OPTIMIZATION AND VALIDATION OF PROTEIN HYDROLYSATE FROM COCKLE (*Anadara granosa*) MEAT WASH WATER USING ENZYMATIC HYDROLYSIS

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ABSTRACT

Protein hydrolysate was produced from cockle meat wash water precipitate, a by-product from the cockles industry, through enzymatic hydrolysis using bromelain. Experiments were carried out to elucidate the optimum condition for different factors which were temperature, enzyme concentration and hydrolysis time using response surface methodology (RSM) based on a central composite rotatable design (CCRD). Results revealed that the optimum values for temperature, enzyme concentration and hydrolysis time were found to be 33.73°C, 1.45% (E/S) and 28.42h, respectively. Enzymatic hydrolysis of cockle meat wash water precipitate using bromelain resulted in nitrogen content (NC) of 0.61% and degree of hydrolysis (DH) of 48.4% under optimized conditions.

***Keywords**: Cockle meat wash water, bromelain, enzymatic hydrolysis, Response Surface Methodology (RSM).*

ABSTRAK

Hidrolisat protein disediakan daripada mendakan air basuhan isi kerang, iaitu produk sampingan daripada industri kerang menggunakan hidrolisis berenzim daripada bromelain. Eksperimen yang dijalankan bertujuan untuk mendapatkan keadaan optimum bagi faktor yang berbeza iaitu suhu, kepekatan enzim dan masa hidrolisis menggunakan Kaedah Respon Permukaan (RSM). Keputusan menunjukkan nilai optimum untuk suhu, kepekatan enzim dan masa hidrolisis masing-masing adalah 33.73°C, 1.45% (E/S) dan 28.42j. Hidrolisis berenzim mendakan air basuhan isi kerang menggunakan bromelain menunjukkan keadaan optimum bagi kandungan nitrogen (NC) adalah 0.61% dan darjah hidrolisis (DH) adalah 48.4%.

Kata kunci: *Air basuhan isi kerang, bromelain, hidrolisis berenzim, Kaedah Respon Permukaan (RSM).*

INTRODUCTION

Cockles (*Anadara granosa*) are edible bivalve commonly found in South East Asia. These bivalves also contain volatile components which are considered as the most important determinant for their flavor quality (Shahidi 1998). Cockles are consumed fresh and also converted into processed products such as sauces. Recycling of wash water from cockles to produce protein hydrolysates may reduce the pollution and give benefits to the food industry. Production of seafood flavors from under-utilized fish species, using protein hydrolysis, is very challenging in order to ensure a high organoleptic quality (Nilsang et al. 2005). The hydrolysis of protein is often accompanied with flavor defects such as bitterness and off-flavor.

Protein hydrolysates used in nutritional formulations are generally categorized into two broad categories, partially hydrolyzed and extensively hydrolyzed proteins. Each category possess different properties that influence their utilization in the final product. Presently there are no precise, widely accepted specifications to differentiate the protein hydrolysates on chemical basis (Taha et al. 2002). Protein hydrolysates have been used, since 1940s for the nutritional management of individuals who cannot digest protein. Food protein hydrolysates have a wide range of applications as ingredients in the areas of nutrition, food industry, health care and cosmetics (Radha et al. 2007).

Hydrolysis significantly impacts the functional and sensory properties of the resultant hydrolysate. The release of bitter tasting peptides is a negative aspect associated with most food protein hydrolysates. Different enzyme-based strategies may be employed in reducing the bitterness associated with hydrolysis of food protein. Bitterness has also been masked in hydrolysates via the addition of polyphosphates, specific amino acids such as Asp and Glu, a-cyclodextrins and by the admixture of hydrolysates with intact protein samples (Fitzgerald et al., 2006). Although there were many studies about the advantage of enzymatic hydrolysis, the process to obtain it has not been designed for industrial scale due to high cost which include pre-extraction, expensive dialysis, and long time thermal incubation for synthesis (Calderón et al., 2000). The objectives of this study were to optimize and validate the optimum conditions in producing protein hydrolysate from cockle (*Anadara granosa*) meat wash water using enzymatic hydrolysis.

MATERIALS AND METHODS

Production of protein hydrolysate

2.5g of precipitate cockle wash water (defatted) were added to different level of enzyme concentration. The solution was hydrolyzed in an incubator shaker for different temperature and time period, cooled and the pH were adjusted to 6. The solution was then centrifuged at 7800g, 30 min. The supernatant was kept frozen at -20°C and freeze dried.

Statistical analysis

Optimization of the hydrolysis conditions were accomplished by employing the RSM with a Central Composite Rotatable Design (CCRD). Different factors and the levels at which they were employed is presented in Table 1. Three different factors (temperature, enzyme concentration and hydrolysis time) were employed at two equidistant levels (-1 and +1). NC (%) and DH (%) were determined as the response variable (X and Y). Each run contained 2.5g sample hydrolyzed at specified concentration, volume and time period. For DH detection, each run after the specified hydrolysis was terminated by 10% trichloroacetic acid (TCA) followed by centrifugation. Total nitrogen in the 10% TCA soluble material and the substrate was estimated by Kjeldahl method.

