilic Tween type from  
399 60 to 80 and an increase in tween concentration from 8% to 10% correspond to an increase in  
400 Z-potential. Interestingly, no further changes are observed beyond this point. This indicates  
401 that these specific conditions result in optimal Z-potential, possibly indicating enhanced  
402 stability of the nanoemulsion.

403 Figure 3 showcases the influence of lipophilic tween type and tween concentration on the  
404 conductivity of the nanoemulsion. As the lipophilic tween type increases from 20 to 80 and  
405 the tween concentration rises from 8% to 12%, conductivity is consistent without any further  
406 changes. This suggests a direct relationship between these factors and the conductivity of the  
407 nanoemulsion. The Contour plot presented in Figure 4 demonstrates the effect of lipophilic  
408 tween type and tween concentration on the nanoemulsion's Poly Dispersity Index (PDI).  
409 Interestingly, an increase in lipophilic tween type from 60 to 80 and a decrease in tween  
410 concentration from 12% to 8% lead to an increase in PDI value without further changes. This  
411 indicates a complex interaction between these factors in determining the homogeneity of  
412 particle size distribution within the nanoemulsion.

413 Figure 5 depicts the pH contour plot of the parijoto fruit extract nanoemulsion. An increase in  
414 lipophilic Tween type from 20 to 80 and an increase in tween concentration from 8% to 12%  
415 result in a consistent increase in pH without any further changes. This observation suggests  
416 that these specific conditions contribute to the alkalinity of the nanoemulsion, which may  
417 have implications for its stability and functionality. Finally, Figure 6 illustrates the viscosity  
418 contour plot of the nanoemulsion. An increase in lipophilic tween type from 35 to 80 and an  
419 increase in tween concentration from 8% to 12% lead to an increase in viscosity without  
420 further changes. This indicates that higher concentrations of lipophilic Tween and Tween  
421 result in a thicker consistency of the nanoemulsion, which affects its flow properties and  
422 application. The presented data highlights the intricate relationship between lipophilic tween  
423 type and tween concentration in influencing various physicochemical properties of the  
424 nanoemulsion derived from parijoto fruit extract. These findings provide valuable insights for  
425 optimizing the formulation and manufacturing process of the nanoemulsion for potential  
426 applications in various industries.

427 Research on the influence of surfactant type and concentration on nanoemulsion indicates  
428 that the selection of surfactant significantly affects the characteristics of nanoemulsion.  
429 Various surfactant types, such as Tween 20, Tween 60, and Tween 80, play different roles in  
430 forming nanoemulsions. The research results show that the particle size of Tween 80  
431 surfactant is the highest, with an average particle size of 107.196 nm. Similar results were  
432 reported by Chang et al. (2013), who obtained the smallest droplets in carvacrol-based  
433 nanoemulsion made with a mixture of food-grade non-ionic surfactants (Tween 20, 40, 60,  
434 80, and 85). Tween, a non-ionic surfactant derived from sorbitan ester, is soluble or  
435 dispersible in water and is commonly used as an oil-in-water emulsifier in the  
436 pharmaceutical, cosmetic, and cleaning industries. Among these surfactants, Tween 80 is one  
437 of the most commonly used. Research by Douglas et al. (2013) confirms that the type of non-  
438 ionic surfactant significantly influences the average particle diameter of the formed colloid  
439 dispersion. The smallest droplets were observed in systems prepared using Tween 80, while

440 the largest droplets formed in systems using Tween 85. The surfactant's Hydrophilic-  
441 Lipophilic Balance (HLB) plays a role in forming small particles. Surfactants with either too  
442 high (Tween 20) or too low (Tween 85) HLB values cannot form optimal nanoemulsions.  
443 Tween types with intermediate HLB values (40, 60, and 80) can form nanoemulsions with  
444 small particle sizes. However, there is no strong correlation between HLB values and particle  
445 sizes produced by these surfactants (Kumar et al., 2008). Small-molecule surfactants have  
446 higher surface activity and form smaller emulsion droplets than large ones (Qian &  
447 McClements, 2011; Teo et al., 2014).

448 Another critical factor for minimal droplet emulsion formation is the Hydrophilic-Lipophilic  
449 Balance (HLB) value of the surfactant (Sagitani, 1981), defined by Griffin as the ratio of  
450 surfactant hydrophilicity to lipophilicity (Griffin, 1949). A high HLB value indicates strong  
451 hydrophilicity, and the HLB values of non-ionic surfactants generally range from 0 to 20  
452 (Gad & Khairou, 2008), such as Tween 20 (HLB 16.7) and Tween 80 (HLB 15) (Dinarvand  
453 et al., 2005). Two polymer and particle surface tension mechanisms influence emulsion  
454 stability: steric stability caused by macromolecules adsorbed on particle surfaces and  
455 electrostatic stability due to repulsion between surface-charged droplets. In nanoemulsions  
456 made with Tween 80 surfactant, the surfactant may not have a charge on the hydrophobic  
457 group, causing the covered droplet surface to be non-charged and resulting in low  $\zeta$ -potential  
458 values, which can lead to increased particle size and PDI (Lian et al., 2016).

459 However, a different study proposed by Alam et al. (2023) suggests that Tween 20 helps  
460 improve PDI and allows for minimum polydispersity. Compared to other nanoparticles, the  
461 ability to maintain particle integrity using Tween 20 is significant. Increasing the Surfactant  
462 content in the formulation increases the polydispersity indices for natural extracts in the 3D  
463 response surface graph. This indicates that the use of Tween types with low and high HLB  
464 values can be applicable when combined with an optimal concentration of co-surfactant.

465 Surfactant concentration is also a critical factor in nanoemulsion formation. Research  
466 indicates that increasing surfactant concentration can result in smaller and more homogenous  
467 size distribution. However, there is a specific limit where surfactant concentration reaches a  
468 plateau level, leading to unadsorbed surfactant aggregation and micelle formation. The results  
469 show that the higher the Tween concentration, the higher the size and PDI. This is confirmed  
470 by Ijat et al. (2016), stating that nanoemulsions prepared with higher surfactant  
471 concentrations significantly increase short-term stability. Systems with 15 or 20% weight of  
472 Tween 80 are highly unstable to increasing dilution, indicating that a medium surfactant  
473 concentration level may be more suitable for stable nanoemulsion preparation. Although the  
474 initial droplet size is small, higher surfactant concentrations can increase raw material costs  
475 and cause undesirable sensory (taste) issues in commercial applications. Therefore, this study  
476 uses a 10% weight of Tween 80 in further experiments.

477 Increasing surfactant concentration increases the number of surfactant molecules migrating  
478 from the oil phase to the emulsion water phase, and nanodroplets form. Frictional forces  
479 applied to the oil-water interface, coated with emulsifier, cause some emulsifiers to sink  
480 parallel to the surface layer while others detach from the surface layer. Hasani et al. (2015)  
481 reported that droplet size increases by increasing surfactant concentration to 20%, and  
482 particles have a broad and non-uniform size distribution. The instability of nanoemulsion at  
483 high surfactant concentrations may be related to the depletion-flocculation mechanism of  
484 adsorbed surfactant. With increased surfactant concentration, additional surfactant molecules  
485 form micelles in the continuous phase rather than orienting on the particle surface. This leads  
486 to an increase in local osmotic pressure, causing the continuous phase between moving  
487 droplets to decrease, reducing the continuous phase between those droplets. As a result,

488 aggregation occurs, causing an increase in particle size. According to Oh et al. (2011) and  
489 Tadros et al. (2004), the average droplet size becomes smaller, and the size distribution  
490 becomes narrower with increasing emulsifier concentration, ultimately reaching a plateau  
491 level. Beyond the plateau level, free or unadsorbed emulsifiers may accumulate to form  
492 micelles. Nanoemulsions are known to be thermodynamically unstable, tending to minimize  
493 interfacial area through coalescence.

494 An increase in the filler extract's concentration can lead to nanoparticles' tendency to  
495 aggregate or form agglomerates and pH nanoemulsion. This phenomenon may occur due to  
496 physical or chemical interactions between nanoparticles and compounds in the filler extract.  
497 Findings by Alab et al. (2021) suggest that an increase in extract concentration results in an  
498 increase in particle size, particularly at the highest concentration of 347.2 nm. On the other  
499 hand, the smallest concentration has the lowest particle size at 86.98 nm. These results  
500 indicate that higher concentrations may increase the likelihood of particle agglomeration.

501 Furthermore, increasing the concentration of parijoto fruit extract can increase the total mass  
502 in the solution, which, in turn, can increase overall viscosity. Additional particles or  
503 molecules from the filler extract can contribute to the increase in viscosity. A study by Olan  
504 et al. (2021) shows that particles with the highest concentration have the highest viscosity and  
505 vice versa. This increase in viscosity may be caused by excess extract loaded into particles.  
506 The physicochemical characteristics of the filler extract may influence the viscosity  
507 properties of nanoparticles, and factors such as changes in pH, temperature, or chemical  
508 composition may also play a role in viscosity increase. Parijoto fruit is rich in active  
509 compounds, such as anthocyanins, which can affect the surface charge of nanoemulsion  
510 particles. At a certain pH, anthocyanins or other components may have specific charges that  
511 can influence the electrostatic stability of particles (Liu et al., 2016). Anthocyanins may  
512 undergo solubility changes at specific pH values, affecting the distribution and stability of the  
513 nanoemulsion's oil or water phase. The same occurs with surfactants, where variations in  
514 charge of the filler extract from parijoto fruit can affect the interaction between nanoparticles,  
515 anthocyanins, and other components in the system. The loading capacity of the extract in the  
516 nanoemulsion likely depends on its solubility in the system used. Anthocyanins tend to  
517 undergo color changes with pH (pH-dependent color shift). Additionally, the antioxidant  
518 activity of anthocyanins can be influenced by pH. This complexity can modulate the overall  
519 physicochemical properties of the nanoemulsion system.

520 Nanoemulsions, despite their promising applications, present challenges related to stability.  
521 The challenge is the propensity for Ostwald ripening, wherein larger droplets grow at the  
522 expense of smaller ones, leading to phase separation and reduced shelf-life. Additionally,  
523 factors such as temperature fluctuations, pH changes, and exposure to light can exacerbate  
524 instability, causing particle aggregation. Surfactant degradation over time is another concern,  
525 as it can compromise the emulsion's ability to maintain a stable dispersion. However, the  
526 industrial application of parijoto fruit or extract holds significant potential. Parijoto fruit,  
527 known for its rich content of bioactive compounds, including anthocyanins, flavonoids, and  
528 phenolic acids, offers various health benefits such as antioxidant and anti-inflammatory  
529 properties. Incorporating parijoto extract into nanoemulsions can enhance its bioavailability  
530 and efficacy, making it suitable for a range of industrial applications especially food  
531 functional and nutraceutical.

532 **3.4 Optimal Point Prediction from RSM in Nanoemulsion Parijoto Fruit Extract**

533 Optimal point predictions from the Response Surface Methodology are obtained by combining optimal conditions based on interactions between  
 534 independent variables. Profiler predictions are obtained if the fitted surface graph is in minimum, maximum, or saddle form. 3D graphics on  
 535 image x. shows a complex interaction between the variable factors of lipophilic tween type and tween concentration on the response. Increasing  
 536 the lipophilic tween type value increases the response somewhat, but the tween concentration value can modify the effect. There is an optimal  
 537 region where the response reaches its peak. The implication for practice is that by setting the variable factors at levels that are estimated to be  
 538 optimum, the research results can achieve the highest optimization in the desired response, which can be seen in Figure 7.

539 Table 3. Prediction of Optimum Conditions for Parijoto Fruit Extract Nanoemulsion

Types of Analysis	Types of Lyphophilic Tweens	Tween Concentration (%)	Parijoto Fruit Extract Concentration (%)	Nanoparticle Size (nm)	Z-potential (mV)	Conductivity (mS/cm)	Poly Dispersity Index	Degree of acidity (pH)	Viscosity (Cp)	Desirability Value
Optimum Condition Prediction	80	12	7.5	61.97	-28.48	0.082	0.691	6.864	5.668	0.74
Maximum Value at Optimum Conditions	80	12	7.5	39.94	-32.48	0.048	0.371	6.82	5.422	
Minimum Value at Optimum Conditions	80	12	7.5	163.88	-26.37	0.115	1.011	6.9	5.913	

540

541 It can be seen in Table 9 that to achieve the maximum desired concentration of nanoparticle size,  $\zeta$ -potential, Conductivity, Poly Dispersity  
 542 Index, degree of acidity, and Viscosity, it is necessary to set the Tween solvent concentration to 80, Tween concentration to 12% and parioto  
 543 fruit extract concentration to 7.5 %. This set of conditions has a desirability value of 0.74. Because the value is almost close to 1 and falls into  
 544 the moderate category, this set of conditions is quite optimal for the aim of this research, namely to maximize the response.

