

The Extraction of Protein from Superworm (*Zophobas morio*) using Saline Treatment (NaCl) Method

Tan Kian Meng, Noor Akhmazillah Mohd Fauzi and Angzzas Sari Mohd Kassim
Department of Chemical Engineering Technology, Faculty of Engineering Technology,
Universiti Tun Hussein Onn Malaysia, 86400 Batu Pahat, Johor, Malaysia

Abstract: The protein extraction using saline treatment method (NaCl) from superworm (*Zophobas morio*) was investigated. The effect of four independent variables, namely reaction time (10-20 min), pH (10-12), rotation speed level (6-9) and NaCl concentration on the protein extraction was designed by Box-Behnken and analysed using Response Surface Methodology (RSM). The protein extracted was quantified and determined using Bradford protein assays. A second-degree equation for independent and response variables was obtained. The optimum conditions for the highest protein extraction (49.20 mg/mL) were found to be at 0.3 M NaOH in 20.0 minutes reaction time with a rotation speed level of 6 and pH 12. Longer reaction time and higher pH shows significant increase for protein extraction from superworm. The results obtained will contribute to the information on the productivity and quality of the protein yield extracted from superworm.

Key words: Superworm, protein extraction, saline treatment, response surface methodology, productivity, quality

INTRODUCTION

The dietary changes towards higher meat intakes are known to be one of the factors on food demand growth. With a growing world population and increasingly demanding consumers, the production of sufficient protein from livestock, poultry and fish represents a serious challenge for the future. Approximately 1,900 insect species are eaten worldwide, mainly in developing countries (Van *et al.*, 2013). The recent study shows that insect species have high and comparable protein content with conventional meat products (Van *et al.*, 2013; Yi *et al.*, 2013).

This unique characteristic of insects has motivated the researcher to expand the research on superworm and mealworm focusing on their protein as an alternative to human food protein sources.

However, human consumption of insects is infrequent and culturally inappropriate, resulting in the nutritive potential of insects could not be fully utilized for human welfare although its nutritional values are proven to be comparable with conventional meat (Van *et al.*, 2013). Generally, insects can be consumed as whole. However, they can also be processed in less recognizable forms and incorporated in food which may increase consumer acceptability.

Recent years, processing edible insects into conventional consumer products seems to encourage

entomophagy (human consumption of insects) as was shown in Kenya where termites and lake flies (Chaoboridae and Chironomidae) were baked, boiled, steamed and processed into crackers, muffins, sausages and meat loaf (Ayieko *et al.*, 2010). These limitations and current approaches motivate the search for new methods and alternatives to utilize insect's protein for food applications.

Generally, the protein extraction process from various sources involved three basic steps; cell disruption, solubilisation and protein enrichment (Samadi *et al.*, 2016). However, there is limited information available for insect protein extraction process. At present, acid extraction protocols were used to extract protein from insects (Yi *et al.*, 2013). Conventional animal protein extractions were mainly done through alkaline extraction protocols so-called Osborne method (Kirkwood *et al.*, 1943; Kristinsson *et al.*, 2005). However, there is a limitation of this method in terms of protein's stabilization. Recent studies report the use of mainly sodium and calcium salts to extract proteins from different vegetal foods (Ghaly and Alkoik, 2010; Nadal *et al.*, 2011). These extraction methods are simple because the agents required are easily available.

This study therefore, determines the effects of extraction conditions (reaction time, pH, rotational speed and NaCl concentration) on the protein extracted from superworm using saline treatment method. NaCl is used as

the extraction solvent with the assistance of mechanical separation using centrifugation technique. The optimum extraction process helps to reduce cost in manufacturing various insect's protein products. Consequently, enhance the acceptability of consumers to entomophagy.

MATERIALS AND METHODS

Sample: Superworm (*Zophobas morio*) were purchased freshly and alive from the worm breeder farm located at Layang-layang, Johor Darul Takzim, Malaysia.

Pretreatment: The worms were sieved using plastic garden sieve (with 35 cm diameter and 12 mm hole size) to get rid of feed which consisted of wheat bran, oats, carrot and pumpkin. The worms were then starved for 3 days to get rid of internal waste and rinsed with distilled water before being stored alive at -20°C. The worms were processed after 30-45 min.

