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Response surface optimization of the forward extraction of jacalin from jackfruit seeds using AOT/isooctane reverse micellar system

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Abstract. Jacalin is the major protein contained in the crude extract of jackfruit (*Artocarpus heterophyllus*) seed that specifically recognizes and binds reversibly to galactose. Conventionally, purification of jacalin is carried out using the tedious and costly chromatographic techniques. In this study, extraction of jacalin from jackfruit seed crude extract were done using the sodium bis(2-ethylhexyl) sulfosuccinate (AOT)-based reverse micellar system. Reverse micellar extraction is an attractive alternative for downstream processing of various proteins. A successful reverse micellar extraction consists of two basic steps: forward and backward extraction. Forward extraction transfers a target protein from an aqueous solution into the reverse micellar solution, while backward extraction releases the protein from the reverse micelles structure into a new aqueous solution. The effects of the aqueous phase pH, NaCl concentration and AOT concentration on the forward extraction efficiency (FEE) are investigated using the response surface methodology (Box-Behnken Design). The main effects and interactions of the parameters are analyzed through the 3D surface plots. The optimum conditions for forward extraction were determined as follows: aqueous phase pH 4.58, 125 mM NaCl and 40 mM AOT. Under the optimal conditions, the FEE reached 88.04±1.30%, closer to 87.99% predicted by the model. The results indicated that AOT/isooctane reverse micelle system is effective in extracting jacalin from the jackfruit seed crude extract and verified the practicability of the BBD model for optimizing the main parameters in the forward extraction of jacalin.

Keywords: Optimization; Reverse micelles; Forward extraction; Jacalin; sodium bis(2-ethylhexyl) sulfosuccinate (AOT)

1. Introduction

Jacalin is a tetrameric two-chain lectin with the molecular weight of 65-66 kDa isolated from the jackfruit seed *(Artocarpus heterophyllus)* [1]. Jacalin has been widely investigated due its numerous biological properties, such as inhibition and binding with specific regions of HIV [2], ability to recognize particular immunoglobulin A1 from human serum [3], stimulation of different T cell functions [4,5] and specific binding with tumor associated Thomsen-Friedenreich (TF) antigen [6]. Conventional approaches to purify jacalin from the jackfruit seeds involved various chromatographic techniques, i.e. affinity chromatography on immobilized IgA-Sepharose column [4], Minileak-melibiose [3] or cross-linked guar gum matrix [7], ion-exchange chromatography [8] and partition chromatography on analytical C4 reversed-phase high performance liquid chromatography [9]. The chromatographic techniques are mostly preferred due to the ability to produce highly purified jacalin, however the methods are laborious, time-consuming and difficult to scale-up for industrial application.

Reverse micelles are thermodynamically stable, spontaneous aggregates formed when amphiphilic surfactant molecules were dissolved in organic solvent [10]. Their inner core contains nanometer-sized water pools that can solubilize biomolecules such as enzymes and proteins [11,12]. Extraction by reverse micelles or reverse micellar extraction (RME) has been one of the most reliable alternative method for downstream processing of various types of proteins. Compared to the conventional purification method of jacalin, RME has many attractive features, including large interfacial area, less energy requirement, continuous mode of operation, low-cost and easy to scale-up [10]. A reverse micellar extraction can be conducted by phase transfer method consisting of two basic steps, forward and backward extraction. Forward extraction transferred a target protein contained in an aqueous solution into a reverse micellar solution while backward extraction released the protein from the reverse micelles structure into a new aqueous solution [13].

The efficiency of the reverse micellar system to selectively transfer target protein from its mixture was controlled by several parameters, such as concentration and type of surfactant, aqueous phase pH, concentration and type of salt, water content, size of reverse micelles, temperature and many more [14,15]. Hence, optimization of the process parameters is critical in maximizing the extraction efficiency of the target protein in reverse micellar system. Response surface methodology (RSM) is a collection of statistical methods based on multivariate nonlinear model that has been extensively applied in various fields of research to determine the effects of several variables and to find the optimum process conditions for the selected process [15,16]. RSM has been applied recently to optimize variables affecting the extraction efficiency of *Phaseolus vulgaris* lectin in AOT/isooctane reverse micellar system [17]. However, until now, there has been no such report focusing on the screening and optimization of the process parameters affecting the forward extraction of jacalin from the crude extract of jackfruit seeds.

Therefore, this study was aimed to maximize the forward extraction efficiency and optimize the forward extraction conditions of jacalin isolated from the jackfruit seed crude extract by response surface methodology (RSM). To do that, a Box-Behnken design (BBD) is implemented and a second order model is developed to predict and optimize the desired response.

