Malays. Appl. Biol. (2019) 48(1): 241–249

BIOCONVERSION OF COCONUT-RESIDUE TO SOLUBLE PROTEIN BY *Aspergillus awamori*

AIZAT MOHD-RAZALI[1], MARIAM TAIB[2], MASOUKI MURNI[3] and AZIZ AHMAD[2*]

[1]*Institue of Medical Science Technology, University Kuala Lumpur, 43000 Kajang, Selangor, Malaysia*
[2]*School of Fundamental Sciences, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*
[3]*Centre for Fundamental and Liberal Education, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*
[*]*E-mail: aaziz@umt.edu.my*

Accepted 30 January 2019, Published online 20 March 2019

ABSTRACT

Protein is the most expensive and important nutrient component in feed formulation. An alternative protein source should be employed to reduce the dependency on fish meal. Limited reports are available regarding the bioconversion of coconut-residue derived-carbohydrate to soluble protein. The objective of this study was to determine the soluble protein and amino acid contents of coconut-residue after solid-state-fermentation by *Aspergillus awamori*. The complete randomised design (CRD) with three parameters; the inoculum-size (10%, 20%, and 30%), incubation temperature (30°C, 35°C and 40°C) and salt concentration (1x, 2x, 3x) were tested. Response surface method (RSM) was used to optimise the fermentation conditions. As a result, fermentation was increased and showed that the soluble protein content of the coconut-residue, to be 1.13-folds higher than the control. RSM analysis displayed that the best fermentation conditions comprised of 21.29% of inoculum size, 34.39°C of incubation temperature and 2.7-times of salt concentration after nine days of fermentation. Essential amino acids namely; histidine, valine, methionine, isoleucine, as well as three non-essential amino acids like the aspartic acid, serine and proline were significantly improved in the fermented coconut-residue. The current findings suggested that fermented coconut-residue is a feasible source of protein and amino acids in feed formulation.

Key words: Coconut, protein, fermentation, histidine, methionine, threonine

INTRODUCTION

One of the major challenges in modern era is food security, safety and sustainability. The global demand for protein meals or protein feed for aquaculture diet is expected to increase as a measurable consequence of ongoing growth in the world population. If no action is taken, the rise in consumption will lead to increased prices, thus putting pressure on animal production and also on food security eventually. Aquaculture represents a solution to the future world demand for healthy protein. The growth of aquaculture has been the fastest of all food production sectors, about 6% per year (FAO, 2016). Increase in aquaculture industry especially in Asia has induced tremendous demand for aqua-feed with the production rate exceeding 30% every year. However, the rising costs of feed protein and massive imports are among the challenges in feed production that requires an urgent solution. The cost of feed alone represents up to 60% of the total cost for industry run and the demand is continuously incresing. Proteins are the most expensive nutrients in feed, which subsequently causes cost increase of feed production, growth of fishes, industrial maintenance and finally the price of fishes (Gaylord & Barrows, 2009). Soybean meal and fishmeal are the two main sources of proteins in livestock diet (Smarason *et al*., 2018). The protein requirement is species-specific with regards to herbivorous, omnivorous and carnivorous fishes. To compute adequate amount of protein in feed, the content of essential amino acids must meet the requirements of the species to be fed. Protein supplementation in feed depends on their availability, digestibility, content of amino acids

* To whom correspondence should be addressed.

and nutrients, anti-nutritional factors as well as the cost of production (El-Sayed, 1999; Hossain *et al.,* 2002). Alternative protein sources are strongly recommended to sustain the growing aquaculture industry to reduce the cost of feeding.

Over the years plant-derived proteins such as soybean meal, corn meal, sunflower oil meal, palm plant by-products such as palm kernel cake and coconut residues have been introduced to minimize the cost of fish feeds (Francis *et al.*, 2001). However, plant origin protein can only be used in limited amounts due to amino acid compositions and the presence of anti-nutritional substances (Smarason *et al*., 2018). The use of microorganisms such fungus, to convert plant carbohydrate to 'single-cell protein' is a useful approach (Alriksson *et al*., 2014; Smarason *et al*., 2018). The re-cycling of the waste matters was proven to be useful for environment not only in terms of disposing waste but also may reduce the cost of production of feed up to 20%. *Aspergillus* is known as one of the most common fungi used in improving the protein content of *cassava* and sugar cane bagasse (Moo-Young *et al*., 2000). In most cases, the substrates that best fit as substrates of *Aspergillus* sp. are reported as the key factor in the better-quality protein production (Pandey, 2003). Therefore, the current study aims to develop a protein rich coconut-meal from the agro-waste by-products. The objective of this study was to determine fermentation conditions for bioconversion of carbohydrate in coconut-residues to soluble protein by *Aspergillus awamori* strain A1.

MATERIALS AND METHODS

Fungal Culture

The fungal culture of, *A. awamori* was isolated from coconut-residues obtained from a local wet market at Kuala Terengganu, Malaysia. The fresh coconut-residues have been exposed to open air for 7 days at room temperature prior to fungal isolation. Two grams of the residue was transferred onto sterile potato dextrose agar (PDA) and Vogel's (1956) minimal agar media (VMM) plates, which were prepared according to manufacturer's protocol (Difco™, France). Subsequently, both plates were incubated at room temperature and daily observed for 7 days or until fungal colony appeared. The colony was isolated and sub-cultured onto new agar medium plates and incubated at room temperature for 3 to 5 days. The process was repeated several times until a pure colony was obtained. Pure culture was inoculated on a slant medium and stored at 4°C as stock culture. The fungus was identified based on spores, colour and size of colony examined under light microscope based on key morphology (Samson *et al.*, 2007) and molecular methods as described by Hinrikson *et al*. (2005).