545 The optimization of nanoemulsion formation from parijoto fruit extract using Response  
546 Surface Methodology (RSM) has been conducted in this study. RSM is a statistical method  
547 used to design experiments and analyze the impact of multiple independent variables on a  
548 measured response. As an output of this research, the synthesis process conditions of  
549 nanoemulsion from parijoto fruit extract can be optimized to achieve particle size,  
550 polydispersity index (PDI),  $\zeta$ -potential, conductivity, pH, and viscosity levels. RSM  
551 determines the optimal extraction time and temperature to maximize the response variable  
552 outcomes (Granato et al., 2014). In line with this, predictions and observations are within a  
553 narrow range and do not show significant differences at a 5% significance level, indicating  
554 the model's suitability for optimization and process efficiency purposes.

555

556 The optimal point prediction from the Response Surface Methodology is obtained by  
557 integrating optimal conditions and depends on the interaction between independent variables,  
558 as Ratnawati et al. (2018) explained. The prediction profile is formed when the adjusted  
559 surface graphs show a minimum, maximum, or saddle shape. The optimization process can  
560 achieve optimal responses by analyzing each response beforehand, ultimately reducing effort  
561 and operational costs, as Nurmiah et al. (2013) stated. Desirability, with a range of values  
562 from 0 to 1, is used as the optimization target value, with low (0-0.49), moderate (0.5-0.79),  
563 and high (0.8-1) categories. The closer the value of 1 is, the greater the desirability, which  
564 indicates the suitability of the combination of process parameters to achieve optimal response  
565 variables.

566

567 Table 3 shows that to achieve the desired concentrations of nanoparticle size,  $\zeta$ -potential,  
568 conductivity, polydispersity index, acidity level, and viscosity, Tween 80 with a Tween  
569 concentration of 12% and parijoto fruit extract concentration of 7.5% is necessary. This set of  
570 conditions has a desirability value of 0.740349. Since its value is close to 1 and falls into the  
571 moderate category, this set of conditions is optimal for this research to maximize the  
572 response.



573 **4 Conclusion**

574 In this series of experiments, nanoemulsion from parijoto fruit has been characterized, considering  
575 various physicochemical parameters such as particle size, polydispersity index,  $\zeta$ -potential,  
576 conductivity, pH, and viscosity respectively ranged from  $14,603 \pm 16.73$  nm to  $118,053 \pm 4.5825$  nm,  
577  $0.402 \pm 0.038$  to  $0.874 \pm 0.100$ ,  $-22.197 \pm 0.738$  mV to  $-28.207 \pm 1.598$  mV,  $0.064 \pm 0.013$  to  $0.090 \pm 0.010$   
578 mS/cm, and  $6.747 \pm 0.035$  to  $6.897 \pm 0.006$ , and  $3.827 \pm 0.021$  to  $5.633 \pm 0.058$ . The research results  
579 indicate significant variations in the physical characteristics of both nanomaterials regarding changes  
580 in surfactant and parijoto extract concentrations. Increased surfactant concentration tends to produce  
581 smaller particle sizes and a more homogeneous distribution, although certain limitations were found  
582 that lead to surfactant aggregation and micelle formation. The nanoemulsion characteristics include  
583  $\zeta$ -potential, polydispersity, particle size, conductivity, pH, and viscosity. The type and concentration  
584 of surfactants played a crucial role in determining the properties of the nanoemulsions. Variations in  
585 surfactant parameters resulted in observable differences in emulsion characteristics, highlighting the  
586 importance of surfactant selection and optimization. To achieve optimal nanoemulsion process  
587 conditions, it is recommended to use Tween 80 with 12% Tween concentration and 7.5% parijoto  
588 fruit extract concentration, resulting in a desirability value of 0.74, into the moderate category.

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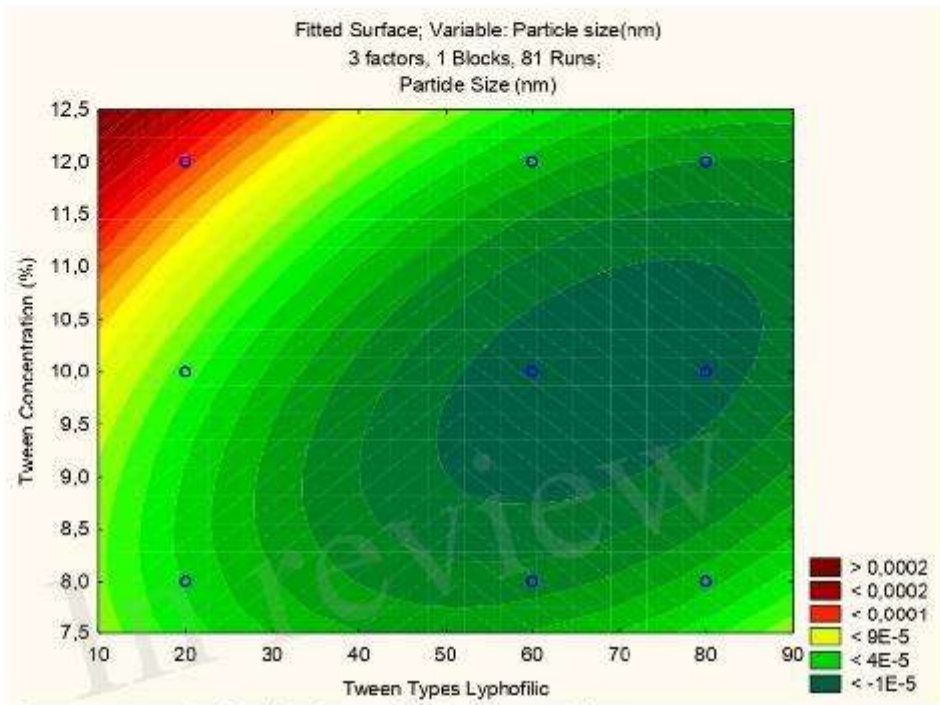


Figure. 1 Contour plot of Particle Size as a function of Nanoemulsion

Figure 2.JPG

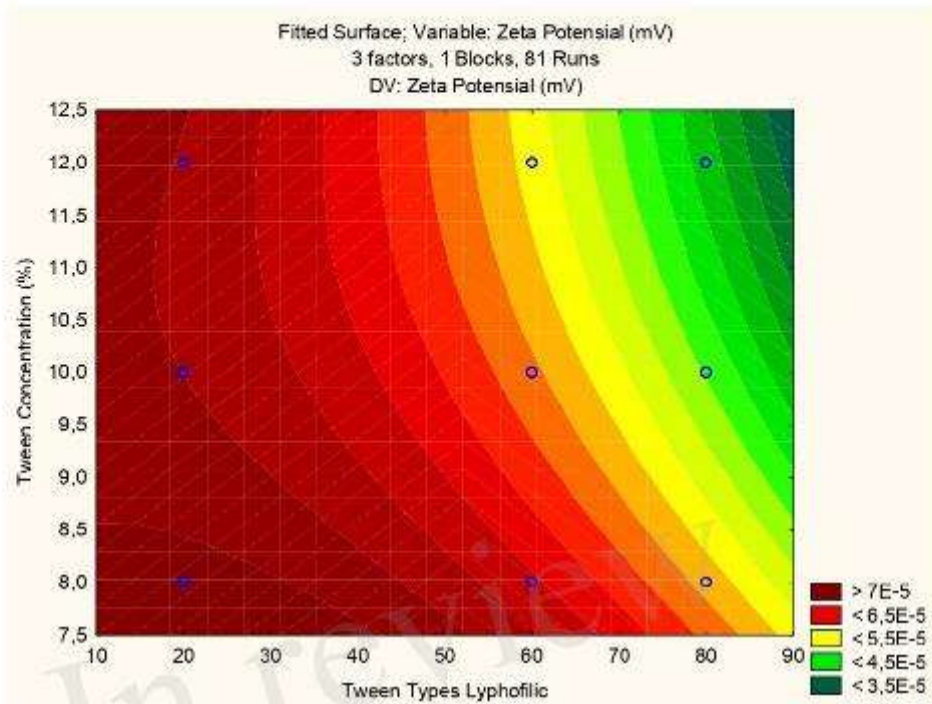


Figure. 2. Contour plot of Zeta Potential as a function of Nanoemulsion Parijoto Fruit Extract.



Figure 6.JPEG

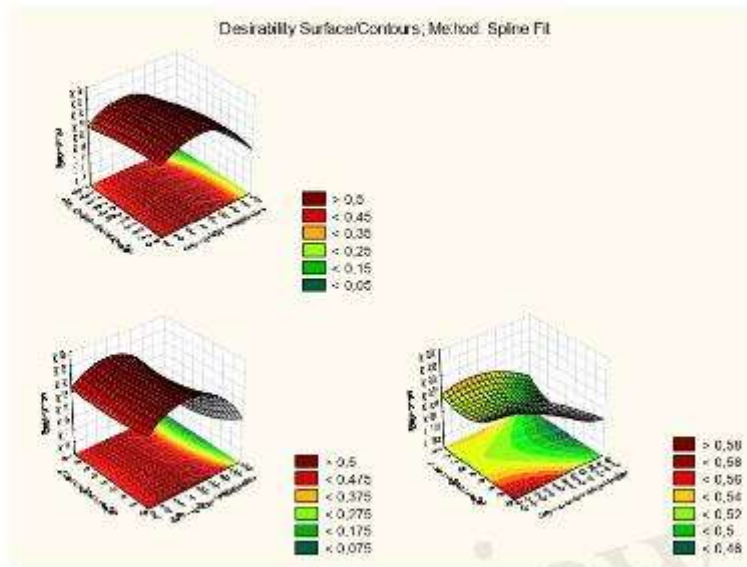


Figure. 7 3D Desirability Profile and Response Graphs for Nanoemulsion Parijoto Fruit Extract

Figure 5.JPG

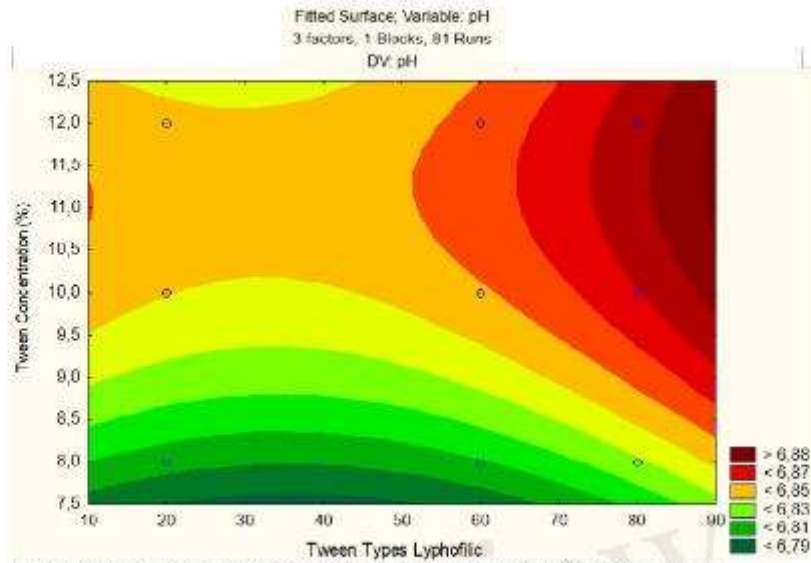


Figure. 5 Contour plot of pH function in Nanoemulsion of Parijoto Fruit Extract.