2. Methods

2.1. Sample collection and preparation of jackfruit seed crude extract

Jackfruits (*Artocarpus heterophyllus* Lam.) used in this study were of local variety known as Mastura, bought from a fruit market in Kuantan, Pahang, Malaysia. Jackfruit seeds were manually separated

from the ripe flesh, washed, and thinly sliced before dried in an oven at 37°C for about 24 h. The dried seeds were ground into powder using a mixer-grinder and passed through an 80-mesh sieve. The jackfruit seed crude extract was prepared by mixing the fine jackfruit seed powder with 10 mM phosphate-buffered saline (PBS) containing 0.15 M NaCl (1:10 w/v, pH 7.4) and gently stirred at 4°C for 24 h. After 20 min centrifugation at 10000 rpm, the clear supernatant was collected, filtered through a 0.45 µm membrane and used in the next step as the crude extract.

2.2. Forward extraction procedure

Forward extraction was carried out using the phase transfer method in screw-capped centrifuge tubes. In this study, 5 ml of organic (AOT dissolved in isooctane) and 5 ml of aqueous solutions (dilutions of crude extract in buffer solution) was slowly mixed with a magnetic stirrer at 300 rpm for 20 min in room temperature. After mixing, the solution was centrifuged at 5000 rpm for 10 min to achieve a clear phase separation between two phases. Both the organic and aqueous phases were collected and the protein concentrations in each phase were measured.

2.3. Optimization of forward extraction by RSM

A response surface methodology (RSM) based on a Box-Behnken design (BBD) was applied to study the optimum conditions for forward extraction of jacalin using AOT reverse micellar system. Based on an one factor at a time (OFAT) study and a factorial analysis [18,19], the aqueous phase pH (pH 4.2 – pH 5.2), AOT concentration (10 – 40 mM) and NaCl concentration (100 – 150 mM) were examined in three different levels (-1, 0 and +1) as shown in Table 1.

Table 1. Independent variables and their respective levels.

Independent variables	Symbols	Coded Factor Levels		
		−1	0	+1
pH of the aqueous phase	A	4.2	4.7	5.2
AOT concentration (mM)	B	10	25	40
NaCl concentration (mM)	C	100	125	150

A total of 17 experiments including 5 repetitions of the center points were conducted in triplicates. All experimental results expressed as mean ± standard deviation was used in the data analysis. The experimental design matrix, and data analysis for optimization procedure were performed using the obtained using the Design Expert Version 10 software® (Version 10.0.1.0 64-bit Stat-Ease Inc., Minneapolis, USA). Mean values were considered significantly different when $p<0.05$. The effects of these independent variables on the response, Y can be predicted using a polynomial regression model of second order (Equation 1) as follows:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i<i=1}^{3} \beta_{ii} X_i X_j \quad (1)$$

where Y is the response calculated by the model, β_0, β_i, β_{ii} and β_{ij} are the coefficients of intercept, linear, quadratic and interaction terms, respectively, while X_i and X_j indicated the independent variables.

2.4. Analysis of jacalin concentration

Jacalin concentration in both feed and aqueous phase after forward extraction were quantified using Lowry assay with bovine serum albumin (BSA) as a standard [20]. Mass balance was used to estimate the amount of jacalin in the organic phase. The samples were analyzed against respective blanks performed with an aqueous phase voided of crude extract. Jacalin concentrations were expressed in

mg/ml. Average values of duplicate readings of jacalin concentrations were used to estimate the efficiency of forward extraction (FEE) using the following Equation (2).

$$FEE(\%) = \frac{C_{org}}{C_0} \times 100 \quad (2)$$

where C_{org} is the concentration of protein in the organic phase after forward extraction and C_0 is the initial concentration of protein in the aqueous phase before forward extraction was performed.

3. Results and Discussion

3.1. Statistical analysis and model fitting

The effects of three forward extraction parameters: aqueous phase pH (A), AOT concentration (B) and NaCl concentration (C) on the forward extraction efficiency (FEE) of jacalin were studied using a Box-Behnken design of experiment. Table 2 shows the experimental matrix design of the total 17 runs, along with the experimental and predicted results obtained.

Table 2. Design matrix and experimental responses of the BBD.