Preparation of Inoculum

The fungal was grown on VMM agar plate for three days and the mycelium was used as inocula. One cm diameter of fungal disc obtained using a cork borer was then transferred into a 50 mL Vogel's liquid media (Vogel's, 1956) in a 250 ml Erlenmeyer flask and sealed. The inocula was grown at ambient temperature with continuous shaking at 180 rpm on a rotary shaker (Barnstead/Lab Line, USA) for three days and used as fresh inocula. Subsequently, 2 mL of the inoculum was transferred into 50 ml fresh Vogel's liquid media in a 250 mL Erlenmeyer flask in triplicates. The cultures were grown at ambient temperature with shaking at 180 rpm on a reciprocal shaker (Barnstead/Lab Line, USA). The growth of the cultures, based on fresh weight was daily measured. At harvest the fungi cultures were filtered through Whatman No. 1 filter paper, blotted on a tissue paper and measured for fresh weight using electronic balance. Subsequently, the samples were oven dried (Memmert, Germany) at 60°C for 24 h or until constant weighed.

Substrate Preparation and Fermentation Conditions

The fermentation process was conducted using solid-state fermentation (SSF; Barrios-Gonzalez, 2012) conditions. The coconut residues were incubated in oven at 70°C for 24 hrs to reduce the moisture to 11% prior to grinding in powdered form. Twenty-five grams of powdered coconut residue were placed into a 250 mL Erlenmeyer flask, capped with aluminium foil and autoclaved at 121°C for 30 min. Subsequently, the residues were inoculated with fresh inocula of fungus and manually mixed. A Complete Block Randomized Design (CBRD) was used (Table 1) with three growth factors; the size of inocula, incubation temperature and salt concentration supplemented. The sizes of inocula were 10, 20 and 30% of total substrate (w/w). Incubation temperatures were at 28, 30 and 40°C. Salt was supplemented at one [1x], two [2x] and three [3x] times concentration based on Ramachandran *et al.* (2004). Salt concentration of [1x] consist of 1% (w/v) NH_4SO_4 and 0.3% (w/v) $KHPO_4$. The salt was added at 5 mL for each combination to maintain the initial moistures approximately at 50%. The fermentation process was allowed for 5 days and stopped by heat treatment at 60°C for 24 hrs. Subsequently, 25 g sample was defatted with 20 mL n-hexane for 3 hrs filtered through Whatman No. 1 and dried in fume-hood overnight. The total soluble proteins were extracted and quantified. The data was optimized and statistically analyzed using

Table 1. The total soluble protein content of coconut residue after five days fermentation with *A. awamori* at different combinations of inoculums size, incubation temperature and salt strength. Salt-free of unfermented dried coconut-residues was used as control (Mean ± Standard Error; n = 3; $p < 0.05$)

Treatment	Size of Inoculums (% w/w)	Incubation Temperature (°C)	Salt Concentration	Total Soluble Protein Content (mg/g dry wt. of sample)
CONTROL	–	–	–	32 ± 2^{a}
A1	10%	28°	[1X]	31 ± 2^{a}
A2	10%	28°	[2X]	32 ± 1^{a}
A3	10%	28°	[3X]	35 ± 3^{ab}
A4	10%	30°	[1X]	47 ± 2^{b}
A5	10%	30°	[2X]	49 ± 4^{b}
A6	10%	30°	[3X]	59 ± 2^{c}
A7	10%	40°	[1X]	38 ± 1^{b}
A8	10%	40°	[2X]	42 ± 4^{b}
A9	10%	40°	[3X]	40 ± 4^{b}
B1	20%	28°	[1X]	56 ± 2^{c}
B2	20%	28°	[2X]	55 ± 2^{c}
B3	20%	28°	[3X]	55 ± 5^{c}
B4	20%	30°	[1X]	61 ± 2^{cd}
B5	20%	30°	[2X]	64 ± 4^{cd}
B6	20%	30°	[3X]	60 ± 3^{cd}
B7	20%	40°	[1X]	58 ± 6^{cd}
B8	20%	40°	[2X]	64 ± 2^{d}
B9	20%	40°	[3X]	59 ± 4^{cd}
C1	30%	28°	[1X]	39 ± 3^{b}
C2	30%	28°	[2X]	43 ± 2^{b}
C3	30%	28°	[3X]	47 ± 7^{b}
C4	30%	30°	[1X]	31 ± 1^{a}
C5	30%	30°	[2X]	38 ± 1^{b}
C6	30%	30°	[3X]	41 ± 2^{b}
C7	30%	40°	[1X]	39 ± 4^{b}
C8	30%	40°	[2X]	39 ± 4^{b}
C9	30%	40°	[3X]	57 ± 7^{cd}

Response Surface Methodology. Three best combination of fermentation parameters that yielded highest protein were repeated with the incubation period at 5, 7, 9, 11 and 13 days. Triplicates were applied for each condition. At harvest, the total proteins and the anti-nutritional contents were quantified.

