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Optimization of Protein Hydrolysate Production Process from *Jatropha curcas* Cake

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***Abstract*—**This was the first document revealing the investigation of protein hydrolysate production optimization from *J. curcas* cake. Proximate analysis of raw material showed 18.98% protein, 5.31% ash, 8.52% moisture and 12.18% lipid. The appropriate protein hydrolysate production process began with grinding the *J. curcas* cake into small pieces. Then it was suspended in 2.5% sodium hydroxide solution with ratio between solution/ *J. curcas* cake at 80:1 (v/w). The hydrolysis reaction was controlled at temperature 50 °C in water bath for 45 minutes. After that, the supernatant (protein hydrolysate) was separated using centrifuge at 8000g for 30 minutes. The maximum yield of resulting protein hydrolysate was 73.27 % with 7.34% moisture, 71.69% total protein, 7.12% lipid, 2.49% ash. The product was also capable of well dissolving in water.

***Keywords*—**Production, protein hydrolysate, *Jatropha curcas* cake, optimization.

I. Introduction

JATROPHA CURCAS, physic nut or purging nut, is a tropical plant in the Euphorbiaceae Family. Usually, it is cultivated in Latin America, Asian and African. The *J. curcas* oil shows high oil yield percentage as 40-60%. Its fatty acid compositions are very similar to those from typical vegetable oils. The cake after oil extraction contains as high protein as 19-27% which is suitable as a protein source for either food or non-food products. However, *J. curcas* cake also contains several toxic and anti-nutritional compounds such as trypsin inhibitors, lectins, saponins and phytate those can cause serious health problems after long time of contact. Thus, utilization of *J. curcas* cake as protein sources for food purposes still needs toxic substance removal. Typically, toxic could be almost removed by different solvent extractions, however, large amount of chemical solvents would be discharged as waste and it needs to be treated. As a result, non-food related products could be an alternative way of *J. curcas* cake utilization.

Sanchez-Vioque and his colleagues (2001) enzymatically produced hydrolysate from rapeseed protein after oil extraction [1]. The obtained product contained amino acid mixture with an average size of 5.4 amino units. After that, this protein hydrolysate was chemically acylated in order to improve its foaming property. Wani *et al*. (2006) optimized the extraction condition for protein in watermelon seed using sodium hydroxide solution as protein source for human feed and animal feed [2]. The Response Surface Methodology (RSM) was applied. The most appropriate condition to obtain high protein yield as 86.08% was when the reaction ran with 1.2% sodium hydroxide solution, 70:1 base solution:watermelon seed, 40 °C for 15 minutes. Bera and his colleagues (2007) studied the utilization of *Terminalia belerica Roxb* cake after oil extraction, containing higher nitrogen content as 8.24% relative to that in coconut cake (6.50%), as organic fertilizer [3]. When applied for spinach, the fertilizer showed well support to the growth of spinach and its resistance to some insects.

In this study, RSM was employed to optimize the parameters for maximum protein extraction from *J. curcas* cake for non-food products.

II. Materials and Methods

A. Preparation and Analysis of Raw Material and Products

The *J. curcas* cake after oil extraction used in this study was obtained from KU Biodiesel Project, Kasetsart University, Thailand. It was ground into fine size using Vita mix® and stored in sealed plastic bottle at -20 °C until use. Raw material and all products were sampled for proximate analysis; moisture, protein and lipid as described in [4]. Total amino acid was analyzed according to AccQ. Tag Amino acid analysis method (1993).

B. Protein hydrolysis of J. curcas *cake*

The effect of lipid on protein hydrolysis

J. curcas cake was put in sealed cloth and then subjected into the Soxhlet apparatus for 12-hour oil extraction twice. Petroleum ether was used as extraction solvent. After that, extracted *J. curcas* cake was vacuum dried at 35 °C for 24 hours. The resulting cake was kept in sealed plastic bag at 4 °C until use.

