Optimization of enzymatic hydrolysis condition of edible bird's nest crumbs by response surface methodology and determination of biochemical characteristics of the hydrolysate

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ARTICLE INFO

Research Paper

Received: September 08, 2022
Revised: December 01, 2022
Accepted: December 30, 2022

Keywords

Degree of hydrolysis
Edible bird's nest crumbs
Enzymatic hydrolysis
Optimization
Protease

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ABSTRACT

Edible bird's nest (EBN) crumbs are the by-product of the bird's nest industry. Despite having lower economic value compared to the original material, EBN crumbs still maintain high protein and carbohydrate content. Therefore, this study aimed to determine the optimal hydrolysis condition for EBN crumbs using protease to achieve the maximum degree of hydrolysis (DH). Plackett Burman design was employed to identify the important factors. The results showed that enzyme loading, temperature and hydrolysis time had the strongest effect on the DH. These factors were subsequently subjected to the optimization study using central composite design (CCD) of response surface methodology (RSM). The optimized conditions for the enzymatic hydrolysis of EBN crumbs were at an enzyme loading of 4%, temperature of 51°C, and hydrolysis time of 90 min. The experimental DH obtained at the optimized condition (63.5%) was close to the predicted DH (64.1%). The enzymatic hydrolysate prepared at the optimal condition showed relatively high amino acid concentration (151.6 ± 1.29 µg/mL) and radical scavenging activity (64.97 ± 0.79%) compared to the boiled sample with values of only 50.1 ± 2.43 µg/mL and 18.36 ± 0.17%, respectively. The resultant hydrolysate had no effect on some of the microorganisms employed in this study. The EBN crumbs hydrolysate inhibited tyrosinase activity with an IC_{50} of 70.22 µg/mL, greater than that of boiled EBN (IC_{50} = 108.9 µg/mL). The results indicated that the EBN crumbs hydrolysate could be further applied in the cosmetic industry as a rich source of nutrients and bioactive compounds for the formulation of beauty products.

Cited as: Truong, H. P. T., Vo, T. T. Q., Tran, H. T. T., Vo, T. S., Luong, H. T., & Le, T. P. (2023). Optimization of enzymatic hydrolysis condition of edible bird's nest crumbs by response surface methodology and determination of biochemical characteristics of the hydrolysate. *The Journal of Agriculture and Development* 22(3), 19-30.

1. Introduction

Edible bird's nest (EBN) is made from the saliva of swiftlet species (*Aerodromus fuciphagus*). It is widely consumed as a health food due to its high beneficial effects on human health and has been considered to be one of the most precious food items in China for thousands of years. The main compositions of EBN are protein (62 - 63%), carbohydrate (25.6 - 27.8%), low amount of lipid (0.14 - 6.28%) and ash (2.1%), (Marcone, 2005). EBN contains 18 amino acids, the most abundant amino acids in the nest are valine, threonine, glutamic acid, aspartic acid, and proline (Ali et al., 2019). It has been used in traditional Chinese medicine as a treatment for malnutrition, a boost to the immune system as well as enhancement to the metabolism and skin complexion (Ma

& Liu, 2012).

In swiftlet farming, EBN crumbs are small fragments obtained from the collecting and processing of EBNs, which are considered as by-products with low economic value. However, several studies have shown that EBN crumbs maintain the important nutrients found in the original material. According to Zainab et al. (2015), EBN by-products contain high levels of protein (47%) and carbohydrates (2.4%). Chan et al. (2015) reported that the free from of N-Acetylneuraminic Acid (NANA) which was found in EBN was proposed to be one of the major compounds responsible for the skin lightening function. Bioactivities of EBN hydrolysates were repoted previously (Ma & Liu, 2012). Enzymatic hydrolysis of proteins released small peptides and free amino acid, leading to an increased nutritional value for food proteins (Khushairay et al., 2014). Many studies mentioned that the enzymatic hydrolysis of EBN results in an increase in amino acids content as well as antioxidant activity (Muhammad et al., 2017; Ali et al., 2019).

