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Optimization of thiamine chitosan nanoemulsion production using sonication treatment

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ABSTRACT

The production of thiamine chitosan nanoemulsion via sonication treatment has been optimized using response surface methodology (RSM). This study examines the effect of sonication treatment on thiamine chitosan formulation and variations in time, amplitude, and surfactant concentration to distinguish optimum production conditions. Chitosan thiamine nanoemulsion was prepared using the central composite design (CCD) with 3 independent variables (time, amplitude, and surfactant concentration), which generated 20 experimental points. The optimum condition was determined by analyzing the response variable, which, in this study, is viscosity. The analysis was carried out using the Minitab 18 software. The sample as formulated using the optimum conditions derived were then tested for particle size using a Particle Size Analyzer (PSA). The results showed that the 3-factorial design and response surface methodology could determine the optimum conditions for the formulation process of chitosan thiamine nanoemulsion. The optimum viscosity of the thiamine chitosan nanoemulsion determined was 10.5 mPa s which was obtained with 55.2 min sonication time, Tween 80 surfactant concentration of 3.4%, and amplitude of 56.8 m. The chitosan thiamine nanoemulsion produced with these conditions had a particle size of 20.1 nm and a polydispersity index of 0.446, which indicated that it was stable and homogeneous.

1. Introduction

Fish wastes including crab shells from marine catches are continuously being generated in large amounts as the result of increasing fishing activities in coastal areas. Crab shells, in lieu of being waste, can be processed into chitin and chitosan (Fig. 1), which then can be utilized as supporting material in various processes in the industrial sector. The conversion process of crab shells into chitosan involves drying and crushing the shells, and subsequently deproteinizing and demineralizing them into chitin, a linear polysaccharide composed of (1–4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose units [1]. The extensive hydrogen bonding network in native form of chitin causes low reactivity as well as poor solubility in most solvents, which then conversion of this molecule into chitosan becomes necessity to achieve a wider range of applications [2–4]. The chitin produced from the marine waste can be further made into chitosan through a deacetylation process [1,3–5].

Crab shells can contain up to 22.66% chitosan [7], which is considerably high when compared to the shells of other crustaceans. Chitosan derived from crab shells is quite favorable for use in various industries. As a compound, chitosan possesses many free amine groups which determines its polycationic properties [2,8]. This feature can be utilized in food processing [9], biotechnology [10], waste management [11], and drug manufacturing [12].

Chitosan possesses various applications including as a heavy metal and phenol adsorbent, carrier on electrodes, and dye [13]. In the food sector, chitosan is widely used as antimicrobial preservatives [14], materials for films [15,16], juice purifiers [17], flavoring agents [18], and dietary supplements [19]. Chitosan has also been used as a raw

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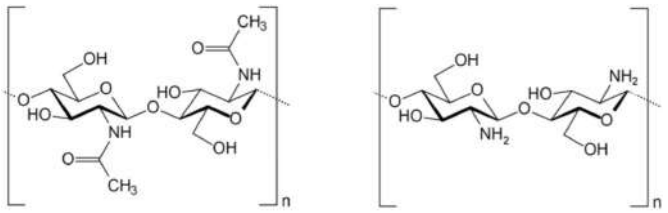

Fig. 1. Molecular structure of (a) chitin and (b) chitosan [6].

material for the manufacture of cosmetics and other beauty products such as hair and skin care products. Moreover, contact lenses as an optical medical aid also use a chitosan mixture, which owing to its high oxygen permeability allows the contact lens to hold water well [8]. In the agriculture field, chitosan is used as an alternative to hazardous chemicals by forming a defense barrier or system against pests within plants [20] through means similar to that of a human vaccine.

Nutrients and hormones are critical in promoting plant growth and reproduction. As to this, nutritional active ingredients and external hormones for plants are often misapplied and, therefore, ineffective. For instance, if vitamins were to be supplied to a plant, a large portion of what is given, often in liquid form, would fall or evaporate, becoming damaged and not being absorbed optimally by the plant. Respectively, thiamine is an element known to help stimulate seedling growth [21–23]. This compound can act as a catalyst and co-enzyme related to plant growth and has been tested for several types of plants [24,25]. Its application, however, requires an adjunct solution to prevent damage or excess release that would lead to wasted use. This can be avoided through a slow-release system that would regulate and control the amount of the vitamin released and consumed by the plants.

Congruously, a nanoemulsion system, consisting of water and oil phases stabilized with surfactants and cosurfactants, can be leveraged to produce a slow-release system. In a slow-release system, an active compound is embedded in a nanoparticle matrix [26]. Active ingredients such as thiamine or vitamin B1 can be formulated into a more stable form, specifically in a chitosan nanoemulsion system. Chitosan, as a natural polymer, can bind thiamine and is of particular interest in this study due to its biodegradability, biocompatibility, and non-toxicity toward the environment. The nanoemulsion system comprises the nanometer-sized (1–100 nm) engineered technology, i.e., nanotechnology, is a dispersion of oil in water that has been stabilized by a surfactant surface layer and has a droplet size smaller than 100 nm. It is thermodynamically stable and transparent or translucent [27].

A nanoemulsion system consists of several components, namely a water phase, an oil phase, a surfactant, and cosurfactants. These components are mixed through sonication, a technique that utilizes sound waves and high-vibration energy to break up particles [28,29]. The size of particles in a nanoemulsion system can be reduced to an optimum by increasing the sonication time and wave amplitude used during the particle formation process [29,30]. Particle size can be made more homogeneous when a mixture is treated at its optimal time range [31]. The sonication method was chosen because compared to conventional methods, it is more efficient in producing nano-sized particles.

