Studies in Fungi 3(1): 176–186 (2018) www.studiesinfungi.org ISSN 2465-4973
Article

Doi 10.5943/sif/3/1/18

Biotechnological potential of agro-industrial wastes for protein enrichment by solid-state fermentation using *Aspergillus niger*

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Adu SK, Yafetto L, Ofosuhene A, Offei-Affedzie T, Adu S, Ntibe TN 2018 – Biotechnological potential of agro-industrial wastes for protein enrichment by solid-state fermentation using *Aspergillus niger*. Studies in Fungi 3(1), 176–186, Doi 10.5943/sif/3/1/18

Abstract

This study is to assess the biotechnological potential of agro-industrial wastes of pineapple (*Ananas comosus*), sweet potato (*Ipomoea batatas*) and watermelon (*Citrullus lanatus*) for protein enrichment by solid-state fermentation using *Aspergillus niger*. Spore suspensions of *A. niger* were prepared with potato dextrose broth that had been supplemented with sodium chloride, ammonium nitrate and thiamine, and adjusted to pH of 5. Initial protein contents of sterile and non-sterile wastes of the pineapple, sweet potato, and watermelon were determined by the Kjeldahl method. The remaining sterile and non-sterile wastes was inoculated with spore suspensions of *A. niger* and incubated at temperature of 24°C under conditions of solid-state fermentation for 14 days. Protein contents of the inoculated sterile and non-sterile wastes were determined after 7 and 14 days. The sterile wastes of pineapple, sweet potato and watermelon recorded initial percentage protein contents of 4.37, 4.39, and 10.89, respectively, whereas their corresponding non-sterile wastes recorded initial percentage protein contents of 3.76, 4.00, and 10.16, respectively. The results further show that percentage increase in protein content of sterile wastes after 14 days of fermentation were pineapple, 35.01%; sweet potato, 27.60%; and watermelon, 64.40%. Percentage increase in protein content of non-sterile wastes after 14 days of fermentation were pineapple, 72.34%; sweet potato, 85.25%; and watermelon, 80.51%. These findings affirm the biotechnological potential of pineapple, sweet potato and watermelon wastes and the importance of fungi as agents for protein enrichment of agro-industrial wastes.

Key words – agro-based wastes – fungal biotechnology – nutrient enrichment – solid-substrate fermentation – value addition

Introduction

Agro-based industries all over the world rely heavily on the supply of raw materials from farmlands. These agricultural, animal feed, confectionary, food, juice, meat, and paper industries, after production generate millions of metric tons of agro-industrial wastes that are improperly disposed of and cause environmental pollution and serious health threats to animals including humans (Rodríguez-Couto 2008, Belewu & Babalola 2009, Ezekiel & Aworh 2013, Sadh et al. 2018). This notwithstanding, agro-industrial wastes such as apple pomace, cassava bagasse, coffee

Submitted 30 April 2018, Accepted 29 June 2018, Published 10 July 2018
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pulp and husk, sugarcane bagasse, sugar beet pulp, among others, are exploited and utilized in large quantities by microorganisms to produce and develop industrial products because of their high nutrient contents (Rivas et al. 2008, Nigam et al. 2009, Paepatung et al. 2009, El-Tayeb et al. 2012, Martin et al. 2012, Weshahy & Rao 2012, Motte et al. 2013, Ballesteros et al. 2014, Sadh et al. 2018). The utilization of these agro-industrial wastes for the production of useful products emphasizes their biotechnological potentials for efficient value addition (Pandey & Soccol 2000, Pandey et al. 2000a, b, c, Soccol & Vandenberghe 2003).

Value addition is mostly accomplished through solid-state fermentation, a biotechnological process that employs microorganisms to grow on non-soluble or solid substrates that contain complex polymers like lignin, pectin, and lignocellulose to produce useful products (Iyayi & Losel 2001, Duru & Uma 2003, Correia et al. 2007, Gélinas & Barrette 2007, Bhargav et al. 2008, Bayitse et al. 2013, Ezekiel & Aworh 2013, Aggelopoulos et al. 2014, Sadh et al. 2018, Yafetto 2018). Some of these useful products that are derived from the agro-industrial wastes through solid-state fermentation using fungi include the following: (i) bioethanol (Ofoefule & Uzodinma 2009, Paepatung et al. 2009, Avci et al. 2013, Duhan et al. 2013, Kumar et al. 2014, 2016, Saini et al. 2015, Maiti et al 2016, Mushimiyimana & Tallapragada 2016); (ii) antioxidants (Duda-Chodak & Tarko 2007, Nigam et al. 2009, Parashar et al. 2014, Sadh et al. 2017a, b, c); (iii) antibiotics (Asagbra et al. 2005, Vastrad & Neelagund 2012); (iv) enzymes (Ellaiah et al. 2002, Kalogeris et al. 2003, Topakas et al. 2004, Negi & Banerjee 2009, Buenrostro et al. 2013, Duhan et al. 2013, Kumar et al. 2013, Mathew et al. 2015, Oliveira et al. 2017, Saharan et al. 2017); (v) mushrooms (Chang 2006, Murthy & Manonmani 2008, Babu & Subhasree 2010, Siqueira et al. 2011, Akinyele et al. 2012, Randive 2012, Kumhomkul & Panich-pat 2013); and (vi) single cell proteins (Mondal et al. 2012, Aggelopoulos et al. 2014).