Quantification of Total Soluble Protein

The soluble protein was extracted using mild-acid extraction method as described by Pickardt *et al.* (2009) with slight modification. Three grams of sample were mild-acid extracted in 21mL McIlvaine Buffer at pH 4.0. The mixture was centrifuged at 7,000 X *g* (Eppendorf, USA), for 30 min at 4°C. Supernatant obtained was determined for total soluble protein content following the Bradford method (1976). The absorbance was read at 595nm using a double beam spectrophotometer (T80 UV/VIS Spectrophotometer, PG Instruments, UK). The protein concentration was calculated based on standard curve of Bovine Serum Albumin (BSA) in range of 2 to 10 ug/ml.

Amino Acid Content

The amino acids content in mycelium of *A. awamori*, the unfermented and fermented coconut-residue were determined following the procedures reported by Denina *et al*. (2010) with some modifications. Samples were hydrolysed in 6 N HCl at ratio 5 mg: 2ml (sample: solution) and 1% (v/v) phenol (Merck, Germany) to prevent the halogenation of tyrosine at 110°C for 24 hrs in a FOSS Tecator™ Digester system was supported with BUCHI-Scrubber (Switzerland) air cleaning system. Amino acids was chromatogram using a Hypersil AA-ODS (2.1 x 200 mm, 5mm; Hewlett-Packard) column attached to HPLC (Agilent, 1100; Hewlett-Packard, Palo Alto, CA) device equipped with an online vacuum degasser (Model G1322A), a quartenary pump (Model G1311A), an autosampler (Model G1329A), a thermostated column compartment (Model G1316A). The amino acids detection was conducted using Diode Array Detector (DAD) (Model G1315A) and Flourescent Light Detector (FLD) (Model G1321A). Mobile phase A consisted of 20 mM sodium acetate

supplemented with 0.018% (v/v) triethylamine (TEA) (pH 7.2) and 0.3% (v/v) tetrahydrofuran (THF). Mobile phase B consisted of 100 mM sodium acetate (pH 7.2): acetonitrile (ACN): methanol (MeOH) at the ratio of 1:2:2. The gradient of mobile phase were set to start at 100% A and gradually decreased to 40% A until 17 minute and to 100% B at 18 to 23.9 min, 100% A at 24 min. Flow rate was 0.45 ml/min until 8.1 minute, increased to 0.8 mL/ min at 18.5 to 23.9 minute at min 24 to 25 the flow rate was 0.45 mL/ min.

Statistical Analysis

Response Surface Methodology (RSM) was performed using Design-Expert Version 6.0.10 (Stat-Ease, Inc. Minneapolis, Minnesota) on the data obtained from the Optimization of Fermentation Condition (Section 3.3). Data obtained was analysed with trend analysis using One-Way Analysis of Variance (ANOVA: SPSS version 16.0, 2007). The One-Way ANOVA was followed with Tukey test for Post Hoc Multiple Comparisons.

RESULTS AND DISCUSSION

Fungal Cultures and Inoculum

Figure 1 shows the fungal colonies grown on culture media inoculated with coconut-residue. Based on the morphological characteristics and 18S rRNA sequence comparison, the isolated-fungus was identified as *Aspergillus awamori* strain A1. It has a normal growth phase with 0.22-fold growth rate during the log phase and reached the optimum growth after nine days of culture (Figure 2). Growth rate is curial in the fermentation process; higher growth rate would reduce the fermentation time, reducing the production cost (Pandey, 2003).

Effects of Fermentation Conditions on Soluble Protein Content

The bioconversion rate of coconut-residues to soluble protein by *A. awamori* (Table 1) was reliant on the combination of fermentation conditions; the inoculum size, salt concentration and incubation temperature. Results showed that higher soluble protein contents were obtained from treatments of B5 (30% inocula, 3X salt concentration, at 40°C), A6 (20% inocula, [2X] salt concentration at 30°C) and C9 (10% inocula, [3X] salt concentration at 30°C), which were 64 ± 4 mg/g, 59 ± 2 mg/g and 56 ± 7 mg/g, respectively. These corresponded to 2.0 to 1.75-fold higher than the control or unfermented coconut residue (32 ± 2 mg/g). The protein increment demonstrated the successful production of fungi-derived protein with fast growth activity of cells on the particular substrates (Pandey, 2003; Punt *et al*., 1991). Results also showed that higher salt concentration, between [2X] to [3X] and incubation temperature at 30 or 40°C was the most preferable condition by *A. awamori* in conversion of coconut-residue to soluble protein. Generally, coconut-residue contains soluble sugars, soluble proteins, starch, lipids and trace amounts of nitrogen (Ramachandran *et al*., 2004). The substrate that best fits the inocula (*Aspergillus*) is the key factor for better-quality protein production (Pandey, 2003). Carbohydrate as substrates has been proven capable