In this study, the enzymatic hydrolysis of EBN crumbs was carried out using protease to increase protein solubility and thus maximize its functionality and bioactivity. Response surface methodology (RSM), is employed to optimize the hydrolysis conditions including enzyme loading, hydrolysis temperature, and time (Bezerra et al., 2008). The biological activities including antimicrobial and antioxidant activity, tyrosinase inhibition of the hydrolysate obtained at optimized conditions were examined. In turn, the data will open up new opportunities for applying the EBN hydrolysate in cosmetic industry and other sectors, thus improving the economic value of EBN by-products.

2. Materials and Methods

2.1. Materials

Impurified EBN crumbs were purchased from swiftlets houses in Binh Phuoc province. The raw material was stored in airtight container at room temperature until further analysis. Protease Alcalase® 2.4 L FG (Unit activity 2.4 AU-A/g) used for enzymatic hydrolysis in this study was purchased from Novo Industry (Denmark).

2.2. Sample preparation

The EBN crumbs were prepared according to the method by Amiza et al. (2019a) with some modifications. Prior to hydrolysis, the original EBN crumbs were swollen by soaking in distilled water, then the remaining impurities (dust, feathers, guano,...) were removed manually by the tweezer. Finally, the purified samples were stored at 4 - 8°C until further use.

2.3. Proximate analysis of the raw material

Proximate analysis including crude protein (TCVN 10034:2013), carbonhydrates (AOAC 986.25 mod), ash (EVN-R-RD-2-TP-3496), fat (EVN-R-RD-2-TP-3498), total acid amin (AOAC 994.12) and moisture (EVN-R-RD-2-TP-3496) content of raw purified EBN crumbs. Samples were analyzed and quantified by Eurofins Sac Ky Hai Dang.

2.4. Enzymatic hydrolysis of EBN crumbs

The enzymatic hydrolysis was performed according to the procedure described by Amiza et al. (2019a) with slight modifications.

Purified EBN crumbs were soaked in distilled water at a ratio of 1:100 and then boiled at 100°C for 30 min. After that, the samples were cooled down to room temperature and adjusted to suitable pH and temperature prior to adding protease. The enzymatic hydrolysis was conducted using a magnetic stirrer at specific pH, temperature, time, enzyme loading, and stirring speed. Double-boiled EBN crumbs without any enzyme treatment served as a control experiment in this study. Following the hydrolysis process, the reaction was stopped by inactivating enzyme at 90°C for 10 min in the water bath. The hydrolysate was then centrifuged at 4000 rpm for 10 min. Finally, the supernatant was filtered using filter papers, and the filtrate was stored at 4 - 8°C for further analysis.

2.5. Experimental design

2.5.1. Determination of main factors affecting the enzymatic hydrolysis of EBN crumbs using Plackett Burman Design

In Plackett-Burman design (PB), the independent variables were enzyme loading (X_1: 1%, 5%),

temperature (X_2: 36°C, 60°C), hydrolysis time (X_3: 60 min, 90 min), pH (X_4: 5, 8) and stirring speed (X_5:600 rpm, 1800 rpm), which were employed at two-level (-1 and +1). These variables as well as their ranges were chosen based on the previous published research (Din, 2020). A total of 12 runs were employed as automatically suggested by Design Expert software (Stat-Ease, Inc). Degree of hydrolysis (DH) was set as a response variable. EBN crumbs were hydrolysed under conditions arranged by PB matrix. Data were then analysed by the Design Expert software to determine significant factors for further optimization experiment.

2.5.2. Experimental design for optimization study

In optimization study, a three-level face-centered central composite design (CCD) was used to examine the effect of significant factors on the DH. The independent variables were enzyme concentration (X_1), hydrolysis time (X_2), and temperature (X_3), which were examined at three levels (-1, 0, -1) as shown in Table 1. The DH served as a dependent variable. The ranges of the independent variables were referred from the other previous research (Khushairay et al., 2014; Din, 2020). A total of 20 runs of EBN crumbs enzymatic hydrolysis (including six replicates at the central point) were employed as suggested by Design Expert software (version 11). The EBN crumbs were hydrolysed under conditions arranged by CCD matrix.

2.5.3. Verification of model

To validate the model, three replications of the EBN crumbs hydrolysis were carried out at the predicted optimum condition. The DH of hydrolysate resulted from the enzymatic hydrolysis were determined. One-sample t-test was then performed to compare experimental values and predicted values of DH given by RSM.