The optimization experiments were carried out through RSM using Design-Expert version 6.0.10 (Stat-Ease 2003). Analysis of the data obtained through the RSM, was carried out to study the optimized conditions for three independent variables (temperature, enzyme concentration and incubation time) which regards to the dependent variables (NC and DH). The three-dimensional (3D) plots were generated by keeping one variable constant at the center point and varying the other variables within the experimental range.

Table 1

Independent factors and levels used in RSM studies for optimizing hydrolysis conditions using bromelain

Factors	Low Coded	High Coded	Low Actual	High Actual
Temperature, (°C)	-1.000	1.000	30.00	50.00
Enzyme concentration, (E/S, %)	-1.000	1.000	0.70	2.50
Hydrolysis time, (h)	-1.000	1.000	16.00	48.00

Data was analyzed using Statistical Analytical System (SAS) version 6.12 for ANOVA test and DUNCAN. All experiments were done using three replication. Validation was done using Root Mean Squared Deviation (RMSD) as described by Pineiro et al. (2008) as formula below:

$$\text{RMSD} = \sqrt{\frac{1}{n-1} \sum_{i=1} (\hat{y}_i - y_i)^2}$$

$\hat{y}_i$ = observed value

y_i = predicted value

RESULTS AND DISCUSSIONS

Response surface for the Fig. I shows the interaction between enzyme concentration and temperature for NC. At fixed temperature, when enzyme concentration increased, NC also increased. Fig. II shows NC as a function of hydrolysis time and temperature for the hydrolysis of cockle meat wash water precipitate with bromelain. The pattern in Fig. II indicated that according to the model, increased hydrolysis time of cockle meat wash water precipitate resulted in a higher NC at fixed temperatures. Fig. III shows NC as a function of hydrolysis time and enzyme concentration for the hydrolysis of cockle meat wash water precipitate with bromelain. The graph illustrated that NC increased constantly when the hydrolysis time increased at fixed enzyme concentration. When hydrolysis time was fixed, NC decreased when the concentration of enzyme was reduced.

Fig. IV shows DH as a function of the enzyme concentration and temperature during hydrolysis of cockle meat wash water using bromelain. It indicated that DH increased constantly when enzyme concentration increased at fixed temperatures. Fig. V shows DH as a function of hydrolysis time and temperature for the hydrolysis of cockle meat wash water with bromelain. When hydrolysis time is fixed, DH increased when temperature increased. Illustration from Fig. VI shows DH as a function of hydrolysis time and enzyme concentration for the hydrolysis of cockle meat wash water with bromelain. DH increased when concentration of bromelain increased at fixed hydrolysis time.

The optimum point was determined based on the highest desirability of the responses. The analysis indicated that optimum NC and DH for hydrolysis of cockle meat wash water precipitate can be achieved using an enzyme concentration of 1.45%, temperature of 33.73°C and hydrolysis time of 28.42h. From the optimization study, NC and DH predicted were 0.61% and 48.36%, respectively with a desirability value of 0.682. The observed values (Table 2) from experimental runs were used to evaluate the validity of the model using Root Mean Squared Deviation (RMSD). The small values (0.0356 for NC) and (4.5724 for DH) of correlation indicate the validity of the model.

Table 2

Nitrogen content (NC, %) and degree of hydrolysis (DH, %) observed during validation of experiments

Run #	NC	DH
1	0.6301	51.44
2	0.6505	47.82
3	0.6322	54.02

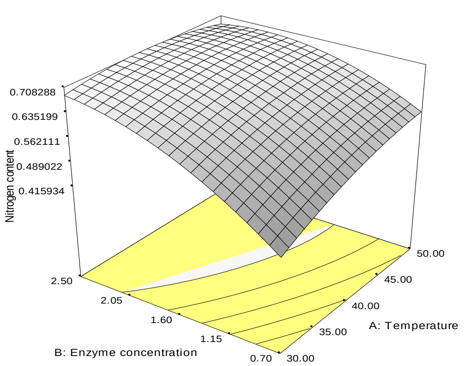

Fig. I Response surface graph for NC as a function of enzyme concentration and temperature during enzymatic hydrolysis of cockle meat wash water precipitate with bromelain

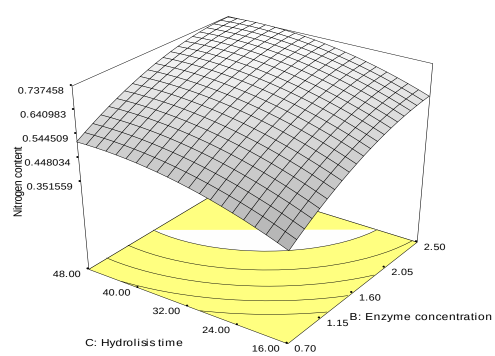

Fig. III Response surface graph for NC as a function of hydrolysis time and enzyme concentration during hydrolysis of cockle meat wash water precipitate with bromelain