The production of a hydrolysate was performed as follows

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(adapted from [5]): ground *J. curcas* cake was mixed with distilled water to obtain optimal protein content with final volume of 100 g. Before subjecting to chemical or enzymatic hydrolysis, the suspension was adjusted to suitable pH with 4 N NaOH. The reaction mixture was incubated at 50 °C with continuous stirring for 90 minutes. After that, the reaction was terminated and centrifuged at 8000 g for 30 minutes to obtain the supernatant as protein hydrolysate. The %degree of hydrolysis (%DH) is defined as the percentage of peptide bonds cleaved during the hydrolysis reaction relative to total peptide bonds [6]. To determine total dissolved nitrogen in the protein hydrolysate supernatant, 20% trichloroacetic acid (TCA) were added to an equivalent of protein hydrolysate and thoroughly mixed for 2 minutes. After that, it was centrifuged at 10000g for 15 minutes before total nitrogen in the supernatant was measured according to [4].

$$\%\,DH = \frac{\text{Total dissolved nitrogen in 10\% TCA}}{\text{Total nitrogen in reaction mixture}} \times 100$$

The experiment was enzymatically carried out following the previous procedure using non-extracted and extracted *J. curcas* cake with the ratio of protein:protease = 5:0.6, pH 8.5, 55 °C for 90 minutes. Each experiment was performed in duplicate. The *J. curcas* cake with highest %DH was selected for further experiments.

Treatment	Raw material
1	Non-extracted Jatropha cake
2	Extracted Jatropha cake

Each treatment was performed in duplicate.

The effect of hydrolysis reagents on protein hydrolysis

The experiment was carried out following the previous procedure using 3 hydrolysis reagents indicated as 1) control (without hydrolysis reagent), 2) 1% (w/w) Alcalase 2.4L FG, Novozymes A/S, Denmark), 3) 1% (w/w) sodium hydroxide solution, and 4) 1% (w/w) hydrochloric acid solution. Each experiment was performed in duplicate. The hydrolysis reagent with highest %DH was selected for further experiments.

Optimization of condition for protein hydrolysis

The experiment was carried out following the previous procedure using non-extracted Jatropha cake and sodium hydroxide solution. The Central Composite Design (CCD) with 3 factors and 5 levels ($-\alpha$, -1, 0, 1, α) was also applied. The first factor was sodium hydroxide concentration varying from 1.7-3.3% (w/w). The second factor was reaction time ranging from 24-56 minutes. The last factor was *J. curcas* cake/reaction volume ranging from 0.010-0.016% (w/v). Each experiment was performed once. The condition with highest %DH was selected for further experiments.

III. Results and Discussion

A. Preparation and Analysis of Raw Material

The proximate analysis of *J. curcas* cake was presented in Table I. It contained 18.98% crude protein, 5.31% ash, 8.52% moisture and 12.18% lipid based on fresh weight. The results indicated rather lower protein content compared to those in Capoverde (45.80%) and Nicaragua (46.60%) varieties. This could be due to the differences of plantation sites or oil extraction methods.

TABLE I
CHEMICAL COMPOSITION OF *J. CURCAS* CAKE

	% On fresh weight		
	1/	2/	3/
Moisture	6.72	-	-
Ash	5.31	9.20	9.00
Crude protein (N x 6.25)	18.98	45.80	46.60
Lipid	12.18	23.20	24.90

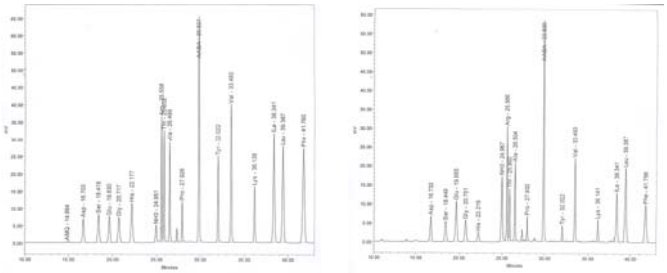
[1]/ Laboratory analysis
[2,3]/ Capoverde variety and Nicaragua variety, respectively [6]
Each experiment was performed in triplicate.

