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ORIGINAL RESEARCH

Encapsulation of *Channa striata* albumin extract: Optimization by Box-Behnken design of response surface methodology

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Abstract *Channa striata* is known to have high albumin content which has beneficial effects for human health. This study aimed to optimize the encapsulation of albumin extracted from *C. striata* and study the effect of independent factors on the encapsulation efficiency (EE) using response surface methodology (RSM). Ionic gelation encapsulation was performed employing chitosan–sodium tripolyphosphate (NaTPP). RSM of Box-Behnken design (BBD) involving three factors, i.e. extract concentration (0.25-1.0 mg/mL), NaTPP concentration (5-15 mg/5 mL), and chitosan concentrations (0.5-1.0 mg/mL) were applied to obtain high EE. The obtained model was quadratic with R^2 of 0.9922 and the difference between adjusted R^2 and predicted R^2 was less than 0.2. The model was significant ($P<0.0001$) with a non-significant lack of fit ($P>0.05$), indicating good fitness of the model. All factors had significant interaction except chitosan-NaTPP. The optimum condition was obtained at 0.25 mg/mL extract, 12.7 mg/5 mL NaTPP, and 0.5 mg/mL chitosan with a maximum EE of 85.50%. Analysis using FTIR exhibited several different functional groups between albumin extract, encapsulated albumin, and blank sample. In addition, scanning electron microscope (SEM) analysis demonstrated a solid, rougher, and more compact surface of encapsulated albumin compared to a blank sample, and transmission electron microscope (TEM) imaging demonstrated a nano-encapsulated albumin extract. Therefore, the RSM Box-Behnken design could be used to optimize the encapsulation of *C. striata* albumin extract that can be used in food and pharmaceutical product applications.

Keywords *Channa striata* . Albumin extract . Encapsulation . Response Surface Methodology . Box-Behnken design

Introduction

Channa striata, known as snakehead fish, is a commercial freshwater fish which contains high protein content. *C. striata* is rich in nutrients such as protein, fat, glucose and some minerals such as Zn, Cu and Fe. The protein content of this fish has been known to be 16.2 g% with 2.17% (weight/volume) albumin as its main fraction. The albumin content of *C. striata* is comparable with other fish (tilapia 0.75%, eel 1.65%, catfish 1.86%, and pangas catfish 2.88%) and *C. striata* has been used for a long time as a source of albumin due to its wide availability. The albumin from *C. striata* has been used for several applications, including food ingredients and food supplements intended for human health (Mustafa et al. 2012). It has also been reported to have some biological activities, such as increasing albumin levels for hypoalbuminemia and post-operative surgery patients, antioxidants, antihypertensive, as well as supporting

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the recovery of stroke patients (Dewita et al. 2018; Rosyidi et al. 2019). Therefore, the albumin from snakehead fish attracted great attention as a source of materials for food and pharmaceutical products (Ramdhani et al. 2019).

Unfortunately, proteins, including albumin, are unstable and can easily change under environmental conditions (Pavlov et al. 2008). Thus, formulation technology is required to protect the proteins and to improve their stability during processing. The challenge of protein formulation is how to increase stability, avoid degradation, increase bioavailability and increase its loading capacity to the targets (Arozal et al. 2021). Lately, the encapsulation techniques have attracted great attention to improve drug and bioactive substance delivery systems. It becomes a promising way to maintain the stability of bioactive compounds such as protein during processing and storage and protect from unwanted interactions (Nedovic et al. 2011). Encapsulation is a process to entrap active agents such as proteins, which commonly use one or more substances as coating materials (Risch and Reineccius 1995). The ionic gelation method, an encapsulation technique employing cross-linking between anionic and cationic polymers, has attracted great interest in protein encapsulation. This method can be applied to protect such proteins due to electrostatic interactions occurring between two ionic species under certain conditions, where one species must be a polymer. It takes advantage of the presence of amino acids with positive and negative charges. These amino acids can interact with one of the counterions. A natural polymer like chitosan combined with ionic tripolyphosphate (NaTPP) as a matrix has been extensively used in the ionic gelation method due to some advantages such as simplicity and low cost due to the lack of complicated machinery, the use of aqueous solvents, and the speed of production. As compared to chemical cross-linking, the physical cross-linking through electrostatic interactions is reversible, which enables the avoidance of potential toxicity and other unfavorable effects in food and pharmaceutical product applications (Pedroso-Santana and Fleitas-Salazar 2020). Chitosan has excellent characteristics for encapsulation due to its properties such as inert, inexpensive, widely available, biocompatible, biodegradable and unique bio-adhesive properties. Acidic amino acids from protein can be attracted to positively charged chitosan chains, while basic amino acids with extra amino groups would interact with highly electronegative TPP phosphate groups (Sacco et al. 2021). This system allows encapsulation in proteins such as protein hydrolysate (Saraswati et al. 2021) or insulin (Nejabat et al. 2021). The spontaneous crosslinking reaction between cationic chitosan and anionic TPP forms a polyelectrolyte complex, which is stabilized by cross-linked chitosan–NH^{3+} and TPP–O^{-}, which then form gel-like particles and encapsulation of protein occurs. Therefore, encapsulation using the ionic gelation method is a promising technique to improve the albumin extract delivery system.