Run No.	Independent parameters			Response (Y)	
	Actual values			Forward extraction efficiency (%)	
	A	*B*	*C*	Experimental (Y_e)	Predicted (Y_p)
1	5.2	25	100	44.44 ± 0.16	43.79
2	5.2	25	150	41.67 ± 0.22	44.12
3	4.2	40	125	75.41 ± 0.24	76.14
4	4.7	25	125	83.03 ± 0.11	81.76
5	4.2	25	100	73.14 ± 0.12	70.70
6	4.2	25	150	70.44 ± 0.22	71.09
7	4.7	25	125	84.16 ± 0.23	81.76
8	5.2	40	125	57.46 ± 0.11	56.39
9	4.7	40	100	81.77 ± 0.91	83.49
10	4.7	25	125	82.24 ± 2.28	81.76
11	4.7	10	100	74.65 ± 0.23	76.03
12	4.7	25	125	80.21 ± 0.34	81.76
13	4.7	40	150	83.89 ± 0.11	82.51
14	4.7	10	150	79.44 ± 0.34	77.73
15	4.7	25	125	79.18 ± 0.80	81.76
16	5.2	10	125	43.81 ± 0.35	43.08
17	4.2	10	125	76.15 ± 0.22	77.22

The experimental results (FEE) obtained from the BBD were analyzed and fitted to the following second-order polynomial equation (Equation 3):

$$Y = 81.76 - 13.47A + 3.06B + 0.18C + 3.60AB - 0.014AC - 0.67BC - 20.54A^2 + 1.98B^2 - 3.80C^2 \quad (3)$$

In this study, the parameters influencing the response of forward extraction of jacalin are in the following order: aqueous phase pH > AOT concentration > NaCl concentration. Furthermore, the lack of fit value of 0.2471 implied that the lack of fit was not significantly correlated with the pure error, hence further validates the model adequacy for predicting the forward extraction efficiency of jacalin.

The coefficient of variation (CV) of the model, defined as the ratio of the standard error of estimate to the mean value of observed response, was calculated as 3.46 %. Generally, a model can be considered reasonably reproducible if its CV is not greater than 10 %. The Adeq. Precision of the test calculated (21.40) was higher than the desired value (4.00), indicating low signal-to-noise ratio and the model can be used to navigate design space. These results proved that the developed polynomial model could adequately describe the real relationship among the parameters chosen and therefore, can be used for the subsequent process optimization.

3.2. Effect of parameters and the interaction between parameters on FEE of jacalin

Figure 1(a) shows predicted values of FEE by varying pH of the aqueous phase (A) and AOT concentration (B), while fixing the NaCl concentration (C) at 125 mM. At all levels of AOT concentration, a significant quadratic effect of pH was observed. Initially, the FEE was found to increase with the increasing pH of the aqueous phase pH until the FEE reached maximum value at pH 4.7. However, further increasing of the aqueous phase pH beyond pH 4.7 resulted in the decreasing of the FEE. Similar results of increase in extraction efficiency with the increase in pH were also observed by He et al. [17] while conducting optimization of forward extraction of lectin from Phaseolus vulgaris [17] using the similar AOT reverse micellar system. This phenomenon may be due to the strong electrostatic interaction in AOT/isooctane reverse micelles, which only solubilizes the target protein when the pH of the aqueous phase was below the isoelectric point (pI) of the target protein [21]. According to He et al. [22], pH values above the pI of the proteins might lead to a decrease in charge density and further yield loss of the proteins.

Figure 1(b) demonstrated that the both aqueous phase pH (A) and NaCl concentration (C) had significant quadratic effects on the FEE, while the AOT concentration (B) was fixed at 25 mM. Overall, at all levels of NaCl concentration, the FEE observed at the lowest pH value (pH 4.2) was higher than the FEE observed at the highest pH value (pH 5.2). Initially, increasing of aqueous phase pH value from pH 4.2 until pH 4.7, resulted in slight increase of FEE until a maximum FEE was found at 125 mM NaCl. These results suggested that the favorable interaction between jacalin and surfactant head groups was enhanced through the addition of NaCl, known as the salting-in effect. However, an opposite trend could be observed with further increase of the NaCl concentration and pH up to the maximum value at pH 5.2. It may have occurred due to the reduction of electrostatic repulsion between charged head groups of the AOT surfactants in high NaCl concentration, thereby decreasing the size of reverse micelles. Consequently, larger protein molecules might be excluded at a higher NaCl concentration, due to the Debye length reduction and size exclusion effect [22].

Figure 1(c) shows the interactive effects of the AOT concentration (B) and the NaCl concentration (C) on the FEE. At all levels of NaCl concentration, the FEE was found to increase with an increasing of AOT concentration Similar trend of increasing extraction efficiency with increasing surfactant concentration was also observed in extraction and purification of 7s globulin subunits from soybean protein [23], probably due to the increase number of reverse micelles and the corresponding size of reverse micelles, as there were more water cores in which jacalin could be solubilized [24].