The slow-release system approach has been employed in previous studies because it can determine the effect of certain experimental variables and the interactions between those variables thus the optimum condition can be reached [32]. Whereas chitosan nanoemulsion optimization is generally carried out one variable at a time. And the optimization can be done with the help of chemometrics, one of which is Response Surface Methodology (RSM). The application of the method on the process optimization analysis was previously conducted by Song et al. (2022) on the preparation of eucalyptus oil nanoemulsion. The RSM method is a statistical and mathematical approach using quadratic polynomial models to study the relationship between one or more response variables and several independent variables [30,33]. The formation of the eucalyptus oil nanoemulsion was confirmed with the optimal process determined by RSM [34]. This method was used because it requires only a specific amount of data to perform evaluation, analysis, and optimization. Thus, experiment can run more effectively as the number of possible courses of action is reduced. In the end, an optimization process can be made that requires less time and thus, more economical. Furthermore, the experimental design used in this study based on RSM is the Central Composite Design (CCD) with the optimal values of three independent variables.

This paper aims to determine the optimal condition of chitosan nanoemulsion synthesis using sonication treatment. The optimization of thiamine chitosan nanoemulsion formulation was assessed by taking into account the effect of three independent variables, namely time, surfactant concentration, and amplitude. It will be observed the influence of those variables on the viscosity of the chitosan nanoemulsion produced. This research aims to determine the effect of sonication treatment on thiamine chitosan nanoemulsion and to obtain the nanoemulsion product with stable physical properties and good viscosity, as well as to perceive how time, amplitude, and surfactant concentration in the sonication process contribute to achieve optimum result using response surface methodology.

2. Research methodology

2.1. Materials

The chitosan was produced from (swimming) crab shells collected from Cirebon Regency, West Java, Indonesia. The chemicals used in this study were sodium hydroxide (NaOH), hydrochloric acid (HCl), sodium hypochlorite (NaOCl), acetic acid (CH_3COOH), Tween 80, polyethylene glycol (PEG) 400, thiamine, distilled water, and filter paper.

2.2. Equipment

The equipment used in this study were glassware, Buchner filter, blender, hotplate stirrer (Thermo), pH meter (Ohaus Starter 2100), centrifuge, pestle and mortar, 60, 100, and 200 mesh sieves, oven (Memmert UN55), analytical balance (AMD HR-200 and Ohaus Scout), ultrasonic processor Sonics & Materials VC 130 PB, Fourier Transform Infrared Spectrometer (FTIR) Shimadzu Prestige-21, Particle Size Analyzer (PSA) Horiba SZ-100, and Ostwald viscometer. While the software used for the response surface methodology analysis was Minitab 18.

2.3. Chitosan production from crab shell

The chitosan synthesis from crab shells consisted of shell preparation (cleaning and crushing), deproteination, demineralization, and deacetylation processes. The (swimming) crab shells used were obtained from fishermans in Cirebon Regency, West Java. The crab shells were washed and dried in an oven at 70 °C for 24 h, crushed by using mortar and pestle, and then ground further using a blender. The powder produced was sieved using a 100-mesh sieve followed by a 200-mesh sieve and then weighed.

The next process was the deproteination stage which was intended to remove protein in the crab shells, including any crab meat remaining. 30 g of the crab shell powder was dissolved in 3% NaOH solution with a ratio of 1:6 (w/v). The mixture was stirred for 1 h at 85 °C. It was then filtered using a Buchner filter and washed with distilled water until the pH became neutral. The powder left was dried in an oven at 35 °C for 24 h. The dried powder was then weighed using an analytical balance.

The crab shell powder obtained from the deproteination was dissolved in 1 M HCl solution with a ratio of 1:15 (w/v). The mixture was then stirred for 1 h at 30 °C. The mixture was filtered using a Buchner

filter, then washed with distilled water until the pH was neutral. The consequent powder was dried in an oven at 60 °C for 24 h and then weighed.

The chitin powder as obtained from the previous procedure was further modified into chitosan through a deacetylation process. The demineralized shell powder was put into a 50% NaOH solution at a ratio of 1:10 (w/v). The mixture was stirred for 1 h at 100 °C, filtered using a Buchner filter, and then washed with distilled water and dilute HCl until the pH was neutral. The final powder was dried in an oven at 80 °C for 24 h. The chitosan powder was later ground and analyzed using FTIR.

2.4. Determination of the degree of deacetylation

Chitin and chitosan produced in this study were characterized using FTIR and the results obtained were used to determine the degree of deacetylation of chitosan. Each powder was pressed into pellets with KBr with a ratio of 1:100 (w/w). Characterization was done at the wave number range of 4000–400 cm^{-1}.

The degree of deacetylation (%DD) was obtained using the absorbance of chitin and chitosan at the wave numbers of the amide group –NH (A 1655) and the primary amine group (A 3450). The value of 1.33 in the equation represents the ratio of the two absorbances of a fully acetylated compound.

$$\%DD = 100 - \left[\frac{A\ 1655}{A\ 3450} \times \frac{100}{1.33}\right] \quad (1)$$

A 1665 = Absorbance at wave number 1655 cm^{-1}
A 3450 = Absorbance at wave number 3450 cm^{-1}
1.33 = Constant obtained from A 1655/A 3450 ratio for fully acetylated chitosan

2.5. Optimization of chitosan thiamin nanoemulsion using 3-varibles CCD-RSM experimental design

The response surface methodology experimental design was carried out using the Minitab 18 model. The model used was a second-order CCD model with 3 independent variables, namely time (minutes), amplitude (m), and surfactant concentration (%). The range of the independent variables and their levels determined in this study are presented in Table 1. This table represents standard value parameter for CCD at second order with the range for each variable (time (minutes), amplitude (m), and surfactant concentration (%)) at the middle (0), minimum (−1), maximum (+1) and axial (- or +) values. The choice of these values is determined based on the orientation experiments by the author and also adapted to the ability of the apparatus (variable 3–amplitude of the sonicator). The response variable used in this experiment was viscosity, while the fixed variable was the amount of thiamine in the nanoemulsion. Data processing was carried out to discern 20 center points of the three variables used. These points consisted of 8 factorial points, 6 center points, and 6 axial points. The results obtained are used to determine the maximum point, midpoint, and minimum point. The experimental design data are presented in Table 2.