In Ghana, there is a problem dealing with large quantities of agro-industrial wastes that are generated from agro-processed raw materials, which the agro-industries essentially do not have any use for. Rather, these wastes are poorly disposed of into the environment, either at the backyards of the agro-industrial plants or are cart away by waste management companies to landfills to rot. This situation of lack of proper management of agro-industrial waste is taken advantage of by some peasant farmers and animal breeders who cart these wastes to their farms for use as manure for their vegetable gardens or as animal feed for their livestock (Fig. 1).

In recent decades, in West Africa, some initial investigations have focused on the potential biotechnological applications of fungi to improve the nutritional and economic values of cassava and cocoyam into animal feed (Iyayi & Losel 2001, Duru & Uma 2003, Bayitse et al. 2013, Ezekiel & Aworh 2013, Bayitse et al. 2015, Yafetto 2018). Meanwhile, corn stalks, coffee pulp, maize cobs, oil palm pulp, sawdust, and peels of mango, pineapple, plantain, sweet potato, and watermelon present themselves as potential candidates for use as agro-industrial wastes whose nutritional and economic values can be increasingly improved to produce useful products like animal feed to support the poultry and fishery sectors of the Ghanaian economy (Kumar et al. 2003, Villas-Boas et al. 2003, Shah et al. 2005, Yafetto 2018). This present study therefore aimed to assess the biotechnological potential of pineapple, sweet potato and watermelon as agro-industrial wastes for protein enrichment by solid-state fermentation using *A. niger*. Findings from this study continues to expand the frontiers of biotechnological application of fungi in the production of protein-enriched agro-industrial products in Ghana and West Africa.

Materials and Methods

Pineapple, sweet potato and watermelon

Agro-industrial wastes of pineapple peels, sweet potato peels and watermelon rind were obtained from local farmers, and small-scale fruit-processing industries in Cape Coast, which once served as the capital of the Gold Coast, now Ghana (Yafetto & Osei-Bonsu 2017).

Aspergillus niger

Aspergillus niger used in this study was isolated through the open plate method. Petri plates with potato dextrose agar (PDA) medium (200 g Irish potato; 20 g dextrose; 20 g agar; 1000 ml distilled water; autoclaved at a pressure of 1.1 kg/cm^2 at 121°C for 15 minutes) were exposed to air for about 5 minutes at the forecourt of the School of Biological Sciences, University of Cape Coast. The exposed Petri plates were covered and incubated at 24°C for 7 days until growth of different fungi were observed. *A. niger* was carefully isolated and sub-cultured on PDA at 25°C–30°C for 7 days to obtain pure cultures. The *A. niger* was identified using morphological and other growth features with the aid of identification manuals (Ellis et al. 2007, Pitt & Hocking 2009, Sharma & Pandey 2010, Watanabe 2010, Campbell et al. 2013). The pure cultures of *A. niger* were then preserved as stock cultures on slants in McCartney tubes at 4°C, and sub-cultured fortnightly. Irish potatoes used to prepare both PDA and potato dextrose broth (PDB) for this study were obtained from a local Ghanaian grocery.

Conidia suspension of *A. niger* as inoculum

PDB medium (200 g Irish potato; 20 g dextrose; 1000 ml distilled water; autoclaved at a pressure of 1.1 kg/cm^2 at 121°C for 15 minutes) was supplemented with 2 g Ammonium nitrate, 8 g Sodium chloride and 400 µg Thiamine with pH adjusted to 5 based on methods used and results obtained from nutritional requirements studies of *A. niger* reported by Yafetto (2018). The PDB-amended media were used to prepare conidial suspension of *A. niger* to inoculate pineapple, sweet potato and watermelon wastes using methods by Guarro et al. (1998), Yalemtesfa et al. (2010).