In review

Figure 4.JPEG

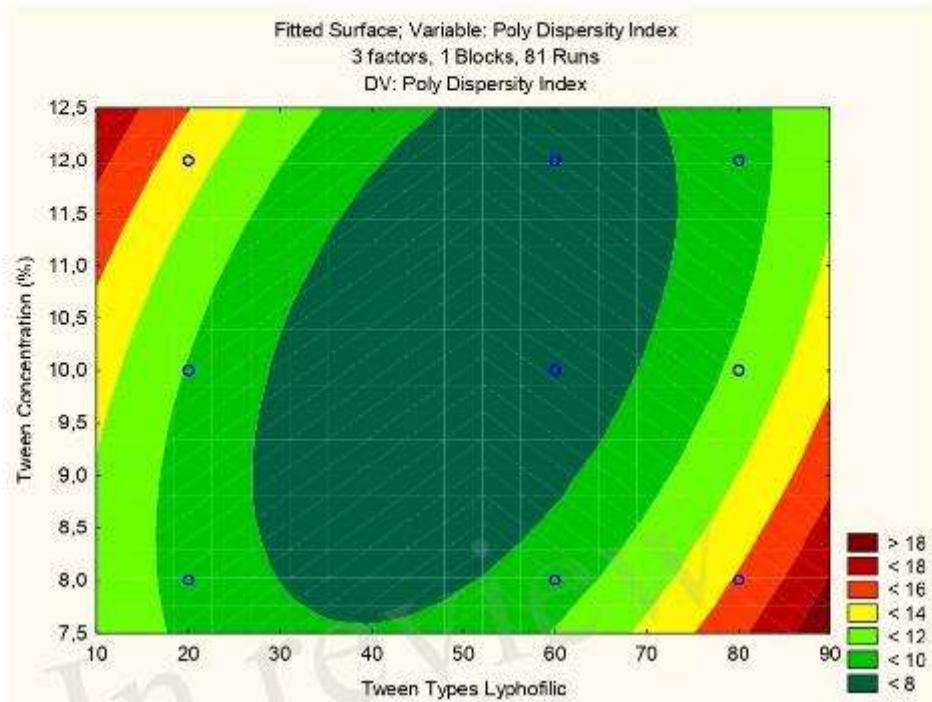


Figure. 4 Contour plot of Poly Dispersity Index as a function of Nanoemulsion of Parijoto Fruit Extract.

Figure 3.JPEG

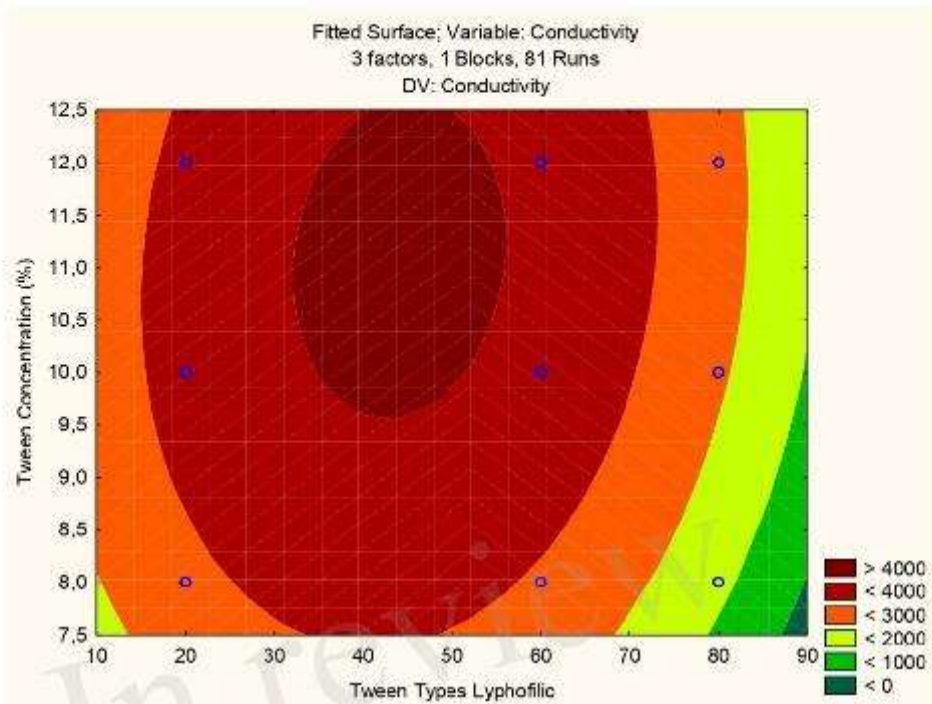


Figure. 3 Contour plot of Conductivity as a function of Nanoemulsion Parijoto Fruit Extract.

Figure 7.JPEG

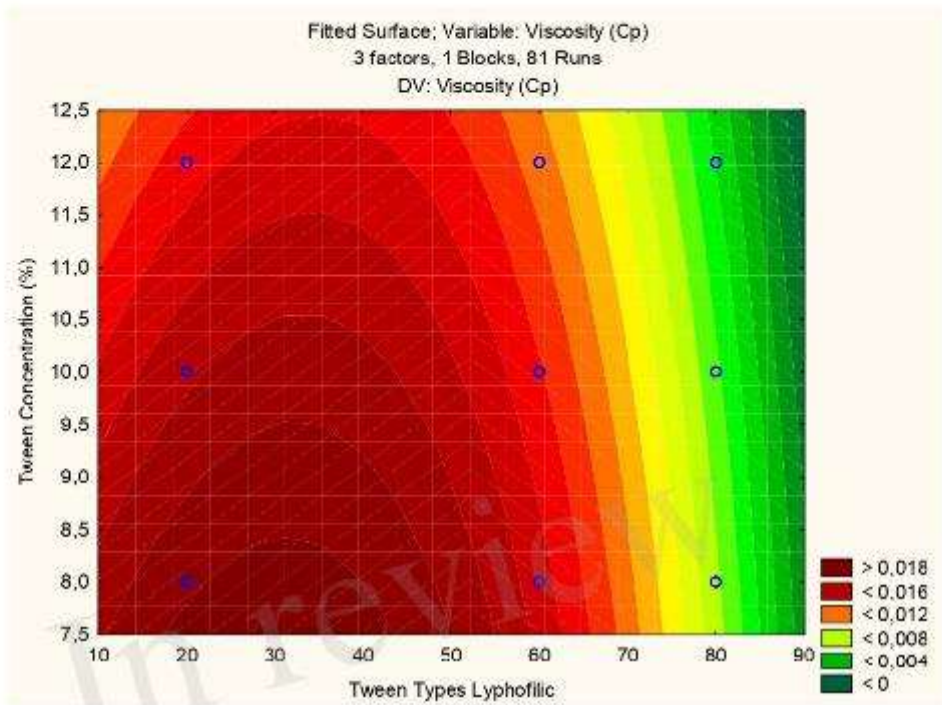






Figure. 6 Contour plot Viscosity function in Nanoemulsion Parijoto Fruit Extract.

## 9. Author Dashboard

### FORMULATION OF NANOEMULSION PARIJOTO FRUIT EXTRACT (*Medinilla speciosa*) WITH VARIATION OF TWEENS STABILIZERS

Victoria Kristina Ananingsih<sup>\*</sup>, Yohanes Alan Sarilita Putra, Alberta Rika Pratiwi and Bernadeta Soedarini  
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Scope Statement: The manuscript aligns seamlessly with the scope ... more  
Keywords: Nanoemulsion<sup>1</sup>, Stabilizers<sup>2</sup>, Tween<sup>3</sup>, Parijoto<sup>4</sup>, RSMS

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## Formulation of nanoemulsion parijoto fruit extract (*Medinilla Speciosa*) with variation of tweens stabilizers

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Nanotechnology has substantial potential for development due to its ability to modify surface characteristic and particle size, facilitating enhanced absorption of functional food compounds and controlled release of active substances to mitigate adverse effects. Nanoemulsion, a stable colloidal system formed by blending oil, emulsifier, and water, was identified as nanotechnology with promising applications. However, investigations into the impact of surfactants on characteristic nanoemulsions need to be more varied. This research gap necessitated further exploration in the advancement of nanotechnology-based foods. The parijoto fruit (*Medinilla speciosa*), an indigenous plant species in Indonesia, has yet to undergo extensive scrutiny for its potential use as a functional and nutraceutical food. Anthocyanin, a principal compound in the parijoto fruit, had exhibited efficacy in reducing the risk of cardiovascular disease diabetes, demonstrating anti-inflammatory and antioxidant properties. This study aimed to investigate the characteristics of nanoemulsion formulations derived from parijoto fruit extract and to evaluate an optimum condition with various tween surfactants. The findings from the investigation could furnish valuable insights for the further advancement of anthocyanin nanoemulsions from parijoto fruit extract. The results comprised the characterization of nanoemulsion particle size, polydispersity index, (-potential, conductivity, pH, and viscosity. Through mathematical modeling and statistical methods, RSM optimizes nanoemulsion by examining the relationships and interactions between independent and response variables. Furthermore, the characterization of nanoemulsion encompassed (-potential, polydispersity, particle size, conductivity, pH, and viscosity. Elevated surfactant concentrations resulted in diminished particle sizes and more uniform size distribution, albeit reaching a plateau where surfactant aggregation and micelle formation ensued. Increased concentrations of surfactant type, concentration, and parijoto extract impacted the physical characteristics of nanoparticle size and polydispersity. The optimal process conditions for nanoemulsion consisting of the type of Tween used are Tween 80, Tween concentration of 12N, and parijoto fruit extract concentration of 7.5N, yielding a desirability value of 0.74, categorizing it as moderate.

### 1 Introduction

Nanotechnology underwent progressive evolution, characterized by measurements on the nanometer scale, approximately  $10^{-9}$  meters (1). Acknowledgment from the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) (2) underscored nanotechnology's significant potential in enhancing food products, attributed to its capacity to modify surface characteristics and particle size. Such modifications facilitate targeted delivery of food compounds to specific organs and the controlled release of active compounds to mitigate adverse effects. The attributes of nanoscale food materials are pivotal in propelling diverse industries, including food, pharmaceuticals, and extensive nutraceutical applications (3). Due to their substantial surface area-to-volume ratio, nanoemulsions exhibit enhanced stability against gravitational separation and aggregation, owing to their distinct physicochemical and biological characteristics compared to conventional emulsions. The droplets or globules inherent in nanoemulsions mitigate gravitational forces and Brownian motion, thereby averting creaming or sedimentation during storage. Nanoemulsions denote a nanotechnological rendition of a stable colloidal system, achieving kinetic stability through the amalgamation of oil, emulsifier, and water (4). Chang et al. (5) conducted research utilizing surfactants as stabilizers in synthesizing nanoemulsions, showcasing the stability of nanoemulsion particle size in curcumin extract. Surfactants can diminish interfacial tension and form a substantially influential steric elastic film on the emulsion results (6).

Renowned for its tropical climate and vast biodiversity, Indonesia harbors at least 30,000 plant species, with 7,000 being herbal plants with documented health benefits (7, 8). Parijoto (*Medinilla speciosa*), an endemic plant species in Indonesia, remains relatively understudied for its scientific potential in pharmacy, functional foods, and nutraceuticals. Analysis has confirmed that the parijoto fruit comprises phytochemical components such as anthocyanin, flavonoids, saponin, tannin, alkaloids, cardenolides, and glycosides. Anthocyanin, a predominant compound in parijoto fruit, demonstrates efficacy in reducing the risk of cardiovascular diseases, diabetes, and inflammation while possessing notable anti-inflammatory and antioxidant properties. Extraction techniques yield varying anthocyanin contents, with the peel extract and whole fruit extract registering 208.75 and 173.7 mg/L, respectively (9). Various factors influence anthocyanin stability, including chemical structure, concentration, solvent, pH, storage temperature, light, oxygen, metal ions, proteins, and flavonoids. Weak stability under high pH, high temperature, and light exposure has been observed, with lower pH contributing to enhanced stability (10, 11). Heating at elevated temperatures accelerates anthocyanin degradation (12).

Response Surface Methodology (RSM) has emerged as a prominent multivariate statistical technique for optimizing various processes in recent years. Initially introduced by Box and colleagues in the 1950s, RSM facilitates examining the relationship and interactions among independent and response variables through mathematical modeling and statistical methods (13). RSM has successfully enhanced and optimized therapeutic extract and drug nanoemulsion (14). In this study, Central Composite Design (CCD) Response Surface Methodology (RSM) was employed to optimize the quality parameters of the nanoemulsion.