Table 1
Second-order CCD ranges and levels for the three independent variables.

No	Variable	Conversion of the real value				
		−1682 (Axial -)	−1 (min)	0 (Middle)	+1 (Max)	+1682 (Axial +)
1	Time (min)	4,77	15	30	45	55,23
2	Surfactant Concentration (%)	0,32	1	2	3	3,68
3	Amplitude (m)	23,18	30	40	50	56,82

2.5.1. Preparation of thiamine-chitosan nanoemulsion

The nanoemulsion was produced by first dissolving chitosan powder in 1% CH_3COOH solution (0.5% (w/v), 40 mL) at room temperature for 24 h. This nanoemulsion formulation was done for all 20 experimental conditions as shown in Table 2. A solution containing chitosan, 2 mL of thiamine, 10 mL of Tween 80 at a concentration as shown in Tables 2 and 10 mL of PEG 400 was made. It was then gradually added with distilled water slowly until it reached a final volume of 100 mL. The solution was then stirred on a hotplate at a speed of 1000 rpm for 20 min. The sample was then made into a nanoemulsion with an ultrasonic homogenizer with time and amplitude as in Table 2. The thiamine chitosan nanoemulsion formed was stored at room temperature for further testing.

2.5.2. Measurement of viscosity as a response parameter

The density of each sample was first determined prior to the viscosity measurement using an Ostwald viscometer. 10 mL of sample was put into the bigger bulb of the viscometer and then suctioned up into the smaller bulb of the viscometer just past the upper mark on the apparatus. Suction was then stopped and the sample was let to flow down. The time required for the sample to flow from the upper mark to the lower mark in the smaller bulb was measured. The flow time was remeasured until a stable viscosity value was obtained. Viscosity test was carried for all 20 experimental conditions with three repetitions. The flow time was recorded to calculate the viscosity using equation (2).

$$\eta_x = \frac{\eta_0 t_x \rho_x}{t_0 \rho_0} \quad (2)$$

η_x = sample liquid viscosity (cP)
η_o = known liquid viscosity (cP)
t_x = sample liquid flow time (s)
t_o = known liquid flow time (s)
ρ_x = density of sample liquid (g/cm^3)
ρ_o = density of known liquid (g/cm^3)

2.5.3. Product quality assessment

The thiamine chitosan nanoemulsion produced underwent an organoleptic test, centrifugation test, as well as pH and specific gravity determinations. The organoleptic test was carried out through visual observation of the thiamine chitosan nanoemulsion sample produced. Observations were made based on shape, color, odor, clarity, homogeneity, and phase separation. The organoleptic test was carried out every seven days for three consecutive weeks.

The stability of the nanoemulsion was observed through the centrifugation test. 40 mL of thiamine chitosan nanoemulsion was put into a centrifugation tube and centrifuged at 1000 rpm for 30 min. Any possible change of the sample was observed for separation, precipitation, creaming, coalescence, and cracking.

Measurement of the pH of the preparation was carried out using a pH meter. Before measurement, the electrodes were calibrated using a standard solution of pH 7. Once calibrated, the nanoemulsion formula pH was measured.

The specific gravity of the thiamine chitosan nanoemulsion was measured using a pycnometer. A clean and dry empty pycnometer was weighed to obtain the weight of the empty pycnometer. The pycnometer was then filled with the thiamine chitosan nanoemulsion sample to the brim and re-weighed to determine the specific gravity of the sample. Specific gravity measurements were carried out for all 20 thiamine chitosan nanoemulsion samples. The specific gravity was then calculated using the following equation.

$$\text{Specific gravity} = \frac{W_2 - W_1}{V_{pycnometer}} \quad (3)$$

W_1 = Empty pycnometer weight

Table 2
Second-order CCD matrix coded value and measured responses of the three variables.

No	Coded Variables			Real Variables		
	X_1	X_2	X_3	Time (minutes)	Surfactant Concentration (%)	Amplitude (m)
1	0	0	1.682	30	2	56.82
2	1.682	0	0	55.23	2	40
3	−1	1	−1	15	3	30
4	−1.682	0	0	4.17	2	40
5	−1	1	1	15	3	50
6	1	1	−1	45	3	30
7	0	0	0	30	2	40
8	0	1.682	0	30	3.68	40
9	0	0	0	30	2	40
10	−1	−1	−1	15	1	30
11	1	−1	−1	45	1	30
12	0	0	0	30	2	40
13	1	−1	1	45	1	50
14	0	0	0	30	2	40
15	0	0	0	30	2	40
16	−1	−1	1	15	1	50
17	1	1	1	45	3	50
18	0	−1.682	0	30	0.32	40
19	0	0	−1.682	30	2	23.18
20	0	0	0	30	2	40

W_2 = Sample-filled pycnometer weight
$V_{pycnometer}$ = Pycnometer volume (mL)

2.5.4. Data analysis

Data analysis with RSM-CCD optimization was carried out with viscosity as the response variable (Y_1). From the analysis, a linear model would be obtained, which would later be tested for significance (P value) and suitability of the regression model (lack of fit). At this stage, the significance, correlation, identical, and normal distribution test were carried out. Residual and normality analysis were performed to check the adequacy of the model. Data analysis was performed by analyzing multiple regressions on Minitab 18 software to obtain the optimal model. Based on the results of the data processing, the optimal response is the stationary point as indicated by the response surface plot in addition to the contour plot.