2.6. Determination of degree of hydrolysis (DH)

Degree of hydrolysis was determined using trichloroacetic acid (TCA) method according to Morais et al. (2013). The DH was identified by the percentage of soluble protein in TCA 10% in relation to the total protein content of the sample.

Table 1. Experimental design of central composite design

Independent variables	Symbol	Range and levels				
		$-\alpha$	- 1	0	1	$+\alpha$
Enzyme concentration (%)	X_1	2.3	3	4	5	5.7
Hydrolysis time (min)	X_2	50	60	75	90	100
Temperature (°C)	X_3	33.2	40	50	60	66.8

Briefly, 2 mL of hydrolysate aliquot was mixed with 2 mL of TCA 20% solution to obtain the soluble and insoluble fractions in TCA 10%. After 30 min, the mixture was centrifuged at 4000 rpm and the soluble protein content of the supernatant was determined by the Lowry (1951) method and the result was expressed as mg of protein. Bovine Serum Albumin (BSA) was used as the standard. The DH was calculated as follows:

$$\%DH = \frac{Solubleproteincontentin TCA10\%(mg)}{Totalproteincontent(mg)} \times 100$$

2.7. Determination of antioxiant activities

The radical scavenging activity of different EBN crumbs samples was determined using the DPPH assay according to Brand-William et al. (1995) with some modifications.

The EBN crumbs hydrolysates (2 mL) were added to 1 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma, St. Louis, USA) 0.1 mM. The mixture was left for 30 min at room temperature in the darkness. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured using a spectrophotometer at 517 nM. Three mL of DPPH was taken as the negative control and Ascorbic acid at concentration of 5 µg/mL was used as the positive control. The EC_{50} value, the concentration of of different EBN crumbs that could decrease the concentration of DPPH by 50% was then determined. Free radical scavenging activity (RSA) was expressed as inhibition percentage and was calculated using the following formula:

$$\%RSA = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Where:

Abs control: the absorbance of DPPH + Ethanol

Abs sample: the absorbance of DPPH + sample

2.8. Investigation of tyrosinase inhibition ability of EBN hydrolysate

Tyrosinase converts L-tyrosine into an orangered DOPA chrome with a 475 nM absorbance. Samples added to the buffer containing enzymes affect the enzyme activity. The decrease in L-tyrosine metabolism is accompanied by a decrease in optical absorption intensity. The percentage inhibition and IC_{50} values of the samples were calculated from the absorbance measurement at different concentrations.

Investigation of tyrosinase inhibition ability of EBN hydrolysate according to Vanitha & Soundhari (2017) with some modifications. Enzyme tyrosinase from mushroom T3824-25KU (enzyme activity ≥ 1000 unit/mg solid) was provided by Sigma chemical. Pipetting out 100 µL of 100 U/mL tyrosinase into test tubes containing 1.9 mL of the samples and incubate for 30 min at 30°C. Add 1000 µL of 2 mM tyrosine to the mixture, then incubate for 7 min at 30°C. The optical density of the mixture was measured at 475 nM. The positive control is kojic acid. Negative control is prepared by mixing the sample with 100 µL of 0.1 mM phosphate buffer (pH 6.8) without an addition of tyrosinase. Each experiment was carried out in triplicate. An IC_{50} value was used to estimate enzyme inhibition of the sample. The effectiveness of tyrosinase inhibition increases with decreasing IC_{50} values. The percentage of inhibition (I %) was calculated by the following equation:

$$I(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where:

$A_{control}$: OD value of the solution without the test sample.

A_{sample}: OD value of the solution in the presence of the test sample.

The IC_{50} value (µM) is the concentration of a test sample at which the sample can inhibit the activity of the tyrosinase by 50%. This value was calculated based on the equation y = aln (x) + b (y is the percentage inhibition I% and x is the sample concentration) with two values a and b derived from the graph. Substituting y = 50 into the equation we get the value of x.

3. Results and Discussion

3.1. Proximate analysis result

The quantity of protein, lipid and carbohydrate in raw EBN was shown in Table 2. The nutritional value of raw EBN was lower than those found in some regions in Malaysia and Indonesia due to its high moisture content. The highest composition of the EBN collected in Long An, Kien Giang, and Khanh Hoa, was protein (49.4 - 51.17%), followed by carbohydrate (36.93 - 38.53%) (Than et al., 2019). The study performed by Marcone

(2005) revealed that protein was the most abundant EBN component, accounting for 62 - 63%. Besides, the content of carbohydrates and lipids was determined to be 5.62 - 27.26% and 0.14 - 1.28%, respectively.