The combination of different formulations would affect the optimal EE of albumin. By determining the optimum conditions, the successful encapsulation can be reached without much time consuming (Mandal et al. 2019). The use of advanced statistical tools such as response surface methodology (RSM) has been widely applied for many purposes, including optimization of the encapsulation process. RSM is a method in statistical design that consists of a combination of experimental design and regression analysis. Moreover, RSM could be used to investigate the effect and interaction among factors. The most frequently used designs in research are central composite design (CCD) and Box-Behnken design (BBD) (Said and Amin 2015). BBD gave a smaller number of experiments that make the consumption of reagents more efficient (Ferreira et al. 2007). It has been applied for many optimization purposes. To the best of our knowledge, the use of RSM BBD for optimization of albumin encapsulation from *C. striata* fish using the ionic gelation method to improve its stability and bioavailability has not been reported yet. The encapsulated product of albumin provides an added value compared to the raw materials because it can protect the albumin from degradation thereby improving its stability. In addition, encapsulation can improve the delivery system of albumin such as maintaining the release to its correct target in the body. Therefore, the main purpose of this study was to obtain an optimum condition for the encapsulation process of albumin extracted from *C. striata* employing RSM Box-Behnken design using three factors, i.e., chitosan, NaTPP, and extract concentrations to obtain high EE of albumin. The specific objective was to investigate the effect of each factor on the EE of albumin and their interactions.

Materials and methods

Snakehead fish (*C. striata*) was obtained from Yogyakarta, Indonesia. Chitosan with low molecular weight (95% deacetylation), sodium tripolyphosphate (NaTPP) and glacial acetic acid were purchased from Merck, Germany. Other chemicals and all reagents used in this work were of analytical grade.

Albumin extraction from *C. striata*

The meat of snakehead fish (*C. striata*) was collected from mature fish (8-12 months) and obtained in local fisheries in Yogyakarta, Indonesia. Before extraction, fish meat (free from skin and other fish body parts) was chopped into small pieces and homogenized. The procedure for albumin extraction from *C. striata* was performed according to the method of Asikin & Kusumaningrum (2018) and Romadhoni et al. (2016) with slight modifications. Albumin was extracted from 250 g of *C. striata* meat using distilled water (1:2 w/v) for 1 hr in a water bath (GFL 1083, Germany) at 50°C. The supernatant was collected and filtered using a Buchner vacuum and followed by liquid-liquid extraction for 30 mins using n-hexane to remove lipid compounds. After the organic phase was removed from the mixture, the aqueous extract was concentrated using a vacuum rotary evaporator (Buchi, Switzerland) at 50°C and lyophilized using a freeze dryer for 24 hrs. The yield was calculated according to the equation 1 as follows:

$$\text{Yield (\%)} = [W_1/W_0] \times 100\% \quad (1)$$

where W_1 represents dry extract (g) and W_0 represents fish meat (g).

Albumin extract encapsulation

Encapsulation of albumin extract was performed using the ionic gelation method. Initially, chitosan was dissolved in 25 mL of 0.35% acetic acid and stirred (IKA C-MAG HS 7, Germany) at 900 rpm for 15 hrs at room temperature. After the chitosan solution was obtained, albumin extract was added to the solution and stirred at 900 rpm for 1 hr at room temperature until the extract completely dissolved. Subsequently, the NaTPP solution was gently dropped into the mixture. The concentration of the chitosan, NaTPP, and albumin extract used in this experiment was made serially according to the experimental design of RSM (Mandal et al. 2019). The EE of encapsulated albumin extract was then determined.

EE determination

EE was evaluated by measuring protein concentration in the supernatant after centrifugation. The encapsulated product of albumin extract was centrifuged at 12,000 rpm for 30 mins at 4°C. The supernatant was collected and determined its protein concentration. Protein was determined using the Lowry method with bovine serum albumin (Merck, Germany) as a protein standard. The EE was calculated according to the equation 2 as follows:

$$\text{EE (\%)} = 100\% - [C_1/C_0 \times 100\%] \quad (2)$$

where C_1 represents concentration (mg/mL) in the supernatant and C_0 represents protein concentration (mg/mL) in the initial extract (Mandal et al. 2019).

Optimization of encapsulation

The experimental design for the encapsulation of albumin extract was carried out using the RSM technique. RSM was applied for optimization of the encapsulation process of albumin extract using three independent factors to obtain high EE. BBD was chosen as a model design with three numeric factors at three levels (-1, 0, +1), as shown in Table 1.

The optimum condition of factors combination to obtain high EE was measured using Derringer's function. The design of the experiment of RSM Box-Behnken Design was carried out using Design Expert 13 software (State-Ease, Minneapolis, Minnesota, USA).

FTIR spectroscopy analysis

FTIR analysis aimed to identify functional groups in the albumin extract, encapsulated albumin extract, and blank sample. The freeze-dried samples were used in this study. Spectra acquisition was carried out using an FTIR spectrophotometer (Bruker Vertex 80, Germany) equipped with the ATR (attenuated total reflectance) technique. The sample was directly placed on an ATR crystal and measured at mid-infrared region (4000 to 600 cm^{-1}) using the resolution of 16 cm^{-1} and 32 scans. The spectra were recorded as absorbance values at each data point. Each sample was analyzed in duplicates and background spectra were measured prior to each sample measurement. Spectra were analyzed using OPUS Software version 8.5 (Bruker, Germany).

SEM analysis

SEM (scanning electron microscope) is conducted to provide detailed surface structure information by tracing the sample in a raster pattern with an electron beam. The morphological structure of freeze-dried non-encapsulated albumin extract and encapsulated albumin extract was evaluated using SEM (JEOL JSM-6510, Japan) operated at an accelerating voltage of 5 kV. Sample in amorphous form was prepared and mounted in a glass substrate, then coated using gold to minimize charging. Analysis was carried out with a working distance of 10 mm.