Protein extraction: The frozen superworm were weighed and mixed with extracting solvent, NaCl with a ratio of 1:10 (established after several preliminary experiments, data not shown). The concentration of salt solution used and the conditions of extraction were based on different combinations which have been designed. The alkali-aided protein extraction from worm was done based on previous method (Yi *et al.*, 2013) with some modifications (Nurdiyana and Fadhilah, 2008). The extraction was done using a blender (MR 540, Braun Multiquick 5 (600 W), Kronberg, Germany) rotate at high speed. The pH of the mixture was adjusted to pH ranged 10-12 by adding in 1M sodium hydroxide, NaOH. Then the blended insect suspension was sieved through a 500 um pore size stainless steel filter sieve. The worm solution was then centrifuged at constant centrifugation speed at 5000 rpm and 4°C for 30 min. The supernatant containing soluble protein was collected and the soluble protein in the supernatant was determined using Bradford protein assays.

Protein concentration determination (Bradford protein assay): A series of protein standards Bovine Serum Albumin (BSA) ranging in concentration from 0.1-1.0 mg/mL was prepared such that the final volume for the assay was 0.1 mL. The worm protein samples were prepared in a similar way such that the final volume was 0.1 mL. Then, 3.0 mL of Bradford reagent was added to each sample and standard, vortex and incubated at room temperature for 5-45 min. The samples were transferred to cuvettes and the absorbance at 595 nm were measured UV-Vis Spectrometer (LABOMED, Inc. USA).

Table 1: Independent variable values of the process and their corresponding levels for saline extraction

Independent variables	Symbols	Range and levels		
		-1	0	1
NaCl concentration (M)	A	0.1	0.3	0.5
Time (min)	B	10.0	15.0	20.0
Rotation speed level	C	3.0	6.0	9.0
pH	D	10.0	11.0	12.0

Design of experiment: Response Surface Methodology (RSM) was used in this study to determine the optimum conditions for the extraction of protein from the superworm. The experiments were based on a Box-Behnken design with a quadratic model in order to study the combined effects of independent variables (time, pH, solvent concentration and mixer rotation speed). The dependent variable (protein yield) was known as response function. The 29 individual runs were employed in the saline extraction as presented in Table 1.

RESULTS AND DISCUSSION

Extracted protein yield and mathematical model: The yield of extracted protein from 29 individual runs was tabulated as in Table 2. The result shows that all runs give a protein concentration of >30 mg/mL. The optimum conditions for the highest protein extraction (49.20 mg/mL) were found to be at 0.3M NaOH in 20 min reaction time with a rotation speed level of 6 and pH 12. Whereas, the minimum protein concentration obtained (35.53 mg/mL) were recorded at 10 min reaction times with pH of 11 at two different NaCl concentration (0.1 M and 0.3 M) and two different speed level (3 and 6).

Regarding the pH, Run 3 at a higher pH resulted in a higher protein concentration (43.08 mg/mL) as compared to a lower pH as indicated in Run 10 with 39.44 mg/mL of extracted protein was obtained. However, no significant differences were reported for the mixer rotation speed when the protein concentration was found to be 39.21 mg/mL and 40.77 mg/mL for Run 16 and Run 27, respectively. Reaction time shows a significant effect on the extracted protein when the concentration was increased from 39.03 mg/mL (at 10 min reaction, Run 2) to 47.30 mg/mL (at 20 min reaction, Run 22).Whereas, a lower concentration of NaCl used resulted in higher concentration of protein extracted as observed in Run 12 and Run 16.

The effect of four independent variables which are NaCl concentration, pH, reaction time and mixer rotation speed level on protein extraction were evaluated using Response Surface Methodology (RSM). Box-Behnken Model was used to observe the optimum

Table 2: Concentration of extracted protein at different conditions designed by Box-Behnken