2.5.5. Particle droplet size test

The particle droplet size was measured using a dynamic light scattering (DLS) particle size analyzer (PSA) instrument for the sample obtained with the optimum conditions. A total of 10 mL of the nanoemulsion sample was put into a cuvette. The cuvette was cleaned so that unwanted particles were present on the cuvette surface that might obscure and cause inaccurate analysis result. The cuvette that had been filled with the sample was then inserted into the sample holder and PSA analysis was performed. Distribution of the particle size was analyzed by determining the average of the particle size. On the other hand, the polydispersity index was calculated by normalization of the second cumulant defined from the polynomial fitting parameters. By using PSA Horiba SZ-100, the calculation is automatically run in the system.

3. Results and discussion

3.1. Chitosan isolation

The yields from the chitosan preparation from crab shells are presented in Table 3. There were 3 processes carried out to isolate chitosan from crab shells, namely deproteination, demineralization, and deacetylation. The deproteination process was carried out to remove protein residues that were still on the crab shells such as meat remnants. In this process, the bond between chitin and protein was broken. Chitin produced from this process was as much as 80.17% of the dry weight of the initial crab shell used. Furthermore, the loss of protein in the crab shell was characterized by a change in the color of the shell powder from orange to whitish orange. This reflected the 19.83% amount of protein that was able to bind with Na^+ ions to form Na-proteinate and be soluble in water.

Table 3
Chitosan yields from the preparation stages.

No	Treatment	Yield (%)
1	Deproteination	80.17%
2	Demineralization	79.50%
3	Deacetylation	40.53%

The mineral content in the powder produced from the deproteination process needed to be removed through the demineralization process. The minerals found in crab shells including calcium carbonate ($CaCO_3$) and calcium phosphate ($Ca_3(PO_4)_2$) were removed and resulted a pure white chitin powder with a yield of 79.50%, meaning that the mineral salts that were initially contained in the powder and dissolved in the demineralization process were 20.5%.

Once chitin was obtained from the deproteination and demineralization processes, a deacetylation process was performed to transform it into chitosan. In this process, the acetyl chain (-$COCH_3$) attached to the amine group bound to chitin is broken off. The reaction that occurs in the transformation of chitin into chitosan is an amine hydrolysis reaction in a basic condition. The deacetylation process produced 15.50 g of chitosan, which was 40.53% of the yield obtained in the demineralization process or 15.83% of the initial crab shell mass used. The yield obtained was similar to the yield values in other studies, which ranged at 13.37–17.39% [35,36]. The low yield of chitosan relates to the initial amount of the shell used which can be attributed to the high concentration of NaOH given during the deacetylation stage. The yield obtained in this study was considered high because during the deproteination, demineralization, and deacetylation stages, both chitin and chitosan were very less washed out in the solvent during the washing process.

The chitin and chitosan obtained were characterized using FTIR to determine the functional groups contained and the degree of deacetylation. The FTIR spectra of chitin and chitosan are presented in Fig. 2. In the FTIR spectra of chitin, the peaks at 3442, 3271, 2963, 1635, 1435, and 1323 cm^{-1} indicate the presence of stretching vibrations of O–H, *N*–H amine, C–H, C═O, C–N amide, and CH_3, respectively. Chitin characteristic peaks were also demonstrated at 1027-1155 cm^{-1} which signified C–O–C vibration and the wave number of 1072 cm^{-1} which signified the presence of a glucopyranose ring in the structure of chitin

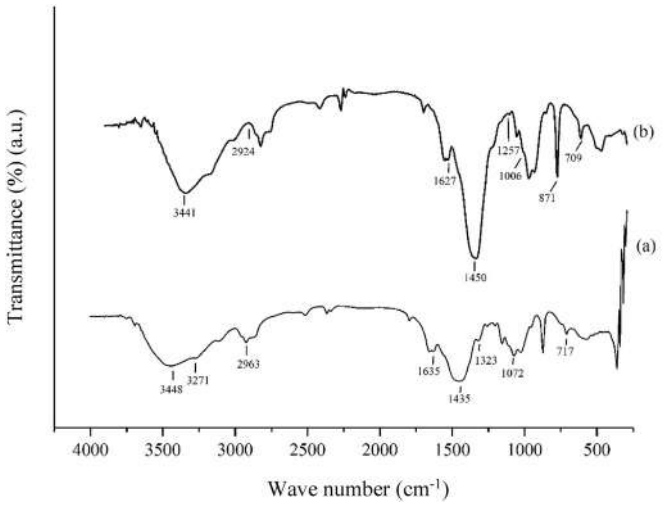

Fig. 2. FTIR spectra of (a) chitin and (b) chitosan.

compound. These absorption spectra agree with the findings of Tanasale et al. [7].

The degree of deacetylation is a parameter of the quality of chitosan. It is a measure of the number of acetyl groups removed from chitin through the deacetylation stage to obtain chitosan. High concentrations NaOH will prompt the amino functional group (-NH_3^+) to substitute the acetyl group in chitin resulting in a high degree of deacetylation. The FTIR spectra of chitosan produced in this study showed the presence of amide and hydroxyl absorption bands which are characteristic for chitosan. The degree of deacetylation achieved in this study was 64.45%, which implied that the powder produced from the three stages of preparation can indeed be said to be chitosan. Deacetylated chitin with a DD value in the range of 40–100% can be said to have transformed into chitosan [37].

3.2. Chitosan thiamine nanoemulsion preparation

An organoleptic test was carried out by observing the physical aspects of the thiamine chitosan nanoemulsion sample, which include color, smell, clarity, and homogeneity. Observations were made every seven days for 3 consecutive weeks. Observations showcased that the nanoemulsion samples were stable initially. All 20 samples of chitosan thiamine nanoemulsion were odorless, clear, and homogeneous. However, in week 3, sample 4 showed slight cloudiness and phase separation, i.e., it was no longer homogeneous.