TEM analysis

TEM (transmission electron microscopy) is conducted to provide nano structural properties in detail which is used for measuring particle size, shape, and distribution. TEM analysis in the present work was performed using a TEM (JEOL JEM-1400, Japan) operating at 250 kV. The encapsulated albumin extract sample was dispersed in water, and then a drop of sample dispersion was mounted on a grid and dried at room temperature. Subsequently, a drop of 1% uranyl acetate solution was added and left for 30 s. Analysis was carried out with a magnification of 40,000–150,000.

Data analysis

The BBD results were analyzed using Design Expert version 13 software. Model fitting for encapsulation of albumin extract was evaluated and confirmed using ANOVA analysis in BBD along with MLRA (multiple linear regression analysis). The model was also evaluated for its goodness of fit and interaction of each dependent variable. All statistical analyses were performed with a confidence level of 95% (p-value = 0.05).

Results and discussion

Albumin extract encapsulation

The protein extract obtained from the extraction process is 4.70% of *C. striata* meat. The extract was then encapsulated using chitosan-NaTPP coating material. Table 2 shows that the EE ranged from 35.88% to 89.62%. The higher the EE, the more the amount of extract can be encapsulated.

Table 1 Factors used for the design of experiment using Box-Behnken design

Factor	Name	Unit	Value		
			-1	0	1
A	Extract	mg/mL	0.25	0.625	1
B	NaTPP	mg/5 mL	5	10	15
C	Chitosan	mg/mL	0.5	0.75	1

Model fitting and model adequacy analysis

A regression model using MLRA was applied to investigate the effects of factors on EE. Box-Cox analysis recommends model transformation, namely power transformation with $\lambda = 2.39$. The transformation was aimed to obtain a normal distribution of data and improve model fitting according to the observations for the optimization (Shen et al. 2012). The model obtained was a quadratic model along with a significant model (p-value < 0.0001) and a not-significant lack of fit (p-value = 0.178) as depicted in Table 3. The equation obtained from MLRA analysis to predict EE is shown in Eq. 2.

The model with a p-value less than 0.05 is considered to be a significant and good model (Kim et al. 2003). The goodness of fit parameters demonstrated in Table 3 showed a high value of R^2 (0.9922). Moreover, the low gap between adjusted and predicted R^2 (< 0.2) and high adequate precision (34.688) indicated that the model had adequate power to predict the response. Instead of a p-value, the model significancy was also shown by a larger F-value (Amini et al. 2008; Kalavathy et al. 2009). Further analysis using ANOVA revealed that the F-value for the model was 98.89, and the p-value was < 0.0001, indicating a significant regression model. Additionally, a lack of fit test could be used to evaluate model deviation. The model with a significant lack of fit is avoided due to its higher deviation; therefore, the lack of fit value must be not-significant (p-value > 0.05) (Rezaee et al. 2014). Moreover, a not-significant lack of fit indicated a good correlation between all factors (concentration of extract, NaTPP, and chitosan) and the response (EE).

Table 2 EE of *C. striata* albumin extract obtained using Box-Behnken design

Run	Extract (mg/mL)	NaTPP (mg/5mL)	Chitosan (mg/mL)	EE (%)
1	0.25	10	0.5	84.22
2	0.625	5	1	54.83
3	0.625	10	0.75	49.47
4	1	10	1	59.58
5	0.625	15	1	64.39
6	0.25	5	0.75	72.43
7	0.625	10	0.75	47.93
8	0.625	10	0.75	47.98
9	0.625	10	0.75	48.44
10	0.25	15	0.75	89.62
11	1	10	0.5	35.88
12	1	15	0.75	45.04
13	1	5	0.75	61.53
14	0.625	5	0.5	67.55
15	0.625	15	0.5	67.47
16	0.25	10	1	66.75
17	0.625	10	0.75	43.53

Table 3 Analysis of variance (ANOVA) of the fitted model in the encapsulation process using Box-Behnken design

Parameters	EE (%)		
	Sum of squares	F-value	p-value
Extract concentration (A)	9.382×10^8	413.92	< 0.0001
NaTPP concentration (B)	2.705×10^7	11.93	0.0106
Chitosan concentration (C)	3.200×10^7	14.12	0.0071
AB	2.019×10^8	89.06	< 0.0001
AC	2.149×10^8	94.78	< 0.0001
BC	9.818×10^6	4.33	0.076
A^2	2.687×10^8	118.56	< 0.0001
B^2	2.271×10^8	100.21	< 0.0001
C^2	4.368×10^7	19.27	0.0032
Model (p-value)	< 0.0001 (significant)		
Lack of fit (p-value)	0.1708 (not significant)		
R^2	0.9922		
Adjusted R^2	0.9822		
Predicted R^2	0.9113		
PRESS	1.804		
Adequate Precision	34.688		

$$EE^{2,39} = 10185.2 - 10829.6*A + 1838.68*B - 2000.11*C - 7104.35*AB + 7328.94*AC + 1566.7*BC + 7989.22*A^2 + 7344.93B^2 + 3220.95*C^2 \quad (2)$$

Model adequacy could be evaluated using several parameters from different diagnostic plots, such as the normal plot of residuals graph, residual versus predicted graph, and residual versus run graph. Observation on the normal plot of the residual graph (Fig. 1A) showed a normal and good distribution data because all the data points were close to the linear regression line, and no visible deviation occurred. The graphs of residuals versus predicted (Fig. 1B) showed that the data points had no obvious pattern and unusual structure, which indicates good model adequacy. To be considered a model with good adequacy, the residual versus predicted graph should be structureless and contain no obvious pattern (Anuar et al. 2013). The residuals versus run graph (Fig. 1C) showed that all the data points were randomly scattered, indicating a good model (Flaifel 2020).