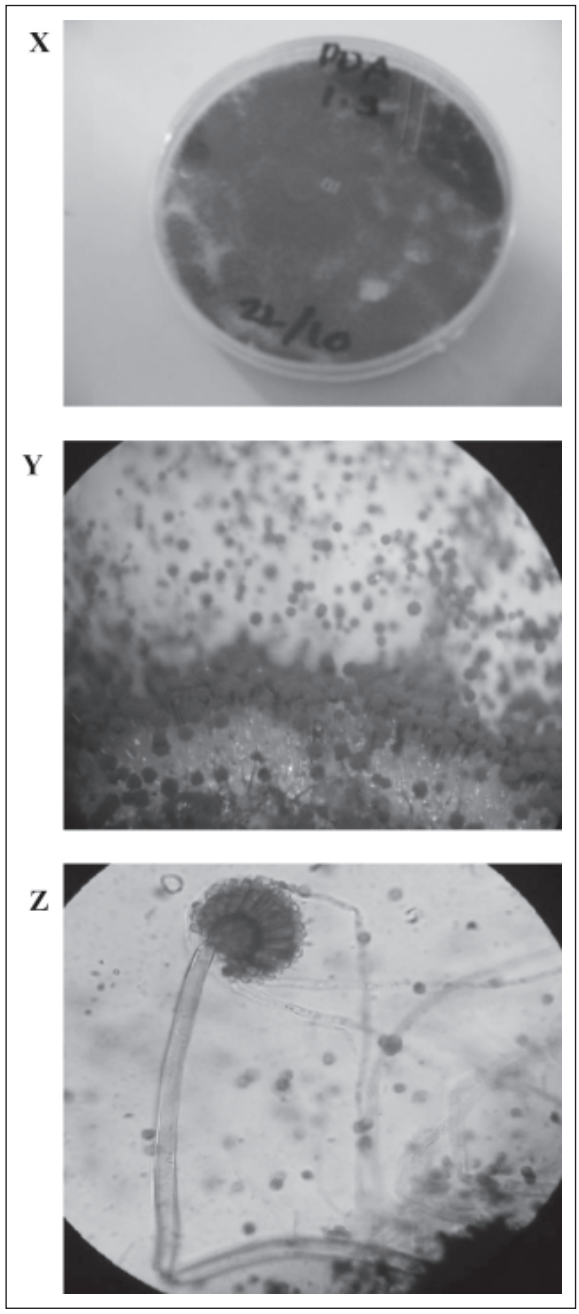

Fig. 1. The fungal colonies (X), spore (Y) and conidial heads (Z) of *Aspergillus awamori* strain A1 grown on agar medium inoculated with infected coconut residues.
Note: X the *A. awamori* strain A1; Y under dissecting microscope at 10 X magnification; Z under light compound microscope at 1000 X magnification.

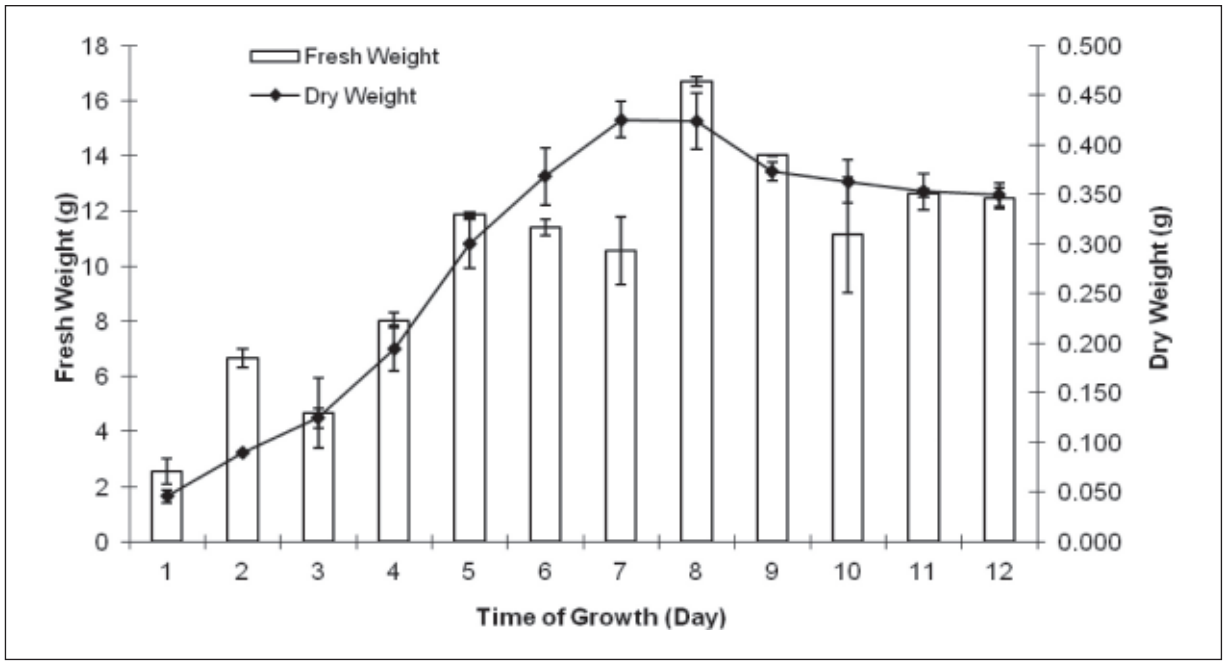

Fig. 2. The growth of *Aspergillus awamori* strain A1 based on fresh and dry weight until 12 days of incubation (day).

to embrace the growth of the fungus with supplementation of salt (Ramachandran *et al*., 2004). Nonetheless, other factors such as particle size, initial moisture, size of inoculums and supplemented nutrients and trace elements also contributed to the bioprocess activity (Moo-Young *et al*., 2000; Punt *et al*., 1991; Pandey, 2003).

