

Glucoamylase Production by *Aspergillus awamori* KT-11 In Solid-State Fermentation Using Cassava Peel as Substrate

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Abstract

Cassava has long been known as one of the main staple food in Indonesia. Whereas the cassava peel contains starch of approximately 72%, it is still underrated as a carbohydrate source for fermentation. The utilization of cassava peel as a substrate in solid state fermentation potentially replaces rice as a carbon source leading to more cost-effective production. This study aims at producing glucoamylase by means of solid state fermentation using *Aspergillus awamori* KT-11 and cassava peel as substrate. The study demonstrated that medium composition and drying technique affected the production of glucoamylase. The highest glucoamylase activities were identified when cassava peel and mineral media was used in fermentation, compared to only cassava peel; the combination of cassava peel, mineral, and rice bran; rice media or a mixture of rice, mineral and rice bran. Freeze-dried glucoamylase, furthermore, exhibited higher specific activity in contrast to the oven-dried one, with 452 U/mL and 365 U/mL, respectively. In conclusion, cassava peel plus mineral is a better substrate for glucosamine production by *A. awamori* KT-11 in solid state fermentation. Besides, powdered glucoamylase had been demonstrated to be capable of hydrolyzing starch-based biomass.

Keywords: glucoamylase, cassava peel, *A. awamori* KT-11, solid state fermentation

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Introduction

Glucoamylases (EC 3.2.1.1) are hydrolase enzymes that degrade starch into sugar. Glucoamylases random lyhydrolyze α -1,4 and α -1,6 bond of starch. The enzymes are of paramount importance for manufactures, including glucose syrup, cakes, and pharmaceutical industries.

The widely-used fermentation techniques to produce glucoamylase are submerged fermentation (SMF) (Wang *et al.*, 2008), solid state fermentation (SSF) (Anto *et al.*, 2006; Lawal *et al.*, 2014; Bhatti *et al.*, 2007) and liquid fermentation (Fujio & Morita, 1996). Solid state fermentation (SSF) is fermentation using solid media with little or no water to support the growth and metabolism of microorganisms (Pandey, 2006). Additionally, the microorganisms commonly used for producing such glucoamylase are *Aspergillus* sp., *Rhizopus* sp., and *Trichoderma reesei* (Pandey *et al.*, 2000). *Aspergillus awamori* is indigenous isolate from Indonesia that has been

identified to produce glucoamylase (Matsubara *et al.*, 2004).

Fermentation of solid substrate to produce glucoamylase by *Aspergillus* has been improved in recent year. Some substrates have been examined, for example, rice flake waste (Anto *et al.*, 2006), wheat bran (Bertolin *et al.*, 2003), and even food waste from fast-food restaurants (Lam *et al.*, 2013). Nevertheless, the efficiency of enzyme production from particular biomass requires to be investigated further in order to decrease production cost.

Indonesia has abundant carbohydrate source, and one of which is cassava (*Manihot esculenta*). Central Bureau of Statistics recorded that cassava production in Indonesia during 2015 as many as 21,801,415 ton. Cassava is a staple food, source of carbohydrates, in most parts of Indonesia. Cassava peel waste is currently processed and consumed as snack; while some research indicated cassava peel waste can be transformed to generate high-value products. Simate & Ndlovu (2015) studied the biosorption of heavy metals using cassava peel.

Amylase production employing cassava peel in submerge fermentation by *Aspergillus niger* resulted in higher activity compared to amylase production using cassava flour (Sani *et al.*, 1992). Static production of the enzyme has advantages over submerge fermentation (Vinegra-Gonzales *et al.*, 2003). A number of studies assigned cassava peel to research subject, since it is agro-industrial waste with high carbon content. Proximate analysis of rice showed the main composition was carbohydrate, which is around 75%–80% (Verma & Srivastav, 2017). On the other hand, Okpako *et al.*, (2008) confirmed carbohydrate content in cassava peel was 72.5%. Accordingly, cassava peel potentially replaces the utilization of rice as a carbon source in solid state fermentation of glucoamylase production, which results in more cost-effective process. Therefore, the purpose of this research is to produce glucoamylase using cassava peel as the substrate by means of solid fermentation technique using *Aspergillus awamori* KT-11.

Materials and Methods

Microorganism.

Microorganism used in this study was *Aspergillus awamori* KT-11. The fungi were refreshed on Potato Dextrose Agar (PDA) and incubated for 5 days at room temperature. Subsequently, the spores on the media were dissolved by adding sterile distilled water as much as 5 mL. A total of 1% suspension of spores was used to inoculate solid medium.