Run	Variables (actual level)				Response Protein conc. (mg/mL)
	A: NaCl conc. (M)	B: Time (Min)	C: Mixer rotation speed level	D: pH	
1	0.30	15	6	11	41.20
2	0.50	10	6	11	39.03
3	0.50	15	6	12	43.08
4	0.30	10	6	12	37.82
5	0.10	15	6	12	42.70
6	0.30	15	3	12	40.29
7	0.30	10	6	10	36.19
8	0.30	15	6	11	41.98
9	0.30	15	3	10	39.88
10	0.50	15	6	10	39.44
11	0.30	20	3	11	48.01
12	0.10	15	9	11	42.35
13	0.30	15	9	10	40.60
14	0.30	20	6	12	49.20
15	0.10	15	6	10	42.08
16	0.50	15	9	11	40.77
17	0.30	15	9	12	42.02
18	0.30	15	6	11	40.45
19	0.30	10	9	11	37.96
20	0.10	20	6	11	48.63
21	0.30	20	6	10	47.52
22	0.50	20	6	11	47.30
23	0.30	15	6	11	37.80
24	0.10	10	6	11	35.51
25	0.30	10	3	11	35.51
26	0.30	20	9	11	48.30
27	0.50	15	3	11	39.21
28	0.10	15	3	11	41.68
29	0.30	15	6	11	39.78

Mixer rotation speed level 3 = 65-80 rpm; 6 = 250-350 rpm; 9 = 650-750 rpm

condition between independent variables. Whereas, mathematical model for protein extraction was developed by Design-Expert Version 9 as Eq. 1:

$$\begin{aligned} \text{Extracted protein concentration (mg/mL)} = & \\ 40.90 - 0.34 A + 5.60B + 0.62C + 0.78 & \quad (1) \\ D - 1.21 AB + 1.68 B^2 & \end{aligned}$$

where, A-D were the coded variables for concentration of NaCl, time, mixer rotation speed and pH respectively. The results of Analysis of Variance (ANOVA) gave a coefficient of determination (R^2) of 0.9403, indicating the adequacy of the applied model. The probability (p) of the regression model significance was 0.0001 which was <0.05 and the model F-value was 57.80, implied the model is significant. There was only a 0.01% chance that an F-value this large could occur due to noise. Therefore, the developed models could adequately represent the real relationship among the parameters chosen.

Effects of NaCl concentration and reaction time on extracted protein yield: The extraction process was

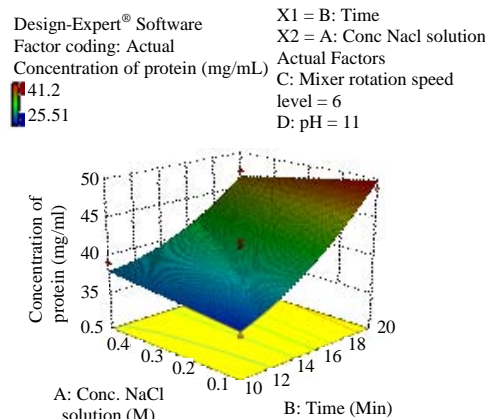


Fig. 1: Response surface plot for the effect of NaCl concentration and reaction time on the extracted protein (at pH 11 with rotation speed level 6)

carried out in different concentrations of NaCl (0.10, 0.30 and 0.50 M) with different extraction time (10, 15 and 20) (min). The plot shows that as the reaction time increases, the concentration of extracted protein was increased significantly, particularly at a very low NaCl concentration (0.1 M) as presented in Fig. 1. However, the trend was not similar at a higher NaCl concentration.

Higher NaCl concentration of 0.50 M at 20 min reaction time resulted in low protein yield. This is because at low concentrations of salt, solubility of the proteins usually increases slightly (salting in). However, at high concentrations of salt, the solubility of the proteins drop sharply (salting out). Initial salting in at low concentrations was explained by the Debye-Huckel theory. Theoretically, proteins were surrounded by the salt counter ions (ions of opposite net charge) and this screening resulted in decreasing electrostatic free energy of the protein and increasing activity of the solvent which in turn, leads to increasing solubility. On the other hand, the behavior of proteins in solutions at high salt concentrations was explained by Omana *et al.* (2010). The abundance of the salt ions decreases the solvating power of the salt ions decreases the solubility of the proteins decreases and precipitation results. Water molecules are strongly bound to the salt and there is competition between the salt ions and the protein molecules for the water molecules.

Effects of pH and NaCl concentration on extracted protein yield: The influence of pH and NaCl concentrations on protein extractability was tested by a series of experiments at a pH ranging from 10-12 and NaCl concentrations varying from 0.10-0.50 M. The results demonstrated that the protein extractability increased with the increase of pH and reached maximum values in alkaline medium at pH 12 (Fig. 2).