Table 2. Proximate analysis of raw edible bird's nest[1]

Factor	Unit	Result
Carbohydrates	%	3.42
Total crude protein	%	8.30
Total Fat	%	Not detected (Limit of detection = 0.1)
Ash	%	0.38
Moisture	%	87.9

[1]Results were provided by Eurofins Sac ky Hai Dang.

3.2. Determination of important variables affecting the enzymatic hydrolysis using Plackett-Burman Design

The experiments resulted in a wide range of DH (31.1- 43.3%). The highest DH (43.3%) was obtained at the enzyme loading of 5% at 60°C for 90 min at pH of 5 and rotation speed of 600 rpm. Meanwhile, the lowest DH (31.1%) was obtained at the enzyme concentration of 1% at 36°C for 60 min, pH 5 and at a speed of 600 rpm. ANOVA analysis indicated that three factors including enzyme rate, temperature, and hydrolysis time gave a confidence level greater than 95 % ($P < 0.05$), thus gave a significant impact on the DH (Table 3). In contrast, variables including pH and stirring speed with a confidence level below 95% were considered insignificant. All five factors have a positive effect on DH. Noticeably, DH was most affected by temperature, with an effect value of 4.9, followed by hydrolysis time (4.1) and enzyme loading (3.5).

The PB model suggested that enzyme concentration, temperature and hydrolysis time were three of the most important factors influencing the degree of hydrolysis (DH). As a result, these variables were incorporated in the experimental design of RSM-CCD to evaluate the interaction between these factors as well as determine the optimal condition for the highest DH.

Table 3. ANOVA analysis of Plackett Burman matrix

Factors	Symbols	Levels		Level of influence	
		Low (-1)	High (+1)	Effect	P - value
Enzyme rate (%)	X_1	1	5	3.5	0.0061*
Temperature (°C)	X_2	36	60	4.9	0.0012*
Hydrolysis time (min)	X_3	30	90	4.1	0.0029*
pH	X_4	5	8	0.6	0.5283
Stirring speed (rpm)	X_5	600	1800	0.6	0.3456

*: significant at $P < 0.05$

3.3. Optimization of hydrolysis conditions for maximum DH using the RSM-CCD model

3.3.1. Experimenal data and ANOVA for degree of hydrolysis

Table 4 showed that DH of EBN crumbs hydrolysate ranged from 42.9 to 63.9%. The highest DH (63.9%) was obtained at enzyme loading of 4% for 100 min at temperature of 50°C. Table 5 showed the ANOVA of two-factor interaction (2FI) as suggested by Design Expert software. At a 95 % confidence level, the model was considered significant with a P-value < 0.001. A P-value of 0.0859 (> 0.05) indicates that the lack of fit is not significant. This non-significant lack of fit is favourable in an optimization study, as it illustrates the model's ability to fit well with experimental data. Besides, the calculated regression coefficient R^2 was 0.9567. The predicted R^2 (0.7228) corresponds to the adjusted R^2 (0.9177) with less than 0.2 difference indicating that the model is adequate. Adeq precision (14.664) is greater than 4 is desirable that the model is suitable for practical use. Additionally, the coefficient of variation (% CV) also indicates the accuracy of the experiment, those with low reliability often have a high % CV. In this model, a % CV value of 3.36 indicates that the experiments performed are reliable.

From the ANOVA, it was found that all linear model terms (X_1, X_2 & X_3) with a P-value < 0.05 affected the DH significantly. Quadratic values include X12, X22 and interaction terms X_1X_3 and X_2X_3 also showed a high level of significane. In contrast, X_2^2 and X_1X_2 were insignificant terms at $P = 0.05$. The model reduction was carried out to simplify the equation by excluding insignificant terms.