Medium.

Rice and cassava peel were substrates for solid-state fermentation in this study. The rice was cooked by adding water with a ratio of 10:7 (w/v). The addition of medium composition to rice was according to treatment variations (Table 1).

Cassava peel of Menti variety is a collection of Research Centre for Biotechnology. Cassava peel was washed, finely chopped and then distilled water was added with a ratio of total media to water of 5:1. The amount and combination of minerals added into the rice and cassava peel medium are shown in Table 1. Solid media was sterilized at 121 °C, 1 atm for 15 minutes.

Table 1. Solid medium compositions

No.		Medium Composition Ratio (100%)		Minerals
		Substrate (%)	Rice Bran (%)	
1	Rice	100	0	-
2	Rice	100	0	+
3	Rice	50	50	+
4	Cassava Peel	100	0	-
5	Cassava Peel	100	0	+
6	Cassava Peel	50	50	+

Minerals added per 20 gr media were (NH₄)₂SO₄ 1 g, KH₂PO₄ 0,1 g, K₂HPO₄ 0,1 g.

Glucoamylase Production.

A suspension of *Aspergillus awamori* KT-11 spore was added into the solid medium in the jam jar at a final concentration of 1% (w/v). The culture, then, was mixed well together with the solid medium. Afterward, the jar's mouth was covered with paper. Fermentation was carried out for 5 days at room temperature. The drying process of fermentation media was performed in two schemes: oven-drying at a temperature of 55 °C for 2 days and freeze-drying for 14-25 hours. Fermented media was weighted before and after the drying process to measure the biomass yields. Following the drying process, the media were crushed and stored at 4 °C.

Glucoamylase Extraction.

Crude enzyme was extracted by diluting the dried powder in 9 mL of 0.2 M acetate buffer, pH 4.8. The extract was agitated for 15 min at 22 °C and 120 rpm. Afterward, the sample was centrifuged at 10,000 g and 4 °C, for 20 min (Pirota *et al.*, 2013). The supernatant was collected as glucoamylase extract and used for subsequent analysis of glucoamylase activity.

Glucoamylase Assay.

Five % soluble starch in 50 mM phosphate buffer pH 7 was used as the substrate for the analysis of glucoamylase activity. 1 mL of substrate solution was added to 100 µL of the glucoamylase extract and incubated at a temperature of 60 °C for 1 hour. The concentrations of reducing sugars were analyzed subsequently using Somogyi-Nelson method (Somogyi, 1952). One unit glucoamylase activity is defined as the amount of enzyme which released 1mg/mL equivalent of glucose per hours. The solid medium without *Aspergillus awamori* KT 11 was used as negative control.

Protein Assay.

The protein concentration was measured spectrophotometrically, using spectrophotometer Biochrom Libra S70 at absorbance 280 nm (Hanson & Philips, 1981).

Sweet Potato Hydrolysis.

In this study, sweet potato powder was used as a substrate to examine the ability of glucoamylase powder or glucoamylase extract to hydrolyze particular biomass. The investigation schemes were as follows,

- a. Sweet potato (1%) was mixed with glucoamylase powder in various concentrations (5%, 4%, 3%, 2%, 1%, 0,5%, 0,25%, and 0,1%) in total of 10 mL of distilled water and incubated for 1 h at 60°C. To measure the concentrations of reducing sugars, 1 mL of sample solution was taken following the incubation.
- b. Sweet potato (1%) was mixed with glucoamylase extract in various concentrations (5%, 4%, 3%, 2%, 1%, 0,5%, 0,25%, and 0,1%) in total 10 mL of distilled water and incubated for 1 h at 60°C. To measure the reducing sugar concentrations, the same amount of sample was taken as indicated above.

The concentrations of reducing sugars in samples were measured by Somogyi-Nelson method (Somogy, 1952). In this study, all analyses were replicated, and the data was shown with standard deviation.

Results

Effect of Media Composition

The crude enzyme of glucoamylases generated from the fermentation of a variety of media by *Aspergillus awamori* KT-11 are shown in Figure 1. The medium compositions affected the production of glucoamylase crude enzyme powder. The addition of rice bran into the media leads to a rougher powder of the crude enzyme, compared with those without rice brain supplementation.

