

The Utilisation of Rice Husks as Stand-alone Substrate in the Solid-State Fermentation for Amylase Production by Local Isolate *Aspergillus flavus* NSH9

R. Saat*, M.H. Hussain and A.A.S. Husaini

Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

A variety of agro-waste has been applied as the solid-state fermentation (SSF) substrate for the production of amylase, nevertheless, only a few studies of rice husks with supplements used as the substrate were reported. Hence, this study was conducted as to investigate the feasibility of rice husk alone without additional supplements as the SSF substrate to produce amylase from the local fungal isolate. Different microbial strains or isolate with different fermentation substrates may have different SSF process requirement for achieving high enzyme activity yield. Therefore, the SSF parameters such as initial moisture content of the substrate, incubation period, incubation temperature and substrate particle were studied and optimised. The highest amylase enzyme activity was observed at 70 % of initial moisture content, 144 hours (6 days) and 30°C, with substrate particle size of 2 mm. Starch hydrolysis analysis performed indicated that the strength of amylase activity in the partially purified extracted enzyme was three times higher than the crude enzyme. Furthermore, a negligible or none of major aflatoxin compounds was detected in the crude and partially purified enzyme. The obtained findings strongly suggest the high potential usage of rice husk alone as the substrate in the SSF process for the production of aflatoxin-free amylase by the local isolate *Aspergillus flavus* (*A. flavus*) NSH9.

Keywords: *Aspergillus flavus*; rice husks; solid-state fermentation; amylase; aflatoxin

I. INTRODUCTION

Rice husk or rice hull is the hard-protective covering of rice grain, and it is removed from rice seed as a by-product during the rice milling process. Rice husk is one of the most widely available agricultural wastes in many rice-producing countries around the globe such as Malaysia. On average 20 to 22 % yield of harvested rice is rice husk (Habeeb & Mahmud, 2010; Ugheoke & Mamat, 2012) which means for every 1 kg of paddy milled, about 200 g to 220 g of husk is produced. In 2023, Malaysia produced at estimated of 1,503,151 metric tonnes of rice where approximately 330,693 tonnes of rice husks were generated, and 15,598 tonnes of rice husks were produced in the state of Sarawak alone (Booklet Statistik Tanaman Pertanian, 2024). Despite the abundant availability of the rice husks, industrial applications of rice husk are still limited whereby in the majority of rice-

producing countries most of rice husk produced is either burnt or dumped as solid waste (Lim *et al.*, 2012). In Malaysia, commercially produced rice husks were ground with broken rice to be used as animal feeds (Johnson & Yunus, 2009). However, due to its low nutrients' digestibility with high silica, ash content and abrasive characteristics, limits its usage (Ghadi *et al.*, 2011). A large part of this agricultural by-product is burnt as fuel during rice processing and when this husk is burnt in the boilers, 25 % of rice husk ash is generated. Although the resulting ash is used or sold as fertiliser, still much of the ash is a dumped waste. If the ash is not properly disposed it could be a great environmental threat which can cause damage to the land and surrounding areas (Kumar *et al.*, 2013; Johnson & Yunus, 2009). Despite having high potential and suitability in many uses as well as industrial applications such as in ceramic, concrete and board production, bio-sorbent production and petroleum industry (Noor Syuhadah & Rohasliney, 2012; Johnson & Yunus, 2009; Manpreet, 2005) still a

*Corresponding author's e-mail: srosma@unimas.my

little portion of rice husks in Malaysia is actually being utilised. Most of these rice husks are simply burnt or disposed as solid waste and thus, creating environmental problems (Ludueno *et al.*, 2011). Commercial utilisation of the agro-wastes could solve the disposal problem and subsequently, the cost of waste treatment could be reduced. Agro-industrial waste such as rice husks which are cheap and abundantly found in Malaysia could readily be used as substrates for SSF process in cultivation of numerous microorganisms for the production of various secondary metabolites, such as enzymes, which are important for industrial applications (Ibrahim *et al.*, 2012).