The final equation in terms of coded factors given by Design Expert software was:

$$Y = 61.5 + 1.22X_1 + 2.76X_2 + 1.81X_3 + 1.93X_1X_3 \breve{} 1.9X_2X_3 \breve{} 5.02X_1^2 \breve{} 3.81X_3^2$$

Where:

Y: degree of hydrolysis (% DH);

X_1, X_2, X_3 are enzyme loading (%), hydrolysis time (min), and temperature (°C), respectively.

The quadratic regression equation implies that enzyme loading, hydrolysis time and temperature have significant impacts on the enzymatic hydrolysis of EBN crumbs using protease enzyme. These variables showed positive coefficients, indicating that each factor contributed to an increased DH. According to the equation, the DH is most affected by hydrolysis time (2.76), followed by temperature (1.81) and enzyme loading (1.22). Additionally, the interaction between enzyme concentration-temperature (X_1X_3) and hydrolysis time-temperature (X_2X_3) also contribute to the improvement of hydrolysis efficiency.

3.3.2. Response surface plots for interaction effect

Figure 1(a) illustrated the impact of the interaction between enzyme concentration (X_1) and temperature (X_3) on DH. The maximum of DH is obtained at intermediate enzyme loading and temperature while hydrolysis time reached 90 min. On the other hand, lower or higher than the intermediate level of these two factors result in decreased DH. It's assumed that more active sites are available in the enzyme at higher enzyme concentration, thus resulting in great cleavage of the peptide bonds and provided higher DH. However, when increasing the enzyme loading to over 5%, the DH no longer increased because the enzyme might be saturated with the substrate, (Mackei, 1982). For most enzymatic hydrolysis reactions, the rate of hydrolysis increased when the temperature increases. In this study, the lower DH of 40 to 50 may be due to the insufficient energy provided for the protease to bind with the substrate. However, DH was not significantly affected by temperature from 50°C - 60°C, this is in agreement with the research carried out by Silva et al. (2010) using the Protamex.

Figure 1(b) showed that the DH increases at intermediate enzyme loading throughout the hydrolysis time used in this study. An increase in hydrolysis time allowed the enzyme to act more extensively on the protein, thereby increasing DH. This is in agreement with Amiza et al. (2019b) and Ovissipour et al. (2010). However, Khushairay et al. (2014) and Nurfatin et al. (2016) reported that, after an initial increase in DH, a decrease in the rate of hydrolysis of EBN was observed when incubated for more than 1 - 1.5 h and the conversion was entering a stationary phase. Despite having more cleavage sites, the extent of hydrolysis of EBN depends on the cleavage specificity of the enzyme and the accessibility of peptide bonds to each enzyme. Besides,

Table 4. DH values according to Central composite design-Response surface methodology

Run	Factors			Degree of hydrolysis (% DH)	
	X_1	X_2	X_3	Experimental	Predicted
1	3	90	40	58.9	56.9
2	4	75	50	62.6	62.0
3	4	75	50	62.3	62.0
4	4	75	50	63.1	62.0
5	5	90	60	58.1	57.6
6	5	90	40	53.8	53.9
7	4	75	50	62.6	62.0
8	3	60	40	47.3	46.0
9	3	90	60	53.2	52.6
10	4	50	50	54.1	55.7
11	5	60	60	56.8	57.2
12	5.7	75	50	49.8	49.7
13	4	75	33	46.1	48.0
14	5	60	40	47.2	45.9
15	2.3	75	50	42.9	45.6
16	4	100	50	63.9	64.9
17	4	75	67	53.4	54.1
18	4	75	50	62.1	62.0
19	3	60	60	51.5	49.6
20	4	75	50	59.7	62.0

Table 5. ANOVA of Central composite design-Response surface methodology

Source	Sum of Squares	Mean square	F-value	*P*-value	
Model	768.09	85.34	24.54	< 0.0001*	significant
X_1 – Enzyme rate	20.19	20.19	5.81	0.0367*	
X_2 – Time of hydrolysis	103.97	103.97	29.90	0.0003*	
X_3 – Temperature	44.59	44.59	12.82	0.0050*	
X_1X	3.65	3.65	1.05	0.3300	
X_1X_3	29.65	29.65	8.53	0.0153*	
X_2X_3	28.88	28.88	8.31	0.0163*	
X_1^2	371.29	371.29	106.78	< 0.0001*	
X_2^2	5.25	5.25	1.51	0.2475	
X_3^2	216.25	216.25	62.19	< 0.0001*	
Lack of fit	27.48	5.5	3.77	0.0859	not significant

$R^2 = 0.9567$; C.V. % = 3.36; adjusted $R^2 = 0.9177$; predicted $R^2 = 0.7228$.
*: significant at = 0.05.