A. awamori KT-11 growth in media containing rice, mineral and rice bran exhibited slower growth compared to that in other media. Table 2 shows the dry weight of glucoamylase powder. The initial weight of all media before fermentation was identical. Following the drying process, it was obvious that the addition

of rice bran to the rice or cassava peel media increased the total weight of dried media.

The higher mass of the media containing rice bran is certainly due to the inability of *Aspergillus* to use rice bran as substrate. They only consume rice or cassava peel, and it is noticeable from intact rice bran at the end of the fermentation process.

The total biomass of solid fermentation using rice media was higher than that of cassava peel. Contrary to the total biomass, the higher growth of *A. awamori* KT-11 was identified in cassava-containing media (Figure 2).



Figure. 1. Glucoamylase crude powders from various fermentation media (Top: freeze dried, bottom: oven dried : (1) cassava peel, (2) cassava peel+mineral, (3) cassava peel+mineral+rice bran, (4) rice, (5) rice+mineral, (6) rice+mineral+rice bran)

High yields of biomass did not correlate with the growth of *A. awamori* KT-11. According to the protein concentration of biomass in Table 3, dried biomass from media consisting of rice + mineral + rice bran had the lowest protein concentration compared to other fermentation media. The data further confirmed that the total fermentation biomass did not correlate with the growth of isolate.

Table 2. The effect of media compositions and drying techniques on biomass dry weight of glucoamylase powder

Media	Oven (%)	Freeze dry (%)
Rice	28	30
Rice + mineral	55	55
Rice + mineral + rice bran	75	76
Cassava peel	14	12
Cassava peel + mineral	16	16
Cassava peel + mineral + rice bran	46	60



Figure 2. Fermentation in solid media by *A. awamori* KT 11: (1) rice+ mineral+rice bran, (2) rice+mineral, (3) cassava peel+mineral+rice bran, (4) cassava peel+mineral, (5) cassava peel, (6) rice

From the protein concentration analysis (Table 3), the addition of rice bran to both rice and cassava peel-containing control media only slightly increased their protein concentration. Furthermore, the protein concentration correlates positively with glucoamylase activities (figure 3) derived from rice-based fermentation. Conversely, it did not always happen for glucoamylase produced from cassava peel-based fermentation. It is apparent that both protein concentrations and glucoamylase activities were high when *A. awamori* KT-11 cultivated on media composed of cassava peel and mineral. Yet, a high protein concentration of fermented cassava peel biomass was inversely correlated with low glucoamylase activities.

Effect of Drying Techniques

The critical point in the production of enzyme powder is the drying process. Two drying methods, oven drying and freeze drying, were investigated. The drying process of samples by oven produced darker powder than using freeze dry (Figure 1).

Percentage yield of crude glucoamylase powder in Table 2 shows slight differences, either by drying oven or freeze dry (2-14%). It indicated that water content in the product has been reduced.

The effects of drying method on protein concentrations in this research showed only slight differences in a range of 0-2 mg/mL (Table 3), except for glucoamylase powder of fermented cassava peel and mineral. Drying method using freeze dryer generated higher protein concentrations than that by oven.

Even though the protein concentrations of glucoamylase powders were not significantly different between two drying methods; freeze drying process produced higher glucoamylase activity than the other one (Figure 3).

Table 3. The effects of media composition and drying techniques on protein concentrations

	Protein Concentration (mg/mL)			
	Oven		freeze dry	
	Control medium	<i>A. awamori</i> KT-11	Control medium	<i>A. awamori</i> KT-11
Rice	0.70035 ±0.085	27.0715 ±6.9003	0.7917 ±0.006	24,911 ±1.661
Rice + mineral	1,37025 ±0.046	9,512 ±0.1435	1,827 ±0.06	11,571 ±0.533
Rice + mineral + rice bran	2,42005 ±0.070	8,1635 ±0.1332	2,32145 ±0.001	8,1635 ±0.2768
Cassava peel	2,39685 ±0.05	22,446 ±0.0820	2,49835 ±0.023	24,6065 ±0.9945
Cassava peel + mineral	2,4389 ±0.08	12,296 ±0.4921	2,43165 ±0.078	34,858 ±0.4716
Cassava peel + mineral + rice bran	2,55345 ±0.575	12,267 ±0.2461	2,54185 ±0.054	14,732 ±0.3691

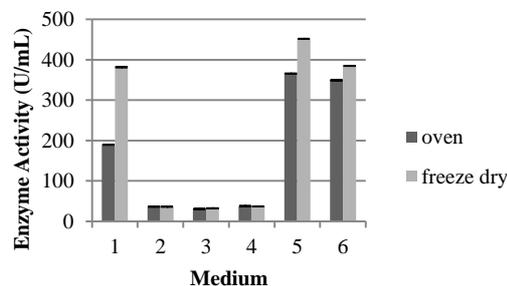


Figure 3. Glucoamylase activities from different fermentation media. Note: (1) rice; (2) rice + mineral; (3) rice + mineral + rice bran; (4) cassava peel; (5) cassava peel + mineral; (6) cassava peel + mineral + rice bran.