Global enzymes market size was around USD 6.3 billion in 2017 and estimated to exceed USD 10 billion by 2024 (Verma & Iqbal 2017). The world enzyme demand is increasing as enzymes are applied in diverse arrays of applications, including technical use, food manufacturing, animal nutrition, cosmetics, medication and as tools for research. Also, due to the fast growth in the pharmaceutical industry to diagnostics enzyme with expanded access to medical care in developing countries (Nirmala & Muralikrishna, 2003). The majority of industrial enzymes being used are hydrolases or hydrolytic enzymes (Saranraj & Stella, 2013). Hydrolytic enzymes are of great significance as these enzymes confer several advantages over the use of chemicals such as acids. One of the advantages is the specificity of enzymes which allows the production of well-defined physical and chemical properties products. Furthermore, the 'mild' enzymatic hydrolysis will result in a few side reactions in compared to chemical hydrolysis (Shafiei *et al.*, 2011; Vengadaramana, 2013; Vaidya *et al.*, 2015). Amongst the different hydrolases, amylases possess the highest potential use in a variety of industries as well as biopharmaceutical and medicinal purposes (Gopinath *et al.*, 2017). Apart from agriculture, Malaysia is also known for its variety of industries such as food and beverages, detergents, animal feed, baking, edible oil and palm oil products, and manufacturing that utilise hydrolytic enzymes, particularly, amylases at some stage or the other (Ibrahim *et al.*, 2012). Most of the industrial enzymes used in Malaysia are imported from other countries, especially, from Denmark, Netherlands and Belgium as there is still yet a local commercial production of these enzymes.

In 2008, the import of industrial enzymes amounted about US\$ 3.5 million annually with the quantum of more than 1 million kg of crude enzyme preparation (Ibrahim *et al.*, 2012). Imported enzymes generally are not cheap, which may result in a higher production cost for industries, especially, those with large scale applications of enzymes. Moreover, the application of industrial enzymes such as amylases in Malaysia is expected to increase exponential due to its vast applications and growth potentials in other areas such as bioconversion as well as green technology (Ibrahim *et al.*, 2012; Vaidya *et al.*, 2015). Thus, this study was conducted as to outsource and produce amylase enzyme from local fungal isolate *Aspergillus flavus* (*A. flavus*) NSH9 via solid-state fermentation (SSF) applying local rice husks alone without addition of any media supplements or nutrients.

Rice husk was shown to have a potential usage as a substrate in the SSF process for producing a list of important industrial enzymes by a variety of microorganisms (Oyeleke *et al.*, 2010; Zambare, 2010; Fadaei & Salehifar, 2012; Masutti *et al.*, 2012). Although rice husk was applied in the SSF process of *A. flavus* for the production of amylase, the rice husk used was supplemented with minerals (Yasmin *et al.*, 2016a). Currently, none on the production of amylase by *A. flavus* applying rice husks alone without any addition of any kind of supplements is observed.

II. MATERIALS AND METHOD

A. A. flavus NSH9

A total of 13 fungal isolates were obtained from sago humus found at sago plantation in Mukah, Sarawak, Malaysia. Preliminary screening for amylolytic activity showed that one of the isolates which was isolate number 9 had the highest activity, and this isolate was subsequently subjected to the DNA sequencing analysis. Sequencing data obtained suggested that the fungal isolate is *Aspergillus flavus* and hence, it is named as *A. flavus* NSH9 (Rosmawati *et al.*, 2012). This isolate was then cultured and maintained on a PDA as the stock culture (Rosmawati *et al.*, 2012) of the fungal collections of Molecular Genetics Laboratory at the Faculty of Resource Science and Technology, UNIMAS. This stock culture was used in the subsequent fungal sub-culturing.

B. Sub-culturing of *A. flavus* NSH9

A. flavus NSH9 was sub-cultured on a potato dextrose agar (BD Difco, USA) containing 50 µg/ml of ampicillin (Nacalai Tesque, Japan) under a sterile condition. Incubation was then conducted at room temperature for about 5 to 6 days until full fungal growth was observed. The matured culture was then maintained at 4°C prior to use for this study.