Centrifugation test was carried out in the first week to determine whether phase separation in the nanoemulsion would occur due to gravitational force. Centrifugational force can be employed such that matters are pushed or pulled from the center of rotation. If phase separation happened in the sample, then the phase with higher density will be at the bottom while the phase with lower density will go up. The tests performed toward 20 samples showed that 19 thiamine chitosan nanoemulsion samples did not experience phase separation and thus were stable. Phase separation was observed in sample 4 only.

pH measurement was conducted for the 20 samples with similar procedures. The measurement was carried out to assess whether there were significant changes that occurred in the acidity level of the samples. Results showed that there were no significant changes in pH, meaning that the samples were stable in their level of acidity.

Finally, the specific gravity of the samples was determined using a pycnometer. Measurement results showed that the 20 nanoemulsion samples had an average specific gravity of 1.012–1.015 g/cm^3.

3.3. Nanoemulsion formulation optimization with RSM

3.3.1. Preparation of chitosan thiamine nanoemulsion

The formulation of chitosan nanoemulsion was carried out according to the treatment and sequence obtained from the CCD experimental design results for 3 independent variables. Some of the nanoemulsion examples produced are shown in Fig. 3 for the experiments number 17, 18, 19 and 20.

Chitosan powder isolated from crab shells was dissolved in acetic acid to transform the amine group (-NH_2) to be positively ionized to (NH_3^+). The formation of these ions allows chitosan to be dissolved. Chitosan would then be mixed with other materials such as thiamine, Tween 80, and PEG 400. Tween 80, a non-ionic and low-toxicity surfactant, was used to lower the interfacial tension so that a nanoemulsion can be formed. The hydrophilic-lipophilic balance (HLB) value possessed by Tween 80 is quite high, i.e. > 10. However, the use of Tween 80 alone would not able to sufficiently reduce surface tension to form a nanoemulsion. Cosurfactant PEG 400 was added to assist the work of Tween 80 in lowering the surface tension so that nanoemulsion formation can occur spontaneously [38,39]. While PEG 400 has a high HLB value of 11.6 which makes it suitable for forming nanoemulsion.

The sonication treatment given to the sample was intended to break down the size of the particles in the nanoemulsion. In addition, if sonication is carried out at high intensity, emulsification will occur and homogeneous sample will be obtained. In addition, large amplitudes can induce and increase the occurrence of cavitation which will lead to the breaking up of particles into smaller sizes. A sonication process is favorable due to its simplicity and time efficiency, and does not require the addition of other chemicals. This will ensure that there will be no significant consequence on the chemical structure of the particles from external factors.

From the 20 samples of nanoemulsion produced, some of them exhibited a slightly cloudy appearance, though they were still transparent such that light could still pass through, while others displayed clear and transparent properties. This implied that the nanoemulsion consisted of sufficiently small particles that allowed the light beam to pass through. Samples that were objected to longer duration, high surfactant concentrations, and high amplitudes showed a clear appearance, i.e., not cloudy nanoemulsion, and did not form agglomerates.

3.3.2. Measurement of viscosity as a response parameter

Viscosity depicts the thickness of a dispersed medium in a nanoemulsion system. The greater the value of viscosity, the harder the emulsion will flow. As a property, viscosity is influenced by the particle size of a sample. The viscosity test on the samples demonstrated varying results. This variation can be attributed to the differences in treatment as by the CCD experimental design for the 3 independent variables. The test was carried out 3 times for each sample and the average value was

Fig. 3. Chitosan thiamine nanoemulsion samples (experiments number 17, 18, 19 and 20).

calculated, which is presented in Table 4.

Differences in the viscosity values indicated differences in the particle size among the samples. The higher the viscosity, the smaller the particle size. A good chitosan thiamine nanoemulsion would exhibit a high viscosity which corresponds to a more stable system at due to the small possibility of particle coalescence. The viscosity values of the samples obtained were in the range of 7–9 mPa s with the lowest viscosity of 7.12 mPa s and the highest viscosity of 9.82 mPa s.

3.3.3. Response surface Methodology analysis

Response analysis was performed using ANOVA to obtain a linear model that was later tested for significance (P value) and suitability of the regression model (lack of fit). Subsequent to the data processing, a significance, correlation, identical, and normal distribution test were carried out. The significance test was aimed to discern the independent variables that had a chemometrically significant effect on the response variable. The significance level used in this study was 0.05 with a 95% confidence level. If the P value of the variable is <0.05 then the variable can be said to have a significant influence on the response variable.

The P values obtained imply that the variables X_1 (time), X_2 (surfactant concentration), and X_3 (amplitude) had significant effects on the response variable, namely viscosity. The P values for all three were P <0.05; variable X_1 (time) had P value of 0.001, X_2 (surfactant concentration) of 0.0006, and X_3 (amplitude) of 0.006. The lack of fit test demonstrated a value of more than 0.05 with 0.088. This indicated that the obtained model can describe the viscosity response variable data and was suitable to be used to produce the optimum response.

The coefficient of determination, R^2 value, obtained from the ANOVA test was also used to determine the influence of the independent variable on the response variable. The R^2 value obtained in this study was 0.8364 or 83.64%, which indicated that the time variable, surfactant concentration, and amplitude had an 83.64% effect on the resulting viscosity value. Meanwhile, the remaining 16.36% represented other factors that were not taken into account in this model, for examples the homogenity of the length chain of chitosan polimer [27]. The adjusted R^2 was obtained at 0.6892 or 68.92%, indicating the close relationship between the independent variables.