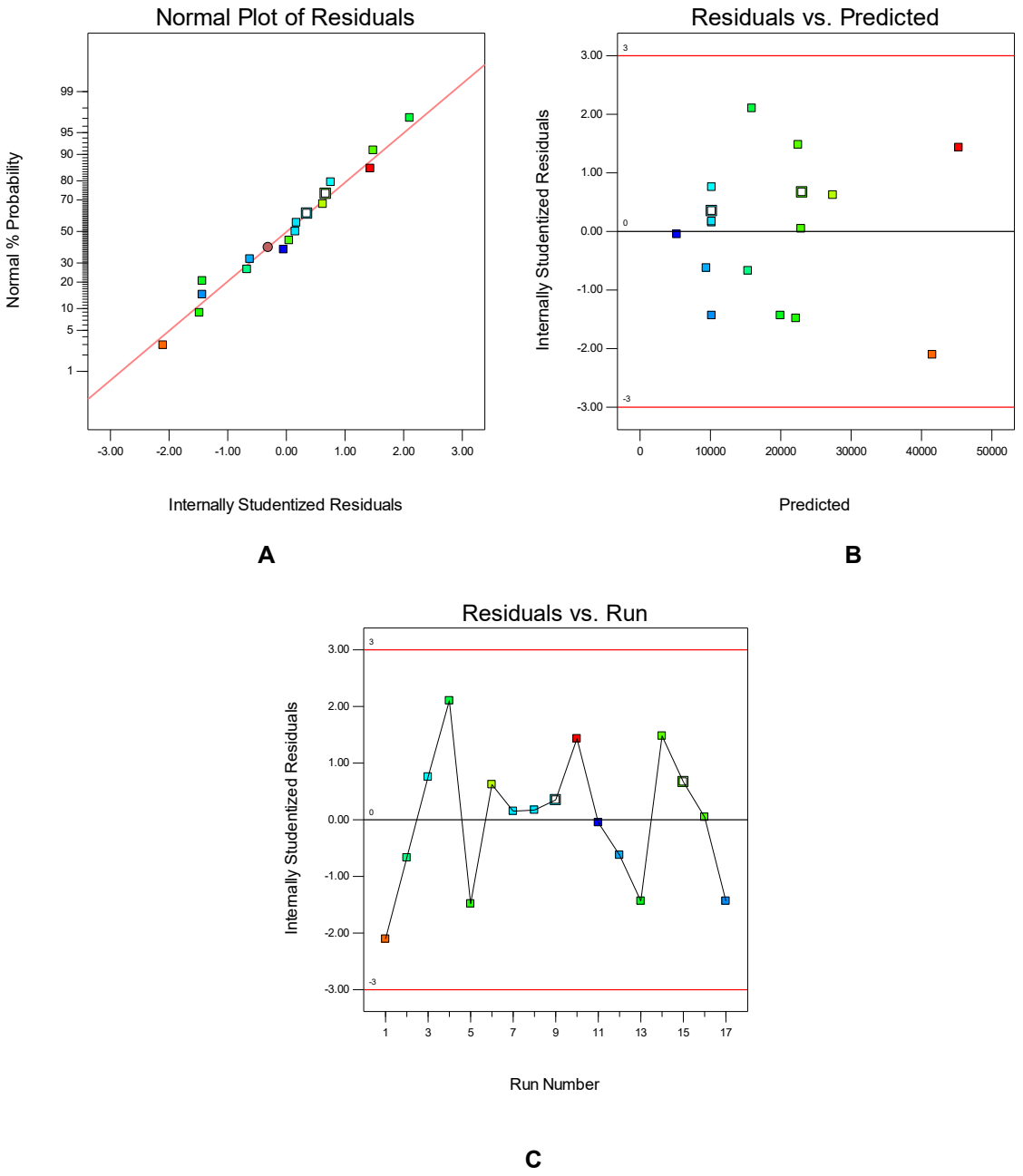

Fig. 1 Diagnostic plots for the adequacy of the proposed model: Normal Plot of Residuals (A) Residuals vs Predicted (B) Residuals vs Run (C)

Effects of independent variables on the EE

According to the statistical parameters in Table 3, all three independent factors were significant to the response (EE). Measured using the coefficient of regression, albumin extract concentration had the highest contribution (22.05%) on EE compared to NaTPP concentration (3.74%) and chitosan concentration (4.07%). A higher contribution of extract concentration should be considered for the encapsulation process to obtain high EE. The varying albumin extract concentration, significantly changes the EE. Additionally, the two-level interaction (AB, AC, BC) effect on the encapsulation process also provides useful information to know the interaction between two factors and its effect on the EE (Jarudilokkul et al. 2011). According to the statistical parameters on EE (Table 3), all factors were significant (p-value < 0.05). In addition, the interactions were also significant, except in NaTPP-chitosan concentration (p-value > 0.05). This result was in accordance with the low contribution of NaTPP-chitosan concentration (3.19%) to the EE. Meanwhile, the contribution of albumin extract-NaTPP concentration and albumin extract-chitosan concentration to the EE were similar: 14.46% and 14.92%, respectively. Moreover, the interaction between factors and the effects on the EE could also be observed through the interaction plot and contour plot. Fig. 2 shows the interaction plot among three independent factors. Clear intersection lines between two factors demonstrated significant interaction between factors. It can be observed that factors of extract-NaTPP and extract-chitosan had clear intersection lines, but not in chitosan-NaTTP. These results were in accordance with statistical analysis that all factors demonstrated significant interaction except chitosan-NaTPP.