The substrate composition and inoculum dosage were also reported to affect the quality and quantity of the fermentation products (Alriksson *et al*., 2014). At low size of inoculums (10%), the fungi growth is insufficient for the fermentation process on 25 g of coconut residues, as it requires longer period of incubation to maximize the usage of nutrient provided for its growth. The under-inoculated fermentation causes the formation of pellet where the mycelia growth is limited to a certain radius hence reducing the cell mass and protein production (Moo-Young *et al*., 2000). While, at larger inoculum size > 30%, the fungus must compete for nutrients and needs to sporulate earlier for survival and sustainability (Punt *et al*., 1991). Instead of nutrient remodelling, the fungi produces too much biomass resulting in poor protein secretion (Moo-Young *et al*., 2000). Therefore, 20% was the best inoculum size of *A. awamori* for bioconversion process of 25 g coconut-residues. At this inoculum size, fertile growth of *A. awamori* was achieved with higher amount of substrate utilization and higher protein production by the mycelium's single cell protein. Generally, fungus may contain 35-40% of crude protein (intracellular) and has the ability to produce extracellular protein (Punt *et al*., 1991).

In a separate experiment the effects of fermentation period on soluble protein content was also tested. Our results showed that the bioconversion capability of *A. awamori* on coconut-residue was influenced by the fermentation period and conditions (Figure 3). Treatment A6 with lower size of inoculum (10%) exhibited higher soluble protein content (39 ± 2 mg/g dry wt.) after 9 days of incubation. Meanwhile, treatment with higher size of inoculum (20 – 30%) took longer time of incubation period for high soluble protein content. For instance, treatments B5 and C9 contains 37 ± 3 and 51 ± 1 mg/g dry wt. sample of protein, respectively after 11 days of incubation. Nonetheless, treatment C9 with the highest size of inoculum, incubation temperature (40°C) and salt concentration [3X] exhibits a significantly higher soluble protein content ($p < 0.05$) compared to both treatments A6 and B5 treatments. This finding was in agreement with study by Farinas e*t al.* (2011), where the physical parameters and nutritional requirements play a significant role in the production of protein or enzyme endo-glucanase.

Optimisation of Fermentation Conditions Using Response Surface Methodology

The optimum condition for high protein content was further confirmed by RSM as displayed in Figure 4. Results showed that six different combinations of fermentation conditions reached the desirable level of 1.000, predicted the best conditions for the highest protein production from coconut-residue when using *A. awamori* (Table 2). Computing software such as RSM was able to

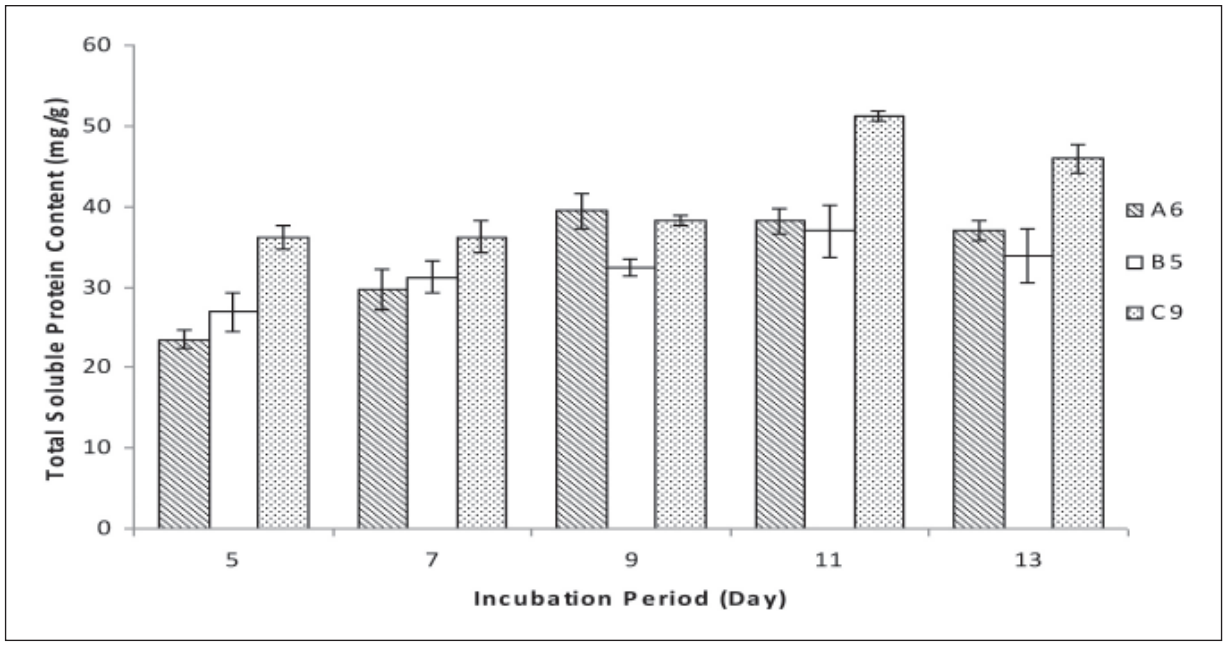

Fig. 3. Total soluble protein contents of treatments A6, B5 and C9 for 13 days of culture period. (Mean ± Standard Error; n = 3; $p < 0.05$).