The model generated from the analysis results was a regression model that depicts the relationship of the three independent variables to the response. The equation of the model is presented in the following equation.

$$Y = 4.56 + 0.0222\,X_1 + 1.025\,X_2 + 0.0619\,X_3 - 0.000794\,X_1 * X_1 - 0.217\,X_2 * X_2 - 0.00039\,X_3 * X_3 + 0.01067\,X_1 * X_2 + 0.000750\,X_1 * X_3 - 0.0023\,X_2 * X_3 \quad (4)$$

Y = viscosity, X_1 = time, X_2 = surfactant concentration, X_3 = amplitude

The correlation test in this study was conducted to determine the relationship between the 3 independent variables to the response variable chemometrically. Correlation is indicated by a correlation coefficient (r) which can have a possible value between -1 and $+1$. A correlation coefficient close to -1 or $+1$ implies that the correlation between the independent variable and the response variable is strong. However, if the correlation value is close to 0, then the correlation is considered weak and does not exist. The results of the correlation test are presented in Table 5.

Degree of correlation can be categorized into 4 levels, namely not correlated (0–0.090), weak (0.1–0.250), strong (0.260–0490), and very strong (0.500–1). Table 5 presented that the correlation coefficient for the time was 0.446; surfactant concentration, 0.441; and amplitude, 0.560. These results indicated that time and surfactant concentration variables had a strong correlation, in addition to the amplitude which had the highest correlation value within the very strong category. The value of the response variable can be predicted as shown in Fig. 4.

Data from observations and the response predictions made are shown in Fig. 4. The R^2 value obtained was 0.8349, i.e., the correspondence between predictive data and observation data was 83.49%. The remaining 16.51% was influenced by other factors that were not taken into account in the model.

3.3.4. Data analysis

Data analysis was carried out to obtain the optimal model through multiple regression analysis. Data processing was carried out to obtain surface response stationary points and contour plots to determine the optimum response value. The response surface model is generated from contour and surface plots with two variables on the response. A contour plot will how the independent variables should be set to achieve the optimum viscosity. The results of data processing for the time variable (X_1) and surfactant concentration (X_2) are presented in Fig. 5. The plots indicated that there was an effect of the independent variable X_1, time, and X_2, surfactant concentration, on the viscosity of the thiamine chitosan nanoemulsion. The highest viscosity values would be achieved from conditions as indicated by the dark green area, with a range of 9.0–9.5. Contour plots and surface plots of the resulting response were reviewed for optimum points. The viscosity response obtained was 9.5 mPa s with a time condition (X_1) of 54 min and a surfactant concentration (X_2) of 3.5%.

Response surface analysis results to determine the effect of time (X_1) and amplitude (X_3) on the response are shown in Fig. 6. In the contour plot shown in Fig. 6 (a), there were color variations generated that described viscosity for the combination of the two variables. The optimum viscosity response was indicated by the dark green region, having a value of 9.5–10 mPa s. The magnitude of the optimum point was determined through further review of the results of the contour plot and the response surface plot. The optimal viscosity response obtained was 10 mPa s with a time (X_1) of 54 min and an amplitude (X_3) of 56 m.

Analysis through contour and response surface plots was also carried

Table 4
Chitosan thiamine nanoemulsion viscosity test results.

Sample number	Average viscosity (mPa.s)
1	9.82
2	9.17
3	8.21
4	7.38
5	8.44
6	8.68
7	9.42
8	9.21
9	8.78
10	7.85
11	8.08
12	8.78
13	8.85
14	8.98
15	8.75
16	8.63
17	9.76
18	7.12
19	7.52
20	8.69
Average result (mPa.s)	8.61

Table 5
Correlation test results between independent and response variables.

Variable	Correlation Coefficient (r)
Time (min)	0.446
Surfactant Concentration (%)	0.441
Amplitude (m)	0.560

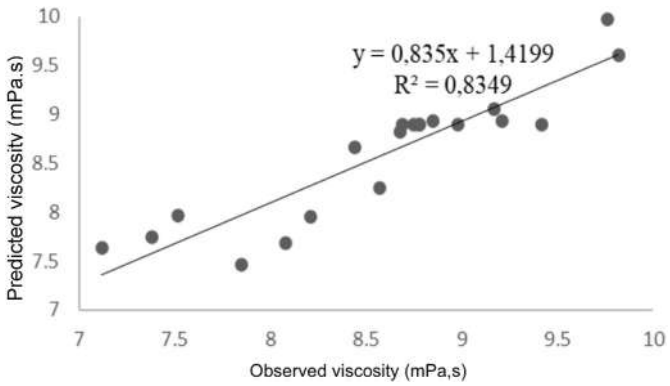

Fig. 4. Graph of the relationship between observed and predicted viscosities.