The effect of extract and NaTPP concentration resulted in a remarkable EE at the lowest extract concentration (0.25 mg/mL) and the highest NaTPP concentration (15 mg/5 mL) as depicted in Fig. 3A. An extra amino group from the basic amino acids of the extract contributed to the interaction with its highly

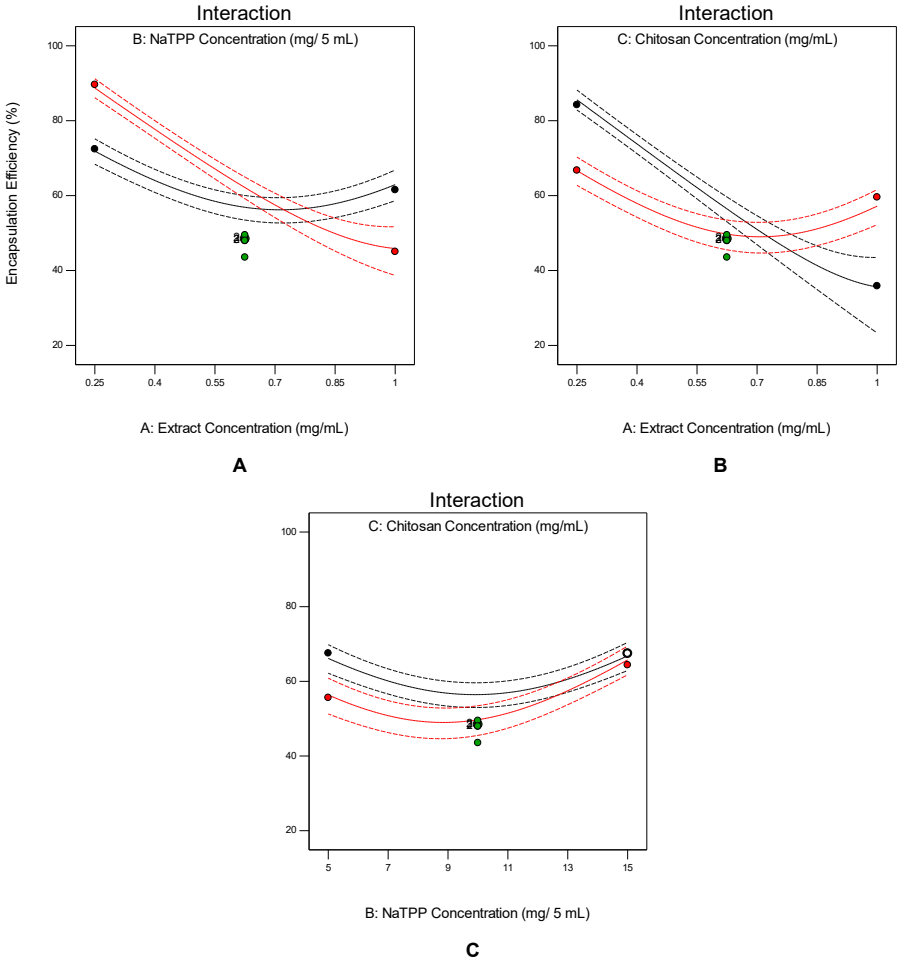

Fig. 2 Interaction plots among factors of extract-NaTPP (A), extract-chitosan (B), and chitosan-NaTPP (C)

electronegative phosphate groups of NaTPP in the system (Sacco et al. 2021). It suggested that a low concentration of albumin extract and a high concentration of NaTPP favored high EE. On the other hand, low concentrations of both albumin extract (0.25 mg/mL) and chitosan (0.5 mg/mL) demonstrated a high EE (Fig. 3B). This EE might be associated with the attraction of the acidic amino acids of extract with positively charged chitosan chains (Sacco et al. 2021). Moreover, the effect of NaTPP and chitosan provided high EE at the low chitosan concentration (0.5 mg/mL) and high NaTPP concentration (15 mg/5 mL), as shown in Fig. 3C. Based on the graph, the concentration of NaTPP and chitosan did not give a clear color gradation which means that these two factors did not contribute most to the EE. This condition was in accordance with the statistical result for interactions between B (NaTPP) and C (chitosan), and the interaction was not significant (p-value > 0.05).