C. SSF of *A. flavus* NSH9

1. SSF substrate

Rice husks obtained from the rice milling factories around Samarahan division in Sarawak, Malaysia were used as the SSF substrate.

2. Pre-treatment of the substrate

Prior to use for SSF process, the rice husks were mechanically pre-treated by grinding into smaller size particles using a cutting mill (Retsch SM 100, Germany). The pre-treated rice husks were then stored in an air-tight container at room temperature prior to use.

3. SSF process

The SSF process was performed based on the method described by Singhanian *et al.* (2009). Prior to SSF process, ultra-pure water (Barnstead water purification system, ThermoFisher Scientific) was added to 5 g of pre-treated rice husks (in an autoclaved 250 ml Erlenmeyer conical flask) to achieve an initial moisture content of 50 %. The water was let to be absorbed by the substrate at room temperature for a while. Subsequently, three plugs of matured *A. flavus* NSH9 (approximately 0.5 cm² in size) were cut out from the PDA and inoculated onto the rice husks (Figure 1). The inoculation was performed under a sterile condition. Incubation was subsequently conducted at room temperature with a static condition for 144 hrs (6 days).



Figure 1. Rice husk inoculated with 3 plugs of *A. flavus* NSH9

4. Optimisation of SSF parameters

The parameters optimised were the size of substrate particles, initial moisture content, temperature of incubation and period of incubation. The substrate particle size was increased from 1 mm to 2 mm. The initial moisture content was optimised from 50 % to 70 %. As for the temperature of incubation, it was increased from room temperature to 30°C. The optimal SSF incubation period was determined by conducting the fermentation process at 30°C under a static condition in an incubator (Taitec Bioshaker BR-43FL), with 70 % of initial moisture content for 48 hrs (2 days), 96 hrs (4 days), 144 hrs (6 days) and 192 hrs (8 days), separately.

D. Crude Enzyme Extraction

The extraction of the crude extracellular enzyme from the fungus was performed using the following steps (Rosmawati *et al.*, 2012). A volume of 20 ml of cold sodium acetate buffer (0.1 M; pH 5.8) was added into the flask (containing the substrate and fungi mixture) upon completion of SSF process and followed with shaking at 120 rpm (Taitec Bioshaker BR-43FL) for 30 min at a room temperature. The homogenised culture was then filtered through a muslin cloth to separate the solid biomass residues. Subsequently, the filtrate was centrifuged at 6,000 rpm for 30 min at 4°C (Himac CR 21G High-Speed Refrigerated Centrifuge, Hitachi, Japan). The collected supernatant was then filtered twice through Whatmann paper No. 1. Both filtration processes were performed on ice. The collected filtrate was used as the crude enzyme.

E. Partial Purification of the Crude Enzyme

The crude enzyme obtained was then filtered again using V1 Filtration glass set with ROCKER 610 vacuum pump (Rocker, Taiwan) through cellulose acetate filter with the pore size of 0.45 µm (Sartorius Stedim, Germany). The process was performed on ice. The enzyme was then further purified and concentrated using Vivaspın20 with MWCO of 10,000 kDa and polyether sulfone as the membrane (Sartorius Stedim, Germany) with centrifugation of 7,000 xg at 4°C (Himac CR 21G High-Speed Refrigerated Centrifuge, Hitachi, Japan) according to the manufacturer's protocol. The concentrated enzyme was then kept at 4°C prior to further use.

F. Measurement of Enzymatic Activity

The enzyme activity of extracted enzymes was assayed using a reaction consists of 1% (w/v) of soluble starch (Sigma-Aldrich) and extracted enzyme (1:1). The mixture was then incubated at 37°C for 30 mins. Total reducing sugars released from the assay was determined using standard DNSA method (Miller, 1959) with glucose as the standard. The enzyme activity was calculated using the following formula.

Enzyme activity (U):

$$\frac{\text{Concentration of glucose released} \times \text{Total assay volume} \times \text{Dilution factor}}{\text{Volume of enzyme used} \times \text{Diameter of cuvette} \times \text{Incubation time}}$$

One unit of enzyme activity (U) was defined as the amount of enzyme that released 1 mg/ml of glucose per minute from a soluble starch under the standard assay condition.