Estimation of percentage protein content of pineapple, sweet potato and watermelon

Sterile and non-sterile pineapple, sweet potato and watermelon wastes used in the estimation of protein contents were cleaned and prepared as described by Yafetto (2018). Initial percentage nitrogen (% N_2) of the sterile and non-sterile agro-industrial wastes were determined using the Kjeldahl method (Kjeldahl 1883), after which the percentage N_2 (% N_2) contents of the wastes were determined after 7 and 14 days of fermentation and subsequently used to estimate the percentage protein contents (% Protein) of the substrates as follows:

$$\% \text{ Protein content} = \% N_2 \times 6.25,$$

where 6.25 is the protein conversion factor.

Percentage increase in the protein content of the sterile and no-sterile wastes of the pineapple, sweet potato and watermelon were determined as follows:

$$\text{Percentage Increase of Protein Content} = \frac{\text{Final Protein Content} - \text{Initial Protein Content}}{\text{Initial Protein Content}} \times 100\%$$

Results

Protein enrichment of fermented sterile substrates of pineapple, sweet potato and watermelon

Sterile wastes of pineapple, sweet potato and watermelon recorded initial % protein contents of 4.37, 4.39, and 10.89, respectively (Fig. 2). Percentage protein contents of sterile wastes of pineapple and sweet potato increased after 7 days of fermentation, but decreased after 14 days (Fig. 2). However, the percentage protein contents of watermelon waste steadily increased up to 14 days of fermentation (Fig. 2). The highest % protein content was determined in watermelon waste (17.90), whereas the lowest % protein content was determined in sweet potato wastes (5.60) after 14 days of fermentation (Fig. 2).

Percentage increase in protein contents of sterile pineapple, sweet potato and watermelon wastes after 14 days of fermentation were 35.01%, 27.60% and 64.40%, respectively (Table 1).

Interestingly, there was an overwhelming increase in percentage protein content in sterile sweet potato waste (171.10%) after 7 days of fermentation, which dropped drastically to 27.60% after 14 days of fermentation (Table 1).

Protein enrichment of fermented non-sterile substrates of pineapple, sweet potato and watermelon

Non-sterile wastes of pineapple, sweet potato and watermelon recorded initial % protein content of 3.76, 4.00, and 10.16, respectively (Fig. 3). Percentage protein content of the non-sterile wastes increased after 7 days of fermentation; the percentage protein content in the three agro-industrial wastes decreased after 14 days of fermentation (Fig. 3). The highest % protein content was determined in watermelon waste (21.70), whereas the lowest % protein content was determined in pineapple (6.48) (Fig. 3). Percentage increases in protein content of non-sterile pineapple, sweet potato and watermelon wastes after 14 days of fermentation were 72.34%, 85.25%, and 80.51%, respectively (Table 1).

Table 1 Percentage (%) increase in protein content of agro-industrial wastes after 7 days and 14 days of fermentation.

	Percentage increase in protein content					
	Pineapple		**Sweet Potato**		**Watermelon**	
Nature of Substrate	**7 Days**	**14 Days**	**7 Days**	**14 Days**	**7 Days**	**14 Days**
Sterile wastes	42.60	35.01	171.10	27.60	51.51	64.40
Non-sterile wastes	81.65	72.34	108.75	85.25	113.60	80.51

Fig. 1 – Pineapple wastes carted from a small-scale pineapple juice factory in a tricycle by a farmer to a piggery. Photo credit: Levi Yafetto (March, 2018).