Optimization and verification of the model

After performing statistical analysis of the model, optimization analysis was carried out using Derringer's design methodology. The algorithm searches for a combination of independent factors which are expected to give a high EE value as the response. In this research, two predicted combinations with a desirability value of 1 were chosen for optimization to obtain a high EE value, as follows: A = 0.250 mg/mL; B = 12.649 mg/5 mL; C = 0.504 mg/mL for optimization 1, and A = 0.253 mg/mL; B = 15 mg/5 mL; C = 0.701 mg/mL

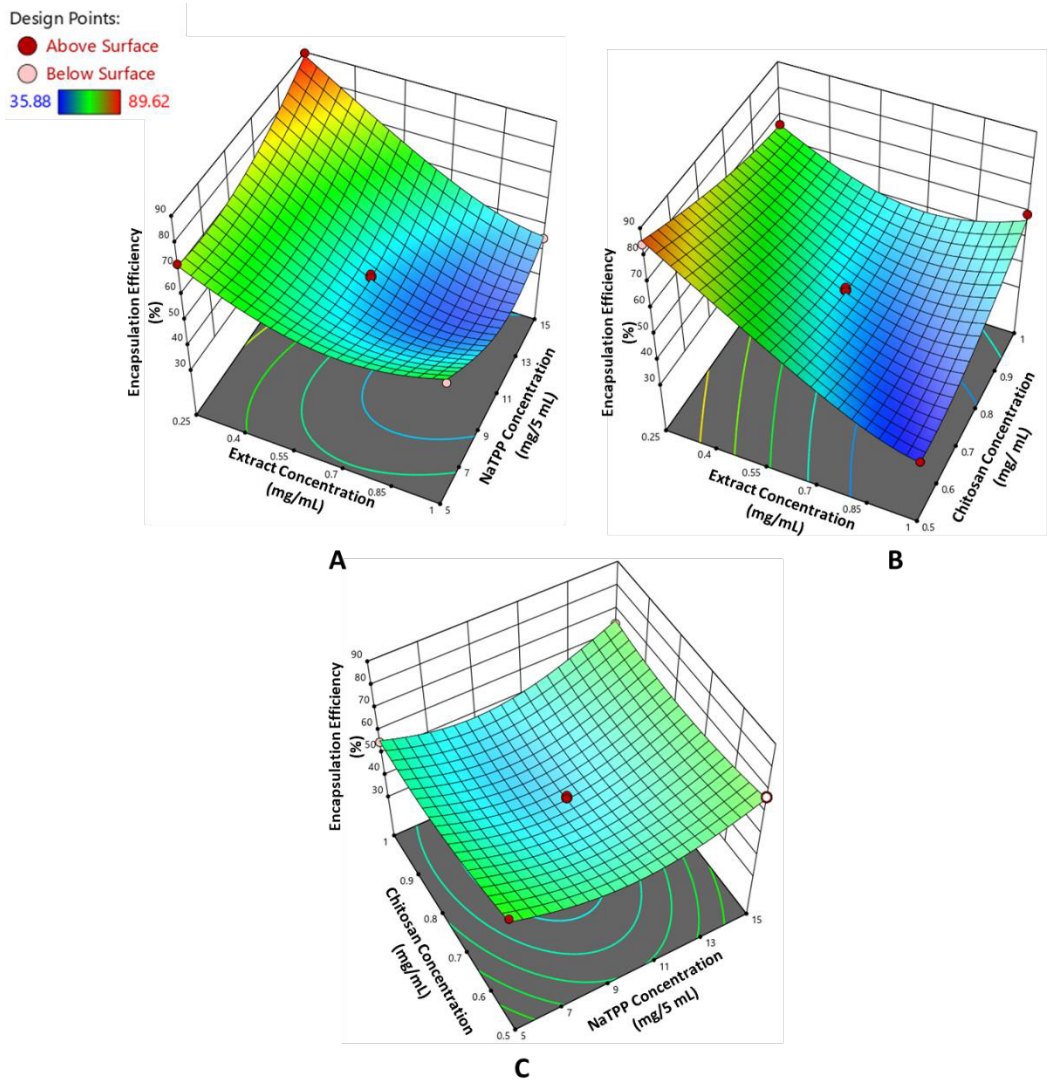

Fig. 3 Three-dimensional contour plot to investigate the effect of extract-NaTPP (A), extract-chitosan (B), and chitosan-NaTPP (C) on the EE

for optimization 2. Duplicate experiments were performed to verify and validate the optimized conditions, resulting in EE of 85.50% and 84.83% for optimization 1 and optimization 2, respectively, with the relative error between the predicted and experimental values being 5.60% (optimization 1) and 5.91% (optimization 2). The obtained verification value of EE was within 95% of the predicted value, which clearly indicated that the model fits the experimental data well and suggested that BBD incorporated with statistical analysis of desirability can be used for optimization factors affecting EE of albumin extract from *C. striata*. This result gives information that for optimum encapsulation of albumin, a low concentration of extract (0.25 mg/mL) is required. It also becomes one advantage, because in the development of a drug or active ingredient delivery systems, the use of a small amount of active ingredients with high stability and efficacy is wanted. In addition, it can be a piece of basic knowledge for further research, such as stability, time release, etc.

FTIR spectroscopy analysis

In this study, there were some differences observed in the FTIR spectra pattern among albumin extract without encapsulation, encapsulated albumin extract, and blank as depicted in Fig. 4. The FTIR spectra of albumin extract without the encapsulation process demonstrated more peaks than the blank and encapsulated samples. Broad peaks around 3063 cm^{-1} and 3274 cm^{-1} corresponded to the stretching vibration of hydroxyl (O-H) and N-H_2 group. The peak at 2930 cm^{-1} belongs to the stretching vibration of C-H_3, C-H_2, and C-H. The strong bands at 1631 cm^{-1} and 1540 cm^{-1} were correlated to the stretching vibration of amide I (C-O stretching vibration of the acetyl group) and vibration of amide II (N-H bending and stretching), respectively. Peaks at 1451, 1379, 1304, 1240, and 929 cm^{-1} corresponded to the bending vibration of C-H_3, C-H_2, and C-H. The peaks observed at 1078, 1067, and 1024 cm^{-1} were associated with the stretching vibration of C-O (Kalavathy et al. 2009; Shen et al. 2012; Said and Amin 2015).