G. Detection of Amylolytic Activity in the Crude and Partial Purified Enzymes

A titre of crude enzyme and partial purified enzyme each was inserted into a starch agar. The surface of the agar was then stained with Lugol's iodine solution. The change in colour was observed.

H. Aflatoxin Analysis

The crude enzyme and partially purified enzyme were sent to the Malaysian Pepper Board Kuching, Sarawak for the aflatoxin analyses. The analysis was performed using American Spice Trade Association (ASTA) analytical method 24.2. in determining the levels of aflatoxin compounds B1, B2, G1 and G2 with the use of HPLC (Waters). The generated data was then analysed.

III. RESULT AND DISCUSSION

A. Solid-state Fermentation of *A. flavus* NSH9

Several fermentation methods can be applied for the production of hydrolytic enzymes such as amylases by micro-organisms. Nevertheless, SSF which utilises solid particles as the substrate for the microbial growth and its metabolic processes is best suited for the filamentous fungi, particularly, *Aspergillus* spp. such as *A. flavus* (Li *et al.*, 2012). SSF process applying agro-waste is said to be the cheapest amongst other fermentation methods as well as environmentally friendly in the value-added industrial productions such as hydrolytic enzymes (Li *et al.*, 2012). Furthermore, SSF method is shown to produce higher yields or better product characteristics than other fermentation methods such as Submerged fermentation and Liquid-state fermentation (Masutti *et al.*, 2012). Agro-industrial residues such as rice husk which are natural could serve as the best substrates for SSF process due to their rich organic nature apart from being abundantly available and cheap (Lim *et al.*, 2012). Rice husks are regarded as under-value residues that lack of nutrients and commercial values. In fact, this agro-industrial waste consists more than just the three polymers of cellulose, hemicelluloses and lignin. Studies have shown that it also contains ash, carbon, hydrogen, oxygen, nitrogen, sulphur, moisture, crude protein, crude fibre, minerals, oil and starch (How & Che Omar, 2004; Fadaei & Salehifar, 2012; Kumar & Kanwar, 2012; Kumar *et al.*, 2013). Furthermore, the rice husk ash constitutes a list of minerals such as potassium, magnesium, calcium, natrium, ferum, aluminium, phosphorus, manganese and zinc (Chiang *et al.*, 2008; Kumar *et al.*, 2013) that can support fungal growth such as *Aspergillus* spp.

In this study, a minimal and simple apparatus was employed in performing SSF of *A. flavus* NSH9 with a conical flask, an incubator, aluminium foil to cover the opening of the flask and parafilm to tighten the cover. Stringent sterility or strict aseptic operation is not essential or required in the SSF process as often SSF processes involve organisms which can grow quite rapidly under the low water conditions and hence, able to out-compete the contaminating organisms (Pandey *et al.*, 2000). The SSF process of *A. flavus* NSH9 in this study, nevertheless, was performed in a clean manner with a minimal sterility or aseptic condition. The pre-treatment of rice husk (grinding) performed prior to SSF process is necessary to generate suitable substrate particle size as to allow the rice husk's chemical components to be accessible and its physical structure to be more susceptible to the penetration of the fungal mycelia (Manpreet *et al.*, 2005). A mature fungal culture with conidia (containing spores) was used in the inoculation as to achieve uniform dispersion through the media with a better yield, better morphology and higher stability of the fungus (Singhania *et al.*, 2009; Mienda *et al.*, 2011). Initially, SSF was performed by inoculating 3 plugs of matured *A. flavus* NSH9 onto 5 g of pre-treated rice husks (average size of 1 mm) with 50 % of initial moisture content and incubated at room temperature. However, the fungal growth on the substrate was observed to be minimal (Figure 2). Therefore, three SSF parameters were optimised. The particle size of substrate was increased to 2 mm, substrate's initial moisture content was increased from 50 % to 70 % and subsequently, the incubation temperature was raised up to 30°C. Fully grown uniform fungal colonies were observed to be covering almost the entire surface of the substrate after six days of incubation at 30°C (Figure 3).