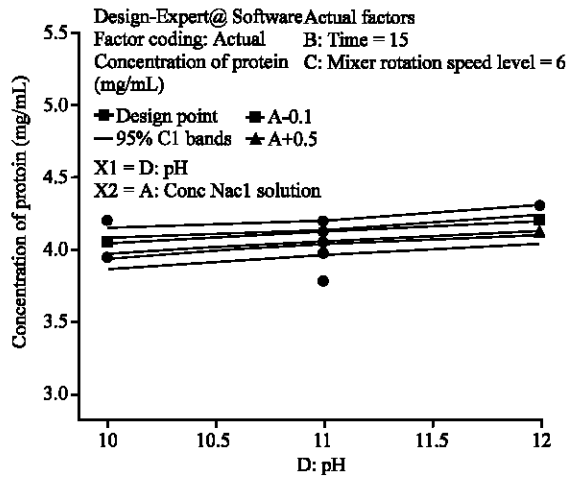


Fig. 2: Effects of pH on the extracted protein at different NaCl concentrations

It has shown that the extracted protein is more pronounced at high alkaline side. Similar finding was reported by Nurdiana *et al.* (2008) where protein solubility increased with increasing pH in freeze dried fish waste. This trend follows the accepted protein monomer aggregation principle in which protein aggregates into an insoluble mass at the isoelectric point due to decrease in electrostatic charge repulsion between the particles as the net charge tends to zero. As the particles come closer, coulumbic forces between positive and negative charges of the protein residues; Van der Waals attraction and hydrogen bonding would then hold the mass together against dispersing forces. But as pH increases, the net negative charge increases and thus desegregation (solubility) progressively increases (Omana *et al.*, 2010). In line with our results, Tan *et al.* (2011) reported that the protein extraction yield and properties are influenced by the type of extraction process and by different factors such as pH, salts concentration, the ionic strength of the medium, net charge and electrostatic repulsions.

Effects of rotation speed and reaction time on extracted protein yield: The result shows that the protein yield increased with increased reaction time. The longest reaction time of 20 min correlated with the highest protein yield extracted as shown in Fig. 3. Longer time enhances the diffusivity of the solvent to extract the protein and hence, increase the protein yield (Geankoplis, 2003). This is similar with previous finding where a linear relationship between protein yield and increased reaction time has been found in the extraction of protein from pumpkin seed (Li and Fu, 2005) and gelatin from chicken deboner (Rafieian *et al.*, 2013). The result also suggests that rotation speed did influence the extraction; however the

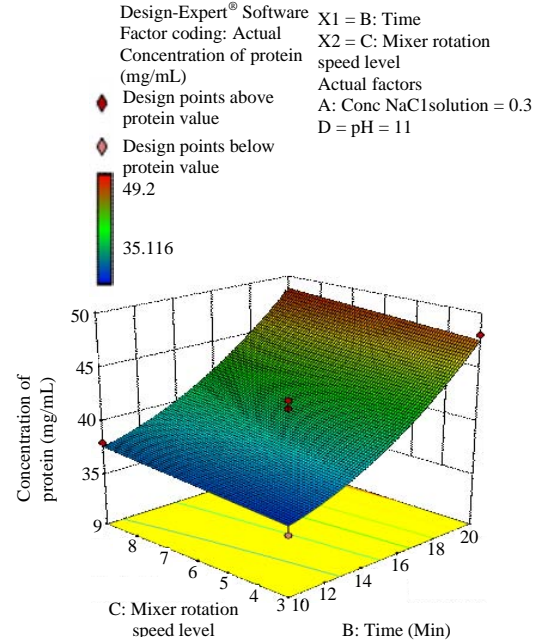


Fig. 3: Response surface plot for rotation speed level and reaction time on the extracted protein

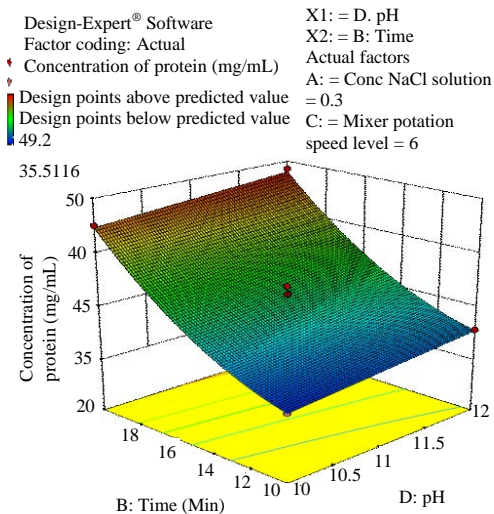


Fig. 4: Response surface plot for pH and reaction time on the extracted protein (at 0.3 M NaCl with rotation speed level 6)

effect was not too significant. It was observed that the maximum yield was obtained at a mixer rotation speed of 9 for 20 min reaction.