The spectra of chitosan-NaTPP (blank) and encapsulated albumin extract in chitosan- NaTPP showed a similar pattern with some changes. The broad band around 3207 cm^{-1} was due to the stretching vibration of the O-H group overlapping with the amine group. Peaks at 2930 cm^{-1} and 2879 cm^{-1} were associated with the stretching vibration of C-H_3, C-H_2, and C-H groups. The vibration of amide I and amide II were observed at 1633 cm^{-1} and 1537 cm^{-1}, respectively. The peaks of amide I and amide II slightly shifted compared to the peaks from albumin extract without encapsulation. The vibration of C-O was observed as one

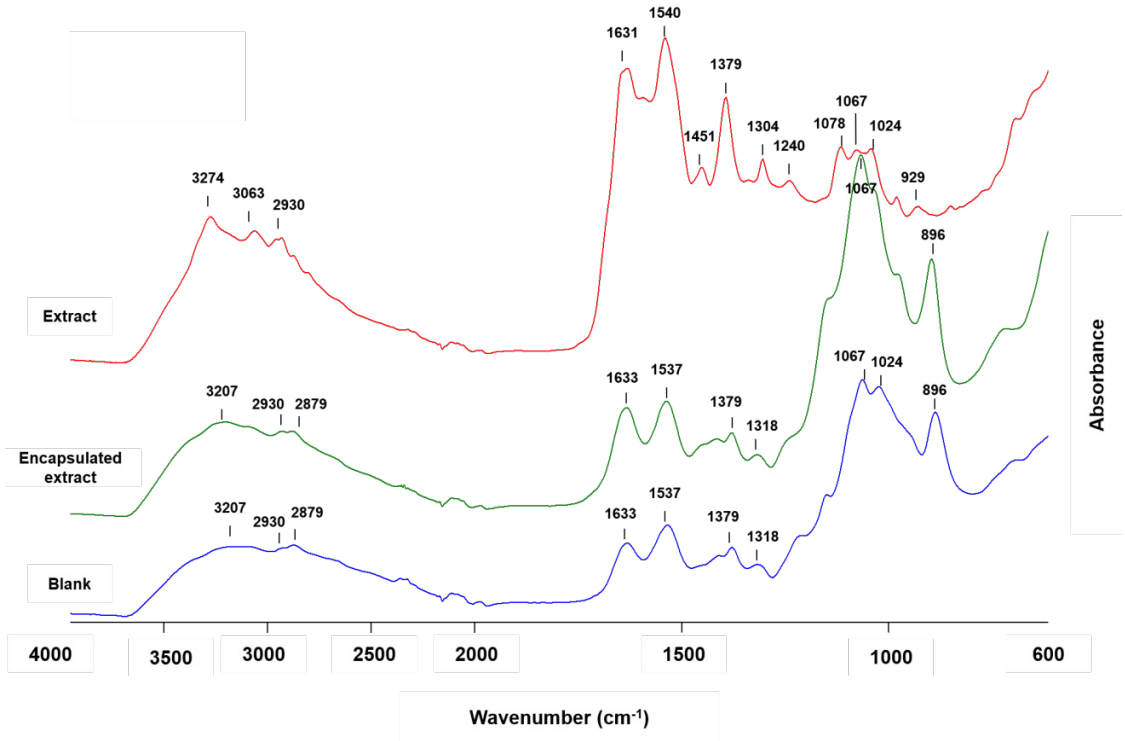

Fig. 4 FTIR spectra albumin extract, blank sample and encapsulated albumin extract

peak at 1067 cm^{-1} in the encapsulated extract whereas two peaks were found at 1067 cm^{-1} and 1024 cm^{-1} in the blank sample. It indicated that the interaction between sample and encapsulation materials (chitosan and NaTPP) changed FTIR spectra patterns. Non-encapsulated albumin extract spectra showed significant differences with encapsulated extracts, indicating successful encapsulation. Chitosan-NaTPP coating materials (chitosan-NaTPP) exhibited dominant FTIR spectra as the outer layer. A small wavenumber shift could be found in several peaks between samples. It might be caused by the difference matrix between blank, extract, and encapsulated extract. Moreover, the interaction between the extract and the encapsulated materials could also cause wavenumber shifts.

SEM analysis

SEM analysis was used to investigate the morphology of encapsulated albumin extract using chitosan and NaTPP. The results of characterization using SEM showed significant differences in morphological structures between blank (without albumin extract) and encapsulated albumin extract, as depicted in Fig. 5. In the blank sample, crosslinked chitosan–NaTPP membrane surfaces were more porous, like molecular chains (Fig. 5A). Open structures with more porosity possessed by blank could be attributed to higher chitosan chain mobility due to their lower crystallinity (Ferreira et al. 2007). It might be correlated with the fact that NaTPP restrains the movement of the molecular chain of chitosan due to the high crosslinking density preventing the reorganization of chitosan chains (Koukaras et al. 2012; Pedroso-Santana and Fleitas-Salazar 2020). On the other hand, the encapsulated albumin extract had a solid, rougher, and more compact surface compared to the blank sample. It suggested that the albumin extract was successfully bound by the crosslinking agent, chitosan–NaTPP (Fig. 5B).