Tables II and III demonstrated that the major amino acid in *J. curcas* cake was 2.82% glutamic acid. The rest were 2.19% arginine, 1.74% aspartic acid and 1.24% leucine. These were consistent with those in watermelon seed in which they were reported as nutritional [2].

TABLE II
AMINO ACIDS IN *J.CURCAS* CAKE

Amino acids	g/100 g sample
Aspartic acid	1.74
Serine	0.91
Glutamic acid	2.82
Glycine	0.81
Histidine	0.43
Arginine	2.19
Threonine	0.71
Alanine	0.87
Proline	0.82
Tyrosine	0.40
Valine	0.86
Lysine	0.75
Isoleucine	0.70
Leucine	1.24
Phenylalanine	0.78
Cysteine	0.51

Each experiment was performed in triplicate.



(a) (b)

Fig. 1 Amino acid chromatogram (a) standard amino acids and (b) *J. curcas* amino acids

B. Protein Hydrolysis of J. curcas cake

The effect of lipid on protein hydrolysis

After oil extraction in *J. curcas* cake, the extracted cake was analyzed for residual lipid compared with that in non-extracted cake. Table III demonstrated the efficient oil extraction as indicating by the obvious decrease of lipid content in extracted cake by 97.95% (from 12.18% to 0.25% before and after extraction, respectively). This also resulted in an increase of protein from 18.98% to 23.18% before and after extraction, respectively. The result was consistent with Aderibigbe *et al*. (1997) who extracted oil from 2 strains of *J. curcas*; Capoverde and Nicaragua strains; using petroleum ether as solvent [7]. A loss of lipid was observed from 23.2% to 1.5% and from 24.9% to 1.8% in Capoverde and Nicaragua strains, respectively. This also suggested an increase in protein contents as shown in Table III. Even though the comparison among these *J. curcas* cakes provided significant differences of lipid and protein contents, the decrease of lipid and an increase in protein in extracted cakes were found to be equivalent by 93-97% and 23-31%, respectively.

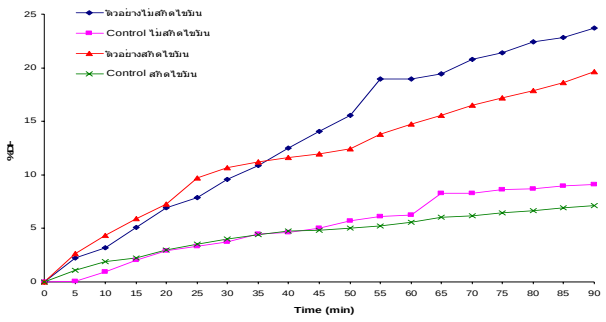


Fig. 2 The effect of lipid on protein hydrolysis
Each experiment was performed in triplicate.

TABLE III
CHEMICAL COMPOSITION OF NON-EXTRACTED AND EAXTRACTED *J.CURCAS* CAKE

	% on dry weight					
	Non-extracted cake			Non-extracted cake		
	1/	2/	3/	1/	2/	3/
Protein	18.98	18.98	18.98	18.98	18.98	18.98
Lipid	12.18	12.18	12.18	12.18	12.18	12.18

[1]/ Laboratory analysis
[2,3]/ Capoverde variety and Nicaragua variety, respectively [7]

Fig. 2 compared the enzymatic hydrolysis efficiency as indicated by %DH of *J. curcas* protein in non-extracted and extracted cakes. DH means the percentage of enzymatically hydrolyzed peptide bonds compared with the original peptide bonds in raw material representing a number of peptide bonds those being degraded during the reaction. In case that DH is high, it indicates high level of protein hydrolysis into smaller peptides and free amino acids [6]. The results clearly showed that protease enzyme improved the %DH of both hydrolysis reactions for non-extracted and extracted cakes. It also demonstrated no effect of high lipid content in *J. curcas* cake on protein hydrolysis as indicating by similar %DH of the reactions.