Effects of pH and reaction time on extracted protein yield: Figure 4 shows the response surface graph for extracted protein as a function of time and pH, when NaCl

concentration and mixer rotation speed level were set at 0.30M and 6, respectively. The graph indicated that the concentration of extracted protein increased as both time and pH increased. The maximum extracted protein obtained at pH 12 for 20 min reaction time. It was observed that the extracted protein was more pronounced at higher alkaline condition.

CONCLUSION

Researcher has shown that the longer reaction time and the higher pH will significantly increase the concentration of protein extracted from superworm. No significant benefit was found when the rotation speed level is adjusted. For the extraction of protein from superworm using saline treatment, it is suggested that the conditions of 0.3M NaCl with 20 min reaction time at pH 12 produced the maximum yield. The finding will contribute to the basic knowledge on the possibility of extracted protein from superworm using saline method which then can be applied for further research particularly in insect-derived food product industry.

REFERENCES

- Ayieko, M., V. Oriaro and I.A. Nyambuga, 2010. Processed products of termites and lake flies: Improving entomophagy for food security within the Lake Victoria region. *Afr. J. Food Agric. Nutr. Dev.*, 10: 2085-2098.
- Geankoplis, C.J., 2003. *Transport Processes and Separation Process Principle*. 4th Edn., Prentice Hall Press, Upper Saddle River, New Jersey, USA., ISBN-10: 013101367X.
- Ghaly, A.E. and F.N. Alkoik, 2010. Extraction of protein from common plant leaves for use as human food. *Am. J. Applied Sci.*, 7: 331-342.
- Kirkwood, J.G., E.J. Cohn and J.T. Edwall, 1943. *Proteins, Amino Acids and Peptides*. Hafner Publishing, New York, USA.,
- Kristinsson, H.G., A.E. Theodore, N. Demir and B. Ingadottir, 2005. A comparative study between acid and alkali aided processing and surimi processing for the recovery of proteins from channel catfish muscle. *J. Food Sci.*, 70: 298-306.
- Li, Q.H. and C.L. Fu, 2005. Application of response surface methodology for extraction optimization of germinant pumpkin seeds protein. *Food Chem.*, 92: 701-706.
- Nadal, P., N. Canela, I. Katakis and O.C.K. Sullivan, 2011. Extraction, isolation and characterization of globulin proteins from *Lupinus albus*. *J. Agric. Food Chem.*, 59: 2752-2758.
- Nurdiyana, H., M.K.S. Mazlina and M.S.N. Fadhilah, 2008. Optimization of protein extraction from freeze dried fish waste using response surface methodology (RSM). *Int. J. Eng. Technol.*, 5: 48-56.
- Omana, D.A., Y. Xu, V. Moayedi and M. Betti, 2010. Alkali-aided protein extraction from chicken dark meat: Chemical and functional properties of recovered proteins. *Process Biochem.*, 45: 375-381.
- Rafieian, F., J. Keramat and M. Kadivar, 2013. Optimization of gelatin extraction from chicken deboner residue using RSM method. *J. Food Sci. Technol.*, 50: 374-380.
- Samadi, S., M. Mohammadi and G.D. Najafpour, 2016. Production of single cell protein from sugarcane bagasse by solid state fermentation in tray bioreactor. *Int. J. Eng. Trans. Appl.*, 29: 1113-1119.
- Tan, S.H., R.J. Mailer, C.L. Blanchard and S.O. Agboola, 2011. Canola proteins for human consumption: Extraction, profile and functional properties. *J. Food Sci.*, 76: R16-R28.
- Van, H.A., V.J. Itterbeeck, H. Klunder, E. Mertens and A. Halloran *et al.*, 2013. *Edible Insects: Future Prospects for Food and Feed Security*. FAO, Rome, Italy, ISBN:978-92-5-107595-1, Pages: 201.
- Yi, L., C.M. Lakemond, L.M. Sagis, S.V. Eisner and V.A. Huis *et al.*, 2013. Extraction and characterisation of protein fractions from five insect species. *Food Chem.*, 141: 3341-3348.