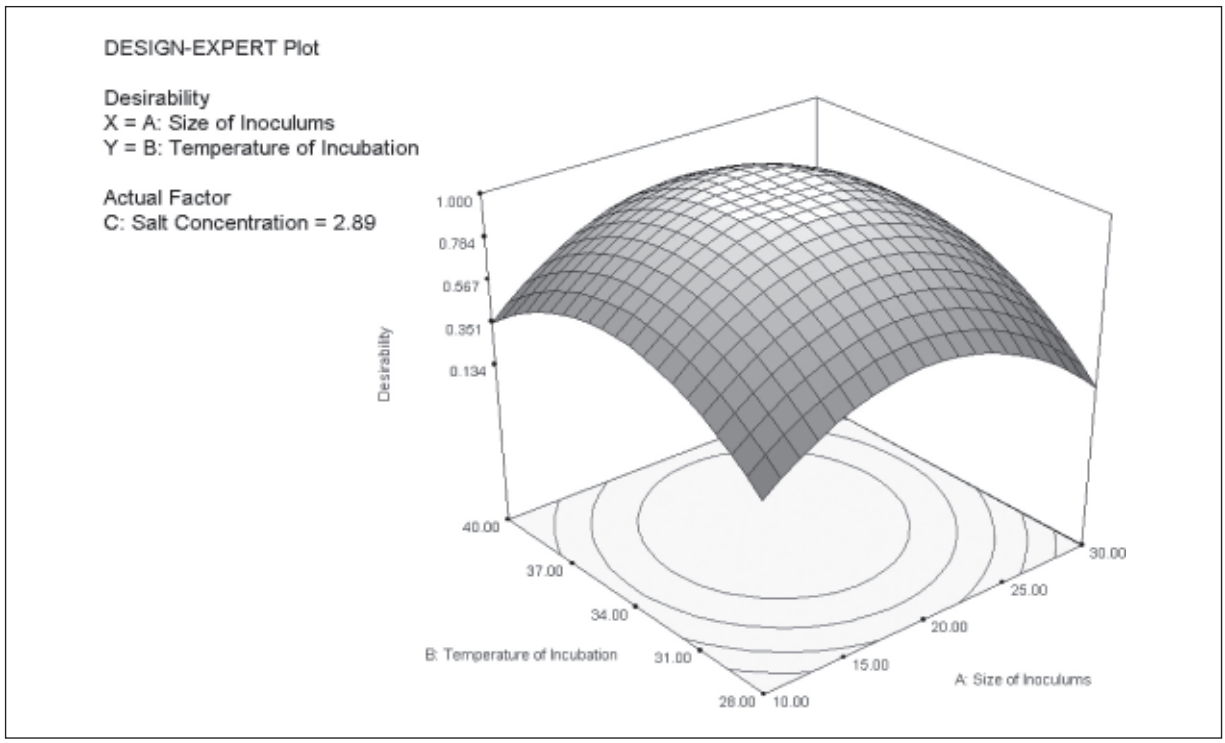

Fig. 4. Three-dimensional graph plotted by Response Surface Methodology using Design Expert 6.0.10 (Stat-Ease).

acquire precise condition for the fermentation optimization (Zhang *et al.*, 2010). The RSM analysis showed that higher protein production could be obtained with slight adjustment (Table 2) of the parameters compared to the earlier experiments (Table 1). For instance, the optimum conditions could be 19.02% (w/w) of inoculums size, with incubation temperature at 33.79°C and [2.94X] salt-concentration. This optimum condition might be applied for up-scaling of the fermentation process. However, the amount of desirability might be decreased referring to a number of unknown

Table 2. The optimum fermentation conditions that produced the highest soluble protein content generated using response surface methodology (RSM)

Num.	Size of Inoculums (%)	Incubation Temperature (ºC)	Salt Concentration	Total Soluble Protein (mg/g dry wt. of sample)	Desirability
1	17.6	35.81	2.89	4	1
2	18.82	33.77	2.97	4	1
3	19.02	33.79	2.94	4	1
4	22.91	34.64	2.88	4	1
5	19.76	34.4	2.56	4	1
6	20.54	34.65	1	4	0.982
#RSM	20	34	2	4.0862	SE= 0.19

**RSM;* Point prediction for the best results on the optimization of fermentation condition by Response Surface Methodology using Design Expert 6.0.10 (Stat-Ease) software.

Table 3. The amino acid contents in the coconut residue, *Aspergillus awamori* and fermented coconut residues