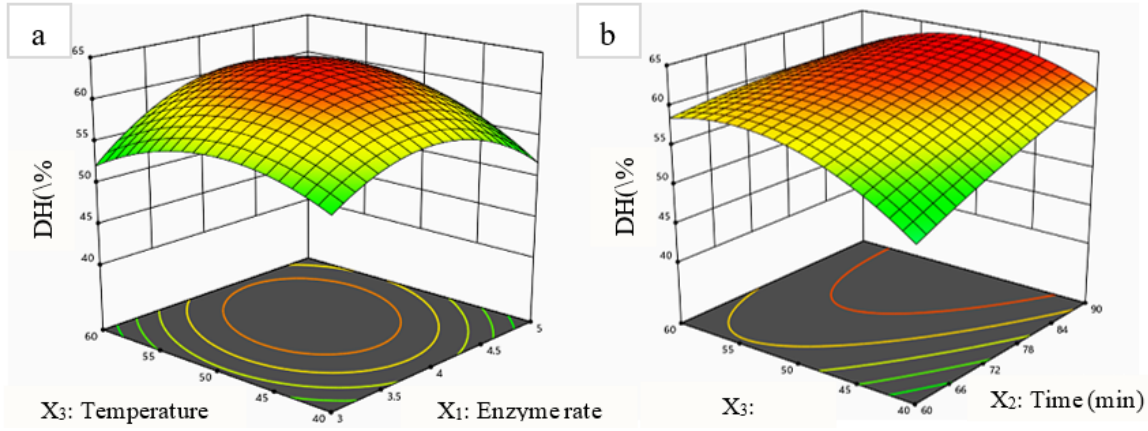

Figure 1. Response surface plot for the interaction between two factors: *(a) enzyme loading (X_1), temperature (X_3) when hydrolysis time was conducted for 90 min; hydrolysis time (X_2) and temperature (X_3) when enzyme loading was fixed at* 4%.

Arihara (2006) stated that, 100% solubility will never achieve which is related to peptide-peptide interactions and partly to the presence of glycoprotein that can not be hydrolyzed. The results obtained from Figures 1(a) & (b) indicated that an increase in DH can be achieved by increasing enzyme concentration, hydrolysis time, and temperature up to certain levels.

3.3.3. Optimization of degree of hydrolysis and verification of the optimal condition

The optimal hydrolysis conditions of EBN crumbs for the highest DH were predicted at 4% of enzyme loading, 51°C of temperature for 90 min. The maximum DH value under this optimal condition was 64.1% .

The maximum DH in this study is higher than that of enzymatic hydrolysis of EBN using alcalase (37.92%), and protamex (33.88%) (Amiza et al., 2019b). However, it is relatively lower compared to that of EBN hydrolysis using Pancretin 4NF (86.5%) and alcalase (82.7%) within hydrolysis time from 1 - 1.5 h. The difference in DH between these studies could be attributed to the difference in source of material used. The major nutrient components of EBN are carbohydrates and glycoproteins. Hydrolysis of glycoprotein often results in a wide range of molecular weight peptides, which are difficult to separate and characterize. According to Muhammad et al. (2015), approximately 40% of the hydroxyl amino acids in EBN are in the position of carbohydrate-peptides linkages, thus making the EBN difficult to hydrolyze thoroughly.

The DH is also depended on the type of enzymes as well as the different range of parameters applied during enzymatic hydrolysis. Each type of enzyme will work differently during proteolysis depending on their preferred amino acid to be cleaved during the hydrolysis process.

To validate the model, three replicates of EBN crumbs hydrolysis were performed under the optimal conditions suggested by the software. According to one-sample t-test, there was no significant difference between the experimental value (63.5% ± 0.76) and the predicted value (64.1%). This means that the equation fits well with the experimental data in this study.