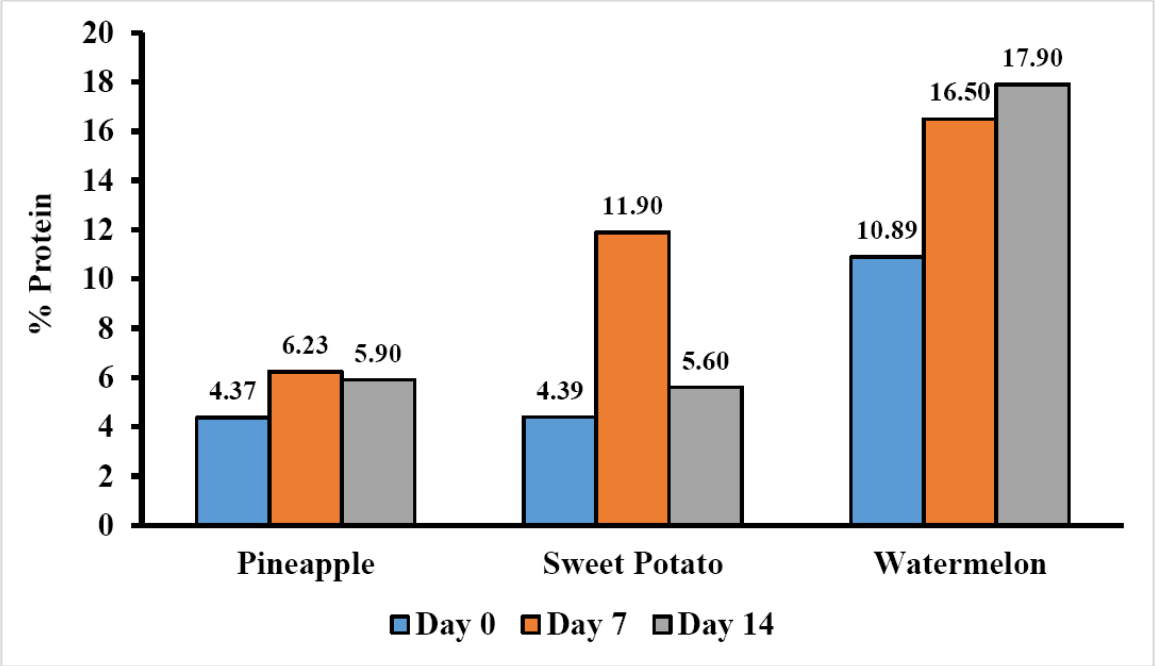

Fig. 2 – Percentage protein contents of sterile pineapple, sweet potato and watermelon wastes after solid-state fermentation with *A. niger*.

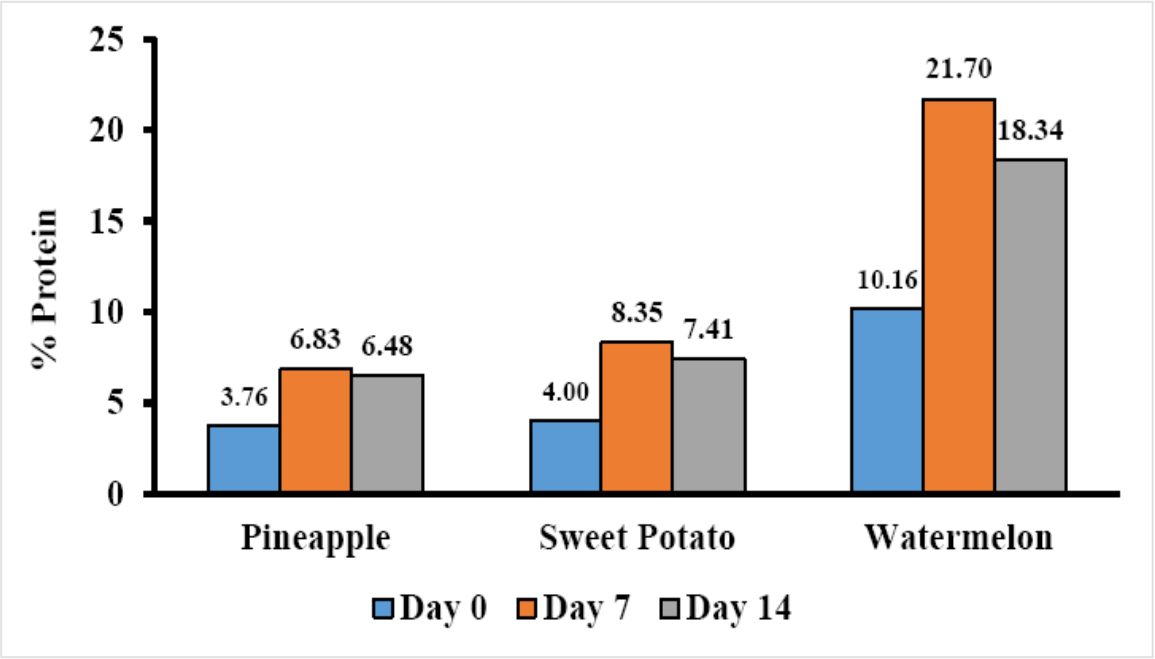

Fig. 3 – Percentage protein contents of non-sterile pineapple, sweet potato and watermelon wastes after solid-state fermentation with *A. niger*.

Discussion

Findings from this study revealed three things: (i) that the three agro-industrial wastes – pineapple, sweet potato and watermelon – are suitable for protein enrichment by solid-state fermentation using *A. niger*, (ii) that the pineapple, sweet potato and watermelon wastes successfully supported growth of *A. niger*, and (iii) that PDB-amended medium was an efficient and a reliable medium that could be used as a source of inoculum to study other agro-industrial wastes as demonstrated by Yafetto (2018). Other studies have employed different fungal species,

media and substrates that have been equally efficient with solid-state fermentation (Mitchell et al. 1988, Tsen et al. 2000, Pandey et al. 2000a, Duru & Uma 2003, Gao et al. 2007, Gao & Liu 2010, Ezekiel & Aworh 2013, Nagadesi & Arya 2013, Gao 2014, Bayitse et al. 2015).