Another important parameter that influences the success of SSF process is the incubation period. Thus, the fermentation processes were conducted in the range of between 2 days and 8 days (with interval of 2 days) at 30°C and with substrate's initial moisture content of 70 %, separately. The highest crude enzyme activity (0.133 U) was recorded at the sixth day of fermentation (Figure 4). The optimal SSF condition for *A. flavus* NSH9 to produce the highest enzyme activity of amylase using rice husks as the substrate is shown as in Table 1. The enzyme activity of concentrated partial purified enzyme (0.346 U) was found to be higher by 2.6 folds than the extracted crude enzyme (0.133 U).



Figure 2. A minimal growth of *A. flavus* NSH9 on rice husk. Several colonies were observed to be scattered on the rice husks.

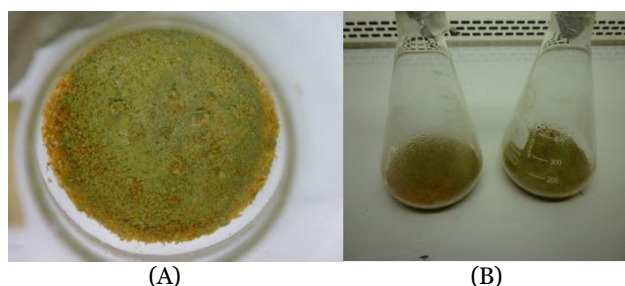


Figure 3. *A. flavus* NSH9 after 6 days of SSF. (A) A top view of the *A. flavus* NSH9 colonies on rice husks (B) *A. flavus* NSH9 colonies on rice husks in conical flasks.

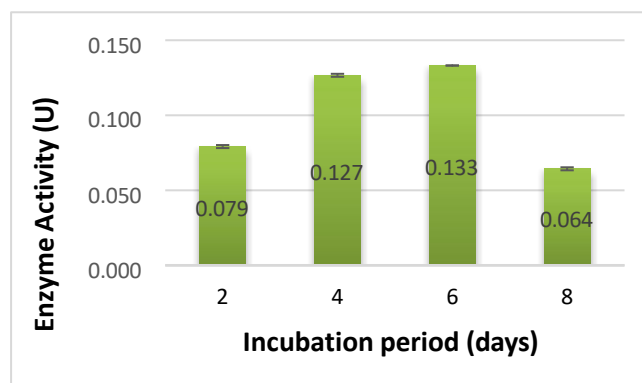


Figure 4. Enzyme activities of the extracted crude enzyme at different incubation period of SSF. Data represent mean $\pm$ S.D. (n=2). One unit of enzyme activity (U) is defined as the amount of enzyme that released 1 mg/ml of glucose per minute from a soluble starch under the standard assay condition.

Table 1. Summary of the SSF parameters for initial SSF and after optimisation.

SSF Parameter	Initial	Optimal SSF condition
Size of rice husks	1 mm	2 mm
Initial moisture content of rice husks	50 %	70 %
Temperature	Room temperature	30°C
Incubation period	6 days	6 days (144 hours)

B. Detection of Amylolytic Activity in the Extracted Crude and Partially Purified Enzymes

The enzyme extracted from the SSF process of *A. flavus* NSH9 was further investigated for the presence of amylase via starch hydrolysis analysis. Colourless zones (halo) were observed around the titres of both crude and partially purified enzymes (Figure 5) indicating that the starch has been hydrolysed to low molecular weight products such as glucose (Nirmala & Muralikrishna, 2003). The loss of intense blue-black stain is because of the iodide ion could no longer slip into the structure of amylose as it was hydrolysed into simpler structure such as glucose. These observations suggested that there were amylolytic activities. A larger diameter of clear zone around the partially purified enzyme titre (about 24 mm) was observed compared to the size of halo around the crude enzyme titre (about 8 mm) in diameter) which indicates that starch has been hydrolysed more by the partially purified enzyme than the crude enzyme (Figure 5). These observations strongly suggested that the enzyme obtained was amylase. The extracted crude enzyme may contain other enzymes apart from amylase as *A. flavus* is known to secrete a list of extracellular hydrolytic enzymes (Amaike & Keller, 2011) and thus, the amylolytic activity was not strong as indicated by the small diameter of halo. Whereas, when the crude enzyme was partially purified and concentrated, most of other hydrolytic enzymes were removed and resulted in a strong amylolytic activity which gave a larger halo size.