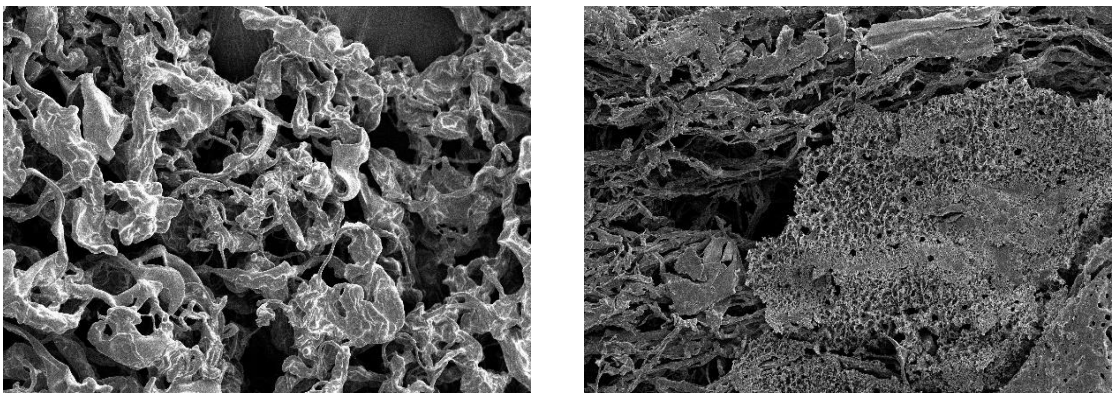

Fig. 5 SEM micrograph (x300, 50 µm) of blank (A) and encapsulated albumin extract (B)

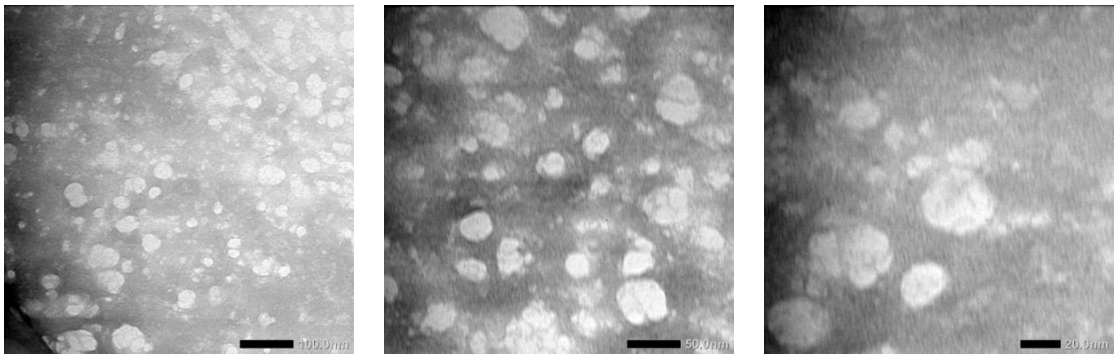

Fig. 6 TEM micrograph of encapsulated extract: micrograph of 100 nm (A), 50 nm (B), and 20 nm (C)

TEM analysis

TEM analysis confirmed the size of the encapsulated products, with microencapsulation producing microparticles with diameters ranging from 1 to 1000 μm and nanoencapsulation producing nanoparticles with diameters 10 to 1000 nm (Suganya and Anuradha 2017). In this study, the encapsulated product of albumin extract was obtained in the diameter range of 136.96 to 151.33 nm (Fig. 6). It demonstrated that this encapsulation method rendered a nanoparticle product (Rahmawanty et al. 2017). Therefore, the obtained encapsulated albumin extract was in nano size. It brings many advantages such as enhancing the transport and distribution of albumin extract to the target as well as to improve bioavailability of albumin extract in the body.

Another important parameter to measure particle size distribution is PDI (polydispersity index). PDI is a heterogeneity parameter used to describe the degree of non-uniformity of the particle size distribution. The value ranges from 0.0 (for samples that are highly uniform or monodisperse with respect to particle size) to 1.0 (for highly polydisperse or non-uniform samples with multiple particle size populations). PDI value > 0.7 indicates that the sample has a very wide particle size distribution and may not be suitable for analysis by dynamic light scattering (DLS) techniques (Rahmawanty et al. 2017). A value ≤ 0.2 is most often considered acceptable in practice for polymer-based nanoparticle materials (Danaei et al. 2018). In this study, the PDI value of the encapsulated product was between 0.28%-0.31% indicating that the encapsulated extract was highly uniform with respect to particle size.

Conclusion

Albumin extract from *C. striata* (snakehead) fish possesses benefits for human health. However, it is susceptible to degradation due to some conditions which could affect its bioavailability. In this study, encapsulation of snakehead fish albumin extract has been effectively performed to improve the stability and bioavailability through the encapsulated product. RSM of BBD was successfully used to optimize the encapsulation process of snakehead fish albumin extract, resulting in high EE of 85.50% at 0.25 mg/mL extract, 12.7 mg/5 mL NaTPP, and 0.5 mg/mL chitosan. Both actual and predicted values were found to coincide nearly, confirming that the estimation model was capable of predicting the dependent variables accurately. Characterization analysis revealed that nano-encapsulated albumin extract from *C. striata* could be used in food and pharmaceutical product applications.

Competing interests The authors declare that they have no competing interests.

Author contributions Endah Noviana Eka Lestari: Investigation, Data curation, Writing – Original draft, Anjar Windarsih: Conceptualization, Methodology, Supervision, Writing – Review & editing, Sunardi: Supervision, Writing – Review & editing, Khoirun Nisa: Supervision, Writing – Review & editing, Funding acquisition

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