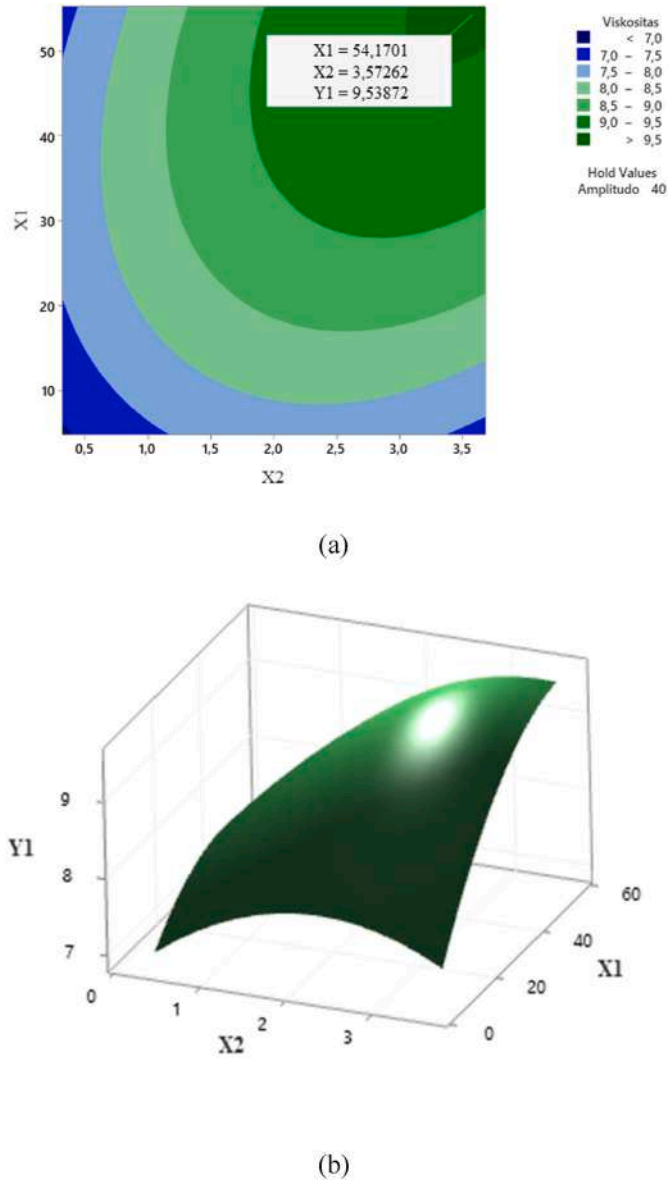

Fig. 5. (a) Contour plot and (b) response surface plot of viscosity response to time (X1) and surfactant concentration (X2).

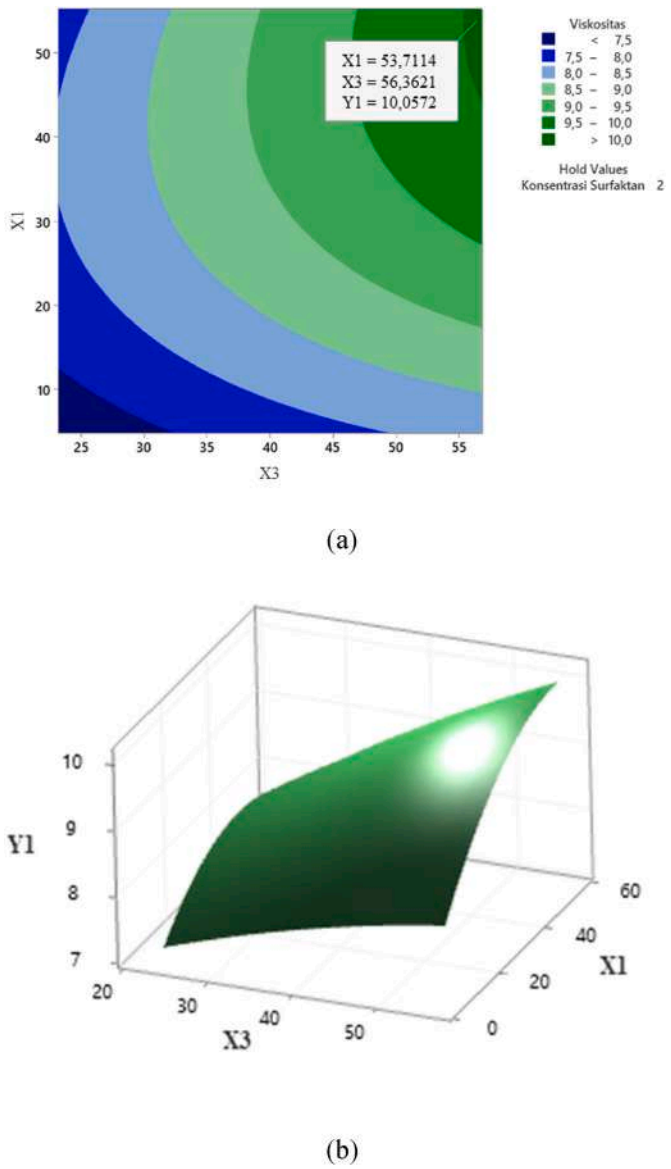

Fig. 6. (a) Contour plot and (b) response surface plot of viscosity response to time (X1) and amplitude (X3).

out to determine the effect of surfactant concentration (X_2) and amplitude (X_3) and the results are in Fig. 7. The optimum viscosity response was indicated in the dark green area of >9.5 mPa s and was computed to be 9.6 mPa s under the treatment conditions of a surfactant concentration (X_2) of 3% and an amplitude (X_3) of 56 m.

Subsequent to the analysis using contour and response surface plots for the three variables, an optimization plot was made. The optimization plot would demonstrate the optimum viscosity response value under the optimum conditions relating to the three independent variables. Based on the optimization plot obtained, the viscosity response would be optimum at a value of 10.5 mPa s. This value would be given by a time (X_1) of 55 min, a surfactant concentration (X_2) of 3%, and an amplitude (X_3) of 57 m. The desirability function (d) obtained a value of 1.00. These results demonstrated favorable values and could thus be recognized as the optimum viscosity value and conditions [40].