	Amino Acid		Unfermented Coconut (µg/g)	Fungus (*A. awamori*) (µg/g)	Fermented Coconut (µg/g)	Δ increases from unfermented (fold)
1	**Aspartic Acid**	ASP	343 ± 13^{b}	372 ± 50^{b}	788 ± 18^{a}	**2.29**
2	Glutamic Acid	GLU	639 ± 6^{a}	818 ± 108^{b}	716 ± 14^{b}	1.12
3	**Serine**	SER	387 ± 68^{a}	337 ± 73^{a}	623 ± 154^{b}	**1.60**
4	**Histidine**	HIS	796 ± 137^{a}	1518 ± 538^{b}	1537 ± 274^{b}	**1.93**
5	Glycine	GLY	41 ± 1^{a}	37 ± 1^{b}	45 ± 1^{c}	1.09
6	Threonine	THR	81 ± 1^{a}	77 ± 1^{b}	85 ± 1^{c}	1.04
7	Alanine	ALA	115 ± 6^{a}	101 ± 5^{b}	156 ± 9^{c}	1.35
8	Arginine	ARG	1299 ± 63^{a}	1155 ± 105^{a}	1395 ± 171^{a}	1.07
9	Tyrosine	TYR	1302 ± 119^{a}	1516 ± 402^{a}	1427 ± 8^{a}	1.09
10	Cystine	CYS	3560 ± 869^{a}	2604 ± 1306^{a}	5056 ± 1903^{a}	1.13
11	**Valine**	VAL	393 ± 4^{b}	372 ± 56^{b}	764 ± 23^{a}	**1.90**
12	**Methionine**	MET	386 ± 13^{b}	378 ± 48^{b}	610 ± 50^{a}	**1.58**
13	Phenylalanine	PHE	7155 ± 1651^{a}	2095 ± 1118^{b}	8237 ± 1793^{a}	1.15
14	**Isoleucine**	ILE	731 ± 21^{c}	139 ± 58^{b}	1825 ± 755^{a}	**2.49**
15	Leucine	LEU	387 ± 7^{b}	403 ± 34^{ab}	424 ± 3^{a}	1.09
16	Lysine	LYS	1060 ± 98^{a}	1071 ± 162^{a}	1112 ± 57^{a}	1.04
17	**Proline**	PRO	3208 ± 784^{c}	17927 ± 8782^{b}	8172 ± 489^{a}	**2.54**

Note: Values with similar alphabet in same row did not significantly different, using post hock test $p = 0.05$; Mean ± Standard Error; n = 3;

possibilities. The application of the statistical optimization software improved the rate of fermentation activity and increased production up to 41% compared to the single variable optimization in the culture medium (Zhang *et al*., 2010). Development of efficient and optimized industrial scale production processes is crucial to ensuring the economic viability of the protein production.

Amino Acids Content in Fungus, Unfermented and Fermented Coconut-residue

Our results showed that the contents of few essential amino acids; the histidine, valine, methionine and isoleucine in the fermented coconut-residues were higher than that of unfermented (Table 3). The increment of histidine, valine, methionine and isoleucine were 1.93-, 1.90-, 1.58-, and 2.49-times, respectively. Three other amino acids that significantly increased were aspartic acid (2.29-fold), serine (1.12-fold) and proline (2.54-fold). Higher accumulation of these amino acids in the fermented coconut-residues consisted of both the fungal mycelium (intracellular) and product (extracellular) of the bioconversion process on carbohydrate substrate. Interestingly, none of the amino acid concentrations in the fermented coconut residue decreased. The presence of essential amino acids in the fermented coconut-residues has a big potential in feed industry. The presence of higher level of essential amino acids may reduce the content of protein requirements in fish feed (Gaylord & Barrows, 2009). Our findings suggest that most of the amino acids in fermented coconut-residue

were comparable to vegetable oil meals. Ironically, methionine and lysine are the most limiting amino acids in feed required for cell growth, tissue regeneration and improvising the quality of animal feed (Riche *et al*., 2001; Cowieson *et al*., 2006; Gaylord & Barrows, 2009). In addition, fermentation also improves the animal feed through digestibility of amino acid content and provides nitrogen balance that enhances the growth rate and utilization for protein synthesis in fish (Bodin *et al*., 2012).

CONCLUSION

The bioconversion of carbohydrate to protein by fungus is an alternative source of protein meal. In this study, *A. awamori* has shown its capability to utilise the carbohydrate in coconut-residues as substrate, increasing the soluble protein and essential amino acids content. The rate of bioconversion was dependent on the inoculum-size, incubation temperature, salt concentration and period of incubation. The current study had opened a new insight in bio-processing of waste into wealth. Agro-waste product of coconut-residue was successfully initialized as a renewable sparing protein and/or energy source into feed.

ACKNOWLEDGEMENT

Authors would like to thank Ministry of Higher Education Malaysia (MOHE) for funding this research under Fundamental Research Grant Scheme (FRGS).

REFERENCES

Alriksson, B., Hörnberg, A., Gudnason, A.E., Knobloch, S., Arnason, J. & Johannsson. R. 2014. Fish feed from wood. *Cellulose Chemistry and Technology*, **48(9–10)**: 843-848.

Bamdad, F., Dokhani, S., Keramat, J. & Zareie, R. 2009. The impact of germination and *in vitro* digestion on the formation of angiotensin converting enzyme (ACE) inhibitory peptides from lentil proteins compared to whey proteins. *Engineering and Technology*, 36-46.

Barrios-Gonzalez, J. 2012. Solid-state fermentation: Physiology of solid medium, its molecular basis and applications. *Process Biochemistry*, **47(2)**: 175-185.

Bodin, N., Delfosse, G., Thu, T.T.N., Boulengé, E.L., Abboudi, T., Larondelle, Y. & Rollin, X. 2012. Effects of fish size and diet adaptation on growth performances and nitrogen utilization of rainbow trout (*Oncorhynchus mykiss W.)* juveniles given diets based on free and/or protein-bound amino acids. *Aquaculture*, **356-357**: 105-115.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochemistry*, **72**: 248-254.

Cowieson, A.J., Acamovic, T. & Bedford, M.R. 2006. Phytic acid and phytase: implications for protein utilization by poultry. *Poultry Science*, **85(5)**: 878-85.