Appropriate nano-encapsulation techniques, such as nanoemulsion, have shown the potential to enhance the stability, bioavailability, and solubility of lipophilic bioactive compounds while also preventing hydrolysis and oxidation (15). The encapsulation process aimed to protect the active substance from oxidation by air and light, thereby increasing the shelf life of the product (16). Nanoemulsions are widely utilized nanoformulations in food-related industries through active or passive targeting mechanisms. Gunasekaran et al. (17) introduced nanotechnology as an effective tool for enhancing the bioavailability and bioactivity of phytochemicals. Nanoemulsion has emerged as a novel technology providing opportunities to address challenges associated with delivering micronutrients in functional food. Shin et al. (18) explored recent advancements in nanoformulation of lipophilic functional foods. Moreover, nanotechnology-based strategies have been explored to associate complex matrices derived from plant extracts, offering promising prospects for developing novel therapeutic formulations (19). Synthesis of nanoemulsion using mangosteen peel extract rich in anthocyanins as the main ingredient of the formulation can increase the dominant penetration of  $\beta$ -mangostin through the stratum corneum (20). Catalchin nanoemulsion showed a remarkable improvement in stability and bioavailability in simulated gastrointestinal conditions (21). Mula et al. (22) showed the optimum results using a high-speed homogenization and Tween surfactant to prepare nanoemulsions with nanoemulsion. Research conducted by Chang et al. (5) used Tween as the surfactant in the stable nanoemulsion synthesis loaded curcumin extract. This highlights the opportunity to develop nanoemulsion formulations for anthocyanins found in parijoto fruit. So far, research on nanoemulsion formulation in parijoto fruit involving



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# Formulation of nanoemulsion parijoto fruit extract (*Medinilla Speciosa*) with variation of tweens stabilizers

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## KEYWORDS

nanoemulsion, stabilizers, tween, parijoto, RSM

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Response Surface Methodology (RSM) has emerged as a prominent multivariate statistical technique for optimizing various processes in recent years. Initially introduced by Box and colleagues in the 1950s, RSM facilitates examining the relationship and interactions among independent and response variables through mathematical modeling and statistical methods (13). RSM has successfully enhanced and optimized therapeutic extract and drug nanoemulsion (14). In this study, Central Composite Design (CCD) Response Surface Methodology (RSM) was employed to optimize the quality parameters of the nanoemulsion.

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## 2 Materials and method

### 2.1 Materials

Grinder (Binder), Erlenmeyer (Pyrex), beaker glass (Pyrex), volume pipette, test tube (Pyrex), test tube rack, funnel (Pyrex), measuring flask (Pyrex), vacuum n filter 0.22  $\mu$ m (Sartorius Stedim 11.694-2-50-06), vial, micropipette (Socorex), blue tip (Biologix 1 nml pipette tips), hotplate (Cimarec et al. SP142025Q), vortex (Thermolyne et al.), Ultrasonic Cleaner (Biobase UC-10SD) modified, UV-VIS spectrophotometer (Shimadzu, UV-1280), aluminum foil, filter paper, 0.22  $\mu$ m filter membrane (Wattman), Cabinet dryer (HetoPowerDry LL1500), rotary evaporator (Biobase RE-2000E), syringe, analytical balance. Fresh parijoto, ethanol pro analysis (Merck, Germany), methanol pro analysis (Merck, Germany), distilled water, F. ciocaltu 10% (Merck, Germany), Na<sub>2</sub>CO<sub>3</sub> 7.5% (Merck, Germany), DPPH solution (Merck, Germany), Quercetin (Merck, Germany), AlCl<sub>3</sub> (Merck, Germany), ammonium acetate 1M (Merck, Germany), acetone (Merck, Germany), acetonitrile (Merck, Germany), standard cyanide (Zigma), delphinidin glu standard (Zigma), Tween 20 (Merck, Germany), Tween 60 (Merck, Germany), and Tween 80 (Merck, Germany).

## 2.2 Preparation of dry samples of parijoto fruit extract

Samples used in this study are fruits from the Parijoto plant (*Medinilla speciosa*) cultivated and harvested on the slopes of Mount Muria, Kudus. The fruits used are ripe fruits harvested when the Parijoto plant reaches full maturity, typically around 90–100 days after flowering. Parijoto, which had been cleaned and sorted, was weighed 200 grams for each treatment. The fruit that has been weighed is then steam-blanching for 3 min. Prepare a citric acid solution with a concentration of 1% for pre-treatment of fruit before drying. After that, soak the parijoto fruit in the citric acid solution for 5 min and drain. The Cabinet Dryer is cleaned before use to maintain hygiene and avoid cross-contamination. The drying temperature used was 70°C for 6 h. The dried Parijoto fruit is then ground into powder using a herbal grinder for 2 min. After that, the sample will be extracted for further testing. The dried Parijoto will be chemically analyzed using UV-Vis spectroscopy.

## 2.3 Making parijoto extract using ultrasonic assisted extraction

Five grams of dry sample powder and 50 mL of 99.5% ethanol were mixed thoroughly for homogeneity in four 250 mL centrifuge bottles. Then, all vials were sonicated using a Bio-Based Ultrasonic Waterbath with a 40 KHz frequency and 100 W power for 30 min. Subsequently, the samples were subjected to shaking for 1 h. The centrifugation step was performed at 4,000 rpm at 4°C for 10 min (Ohaus, United States). The supernatant was then carefully collected, and the remaining solution was evaporated to dryness under vacuum conditions. The residue was dissolved in 99.5% ethanol and diluted to 20 mL. After filtering through a 0.22 µm membrane filter, parijoto fruit extract was obtained and stored at -20°C for UV-Vis analysis.

## 2.4 Preparation of anthocyanin nanoemulsion from parijoto extract

Approximately 3 mL of anthocyanin nanoemulsion with concentrations of 2 mg/mL, 4 mg/mL, and 6 mg/mL, respectively, were prepared by collecting a portion of parijoto extract, and the solvent was removed with nitrogen. The solvent removal process during anthocyanin extraction can be monitored using a combination of visual inspection and periodic weight measurements. Visual inspection involves observing the extract as the solvent evaporates, noting its increasing concentration, evidenced by a thicker and more viscous appearance. Periodic weighing of the container or flask containing the extract allows for weight loss tracking as the solvent evaporates. Once the weight stabilizes or reaches a predetermined target, the desired solvent removal rate has been attained, ensuring the production of a concentrated anthocyanin extract suitable for further analysis. Anthocyanin nanoemulsion was prepared using a combination of surfactants that have low, medium, and high hydrophilic lipophile balance (HLB), namely Tween 20, Tween 60, and Tween 80. Then, surfactant (0.24 g) was added, and the mixture was homogenized entirely. This was followed by adding (2.76 g) deionized water and mixing again for complete dispersion of surfactant in water.

The solution was then sonicated in a sonicator with a temperature of 35°C, frequency of 45 Hz, and 100% power for 60 min. To produce a good nanoemulsion, homogenization was carried out using high shear homogenization at 15,000 rpm with a temperature of 40°C for 15 min.

## 2.5 Characterization of particle size and polydispersity index of nanoemulsion parijoto fruit extract

The particle size analysis tool used in this study was the Zetasizer (Zetasizer Pro; Malvern Instruments, Ltd., Malvern), which operates based on the general principle of dynamic light scattering (DLS). This tool has a detector placed at an angle of 173° from the transmitted light beam and detects size using a patented technology known as noninvasive backscattering. This technique is used for various purposes. One is to reduce the effect known as multiple scattering, making it easier to measure samples with high concentrations. Modifying McClements (2016), the particle size distribution and average particle size of nanoemulsions were determined by dynamic light scattering (DLS) at a wavelength of 633 nm and a temperature of 25°C.

## 2.6 Characterization of Z-potential nanoemulsion parijoto fruit extract

The ζ-potential of Parijoto Fruit Extract Nanoemulsion was evaluated using ζ-potential analysis (Zetasizer Pro; Malvern Instruments, Ltd., Malvern) following the method described by Khalid et al. (2017). The ζ-potential of the samples was evaluated automatically using 10 to 100 analytical runs after equilibration for 120 s at 25°C. The ζ-potential of the particles was measured by phase-analysis light scattering (PLS) using a Zeta dip cell.

## 2.7 Characterization of the conductivity of nanoemulsion parijoto fruit extract

The conductivity of nanoemulsion particles was measured by phase-analysis light scattering (PLS) using a Zeta dip cell with a cuvet electrode. Samples were evaluated automatically using 10 to 100 analytical runs after equilibration for 120 s at 25°C. The detector is placed at an angle of 173° from the transmitted light beam.

## 2.8 pH measurement of nanoemulsion parijoto fruit extract

The pH was determined using a Schott pH meter at room temperature (27 ± 2°C), calibrated with a standard buffer of pH 7. The pH analysis of the Parijoto fruit extract nanoemulsion sample was carried out using a pH meter with a particular electrode. First, the pH meter is set and calibrated with a standard buffer solution at a known pH, generally at pH 4.0, 7.0, and 10.0. Samples were diluted with 10 mM phosphate buffer pH seven before analysis to avoid multiple scattering effects during testing. The pH meter electrode is then carefully inserted into the sample to ensure good contact. Once

the electrode is stable, a pH reading is taken and recorded. This step is repeated as necessary to obtain consistent results. This pH analysis provides essential information regarding the acidity or alkalinity level of nanoemulsion and nanocitosan Parijoto fruit extract, which can affect the stability and quality of products using the nanoemulsion.

## 2.9 Viscosity measurement of nanoemulsion parijoto fruit extract

Viscosity measurements are carried out using a viscometer Brookfield. 14 mL of sample was put into the cup and attached to the solvent trap provided. The viscometer was set at 200 rpm, three rotations, for 30 s. The measurement process begins by activating the viscometer, and this tool automatically measures the time required for a liquid to flow through the viscometer tube at a specific temperature and rpm. This time, a predetermined formula converts the reading into a viscosity value. Repeated measurements can be made to ensure consistent results.

## 2.10 Statistical analysis uses response surface methodology

In this study, primary data in 3 repetitions of extraction and three repetitions of testing were averaged and given a standard deviation value for each treatment combination using Statistica 12.5 by StatSoft. The data is then entered into a statistical application, arranged in a combination of factorial points, axial points, and central points with three repetitions. After that, the data was analyzed, and several test stages were carried out. The basis for testing is studentification from primary data. Studentification means that the scale of the variable is adjusted by dividing it by the estimated population standard deviation. Variability in sample standard deviation values contributes to additional uncertainty in the calculated value. This will cause problems in finding the probability distribution of each statistic studied.

### 2.10.1 Effect summary

This test can summarize the effects of the combination of treatments used. The Longworth value in the results of this test is defined as  $-\log(p\text{-value})$  and is a transformation of the  $p$ -value based on the Pearson Chi-Squared test. The Pearson Chi-Squared test evaluates the possibility of the split being caused by chance. The higher the Pearson Chi-Squared value, the higher the probability of the split being caused by dependency. Generally, if the worth is greater than 2, the statistical model considers the variable necessary.

### 2.10.2 Lack of fit

Model suitability testing (lack of fit) is carried out to review whether the model equation is acceptable or not in predicting responses. In the lack of fit test, the following hypothesis is used:

$H_0$  = no lack of fit (suitable model).

$H_1$  = there is a lack of fit (the model is not suitable).

The hypothesis is concluded by comparing the calculated  $F$  value with the  $F$  table. The calculated  $F$  is obtained from the statistical test results and displayed in the ANOVA table. The  $F$  table value is obtained from the  $F$  Distribution Table. The criteria for the lack of fit test are:

$F \text{ count} < F \text{ table}$ , then  $H_0$  is accepted.  $F \text{ count} > F \text{ table}$ , then  $H_0$  is rejected.

Another parameter that can prove the suitability of the model obtained is by comparing the  $p$ -value with the  $\alpha$  value. If the  $p$ -value of lack of fit is smaller than the  $\alpha$  value, then there is a significant lack of fit, so the model obtained is inappropriate.

### 2.10.3 Summary of fit

The  $R$  square and Root Mean square error values are obtained in this test. Measures the difference in values from a model's predictions as estimates of the observed values.  $R$  square is also known as the coefficient of determination, which explains how far independent data can explain dependent data.  $R$  square has a value between 0 and 1 with the condition that the closer it is to one, the better it is. If the  $r$  square is 0.6, the independent variable can explain 60% of the distribution of the dependent variable. The independent variable cannot explain the remaining 40% or can be explained by variables outside the independent variable (error component).

### 2.10.4 Parameter estimates

The parameter estimates are the coefficients of the linear predictor. This value represents the change in response if you have a certain level of a categorical predictor or a change of 1 unit for a continuous predictor, which means the same thing as in a multiple regression analysis with continuous response.

### 2.10.5 Analysis of variance

The ANOVA test (Analysis of Variance) has the following test criteria:

$H_0$  is accepted if  $F \text{ count} < F \text{ table}$ , which means the model cannot be accepted statistically because no independent variables directly influence the response.

$H_1$  is accepted if  $F \text{ count} > F \text{ table}$ , which means the model is statistically acceptable and at least one independent variable has a real influence on the response.

### 2.10.6 Fitted surfaces

The depiction of the fitted surface is carried out using the Central Composite Design model. The experimental design is factorial, specifically Central Composite Design (CCD). CCD was chosen over Box-Behnken Design because CCD provides more design points in terms of axial points. Additionally, CCDs can run experiments at extreme values, providing better quadratic equations for analysis. CCD contains a factorial or fractional factorial design with a central point augmented by a group of 'axial points' that allow estimation of curvature. If the distance from the center of the design space to the factorial point is  $\pm 1$  unit for each factor, the distance from the center of the design space to the axial point is  $|\alpha| > 1$ . The exact value of  $\alpha$  depends on the properties desired for the design and the number of factors involved. The CCD has twice as many star points due to a factor in the design.