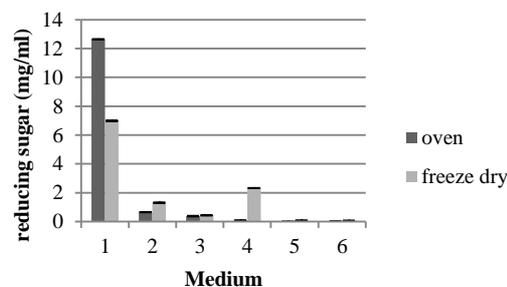


Figure 4. Reducing sugar concentrations in different media. Note: (1) rice; (2) rice + mineral; (3) rice + mineral + rice bran; (4)

cassava peel; (5) cassava peel + mineral; (6) cassava peel + mineral + rice bran.

The effects of drying methods on reducing sugar concentrations presented in Figure 4, it is obvious that there was a correlation between drying process with reducing sugar concentration (Figure 4), except for media containing rice or cassava peel only. The highest reducing sugar concentration (12 mg/ml) was observed in rice medium treated with oven drying, while the lowest concentration (not detected) was detected in cassava peel and mineral media treated by means of oven drying.

Activities of Crude Enzyme Glucoamylase

Fermented media consisting of cassava peel and minerals gave the highest enzyme activities when treated with both oven and freeze dryer. To further investigate the glucoamylase ability obtained from the fermentation of such media to hydrolyze biomass, sweet potato powder was selected as a substrate.

In this study, the sweet potato powder at a concentration of 1% was hydrolyzed by either glucoamylase powder or extract (Figure 5 and Figure 6, respectively). According to Figure 5, in which sweet potato powder hydrolyzed by glucoamylase powder, the higher enzyme concentrations the higher reducing sugar concentrations. From the figure, it is indicated that freeze-dried glucoamylase powder generated a higher concentration of reducing sugars. By contrast, different results were shown in a study of the hydrolysis of sweet potato powder by glucoamylase extract, as indicated in Figure 6. Oven dried-glucoamylase extract apparently released higher concentrations of reducing sugars from hydrolysis of sweet potato powder compared to freeze-dried one.

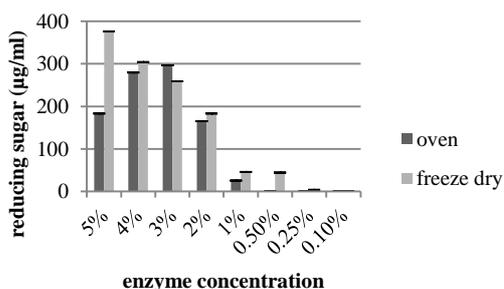


Figure 5. Hydrolysis of 1% sweet potato powder by glucoamylase powder from cassava peel and mineral media

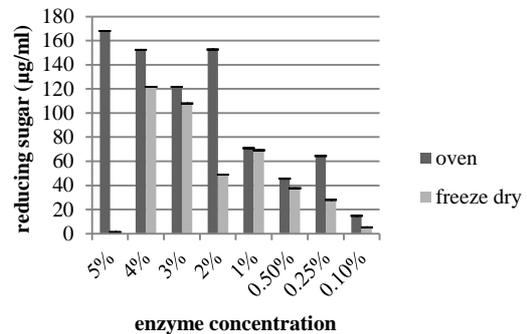


Figure 6. Hydrolysis of 1% sweet potato powder by glucoamylase extract from cassava peel and mineral media

Discussion

Effect of Media Composition

Application of more economic cassava peel waste as a substrate in glucoamylase fermentation provides a solution to rice-base fermentation enabling more cost-effective production. On top of that, cassava peel utilization in glucoamylase aims at increasing its activity (Sani *et al.*, 1992).

On the one hand, the addition of rice bran into the media is intended as a support material for the growth of fungi in the production of glucoamylase (Arasaratnam *et al.*, 2001).