3.3.5. *Particle Droplet size test*

The particle droplet size test was carried out on the optimum thiamine chitosan nanoemulsion sample. This test was aimed to determine the particle size of the resulting sample, whereby the particle size for a

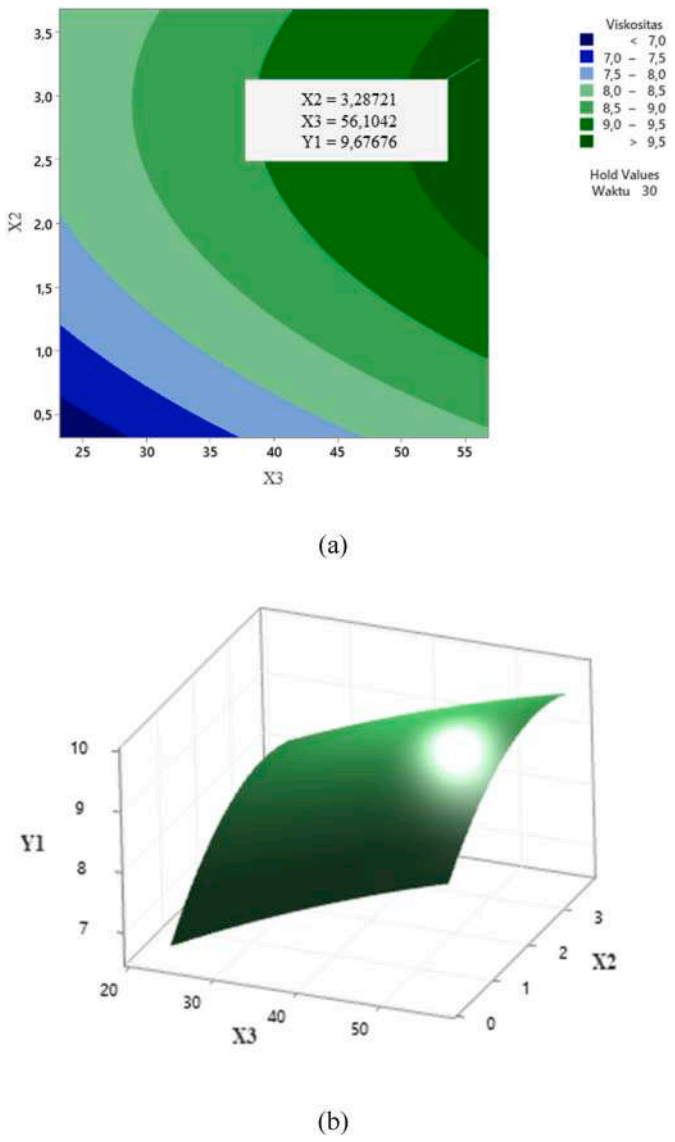

Fig. 7. (a) Contour plot and (b) response surface plot of viscosity response to surfactant concentration (X_2) and amplitude (X_3).

nanoemulsion is 1–100 nm. Nanoemulsion testing was carried out with a dynamic light scattering (DLS) particle size analyzer (PSA) instrument. This instrument works based on the scattering of light from a laser beam that will hit particles in the sample. The scattered light will be detected by a photon detector at a certain angle quickly wherefrom the particle size can be determined [41]. In Fig. 8, we can see the distribution of particle size of the nanoemulsion.

Table 6
PSA test results of samples at optimum conditions.

Measured property	Average
Particle Size	20.1 nm
Polydispersity Index (PI)	0.446

As presented in Table 6, the particle size of the optimum sample was 20.1 nm which was a suitable nanoemulsion size. A nanoparticle has a particle size of less than 100 nm [27].

In addition to particle size, the polydispersity index value was also obtained. PI value describes the particle size distribution that occurs in a sample. The PI value obtained in this study was 0.446, a low value that indicated satisfactory distribution within the range of 0.01–0.7 [42]. This result implied that the optimum nanoemulsion had a narrow or uniform particle size distribution. This could also imply that the nanoemulsion was homogeneous. If a PI value is greater or closer to 1, then the nanoemulsion in question does not have good particle uniformity and will exhibit coalescence that results in a large particle size. From this particle droplet size test, it was concluded that the thiamine chitosan nanoemulsion sample made under the optimum conditions derived had an appropriate particle size and PI value.

4. Conclusion

Response surface methodology with 3 variables CCD was able to derive optimum conditions in thiamine chitosan nanoemulsion formulation with sonication treatment. The response variable, namely viscosity, had a derived optimum value of 10.5 mPa s. The results showed that the optimum viscosity could be obtained with a time (X_1) of 55 min, a surfactant concentration (X_2) of 3%, and an amplitude (X_3) of 57 m.

This research presents an optimum condition that can be applied to synthesize chitosan-thiamin nanoemulsion which then can be implemented in the agricultural sector as a slow-releasing agent system of root growth support hormone. The preparation process by sonication treatment suggests an alternative simple and low-cost pathway of producing relatively homogeneous and stable nanoemulsion with the implementation of green chemistry.

Credit author statement

Iqmal Tahir: Conceptualization, Supervision, Resources, Project administration, Methodology, Data Interpretation, Writing – Reviewing and Editing, Funding Acquisition. Justitia Millevania: Conceptualization, Methodology, Data Interpretation, Writing- Original draft preparation, Software, Data curation. Karna Wijaya: Conceptualization, Supervision, Validation, Methodology. Mudasir: Supervision, Validation. Roswanira Abdul Wahab: Supervision, Validation. Widi Kurniawati: Writing – original draft, writing – review & editing, Visualization.

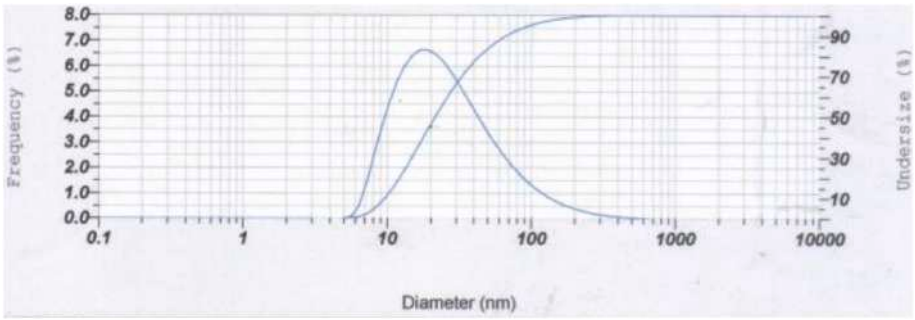

Fig. 8. Particle size distribution of the optimum nanoemulsion product.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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