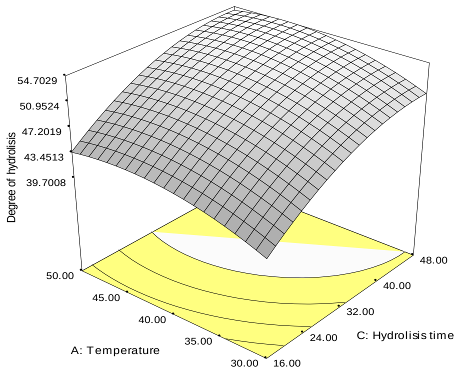

Fig. V Response surface graph for DH as a function of hydrolysis time and temperature during hydrolysis of cockle meat wash water precipitate with bromelain

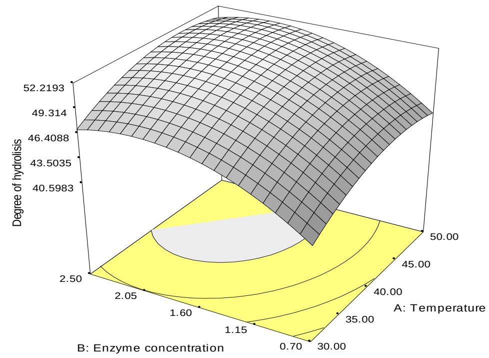

Fig. IV Response surface graph for DH as a function of enzyme concentration and temperature during enzymatic hydrolysis of cockle meat wash water precipitate with bromelain

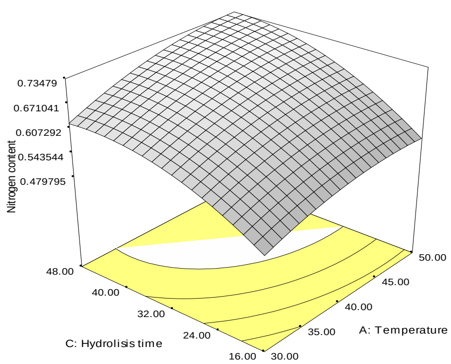

Fig. II Response surface graph for NC as a function of hydrolysis time and temperature during hydrolysis of cockle meat wash water precipitate with bromelain

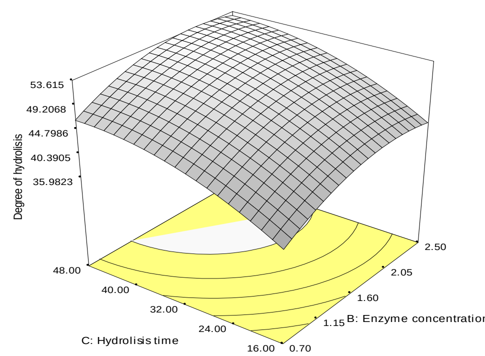

Fig. VI Response surface graph for DH as a function of hydrolysis time and enzyme concentration during hydrolysis of cockle meat wash water precipitate with bromelain

CONCLUSION

The optimum condition for enzymatic hydrolysis of cockle (*Anadara granosa*) meat wash water were found to be temperature of 33.73°C, enzyme concentration of 1.45% (E/S) and hydrolysis time of 28.42h. It also found the validity of the model with NC values of 0.0356 and DH values of 4.5724.

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REFERENCES

Calderón De La Barca, A.M., Ruiz-Salazar, R.A. & Jara-Marini, M.E. (2000). Enzymatic hydrolysis and synthesis of soy protein to improve its amino acid composition and functional properties. *Journal of Food Science,* 65(2), 246-253.

FitzGerald, R.J. & O'Cuinn, G. (2006). Enzymatic debittering of food protein hydrolysates. *Biotechnology Advances,* 24, 234–237.

Nilsang, S., Lertsiri, S., Suphantharika, M. & Assavanig, A. (2005). Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases. *Journal of Food Engineering,* 70, 571–578.

Pineiro, G., Perelmanb, S., Guerschman, J.P. & Parchieloa, J.M. (2008). How to evaluate models: Observed vs. predicted or predicted vs. observed?. *Ecological Modelling,* 216, 316–322.

Radha, C., Kumar, P.R. & Prakash, V. (2007). Preparation and characterization of a protein hydrolysate from an oilseed flour mixture. *Food Chemistry,* 106, 1166-1174.

Shahidi, F. (1998). Flavor of meat, meat products and seafoods. 2nd Ed. Blackie Academic & Professional, UK.

Stat-Ease (2003). Design-Expert Version 6.0.10. Stat-Ease Inc., Minneapolis, MN.

Taha, F.S., Ibrahim, M.A., & El-Zanaty, E.A. 2002. Optimum conditions for enzymatic degradation of some oilseed proteins. *Grasas y Aceites,* 53(3), 267-272.