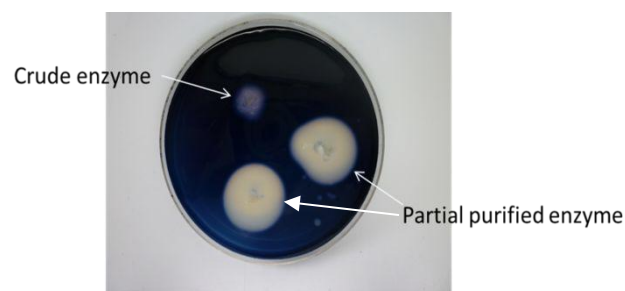


Figure 5. A starch agar stained with Lugol's iodine, showing the halo zones of crude enzyme titre ($\varnothing \sim 8$ mm) and partially purified enzyme titre ($\varnothing \sim 24$ mm).

C. Aflatoxin Analysis

A. flavus is a well-known fungus to produce aflatoxin, a family of mycotoxin, which is not only a threat to crops and plants but also can be harmful to humans and animals when inhale or ingest the aflatoxin-contaminated food or feed (Yang *et al.*, 2016). There are four major aflatoxin compounds known as B₁, B₂, G₁ and G₂ which contaminate agricultural commodities and may pose a potential risk to human health and livestock (Yu *et al.*, 2004). Therefore, aflatoxin analysis was performed on the extracted and partially purified enzyme to detect the presence of any of the major toxic compounds as well as to determine its quantity. There were two types of aflatoxin compounds detected in the enzyme extracted from *A. flavus* of earlier culture grown on PDA which undergone SSF process on rice husks. These aflatoxin compounds of B₂ and G₂ detected, however, were shown to be a very minimal in amount which was considerably almost undetectable. The aflatoxin compounds detected in the crude enzyme were B₂ of 0.272 $\mu\text{g/kg}$ or 0.000272 ppm and G₂ of 0.176 $\mu\text{g/kg}$ or 0.000176 ppm with the aflatoxin total amount of 0.448 $\mu\text{g/kg}$ or 0.000448 ppm (Table 1).

As for the partially purified enzyme of the earlier culture, the total amount of aflatoxin determined was slightly higher with 1.752 $\mu\text{g/kg}$ (0.001752 ppm) which comprised of aflatoxin B₂ of 0.678 $\mu\text{g/kg}$ (0.000678 ppm) and 1.074 $\mu\text{g/kg}$ or 0.001074 ppm of aflatoxin G₂ (Table 2). However, aflatoxin B₁ and G₁, were not detected in those both crude and partial purified enzymes (Table 1 and Table 2). On the other hand, none of the aflatoxin compounds was detected in the crude enzyme as well as in the partially purified enzyme extracted from the SSF fermented *A. flavus* NSH9 of later culture (Table 3 and Table 4).

Different *A. flavus* isolates can vary considerably in their ability to produce any of the aflatoxin compounds (Chang & Ehrlich, 2010). Although most studies have shown that the temperature between 24°C and 30°C favours the aflatoxin production (Obrian *et al.*, 2007), the amount of aflatoxin compounds (B2 and G2 types) detected in the extracted enzyme from *A. flavus* NSH9 which was grown on rice husks at 30°C was very minimal.