3.4. Quantitative analysis of amino acids

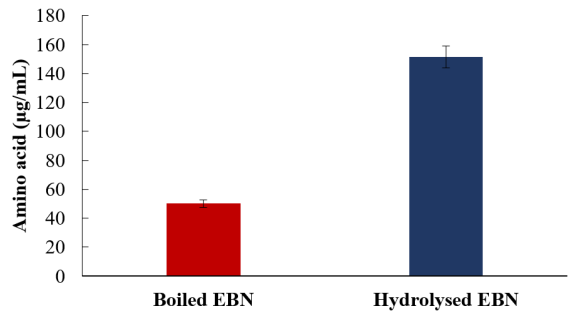

Figure 2. Amino acid concentration in the hydrolyse. EBN: edible bird's nest.

According to Figure 2, hydrolysed EBN crumbs have a total amino acid concentration of 151.6 ± 1.29 µg/mL, whereas boiled EBN crumbs have a concentration of 50.1 ± 2.43 µg/mL. After enzymatic hydrolysis, the total amount of free amino acids in the EBN crumbs is three times higher than that in the doubled-boiled samples. This could be due to double-boiled EBN does not undergo enzymatic hydrolysis and the boiling temperature was not able to break the strong peptide bonds.

The result demonstrated the efficiency of the enzymatic hydrolysis in cleaving peptide bonds to release soluble protein and free amino acids.

3.5. DPPH radical scavenging assay

The EBN hydrolysate exhibited higher DPPH radical scavenging activity 64.97 ± 0.79% than that of double-boiled EBN 18.36 ± 0.17% (Figure 3). As compared with the double-boiled preparations, the EBN prepared with protease may have more active amino acids or peptides, which could scavenge free radicals.

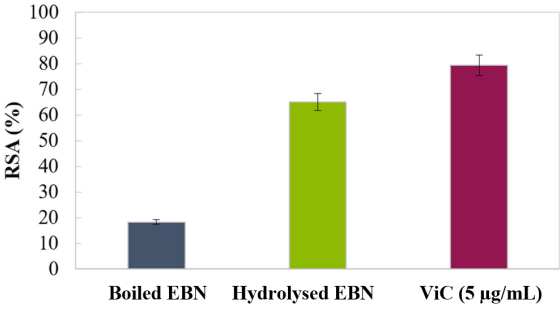

Figure 3. Free radical scavenging activity (RSA) of the hydrolysate. EBN: edible bird's nest.

The EBN crumbs hydrolysate in this study had greater DPPH scavenging activity compared to the hydrolysis process employed by alcalase (RSA of 44.8%) and papain (RSA of 49.78%) (Muhammad et al., 2015). However, it is lower compared to the EBN hydrolysis by Bromelain with antioxidative activity of 82.55 ± 0.73% (Bui, 2020). Previous research also proved that the antioxidative activity of protein hydrolysates depends on the kind of protease enzyme and hydrolysis conditions employed (Jun et al., 2004). During hydrolysis, a wide variety of smaller peptides and free amino acids are generated, depending on en-

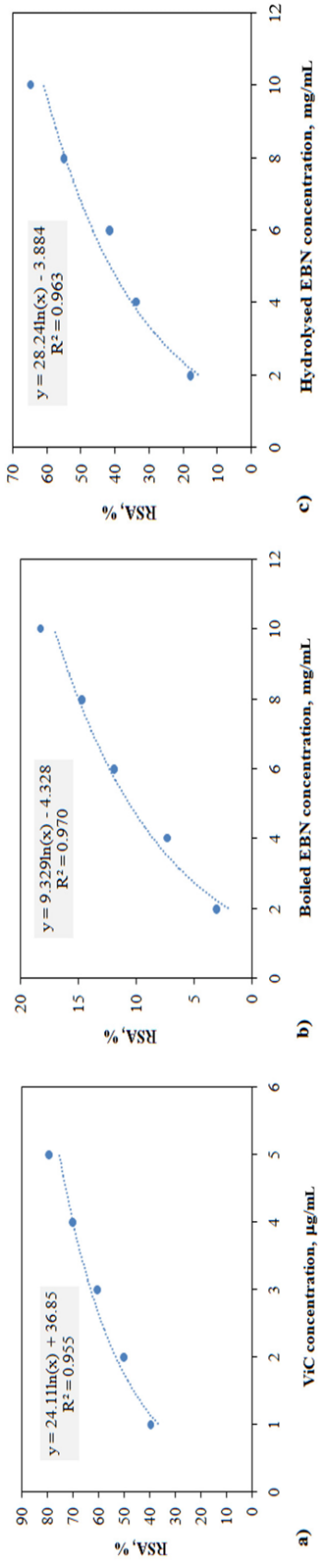

Figure 4. Antioxidant activity of vitamin C and different edible bird's nest (EBN) crumbs hydrolysate (a) Vitamin C, (b) Boiled EBN, (c) Hydrolysed EBN. RSA: radical scavenging activity.