Denina, I., Paegle, L., Prouza, M., Holátko, J., Pátek, M., Nesvera, J. & Ruklisha, M. 2010. Factors enhancing L-valine production by the growth-limited L-isoleucine auxotrophic strain *Corynebacterium glutamicum*. *Journal of Industrial Microbiology and Biotechnology*, **37(7)**: 689-99.

El-Sayed, A.F.M. 1999. Alternative dietary protein sources for farmed. *Aquaculture*, **179**: 149-168.

Farinas, C.S., Vitcosque, G.L., Fonseca, R.F. Neto, V.B. & Couri, S. 2011. Modelling the effects of solid state fermentation operating conditions on endoglucanase production using an instrumented bioreactor. *Industrial Crops and Products,* **34**: 1186-1192.

F.A.O. 2016. The State of World Fisheries and Aquaculture. Contributing to food security and nutrition for all. Rome. p. 200

Francis, G., Makkar, H.P.S. & Becker, K. 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, **199**: 197-227.

Gaylord, T.G. & Barrows, F.T. 2009. Multiple amino acid supplementations to reduce dietary protein in plant-based rainbow trout *Oncorhynchus mykiss* feeds. *Aquaculture*, **287(1-2)**: 180-184.

Hinrikson, H.P., Hurst, S.F. de Aquirre, L. & Morrison, C.J. 2015. Molecular methods for identification of *Aspergillus* species. *Medical Myxcology,* **43**: S129-S137.

Hossain, F., Onyango, B., Adelaja, A., Schilling, B. & Hallman, W. 2002. Uncovering factors influencing public perceptions of food biotechnology. *Food Policy Institute. Working Paper* 0602-003.

Kies, A.K., De Jonge, L.H., Kemme, P.A. & Jongbloed, A.W. 2006. Interaction between protein, phytate, and microbial phytase. *In vitro* studies. *Journal of Agricultural and Food Chemistry*, **54(5)**: 1753-1758.

Loeffler, J., Hebart, H., Cox, P., Flues, N., Schumacher, U. & Einsele, H. 2001. Nucleic acid sequence-based amplication of *Aspergillus* RNA in blood samples. *Journal of Clinical Microbiology*, **39(4)**: 1626-1629.

Moo-Young, M., Xu, J., Wang, L., Ridgway, D. & Gu, T. 2000. Increased heterologous protein production in *Aspergillus niger* fermentation through extracellular proteases inhibition by pelleted growth. *Biotechnology Progress*, **16**: 222-227.

Moorthy, M. & Viswanathan, K. 2009. Digestibility and feeding value of coconut meal for white leghorn layers. *Tamilnadu Journal of Veterinary & Animal Sciences*, **6(5)**: 196-203.

Pickardt, C., Neidhart, S., Griesbach, C., Dube, M., Knauf, U., Kammerer, D.R. & Carle, R. 2009. Food hydrocolloids optimisation of mild-acidic protein extraction from defatted sunflower (*Helianthus annuus L.*) meal. *Food Hydrocolloids*, **23(7)**: 1966-1973.

Punt, P.J., Zegers, N.D., Busscher, M., Pouwels, P.H. & van den Hondel, C.A.M.J.J. 1991. Intracellular and extracellular production of proteins in *Aspergillus* under the control of expression signals of the highly expressed *Aspergillus nidulans* gpdA gene. *Journal of Biotechnology*, **17**: 19-33.

Ramachandran, S., Patel, A.K., Nampoothiri, K.M., Francis, F., Nagy, V., Szakacs, G. & Pandey, A. 2004. Coconut oil cake, a potential raw material for the production of a-amylase. *Bioresource Technology*, **93**: 169-174.

Riche, M., Trottier, N.L., Ku, P.K. & Garling, D.L. 2001. Apparent digestibility of crude protein and apparent availability of individual amino acids in tilapia (*Oreochromis niloticus*) fed phytase pre-treated soybean meal diets. *Fish Physiology and Biochemistry*, **25**: 181-194.

Sadasivam, S. & Manickam, A. 1996. *Biochemical Methods. New Age International* (P) Limited Publishers, ISBN: 81-224-0976-8.

Samson, R.A., Noonim, P., Meijer, M., Houbraken, J. & Frisvad, J.C. 2007. Diagnostic tools to identify black *Aspergilli*. *Studies in Mycology,* **59**: 129-145.

Smarason, B.O., Alrikson, B. & Johannsson, R. 2018. Safe and sustainable protein sources from the forest industry – The case of fish feed. *Trends in Food Science & Technology*, https://doi.org/10.1016/j.tifs.2018.03.005

Vogel, H.J. 1956. A convenient growth medium for *Neurospora crassa*. *Microbial Genetics Bulletin*, **13**: 42-44.

Yin, S., Tang, C., Wen, Q., Yang, X. & Li, L. 2008. Functional properties and *in vitro* trypsin digestibility of red kidney bean (*Phaseolus vulgaris L.*) protein isolate/: Effect of high-pressure treatment. *In Vitro*, **110**: 938-945.

Zhang, X., Zhou, J., Fu, W., Li, Z., Zhong, J., Yang, J. & Xiao, L. 2010. Response surface methodology used for statistical optimization of jiean-peptide production by *Bacillus subtilis*. *Electronic Journal of Biotechnology*, **13(4)**.