The effect of hydrolysis reagents on protein hydrolysis

The commercial protease, NaOH solution and HCl solution were compared for their hydrolysis efficiency under the same condition. The soluble nitrogen content in protein hydrolysate products were analyzed for %DH calculation. Table IV obviously showed the influence of hydrolysis reagents on *J. curcas* protein hydrolysis as indicating by significantly higher %DH than that of the reaction without hydrolysis reagent. Even though, NaOH solution and Alcalase enzyme demonstrated similarity in high %DH (19.93% and 19.14%, respectively) of *J. curcas* cake, NaOH solution was selected as the most appropriate hydrolysis reagent used in the next experiment due to its cheaper cost. However, in other cases the Alcalase enzyme might be utilized. Since the protein hydrolysis reaction using enzyme as catalyst, even consumes high investment, but is very efficient and specific so that the nutritional quality of the amino acids is maintained [6]. For example, protein hydrolysis using alkaline solution not only destroyed some essential amino acids (tryptophane, cysteine or serine), but it also resulted in change of amino acid structure from *L*-form into *D*-form which human beings were not able to consume [8]. Thus, in case that the *J. curcas* cake hydrolysate is to be utilized in sense of nutritional products or in more expensive and complicated end product, the enzymatic hydrolysis technique could be applied.

TABLE IV
THE EFFECT OF HYDROLYSIS REAGENTS ON %DH

Treatments	Condition	%DH
control	50 °C, 90 MINUTES	2.3231
Enzymatic hydrolysis	50 °C, 90 MINUTES	19.1398
Basic hydrolysis	50 °C, 90 MINUTES	19.9257
Acidic hydrolysis	50 °C, 90 MINUTES	5.9299

Each experiment was performed in triplicate.

Optimization of condition for protein hydrolysis

The effects of reaction time, NaOH concentration and reaction volume: *J. curcas* cake ratio on degree of hydrolysis were shown in Figures 3-4. It revealed that all 3 parameters significantly affected the hydrolysis of *J. curcas* protein as demonstrated by an increase in degree of hydrolysis when all 3 parameters were raised. Thus, it could be concluded that the most optimum condition for hydrolysis of *J. curcas* protein was at 50 °C, 2.5% NaOH solution, 0.0125% reaction volume: *J. curcas* cake ratio for 45 min with highest %DH at 19.9257%.

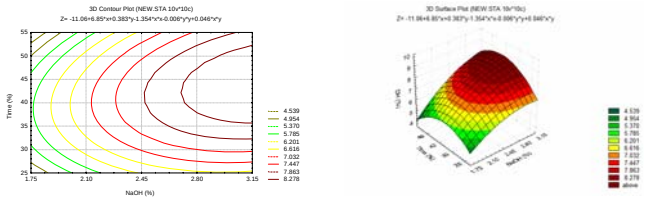


Fig. 3 Response surface for the effects of reaction time (minutes) and NaOH concentration (%) on %DH

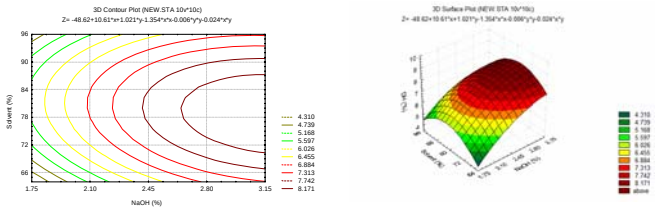


Fig. 4 Response surface for the effects of NaOH concentration (%) and reaction volume:Jatropha cake ratio on %DH

C. Analysis of Product

Table V shows the composition of the hydrolysate product. The protein content was significantly improved from 18.98% in *J. curcas* cake raw material to 71.69% in *J. curcas* hydrolysate. Likewise, the lipid content in *J. curcas* hydrolysate was clearly decreased from 12.18% in *J. curcas* cake raw material to 7.12% in *J. curcas* hydrolysate. Furthermore, the hydrolysate product showed high water solubility indicating its suitability in the application of various products.

Table VI shows types and amounts of amino acids present in the product compared with those in raw material. The results revealed similarity in amino acids profiles between the two samples. Interestingly, loss of cysteine was observed in the hydrolysate. This could be due to the strength of NaOH solution which is known to denature some amino acids—in particular, cysteine and methionine [9].