Findings from this study amply demonstrate that the protein contents of both sterile and non-sterile wastes of pineapple, sweet potato and watermelon have the biotechnological potential to be enriched with *A. niger* under conditions of solid-state fermentation. For example, percentage increase in protein contents of sterile pineapple, sweet potato and watermelon wastes after 14 days of fermentation were 35.01%, 27.60% and 64.40%, respectively; percentage increase in protein contents of non-sterile pineapple, sweet potato and watermelon wastes after 14 days of fermentation were overwhelmingly higher at 72.34%, 85.25%, and 80.51%, respectively. After just 2 days of solid-state bioprocessing, Correia et al. (2007) reported a 22.00% protein content in pineapple waste with *Saccharomyces cerevisiae*. A more decreased crude protein contents have been reported for pineapple wastes after 2, 3 and 4 days of fermentation with *A. niger* and *Trichoderma viride* (Omwango et al. 2013). Also, Yafetto (2018) reported lower % protein content in cassava after 8 days of fermentation; Aruna et al. (2017) reported a range of protein content between 6.60 – 11.08% in yam peels after 4 days of fermentation with *Saccharomyces cerevisiae*. These findings by Correia et al. (2007), Omwango et al. (2013), Aruna et al. (2017), Yafetto (2018) may suggest that protein content of agro-industrial wastes could increase substantially with extended days for fermentation. This suggestion is supported by the higher protein contents reported in this study when fermentation of pineapple, sweet potato and watermelon wastes were extended to 14 days (Table 1, Figs 2, 3).

Bayitse et al. (2015) reported 36.90% and 48.10% protein enrichment of cassava waste after 12 days of fermentation. Similarly, Yalemtesfa et al. (2010) reported protein content of 39.64% and 31.70% in orange waste using *Chaetomium* spp and *A. niger*, respectively, demonstrating the potential of orange waste for protein enrichment under conditions of solid-state fermentation. Findings by Yang (1988, 1993), and Yang et al. (1993) further revealed varying levels of protein content that ranged between 16.11–34.00% with mono- and co-cultures of amylolytic fungi.

Although the highest protein contents were surprisingly reported in watermelon among the agro-industrial wastes used in this study, interestingly, it appears there are no reports on protein enrichment of watermelon wastes available in literature. Rather, the use of watermelon seed and rind for value addition into other products have been reported (Al-Sayed & Ahmed 2013, Wani et al. 2013) This could suggest that the findings on watermelon in this study may be among the first reports in literature on the use of watermelon waste for protein enrichment with fungi using solid-state fermentation. Considering the high protein contents reported here, watermelon wastes could have a huge potential for use in the production of animal feed through the use of fungi.

The increase in protein content recorded for the three agro-industrial wastes in this study gives credence to the ability of *A. niger* to secret enzymes that convert complex starch and non-starch polysaccharides into simple monomer sugars which are metabolized into proteins (Ezekiel & Aworh 2013). The higher percentages in protein contents of non-sterile wastes may be attributed to the presence of other microbes, in addition to *A. niger*, whose metabolic activities collectively contributed to the overall protein enrichment of the wastes. The overall reduction in protein contents reported in both the sterile and non-sterile wastes in this study was observed probably due to the occurrence of proteolysis after some days of fermentation and the depletion of nutrient sources (Correia et al. 2007).

We conclude from this study that pineapple, sweet potato and watermelon wastes have the biotechnological potential for protein enrichment using fungi, and that the findings continue to add to the body of knowledge and the relatively scanty literature available on the use of agro-industrial wastes together with fungi for value addition. It is recommended that future studies should aim to investigate further and explore the biotechnological applications of other fungi such as *T. viride*, and *S. cerevisiae* to enrich agro-industrial wastes using mono- and co-cultures of these fungi. Findings from such studies should provide also more insights into the biotechnological potential of agro-industrial wastes in relation to their use as animal feed.

Acknowledgments

The authors are grateful to the technical staff of the Department of Molecular Biology and Biotechnology and the Department of Animal Sciences, University of Cape Coast, Ghana for the technical assistance provided during the study. Many thanks go also to the local Ghanaian farmers and small-scale fruit-processing industries that provided the pineapple, sweet potato and watermelon wastes for this study.

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