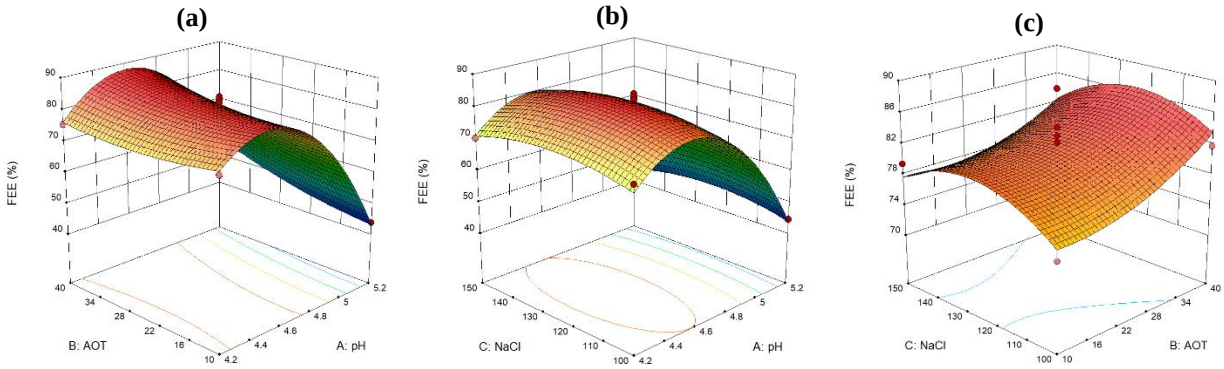

Figure 1. Response surface curves showing the effects of aqueous phase pH (A), AOT concentration (B) and NaCl concentration (C) on the forward extraction efficiency of jacalin.

3.3. Verification of optimum condition

The validation of the regression model equation for predicting the optimum response values was tested by conducting three confirmation experiments using the recommended optimum conditions obtained from the numerical RSM optimization approach. The optimum process conditions for aqueous phase pH (A), AOT concentration (B) and NaCl concentration (C) were pH 4.58, 40 mM and 125 mM, respectively (Table 3). Under these optimum process conditions, the experimental response (%FEE) obtained was 88.04±1.30%, which is closely related to the predicted FEE of 87.99%.

Table 3. Verification of optimum forward extraction condition.

Parameter	Optimum process conditions		Predicated response (% FEE)	Actual response (% FEE)	Deviation (%)
	Coded	Uncoded			
Aqueous phase pH	-0.241	pH 4.58			
AOT concentration	1.000	40 mM	87.99	88.04 ± 1.30	0.06
NaCl concentration	0.024	125 mM			

4. Conclusion

BBD-based RSM was successfully used to optimize the conditions of forward extraction of jacalin using AOT reverse micelles. The developed model for FEE exhibited non-significant lack of fit and R2 value of 0.9879. The surface graphs indicated that maximum forward extraction efficiency could be obtained by extracting jacalin with aqueous phase pH of 4.58, containing 125 mM NaCl and AOT concentration of 40 mM. Under these optimum conditions, the forward extraction efficiency observed was 88.04 ± 1.30%, slightly higher than the predicted value of 87.99%. From these experimental results, it can be concluded that extraction using AOT reverse micelles were effective to extract jacalin from the crude extract of jackfruit seed powder and that the regression model equation was suitable and adequate for predicting the optimum response values in the forward extraction process.

References

[1] Kabir S 1998 Jacalin: A jackfruit (*Artocarpus heterophyllus*) seed-derived lectin of versatile applications in immunobiological research *J. Immunol. Methods* **212** 193–211

[2] Corbeau P, Pasquali J-L and Devaux C 1995 Jacalin, a lectin interacting with O-linked sugars and mediating protection of $CD4^+$ cells against HIV-1, binds to the external envelope