## 3 Result and discussion

### 3.1 Phytochemical profiles of dried parijoto fruit

Drying Parijoto Fruit is carried out using a cabinet dryer at a temperature 70°C for 6 h. The results of drying parijoto fruit were obtained through the preparation process; the antioxidant and

anthocyanin activity profiles were expressed, respectively, in units of % inhibition and ppm. The total anthocyanin content in the dry samples and extracts was  $538.47 \pm \text{ppm}$ . The dried Parijoto exhibited significant antioxidant activity, with a % inhibition value of  $79.14 \pm 34.82$ . This indicates a substantial capacity to neutralize free radicals in various chronic diseases and aging processes. The high antioxidant activity suggests that the drying process did not significantly diminish the antioxidant potential of Parijoto. The total anthocyanin content of the dried Parijoto was  $538.47 \pm 4.67 \text{ ppm}$ . Anthocyanins are a group of pigmented compounds known for their antioxidant properties and potential health benefits. The retention of anthocyanins after drying indicates that cabinet drying effectively preserved these bioactive compounds in the dried Parijoto.

The parijoto fruit extract was obtained through an extraction process using the Ultra-assisted extraction method. The Ultra-assisted extraction method involves the utilization of a modified ultrasonic water bath for the extraction of parijoto fruit. This method harnesses ultrasonic energy to enhance the extraction process by facilitating cell wall breakdown and increasing target compounds' solubility. During extraction, the parijoto fruit is immersed in a solvent within the ultrasonic waterbath, where ultrasonic waves are applied to the sample. This results in intensified agitation and cavitation within the solvent, leading to improved extraction efficiency and higher yields of bioactive compounds from the fruit. The characterization of Parijoto extract as a filler in nanoemulsion involved various analyses to assess its antioxidant properties and phytochemical composition. The extraction method was ultra-assisted extraction, known for its efficiency in extracting bioactive compounds from plant materials. The antioxidant activity of the Parijoto extract was evaluated, yielding a % inhibition value of  $50.776 \pm 6.18$ . This indicates a significant antioxidant capacity, crucial for combating oxidative stress and preventing cellular damage caused by free radicals.

Furthermore, the total anthocyanin content of the extract was determined to be  $94.43 \pm 4.14 \text{ ppm}$ . Anthocyanins are well-known antioxidants in many fruits and vegetables. They are known for their potential health benefits, including anti-inflammatory and anti-cancer properties. The flavonoid content of the Parijoto extract was measured to be  $126.85 \pm 1.15 \text{ g/L}$ . Flavonoids are a class of polyphenolic compounds known for their antioxidant and anti-inflammatory effects. Additionally, the phenolic content of the extract was quantified as  $8.43 \pm 0.70 \text{ GAE/g}$ . Phenolic compounds are another group of bioactive compounds found in plants, known for their antioxidant and anti-inflammatory activities and their potential role in reducing the risk of chronic diseases.

### 3.2 Fitting model for RSM (response et al.) in parijoto fruit extract nanoemulsion

Data recorded for each run included nanoemulsion particle size, polydispersity index,  $\zeta$ -potential, conductivity, pH, and viscosity. Each variable was measured with three repetitions and the measurements three times to get consistent results. This data will be used to analyze the influence of various factors on the characteristics of nanoemulsions using the Response Surface Methodology method, which can be seen in the table.

Table 1 shows that the particle size range of the nanoemulsion is between  $14,603 \pm 16.73 \text{ nm}$  and  $118,053 \pm 4.5825 \text{ nm}$ . The largest and

smallest nanoparticle sizes found are  $126.47 \text{ nm}$  and  $13.72 \text{ nm}$ , respectively, with most nanoparticle sizes falling within the  $50\text{--}100 \text{ nm}$  range. Similar results were confirmed by Noor El-Din et al. (23), who reported nanoemulsion sizes ranging from  $31.58$  to  $220.5 \text{ nm}$ . Studies conducted by Delmas et al., Liu et al., and Mei et al. using ultrasonication and high emulsification methods also confirmed comparable results of  $45\text{--}170 \text{ nm}$ ,  $222.4\text{--}166.4 \text{ nm}$ , and  $170\text{--}280 \text{ nm}$ , respectively (24–26). Conversely, Peng et al. (27) reported a nanoparticle size range of  $21\text{--}684 \text{ nm}$ . Z-potential reflects the surface charge of particles and affects colloidal stability. High  $\zeta$ -potential can prevent particle aggregation due to electrostatic repulsion. The research includes the evaluation and characterization of  $\zeta$ -potential under various treatments. The study obtained  $\zeta$ -potential results for nanoemulsion ranging from  $-22.197 \pm 0.738 \text{ mV}$  to  $-28.207 \pm 1.598 \text{ mV}$ , respectively. Similar results were confirmed by obtaining results of  $+21.5 \text{ mV}$ . Particles with high ZP values, between  $20$  and  $40 \text{ mV}$ , provide system stability and are less likely to aggregate or increase particle size. However, it should be noted that ZP values are not an absolute measure of nanoparticle stability. Furthermore, emulsions with ZP variations  $>10 \text{ mV}$  are suggested to have better stability (28). The ideal potential range for nanoparticle stability is  $(-30 \text{ to } 20 \text{ mV}$  or  $+20 \text{ to } +30 \text{ mV})$  (25). The produced values tend to be harmful due to the influence of acetic acid, resulting in a negative charge. This charge causes electrostatic repulsion forces between formed nanoparticles to prevent aggregation into larger sizes. Higher  $\zeta$ -potential values increase nanoparticle stability due to higher electrostatic repulsion forces between nanoparticles.

Conductivity provides information about the ability of nanoemulsions to conduct electricity. Changes in conductivity can occur with changes in surface particle charge. Table 1 shows that the nanoemulsion conductivity of Parijoto fruit extract ranges from  $0.03458$  to  $0.09987 \text{ mS/cm}$ . Good nanoemulsion conductivity measurements have higher electrical conductivity values ( $10\text{--}100 \mu\text{S/cm}$ ) (29). Electrical conductivity values tend to decrease with decreasing water content in the emulsion. O/W type (Oil-in-Water) nanoemulsions have higher conductivity than W/O type (Water-in-Oil) nanoemulsions. This is because the more extensive water phase provides more pathways for ion conduction.

The type and concentration of surfactant in nanoemulsion can influence conductivity. Surfactants can provide ionic charge or facilitate ion conduction in the system. Viscosity is an essential parameter in evaluating the flow properties of nanoemulsion. Viscosity is one of the parameters used to determine the stability of polymers in a solution because it undergoes reduction during polymer storage due to polymer degradation (30). In this study, as shown in Table 1, the viscosity of nanoparticles ranges from  $3,810 \text{ cP}$  to  $4,433 \text{ cP}$ . Alemu et al. (31) stated that viscosity can depend on particle size and storage time. Appropriate viscosity can affect the applicability and spread of the system. The viscosity of a preparation is related to the consistency and spreadability of the preparation, which will affect ease of use. Viscosity values are influenced by several factors, such as temperature, pH, manufacturing conditions, and the quality and concentration of raw materials. The results of viscosity tests are shown in centipoise (cP). The higher the viscosity value of a preparation, the better the stability of the product, but the preparation will be difficult to apply.

This ANOVA table is essential to evaluate the statistical significance of each model component and determine whether the quadratic model used is good enough to explain the characteristics



TABLE 1. Design of experiment RSM particle size, poly dispersity index,  $\zeta$ -potential, conductivity, pH, viscosity in nanomulsion.

No. run test	Dependent variables			Independent variables									
	Types of lyophilized liposomes	Tween concentration (%)	Purified fruit extract concentration (%)	Nanoparticle size (nm)	$\zeta$ -potential	Conductivity		Poly dispersity index		pH		Viscosity (cP)	
						$\gamma_1$	$\gamma_2$	$\gamma_3$	$\gamma_4$	$\gamma_5$	$\gamma_6$	$\gamma_7$	$\gamma_8$
1	20	8	2	15.53 ± 8.529	-24.34 ± 1.274	0.073 ± 0.003	0.43 ± 0.03	6.70 ± 0.013	0.071 ± 0.005				
2	20	8	3	19.1 ± 9.527	-25.47 ± 2.407	0.067 ± 0.019	0.34 ± 0.06	6.87 ± 0.029	0.067 ± 0.019				
3	20	8	3	14.88 ± 16.739	-32.28 ± 1.423	0.066 ± 0.001	0.27 ± 0.03	6.58 ± 0.039	0.066 ± 0.001				
4	20	8	8	22.67 ± 13.85	-25.93 ± 3.139	0.067 ± 0.001	0.38 ± 0.03	6.88 ± 0.039	0.067 ± 0.001				
5	20	8	8	19.87 ± 8.881	-23.17 ± 2.342	0.069 ± 0.016	0.48 ± 0.07	6.73 ± 0.023	0.069 ± 0.016				
6	20	8	4	41.84 ± 8.738	-22.97 ± 3.738	0.074 ± 0.006	0.63 ± 0.07	6.83 ± 0.032	0.074 ± 0.006				
7	20	8	8	42.57 ± 9.091	-24.65 ± 3.707	0.074 ± 0.016	0.52 ± 0.13	6.97 ± 0.06	0.074 ± 0.016				
8	20	8	8	44.34 ± 3.007	-24.77 ± 3.279	0.074 ± 0.017	0.38 ± 0.03	6.91 ± 0.026	0.074 ± 0.017				
9	20	8	8	46.26 ± 13.013	-24.07 ± 3.699	0.064 ± 0.013	0.48 ± 0.04	6.97 ± 0.023	0.064 ± 0.013				
10	20	10	3	16.77 ± 16.899	-24.67 ± 1.386	0.063 ± 0.013	0.41 ± 0.03	6.78 ± 0.026	0.063 ± 0.013				
11	20	10	3	38.507 ± 9.007	-24.07 ± 1.133	0.069 ± 0.019	0.26 ± 0.08	6.84 ± 0.021	0.069 ± 0.019				
12	20	10	3	16.237 ± 1.887	-24.27 ± 1.873	0.064 ± 0.001	0.41 ± 0.02	6.83 ± 0.029	0.064 ± 0.001				
13	20	10	8	41.94 ± 4.251	-25.33 ± 1.207	0.068 ± 0.019	0.39 ± 0.04	6.87 ± 0.026	0.068 ± 0.019				
14	20	10	4	44.687 ± 9.472	-23.79 ± 1.445	0.075 ± 0.017	0.52 ± 0.08	6.84 ± 0.013	0.075 ± 0.017				
15	20	10	8	44.327 ± 9.666	-23.19 ± 3.082	0.067 ± 0.019	0.39 ± 0.04	6.78 ± 0.029	0.067 ± 0.019				
16	20	10	8	22.007 ± 12.289	-26.127 ± 1.756	0.073 ± 0.019	0.33 ± 0.13	6.84 ± 0.021	0.073 ± 0.019				
17	20	10	8	16.99 ± 1.181	-26.887 ± 0.881	0.083 ± 0.029	0.53 ± 0.03	6.88 ± 0.029	0.083 ± 0.029				
18	20	10	8	27.08 ± 19.072	-26.28 ± 1.238	0.076 ± 0.023	0.48 ± 0.03	6.78 ± 0.019	0.076 ± 0.023				
19	20	12	3	41.49 ± 13.623	-26.007 ± 1.314	0.062 ± 0.023	0.37 ± 0.16	6.89 ± 0.019	0.062 ± 0.023				
20	20	12	3	85.4 ± 14.626	-24.85 ± 1.865	0.066 ± 0.017	0.68 ± 0.27	6.77 ± 0.012	0.066 ± 0.017				
21	20	12	3	46.067 ± 7.607	-24.013 ± 3.298	0.063 ± 0.016	0.44 ± 0.23	6.77 ± 0.012	0.063 ± 0.016				
22	20	12	8	37.49 ± 2.258	-27.477 ± 0.961	0.061 ± 0.016	0.70 ± 0.14	6.81 ± 0.019	0.061 ± 0.016				
23	20	12	8	130.66 ± 18.92	-27.977 ± 1.299	0.061 ± 0.017	0.37 ± 0.27	6.87 ± 0.029	0.061 ± 0.017				
24	20	12	8	136.23 ± 7.094	-27.23 ± 0.939	0.077 ± 0.011	0.72 ± 0.28	6.89 ± 0.017	0.077 ± 0.011				
25	20	12	8	117.667 ± 17.129	-28.287 ± 1.298	0.078 ± 0.019	0.64 ± 0.29	6.87 ± 0.021	0.078 ± 0.019				
26	20	12	8	118.33 ± 4.581	-28.023 ± 0.679	0.073 ± 0.014	0.82 ± 0.19	6.87 ± 0.026	0.073 ± 0.014				
27	20	12	8	124.187 ± 4.582	-28.073 ± 0.634	0.088 ± 0.019	0.73 ± 0.21	6.87 ± 0.013	0.088 ± 0.019				



TABLE 2 ANOVA (analysis of variance) for the RSM quadratic model particle size, poly dispersity index, Z-potential, conductivity, pH, and viscosity in nanoemulsion.