zyme specificity. Changes in size, level, and composition of free amino acids and small peptides significantly affect the antioxidative activity (Wu et al., 2003).

In fact, Figure 4 shows that antioxidant capacity of the EBN crums hydrolysate was higher than the boiled ENB through IC_{50} value. The results indicated that bioactive substances produced by enzymatic hydrolysis of EBN crumbs could be further applied in the cosmetic industry as a rich nutrient for the formulation of beauty products, enhancing both their economic and nutritional value.

3.6. Tyrosinase inhibition of EBN crumbs hydrolysate

According to the results of the tyrosinase enzyme inhibitory activity, kojic acid was significantly inhibited with an IC_{50} of 19.69 µg/mL, boiled EBN had a weak inhibitory activity with an IC_{50} value of 108.9 µg/mL, and EBN crumbs hydrolysate prepared under optimal conditions gave a moderate inhibitory activity with an IC_{50} value of 70.22 µg/mL (Figure 5).

Tyrosinase inhibition of the EBN crumbs hydrolysate (Figure 6) was about 56%, which was comparable to bird's nest extract in Khanh Hoa with 60% of enzyme inhibitory (Le et al., 2017). The results demonstrated a potential use of ENB crumbs hydrolysate in skin care products such as foam, lotion or serum.

4. Conclusions

Optimal conditions for the enzymatic hydrolysis of EBN crumbs were found at an enzyme loading of 4%, temperature of 51°C, and hydrolysis time of 90 min. The experimental DH achieved at the optimized condition (63.5%) was close to the predicted DH (64.1%) suggested by the model. The enzymatic hydrolysate prepared at the optimize condition showed relatively high amino acid concentration (151.6 ± 1.29 µg/mL) and DPPH radical scavenging activity (64.97 ± 0.79%) compared to those of the boiled sample with only 50.1 ± 2.43 µg/mL and 18.36 ± 0.17%, respectively. In addition, the EBN crumbs hydrolysate showed to inhibit tyrosinase at an IC_{50} of 70.22 µg/mL, which demonstrated potential applications in skin care and beauty products.

Figure 5. Tyrosinase inhibition reaction at concentrations ranging from 0 to 100 (µg/mL). (a) Acid kojic; (b) Boiled EBN; (c) Hydrolysed EBN. EBN: edible bird's nest

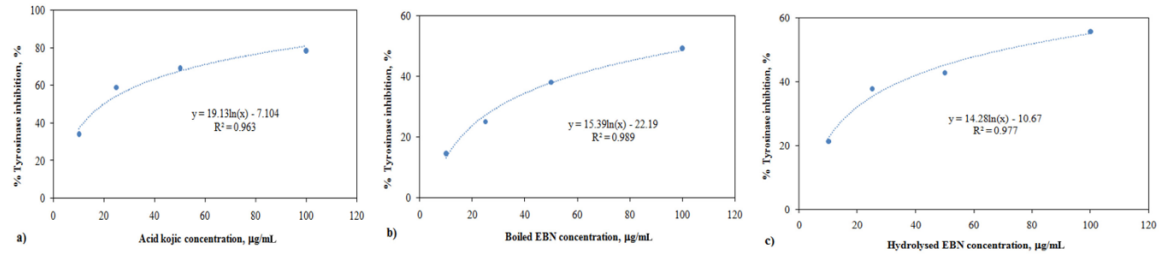

Figure 6. Correlation between substance concentration and tyrosinase inhibitory activity. (a) Kojic Acid, (b) Boiled EBN, (c) Hydrolysed EBN. EBN: edible bird's nest.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgements

The study was funded by Nong Lam University, Ho Chi Minh City, Vietnam (the research code: CS - CB21 - Vien CNSH - 02).

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