TABLE V
CHEMICAL PROPERTIES OF *J.CURCAS* CAKE AND HYDROLYSATE

	Jatropha cake	Jatropha cake hydrolysate
Moisture (%)	6.72	7.34
Protein (%)	18.98	71.69
Lipid (%)	12.18	7.12
Ash (%)	5.31	2.49

TABLE VI
COMPARISON OF THE PRODUCT AND RAW MATERIAL IN TERMS OF TYPES AND AMOUNTS OF AMINO ACIDS

g/100 g sample	*J. curcas* cake raw material	*J. curcas* cake hydrolysate
Aspartic acid	1.74	1.78
Serine	0.91	0.95
Glutamic acid	2.82	2.84
Glycine	0.81	0.89
Histidine	0.43	0.45
Arginine	2.19	2.17
Threonine	0.71	0.60
Alanine	0.87	0.69
Proline	0.82	0.62
Tyrosine	0.40	0.32
Valine	0.86	0.92
Lysine	0.75	0.85
Isoleucine	0.70	0.71
Leucine	1.24	1.53
Phenylalanine	0.78	0.92
Aspartic acid	1.74	1.98
Serine	0.91	1.00
Cysteine	0.51	Not determined

IV. CONCLUSION

Proximate analysis revealed 18.98% high protein in *J. curcas* cake suitable as protein source in various applications. The high content of lipid as 12.18% in raw material had no influence on protein hydrolysis. The degree of hydrolysis reached maximum of 19.9257% to obtain most soluble nitrogen when hydrolysis condition was the best optimized (2.5% NaOH, 45 minutes, 0.0125% reaction volume: *J. curcas* cake ratio). The obtained protein hydrolysate was shown to be well solubilized and contained as high protein as 71.69% which was appropriate for further applications in human food, animal feed or even the non-food ones such as for light-weight concrete industry. Therefore, it is advantageous to consider improving important properties for such industry, for example foaming and stability properties, as well as scaling up the production process for industrial purpose.

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REFERENCES

[1] Sanchez-Vioque, R., Bagger, C.L., Rabiller, C. and Gueguen, J. (2001) Foaming properties of acylated rapeseed (*Brassica napus* L.) hydrolysates. Journal of Colloid and Interface Science 244, 386-393.
[2] Wani, A.A., Sogi, D.S., Grover, L. and Saxena, D.C. (2006) Effect of temperature, alkali concentration, mixing time and meal/solvent ratio on the extraction of watermelon seed proteins-a response surface approach. Biosystems Engineering 94(1): 67-73.
[3] Bera, D., Lahiri, D., De Leonardis, A., De, K.B. and Nag, A. (2007) Biotechnological applications in agricultural: A new source of edible oil and production of biofertilizer and antioxidant from its by-products. Journal of Food Engineering 81: 688-692.
[4] AOAC. 2000. Official Method of Analysis of AOAC International. 17th ed. Association of Official Analytical Chemists. Gaithersburg, Md.
[5] Vaithanomsat, P. and Punyasawon, C. (2008) Process Optimization for the production pf *Philosamia ricini* (Eri Silk) pupae hydrolysate. Kasetsart Journal (Natural Science) 42: 341-352.
[6] Adler-Nissen, J. 1982. Determination of the degree of hydrolysis of food protein hydrolyzates by trinitrobenzenesulfonic acid. Journal of Agricultural Food Chemistry 27: 1256-1262.
[7] Aderibigbe, A.O., Johnson, C.O.L.E., Makkar, H.P.S., Becker, K. and Foidl, N. (1997) Chemical composition and effect of heat on organic matter- and nitrogen-degradability and some antinutritional components of Jatropha meal. Animal Feed Science Technology 67, 223-243.
[8] Hall, G.M. and N.H., Ahmad. 1992. Functional properties of fish protein hydrolysate. pp. 249-270. G.M. Hall, (ed.). *In* Fish processing technology. Blackle Academic. London.
[9] Sarwar, G. 1999. Influence of feeding alkaline/heat processed proteins on growth and mineral status of rats. Adv Exp. Med. Biol. 459: 161-77.