Quadratic model equation	Sources of variation	p-value
Particle size ( $R^2$ : 0.558 $R^2$ , 0.50156) $Y_1 = -0.000008 - 0.000069X_1 + 0.000040X_2 + 0.000032X_3 + 0.000056X_1^2 + 0.000064X_2^2 - 0.000003X_1^2 - 0.000056X_1X_2 + 0.000044X_1X_3 + 0.000005X_2X_3$	Model	0.294*
	Lack of fit	0.185
Poly dispersity index ( $R^2$ : 0.3643 $R^2$ , 0.2471) $Y_2 = 6.23086 + 0.58801 X_1 - 0.75655 X_2 + 84.3654 X_3 + 24.65 X_1^2 + 18.7863 X_2^2 - 20.744 X_1^2 + 23.0025 X_1X_2 + 26.3043 X_1X_3 + 9.5269 X_2X_3$	Model	0.041*
	Lack of fit	0.692
Z-potential ( $R^2$ : 0.54095 $R^2$ , 0.56905) $Y_3 = -0.000062 - 0.000023 X_1 - 0.000010 X_2 + 0.000008 X_3 + -0.000007 X_1^2 + 0.000003 X_2^2 + 0.000008 X_3^2 - 0.000006 X_1X_2 + 0.000008 X_1X_3 + -0.000005 X_2X_3$	Model	0.000*
	Lack of fit	0.980
Conductivity ( $R^2$ : 0.2444 $R^2$ , 0.3464) $Y_4 = 4035.80 - 1198.06X_1 + 833.22X_2 - 1083.49X_3 - 2597.39X_1^2 - 709.42X_2^2 + 881.10X_3^2 + 305.68X_1X_2 + 700.69X_1X_3 - 943.96X_2X_3$	Model	0.0004*
	Lack of fit	0.928
pH ( $R^2$ : 0.832 $R^2$ , 0.797) $Y_5 = 0.003122 - 0.000040X_1 - 0.000060X_2 + 0.000039X_3 - 0.000034X_1^2 + 0.000047X_2^2 + 0.000031 X_3^2 - 0.000006X_1X_2 + 0.000015X_1X_3 + 0.000001 - X_2X_3$	Model	0.000*
	Lack of fit	0.067
Viscosity ( $R^2$ : 0.95976 $R^2$ , 0.95466) $Y_6 = 0.013177 - 0.009573X_1 - 0.003288X_2 - 0.000624X_3 - 0.008334X_1^2 - 0.000266X_2^2 - 20.744 X_3^2 + 23.0025 X_1X_2 + 26.3043 X_1X_3 + 9.5269 X_2X_3$	Model	0.000*
	Lack of fit	0.103

\*The model has a statistically significant effect ( $p \leq 0.05$ ). \*\*Model mismatch or lack of fit occurs ( $p \leq 0.05$ ).

of the nanoemulsion or not. The  $p$ -value is used to determine statistical significance, and the analysis results will help select an appropriate model and interpret the significance of factors that influence the characteristics of nanoemulsions, which can be seen in the table.

Based on the ANOVA RSM analysis of three factors, namely the type of Tween in nanoemulsion, Tween concentration, and Parijoto extract concentration, all ANOVA values show probabilities  $< 0.0001$  ( $p < 0.05$ ). This indicates that the quadratic response surface model used for both responses (dependent variables) is significant and can be used to optimize extraction factors (32). The coefficient of determination, or R square, depicts how independent data can explain dependent data. The range of R square values is between 0 and 1, where values closer to 1 indicate better explanatory power.

In the Central Composite Design analysis, the  $p$ -value indicates the significance of each coefficient in the built polynomial regression model. The lower the  $p$ -value, the more significant the contribution of the coefficient to the overall regression model (33). Using experimental data within the allowed range of variables in this study to create mathematical equations, which may have broader general applications, can provide the ability to predict system behavior when different factors are combined. From the perspective of optimizing the formation of emulsion nanoparticles, there is potential to develop more significant results based on the variables investigated in this study. Additionally, this optimization may be performed using the techniques outlined in this research to test further the effects of time and temperature or other conditions, as needed.

Table 2 shows details of the RSM approach used to assess particle size (nm), Poly Dispersity Index, Z-potential (mv), Conductivity, pH, and viscosity (Cp) in nanoemulsion of Parijoto fruit extract involved in a series of 81 experiments based on factorial design. The coefficients for the second-degree polynomial Equation are determined through experimental results, along with the regression coefficients for Particle Size (Y1), Poly Dispersity Index (Y2), Z-potential (Y3), Conductivity (Y4), pH (Y5), and viscosity (Y6). The Equation presented as Equation (2) shows the full quadratic model, while Table 2 shows the models predicting the response of the independent variables (Y1–Y6).

To assess the extent to which the equation model in RSM fits the data and how strong the influence of the variables is, the coefficient of determination or ( $R^2$ ) is used. Chin (34) has categorized that for model suitability, the R-Square value is substantial if it is more than 0.67, moderate if it is more than 0.33 but lower than 0.67, and weak if it is more than 0.19 but lower than 0.33. pH and viscosity indicate strong model adequacy on these response variables. In contrast, the responses of Particle Size, Poly Dispersity Index, Z-potential, and Conductivity indicate a moderate model for these response variables. A lack of fit test was then performed to assess model fit for each response. With a  $p$ -value exceeding 0.05, it was confirmed that the model adequately fit the experimental data, as seen in Table 2.

### 3.3 Contour plot on particle size, poly-dispersity index, Z-potential, conductivity, pH, and viscosity as a function of nanoemulsion parijoto fruit extract

In this research, the model is created as a Contour plot, showing the response: Particle Size, Poly Dispersity Index, Z-potential, Conductivity, pH, and Viscosity. Continued research shows a significant relationship between particle size and tween concentration and the type of lipophilic Tween in nanoemulsions, as shown in Figures 1–6. The presented data offers valuable insights into the influence of lipophilic tween type and tween concentration on various properties of the nanoemulsion derived from parijoto fruit extract. Each figure depicts the contour plots illustrating the interaction effects between these two factors on different characteristics of the nanoemulsion.

Figure 1, the contour plot demonstrates the interaction between the lipophilic tween type and tween concentration in controlling nanoparticle size. It reveals that as the lipophilic tween type increases from 20 to 80, and the tween concentration rises from 8 to 10%, there is a general trend of increasing particle size, albeit with a slight decreasing trend observed to some extent. This suggests that both

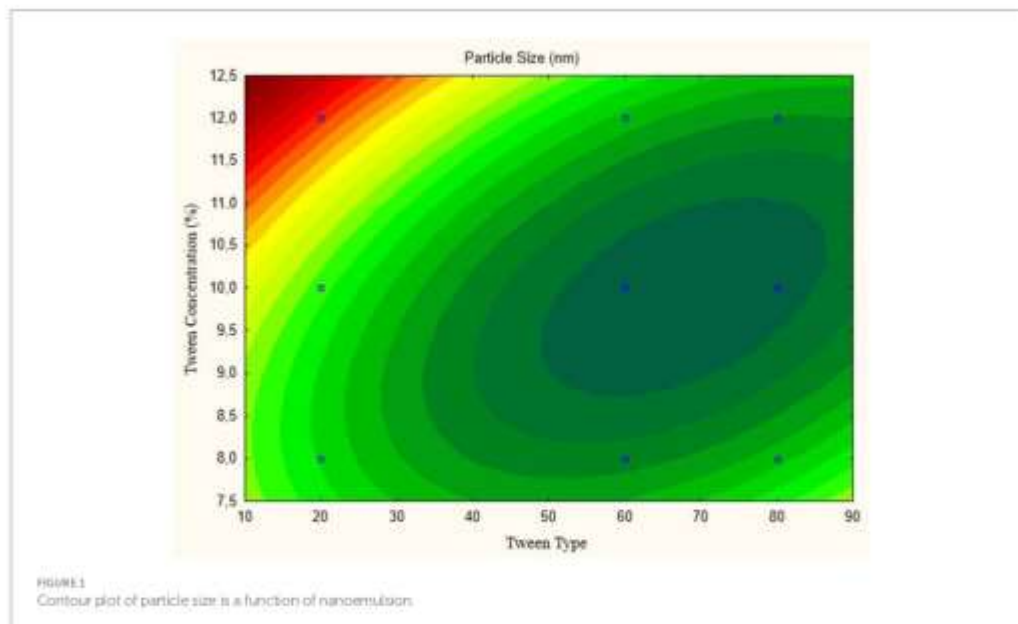
factors play a role in determining the nanoparticle size, with higher concentrations leading to larger particle sizes. Moving to [Figure 2](#), which illustrates the Z-potential of the nanoemulsion, an increase in the lipophilic Tween type from 60 to 80 and an increase in tween concentration from 8 to 10% correspond to an increase in Z-potential. Interestingly, no further changes are observed beyond this point. This indicates that these specific conditions result in optimal Z-potential, possibly indicating enhanced stability of the nanoemulsion.

[Figure 3](#) showcases the influence of lipophilic tween type and tween concentration on the conductivity of the nanoemulsion. As the lipophilic tween type increases from 20 to 80 and the tween concentration rises from 8 to 12%, conductivity is consistent without any further changes. This suggests a direct relationship between these factors and the conductivity of the nanoemulsion. The Contour plot presented in [Figure 4](#) demonstrates the effect of lipophilic tween type and tween concentration on the nanoemulsion's Poly Dispersity Index (PDI). Interestingly, an increase in lipophilic tween type from 60 to 80 and a decrease in tween concentration from 12 to 8% lead to an increase in PDI value without further changes. This indicates a complex interaction between these factors in determining the homogeneity of particle size distribution within the nanoemulsion.

[Figure 5](#) depicts the pH contour plot of the parijoto fruit extract nanoemulsion. An increase in lipophilic Tween type from 20 to 80 and an increase in tween concentration from 8 to 12% result in a consistent increase in pH without any further changes. This observation suggests that these specific conditions contribute to the alkalinity of the nanoemulsion, which may have implications for its stability and functionality. Finally, [Figure 6](#) illustrates the viscosity contour plot of the nanoemulsion. An increase in lipophilic tween type from 35 to 80 and an increase in tween concentration from 8 to 12% lead to an increase in viscosity without further changes. This indicates that

higher concentrations of lipophilic Tween and Tween result in a thicker consistency of the nanoemulsion, which affects its flow properties and application. The presented data highlights the intricate relationship between lipophilic tween type and tween concentration in influencing various physicochemical properties of the nanoemulsion derived from parijoto fruit extract. These findings provide valuable insights for optimizing the formulation and manufacturing process of the nanoemulsion for potential applications in various industries.