glycoprotein gp120. *Immunol. Lett.* **47** 141–143
[3] Hagiwara K, Collet-Cassart D, Kunihiko K and Vaerman J-P 1988 Jacalin: Isolation, Characterization, and Influence of Various Factors on Its Interaction with Human IgA1, As Assessed by Precipitation and Latex Agglutination. *Mol. Immunol.* **25** 69–83
[4] Bunn-Moreno MM and Campos-Neto A 1981 Lectin(s) extracted from seeds of *Artocarpus integrifolia* (jackfruit): potent and selective stimulator(s) of distinct human T and B cell functions. *J. Immunol.* **127** 427–429
[5] Lafont V, Hivroz C, Carayon P, Dornand J and Favero J 1997 The lectin jacalin specifically triggers cell signaling in $CD4^+$ T lymphocytes. *Cell Immunol.* **29** 23–29
[6] Mahanta SK, Sanker S, Rao N V, Swamy MJ and Surolia A 1992 Primary structure of a Thomsen-Friedenreich-antigen-specific lectin, jacalin [*Artocarpus integrifolia* (jackfruit) agglutinin]. Evidence for the presence of an internal repeat. *Biochem. J.* **284** 95–101
[7] Roy I, Sardar M and Gupta MN 2005 Cross-linked alginate-guar gum beads as fluidized bed affinity media for purification of jacalin. *Biochem. Eng. J.* **23** 193–198
[8] Ruffet E, Paquet N, Frutiger S, Hughes GJ and Jaton J-C 1992 Structural and electron-microscopic studies of jacalin from jackfruit (*Artocarpus integrifolia*) show that this lectin is a 65 kDa tetramer. *Biochem. J.* **286** 131–134
[9] Young NM, Johnston RAZ and Watson DC 1991 The amino acid sequences of jacalin and the *Maclura pomifera* agglutinin. *FEBS Lett.* **282** 382–384
[10] Gangadharappa BS, Dammalli M, Rajashekarappa S, Pandurangappa KMT and Siddaiah GB 2017 Reverse micelles as a bioseparation tool for enzymes. *J. Proteins Proteomics* **8** 105–120
[11] Murugesan S, Iyyaswami R, Kumar SV, Surendran A and Vijay S 2017 Anionic surfactant based reverse micellar extraction of l-asparaginase synthesized by *Azotobacter vinelandii*. *Bioprocess Biosyst. Eng.* **40** 1163–1171
[12] Hemavathi AB, Hebbar HU and Raghavarao KSMS 2012 Reverse micellar extraction of bioactive compounds for food products. In: Libovka N, Vorobiev E, Chemat F (eds) *Enhancing Extraction Processing Food Industries* CRC Press, pp 399–436
[13] Leser ME, Wei G, Luisi PL and Maestro M 1986 Application of reverse micelles for the extraction of proteins. *Biochem. Biophys. Res. Commun.* **135** 629–635
[14] Li S and Cao X 2014 Extraction of tea polysaccharides (TPS) using anionic reverse micellar system. *Sep. Purif. Technol.* **122** 306–314
[15] Prabhu AA, Chityala S, Garg Y and Dasu VV 2017 Reverse micellar extraction of papain with cationic detergent based system: An optimization approach. *Prep. Biochem. Biotechnol.* **47** 236-244
[16] Chuo SC, Ahmad A, Mohd-Setapar SH, Mohamed SF and Rafatullah M 2018 Utilization of green sophorolipids biosurfactant in reverse micelle extraction of antibiotics: Kinetic and mass transfer studies. *J Mol. Liq.* **276** 225–232
[17] He S, Shi J, Walid E, Zhang H, Ma Y and Xue SJ 2015 Reverse micellar extraction of lectin from black turtle bean (*Phaseolus vulgaris*): Optimisation of extraction conditions by response surface methodology. *Food Chem.* **166** 93–100
[18] Sy Mohamad SF, Mohd Said F, Abdul Munaim MS and Mohamad S 2019 Effect of key parameters on jacalin extraction from aqueous phase into anionic reverse micelles. *Indones. J. Chem.* **19(3)** 737–746
[19] Sy Mohamad SF, Mohd Said F, Abdul Munaim MS, Mohamad S and Wan Sulaiman WMA 2018 Fractional factorial analysis for identifying significant factors in extraction of jacalin from jackfruit seeds using anionic reverse micellar system. *Int. Conf. in Chemical Science and Engineering (ICCSE)* 7-8 Nov. Melaka, Malaysia.
[20] Lowry OH, Rosebrough NJ, Farr AL and Randall RJ 1951 Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193** 265–275
[21] Nandini KE and Rastogi NK 2010 Single step purification of lactoperoxidase from whey

involving reverse micelles-assisted extraction and its comparison with reverse micellar extraction. *Biotechnol. Prog.* **26** 763–771
[22] He S, Shi J, Walid E, Ma Y and Xue SJ 2013 Extraction and purification of a lectin from small black kidney bean (*Phaseolus vulgaris*) using a reversed micellar system. *Process Biochem.* **48** 746–752
[23] Zhao X, Zhang X, Liu H and Sun H 2018 Optimization of AOT reversed micelle forward extraction of 7S globulin subunits from soybean proteins. *J. Food Sci. Technol.* **55** 4909–4917
[24] Pawar SS, Iyyaswami R and Belur PD 2017 Reverse micellar extraction of lactoferrin from its synthetic solution using CTAB/n-heptanol system. *J. Food Sci. Technol.* **54** 3630–3639

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