The regulation of aflatoxin biosynthesis is complex whereby it may be influenced by several environmental and cultural condition factors such as temperature, pH, amino acid supplement, nitrogen as well as carbon sources (Obrian *et al.*, 2007; Wilkinson *et al.*, 2007). Other study which applied amino acid supplement such as tyrosine into their medium showed significant increase of aflatoxin compounds (Wilkinson *et al.*, 2007). Rice husk applied in this study was free from any media supplements including amino acids as well as other carbon sources. Other factor contributed to the minimal presence of aflatoxin compounds is the ready availability of the substrate (with optimal size), which is the rice husk in SSF process, that provided an easy access and utilisation of the substrate for nutrients for the fungal growth as well as its metabolic processes. Furthermore, there is no competition with other micro-organisms either for food or space. Whereas in nature, for *A. flavus* be able to invade host tissues and out-compete other microorganisms for nutrients as well as for survival, requires the fungus to secrete high dose of aflatoxins. The highest level of aflatoxin is produced when the fungus invades the seed embryo, where the highest levels of simple sugars (glucose and sucrose) are present compared to other parts of the seed (Bhatnagar *et al.*, 2006). In rice husks used in the SSF process, on the other hand, simple sugars were not available.

The enzyme extracted from *A. flavus* NSH9 that had been several re-cultured and maintained on PDA which had undergone SSF on rice husks, however, was found to be free from any of those four major types of aflatoxin compounds. The absence of any of the major toxic compounds in both crude and partially purified enzyme may be due to the loss of capability in producing those aflatoxin compounds caused by several sub-culturing of the fungal isolate on a culture medium particularly PDA (Chang *et al.*, 2007; Yu, 2012).

Table 1. Aflatoxin results of crude enzyme from earlier culture of *A. flavus* NSH9.

Sample code: C1	
Aflatoxin B1 (µg/kg)	ND
Aflatoxin B2 (µg/kg)	0.272
Aflatoxin G1 (µg/kg)	ND
Aflatoxin G2 (µg/kg)	0.176
Total Aflatoxin (µg/kg)	0.448

Note: ND = not detected

Table 2. Aflatoxin results of partially purified enzyme form earlier culture of *A. flavus* NSH9.

Sample code: P1	
Aflatoxin B1 (µg/kg)	ND
Aflatoxin B2 (µg/kg)	0.678
Aflatoxin G1 (µg/kg)	ND
Aflatoxin G2 (µg/kg)	1.074
Total Aflatoxin (µg/kg)	1.752

Note: ND = not detected

Table 3. Aflatoxin results of crude enzyme from later culture of *A. flavus* NSH9.

Sample code: C2	
Aflatoxin B1 (µg/kg)	ND
Aflatoxin B2 (µg/kg)	ND
Aflatoxin G1 (µg/kg)	ND
Aflatoxin G2 (µg/kg)	ND
Total Aflatoxin (µg/kg)	0.000

Note: ND = not detected

Table 4. Aflatoxin results of partially purified enzyme from later culture of *A. flavus* NSH9.

Sample code: P2	
Aflatoxin B1 (µg/kg)	ND
Aflatoxin B2 (µg/kg)	ND
Aflatoxin G1 (µg/kg)	ND
Aflatoxin G2 (µg/kg)	ND
Total Aflatoxin (µg/kg)	0.000

Note: ND = not detected

IV. CONCLUSION

In conclusion, local fungal isolate identified as *A. flavus* NSH9 was shown to be able to produce amylases via SSF using local rice husks alone as the substrate. None of any previous studies has applied rice husks without any addition of nutrient supplements or minerals. This study has proven the feasibility of rice husks alone as a natural substrate for the production of amylases from fungi, particularly, *A. flavus*. Rice husks that are massively generated annually have the potential in providing a better alternative of eco- friendly and much cheaper natural substrate; with every 5 g of rice husks used, approximately 20 ml of crude enzyme can be produced. The findings are important, particularly, to the rice-producing countries such as Malaysia, and can be used in future researchers for the development of local amylases by utilising the under-utilised as well as readily available rice husks to replace the expensive synthetic substrate in the SSF process. This may allow competitive pricing of enzymes to be introduced into the current market and furthermore, will help in reducing dependence on the imported enzymes. Subsequently, sectors of local industries can be expanded. The obtained findings suggest production of aflatoxin compounds free enzymes from *A. flavus* can be achieved and thus, possible applications of the enzyme into the food and beverages industries. Furthermore, apart from providing a platform for solving environmental issues relating to the disposal of the agro-industrial waste, the use of rice husks as SSF substrate will also provide an opportunity for the paddy farmers to generate an extra income.

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