Research on the influence of surfactant type and concentration on nanoemulsion indicates that the selection of surfactant significantly affects the characteristics of nanoemulsion. Various surfactant types, such as Tween 20, Tween 60, and Tween 80, play different roles in forming nanoemulsions. The research results show that the particle size of Tween 80 surfactant is the highest, with an average particle size of 107.196 nm. Similar results were reported by [Chang et al. \(35\)](#), who obtained the smallest droplets in carvacrol-based nanoemulsion made with a mixture of food-grade non-ionic surfactants (Tween 20, 40, 60, 80, and 85). Tween, a non-ionic surfactant derived from sorbitan ester, is soluble or dispersible in water and is commonly used as an oil-in-water emulsifier in the pharmaceutical, cosmetic, and cleaning industries. Among these surfactants, Tween 80 is one of the most commonly used. Research by [Jadhav et al. \(36\)](#) confirms that the type of non-ionic surfactant significantly influences the average particle diameter of the formed colloid dispersion. The smallest droplets were observed in systems prepared using Tween 80, while the largest droplets formed in systems using Tween 85. The surfactant's Hydrophilic-Lipophilic Balance (HLB) plays a role in forming small particles. Surfactants with either too high (Tween 20) or too low (Tween 85) HLB values cannot form optimal nanoemulsions. Tween types with intermediate



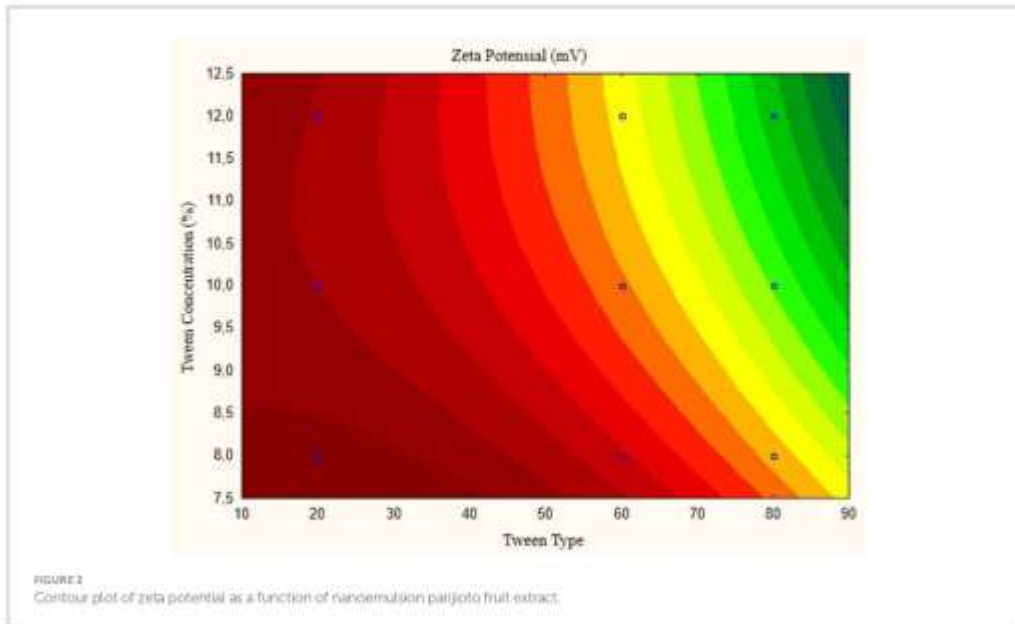


FIGURE 2  
Contour plot of zeta potential as a function of nanoemulsion parijoto fruit extract.

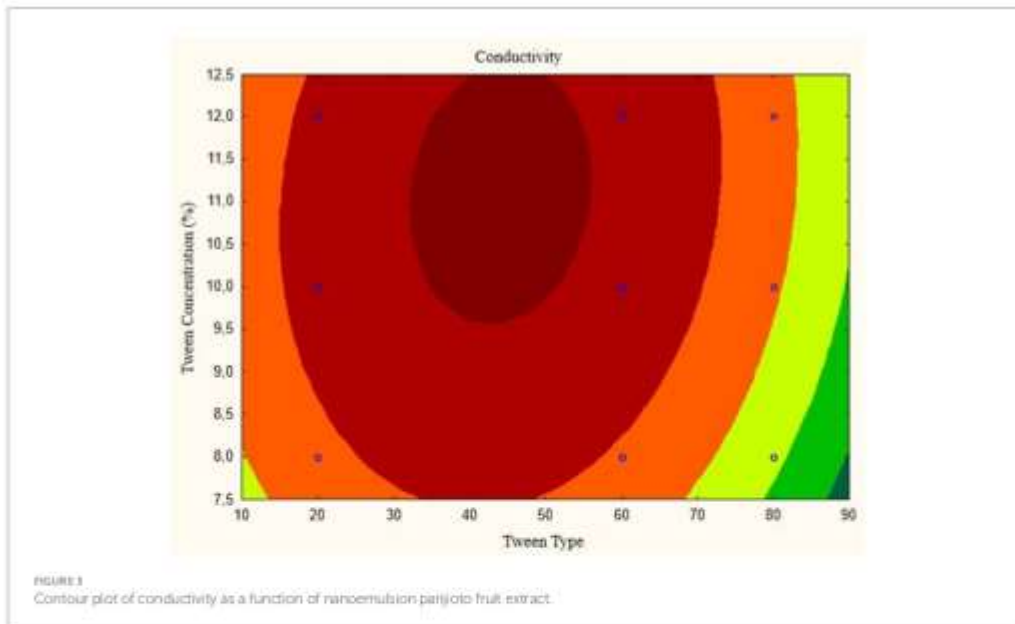


FIGURE 3  
Contour plot of conductivity as a function of nanoemulsion parijoto fruit extract.

HLB values (40, 60, and 80) can form nanoemulsions with small particle sizes. However, there is no strong correlation between HLB values and particle sizes produced by these surfactants. Small-molecule surfactants have higher surface activity and form smaller emulsion droplets than large ones (37).

Another critical factor for minimal droplet emulsion formation is the Hydrophilic-Lipophilic Balance (HLB) value of the surfactant, defined by Griffin as the ratio of surfactant hydrophilicity to lipophilicity. A high HLB value indicates strong hydrophilicity, and the HLB values of non-ionic surfactants

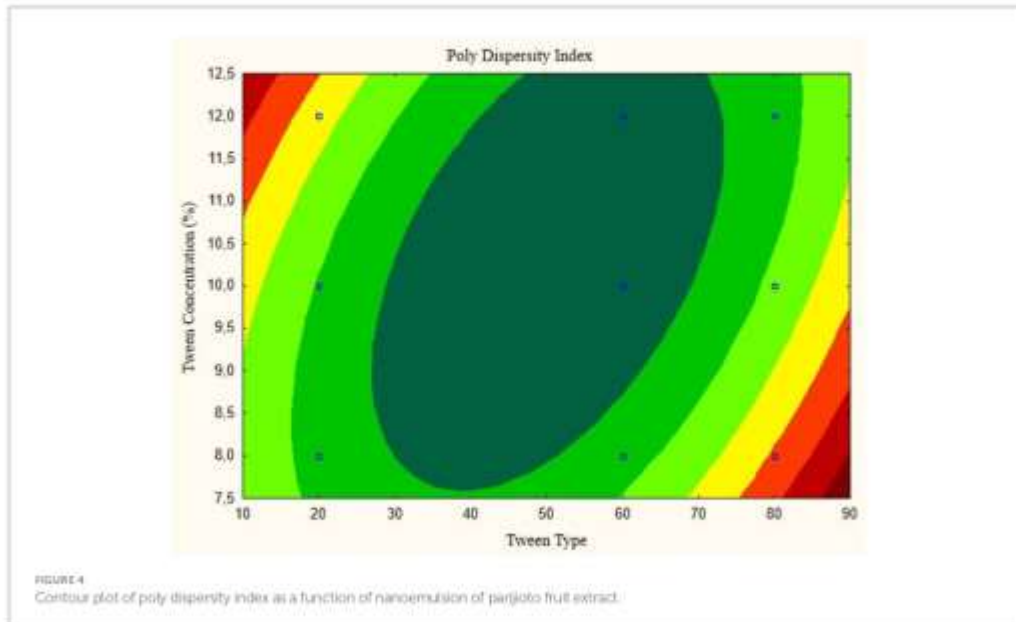


FIGURE 4  
Contour plot of poly dispersity index as a function of nanoemulsion of parjoto fruit extract.

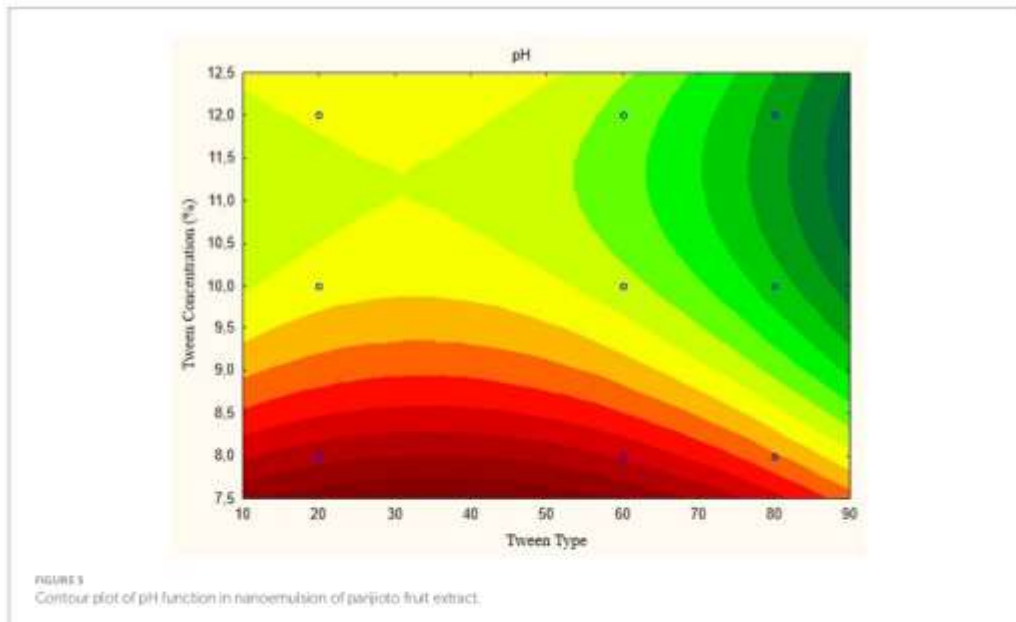
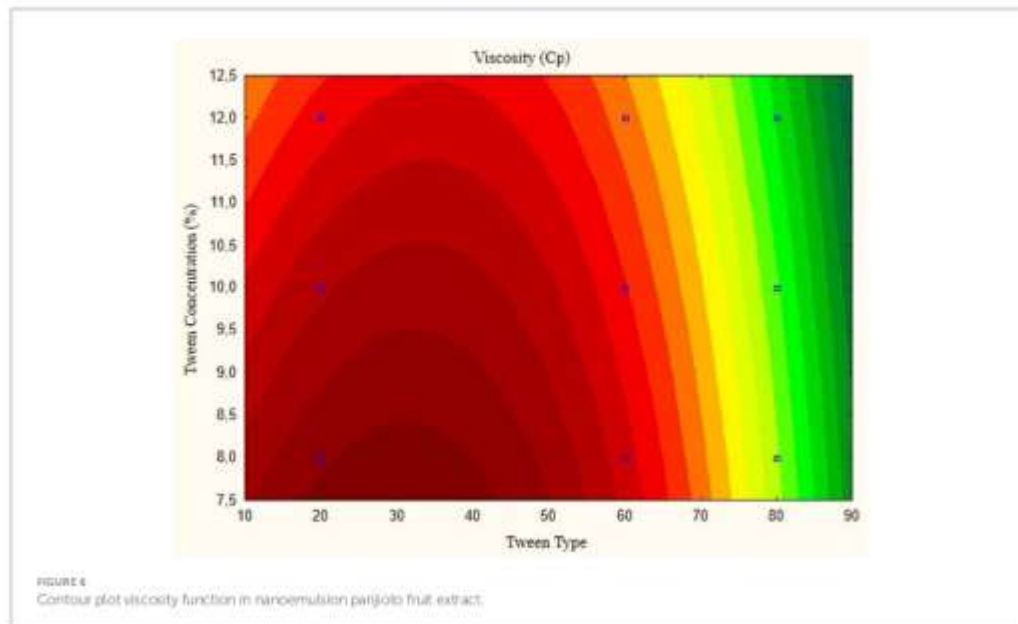


FIGURE 3  
Contour plot of pH function in nanoemulsion of parjoto fruit extract.

generally range from 0 to 20, such as Tween 20 (HLB 16.7) and Tween 80 (HLB 15) (38). Two polymer and particle surface tension mechanisms influence emulsion stability: steric stability caused by macromolecules adsorbed on particle surfaces and electrostatic stability due to repulsion between surface-charged droplets. In

nanoemulsions made with Tween 80 surfactant, the surfactant may not have a charge on the hydrophobic group, causing the covered droplet surface to be non-charged and resulting in low  $\zeta$ -potential values, which can lead to increased particle size and PDI.



However, a different study proposed by Alam et al. (39) suggests that Tween 20 helps improve PDI and allows for minimum polydispersity. Compared to other nanoparticles, the ability to maintain particle integrity using Tween 20 is significant. Increasing the Surfactant content in the formulation increases the polydispersity indices for natural extracts in the 3D response surface graph. This indicates that the use of Tween types with low and high HLB values can be applicable when combined with an optimal concentration of co-surfactant.

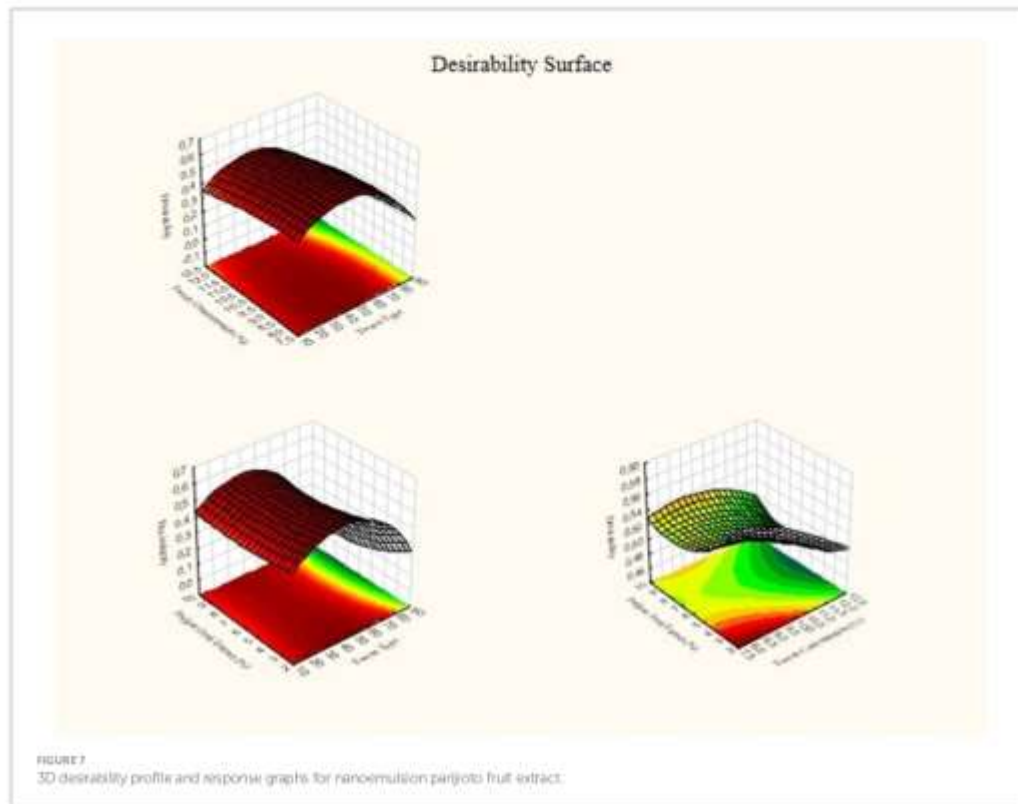
Surfactant concentration is also a critical factor in nanoemulsion formation. Research indicates that increasing surfactant concentration can result in smaller and more homogenous size distribution. However, there is a specific limit where surfactant concentration reaches a plateau level, leading to unadsorbed surfactant aggregation and micelle formation. The results show that the higher the Tween concentration, the higher the size and PDI. This is confirmed by Abaolnaja et al. (40), stating that nanoemulsions prepared with higher surfactant concentrations significantly increase short-term stability. Systems with 15 or 20% weight of Tween 80 are highly unstable to increasing dilution, indicating that a medium surfactant concentration level may be more suitable for stable nanoemulsion preparation. Although the initial droplet size is small, higher surfactant concentrations can increase raw material costs and cause undesirable sensory (taste) issues in commercial applications. Therefore, this study uses a 10% weight of Tween 80 in further experiments.

Increasing surfactant concentration increases the number of surfactant molecules migrating from the oil phase to the emulsion water phase, and nanodroplets form. Frictional forces applied to the oil-water interface, coated with emulsifier, cause some emulsifiers to sink parallel to the surface layer while others detach from the surface layer. Hasani et al. reported that droplet size increases by

increasing surfactant concentration to 20%, and particles have a broad and non-uniform size distribution. The instability of nanoemulsion at high surfactant concentrations may be related to the depletion-flocculation mechanism of adsorbed surfactant. With increased surfactant concentration, additional surfactant molecules form micelles in the continuous phase rather than orienting on the particle surface. This leads to an increase in local osmotic pressure, causing the continuous phase between moving droplets to decrease, reducing the continuous phase between those droplets. As a result, aggregation occurs, causing an increase in particle size. According to Oh et al. (41) and Tadros et al. (42), the average droplet size becomes smaller, and the size distribution becomes narrower with increasing emulsifier concentration, ultimately reaching a plateau level. Beyond the plateau level, free or unadsorbed emulsifiers may accumulate to form micelles. Nanoemulsions are known to be thermodynamically unstable, tending to minimize interfacial area through coalescence.

An increase in the filler extract's concentration can lead to nanoparticles' tendency to aggregate or form agglomerates and pH nanoemulsion. This phenomenon may occur due to physical or chemical interactions between nanoparticles and compounds in the filler extract. Increase in extract concentration results in an increase in particle size, particularly at the highest concentration of 347.2 nm. On the other hand, the smallest concentration has the lowest particle size at 86.98 nm. These results indicate that higher concentrations may increase the likelihood of particle agglomeration.

Furthermore, increasing the concentration of parijoto fruit extract can increase the total mass in the solution, which, in turn, can increase overall viscosity. Additional particles or molecules from the filler extract can contribute to the increase in viscosity. Particles with the highest concentration have the highest viscosity and vice versa. This



increase in viscosity may be caused by excess extract loaded into particles. The physicochemical characteristics of the filler extract may influence the viscosity properties of nanoparticles, and factors such as changes in pH, temperature, or chemical composition may also play a role in viscosity increase. Parijoto fruit is rich in active compounds, such as anthocyanins, which can affect the surface charge of nanoemulsion particles. At a certain pH, anthocyanins or other components may have specific charges that can influence the electrostatic stability of particles (43). Anthocyanins may undergo solubility changes at specific pH values, affecting the distribution and stability of the nanoemulsion's oil or water phase. The same occurs with surfactants, where variations in charge of the filler extract from parijoto fruit can affect the interaction between nanoparticles, anthocyanins, and other components in the system. The loading capacity of the extract in the nanoemulsion likely depends on its solubility in the system used. Anthocyanins tend to undergo color changes with pH (pH-dependent color shift). Additionally, the antioxidant activity of anthocyanins can be influenced by pH. This complexity can modulate the overall physicochemical properties of the nanoemulsion system.

Nanoemulsions, despite their promising applications, present challenges related to stability. The challenge is the propensity for Ostwald ripening, wherein larger droplets grow at the expense of smaller ones, leading to phase separation and reduced shelf-life.

Additionally, factors such as temperature fluctuations, pH changes, and exposure to light can exacerbate instability, causing particle aggregation. Surfactant degradation over time is another concern, as it can compromise the emulsion's ability to maintain a stable dispersion. However, the industrial application of parijoto fruit or extract holds significant potential. Parijoto fruit, known for its rich content of bioactive compounds, including anthocyanins, flavonoids, and phenolic acids, offers various health benefits such as antioxidant and anti-inflammatory properties. Incorporating parijoto extract into nanoemulsions can enhance its bioavailability and efficacy, making it suitable for a range of industrial applications especially food functional and nutraceutical.

### 3.4 Optimal point prediction from RSM in nanoemulsion parijoto fruit extract

Optimal point predictions from the Response Surface Methodology are obtained by combining optimal conditions based on interactions between independent variables. Profiler predictions are obtained if the fitted surface graph is in minimum, maximum, or saddle form. 3D graphics on Figure 7 shows a complex interaction between the variable factors of lipophilic tween type and tween concentration on the response. Increasing the lipophilic tween type



TABLE 3 Prediction of optimum conditions for parijoto fruit extract nanoemulsion.

Types of analysis	Types of lyophobic tweens	Tween concentration (%)	Parijoto fruit extract concentration (%)	Nanoparticle size (nm)	Z-potential (mV)	Conductivity (mS/cm)	Poly dispersity index	Degree of acidity (pH)	Viscosity (Cp)	Desirability value
Optimum condition prediction	80	12	7.5	61.97	-28.48	0.082	0.091	6.864	5.668	0.74
Maximum value at optimum conditions	80	12	7.5	39.94	-32.48	0.098	0.371	6.82	5.422	
Minimum value at optimum conditions	80	12	7.5	163.88	-26.37	0.115	1.011	6.9	5.913	

value increases the response somewhat, but the tween concentration value can modify the effect. There is an optimal region where the response reaches its peak. The implication for practice is that by setting the variable factors at levels that are estimated to be optimum, the research results can achieve the highest optimization in the desired response, which can be seen in Figure 7.

It can be seen in Table 3 that to achieve the maximum desired concentration of nanoparticle size,  $\zeta$ -potential, Conductivity, Poly Dispersity Index, degree of acidity, and Viscosity, it is necessary to set the Tween solvent concentration to 80, Tween concentration to 12% and Parijoto fruit extract concentration to 7.5%. This set of conditions has a desirability value of 0.74. Because the value is almost close to 1 and falls into the moderate category, this set of conditions is quite optimal for the aim of this research, namely to maximize the response.

The optimization of nanoemulsion formation from Parijoto fruit extract using Response Surface Methodology (RSM) has been conducted in this study. RSM is a statistical method used to design experiments and analyze the impact of multiple independent variables on a measured response. As an output of this research, the synthesis process conditions of nanoemulsion from Parijoto fruit extract can be optimized to achieve particle size, polydispersity index (PDI),  $\zeta$ -potential, conductivity, pH, and viscosity levels. RSM determines the optimal extraction time and temperature to maximize the response variable outcomes (44). In line with this, predictions and observations are within a narrow range and do not show significant differences at a 5% significance level, indicating the model's suitability for optimization and process efficiency purposes.

The optimal point prediction from the Response Surface Methodology is obtained by integrating optimal conditions and depends on the interaction between independent variables, as Ratnawati et al. (45) explained. The prediction profile is formed when the adjusted surface graphs show a minimum, maximum, or saddle shape. The optimization process can achieve optimal responses by analyzing each response beforehand, ultimately reducing effort and operational costs, as Nurmiah et al. (46) stated. Desirability, with a range of values from 0 to 1, is used as the optimization target value, with low (0–0.49), moderate (0.5–0.79), and high (0.8–1) categories. The closer the value of 1 is, the greater the desirability, which indicates the suitability of the combination of process parameters to achieve optimal response variables.

Table 3 shows that to achieve the desired concentrations of nanoparticle size,  $\zeta$ -potential, conductivity, polydispersity index, acidity level, and viscosity, Tween 80 with a Tween concentration of 12% and Parijoto fruit extract concentration of 7.5% is necessary. This set of conditions has a desirability value of 0.740349. Since its value is close to 1 and falls into the moderate category, this set of conditions is optimal for this research to maximize the response.

## 4 Conclusion

In this series of experiments, nanoemulsion from parijoto fruit has been characterized, considering various physicochemical parameters such as particle size, polydispersity index,  $\zeta$ -potential, conductivity, pH, and viscosity, respectively, ranged from  $14,603 \pm 16.73$  nm to  $118,053 \pm 4.5825$  nm,  $0.402 \pm 0.038$  to  $0.874 \pm 0.100$ ,  $-22.197 \pm 0.738$  mV to  $-28.207 \pm 1.598$  mV,

0.064 ± 0.013 to 0.090 ± 0.010 mS/cm, and 6.747 ± 0.035 to 6.897 ± 0.006, and 3.827 ± 0.021 to 5.633 ± 0.058. The research results indicate significant variations in the physical characteristics of both nanomaterials regarding changes in surfactant and parijoto extract concentrations. Increased surfactant concentration tends to produce smaller particle sizes and a more homogeneous distribution, although certain limitations were found that lead to surfactant aggregation and micelle formation. The nanoemulsion characteristics include ζ-potential, polydispersity, particle size, conductivity, pH, and viscosity. The type and concentration of surfactants played a crucial role in determining the properties of the nanoemulsions. Variations in surfactant parameters resulted in observable differences in emulsion characteristics, highlighting the importance of surfactant selection and optimization. To achieve optimal nanoemulsion process conditions, it is recommended to use Tween 80 with 12% Tween concentration and 7.5% parijoto fruit extract concentration, resulting in a desirability value of 0.74, into the moderate category.

## Data availability statement

The datasets presented in this article are not readily available because non-commercial use; the dataset is provided solely for academic research purposes. Requests to access the datasets should be directed to [kristina@unika.ac.id](mailto:kristina@unika.ac.id).

## Author contributions

VA: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. AP: Data curation, Formal analysis, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing. BS: Data curation, Formal analysis, Methodology, Validation, Writing – original draft, Writing – review & editing. YP: Data curation, Formal analysis, Investigation, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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