Aspergillus awamori KT-11 exhibited steady growth due to low water content in the media. In the solid-state fermentation, water content in the media is designated as water activity (AW). The water activity depends on the properties of substrate to bind water (Krishna, 2005). The reduction of AW decreases growth speed and in turn reduces the amount of biomass produced (Pandey *et al.*, 1994).

The addition of rice bran into crude media confers rougher structure to enzyme powder than those without. It perhaps be due to the fiber as the main component of rice bran (Kahlon, 2009) cannot be metabolized completely by *A. awamori* KT-11. Until now, *A. awamori* KT-11 is widely recognized for their ability to produce enzymes from the amylolytic type (Matsubara, *et al.*, 2004; Anindyawati *et al.*, 1998a, Anindyawati *et al.*, 1998b. Other studies

demonstrated the activity of cellulase enzymes from *A. awamori* IOC-3914 was only 20 U g⁻¹, endoamylase activity and exoamylase activity were only 197 U g⁻¹, and 106 U g⁻¹, respectively (de Castro *et al.*, 2015). Our study successfully obtained far higher glucoamylase activity, with 452 U/mL. This high activity was showed by freeze-dried glucoamylase powder from fermentation of substrate consisting of cassava peel and mineral supplementations.

The yields of total biomass at the end of fermentation did not correspond to the growth of the mycelium of *A. awamori* KT-11 in solid media. Higher yields of biomass are plausibly owing to the unmetabolized substrates so that their weight is still high. Protein concentrations of the media indicate the growth of *A. awamori* KT-11. From Table 3, fermented media consisting of rice, minerals, and rice bran have lower protein concentration than the other media. It can be found in Figure 2, *A. awamori* KT-11 do not grow on such media. The microorganisms benefit from the substrates as a carbon source for their growth in the way which they degrade the media and thus leads to its weight loss. The high protein concentration of fermentation media has ensued from high *A. awamori* KT-11 growth. It is apparent that the mycelia of *A. awamori* KT-11 are more abundant in media containing cassava peel (fig.2). This corresponds to higher protein concentrations in fermented media of cassava peel after 6 days (Table 3).

The concentrations of protein in control media (without microorganisms) supplemented with rice bran was higher than the control medium without supplementation. It indicated that rice bran serve as a source of nitrogen for microbial growth (Tabel 3). Nonetheless, following fermentation by *A. awamori* KT-11, protein concentration in media plus rice bran is lower than those without rice bran. It is perhaps results from decreased moisture in the rice bran-containing media, which in turn result in declined growth and enzyme production of *A. awamori* KT-11 (Delabona *et al.*, 2013).

In cassava peel-containing media, the protein concentration was high but the activity of glucoamylase was on the contrary. It is plausibly emerged from high contents of protein cell, but not glucoamylase content. These results are supported by the low biomass yield from cassava peel-containing media. The cassava peel as the only nutrient in the media has been completely consumed for cell growth,

but not for enzyme production. Mycelia of *A. awamori* KT-11 also can be seen in Figure 2.

Rice-containing media have higher concentrations of reducing sugars. This media is likely the preferable substrate for *A. awamori* KT-11 growth. Starch, as the main component of rice, will be broken down by the microorganism become monomers such as reducing sugars. Consequently, the concentration of reducing sugar of such media is higher compared to that of other media.

Effect of Drying Techniques

The darker color of materials dried using oven is possibly the results of high temperature and length of drying time (Arslan & Özcan, 2010). Distinct drying techniques do not significantly affect the total biomass yields. It has been demonstrated that both oven and freeze drying techniques are satisfactorily suitable for reducing water contents of the crude enzymes.

Regarding protein concentrations, drying techniques affect this property. High temperatures of oven drying method possibly give rise to denaturation of some proteins and loss of compounds, such as ammonia or other non-protein nitrogenous volatile substances (Alomar *et al.*, 1999).

The concentrations of residual sugars in the fermentation medium are affected by the drying techniques. The oven dried-rice media had higher reducing sugar concentrations than that of freeze-dried one. On the other hand, freeze drying generates a higher concentration of such sugars from cassava peel media than oven drying method. The plausibly explanation for this is that higher complexity of sugar polymer in the rice medium in comparison to that in the cassava peel one. Due to this complexity, the heating process releases monomers from remaining sugar polymers leading to high concentrations of reducing sugars at the end of fermentation process. On the contrary, a less complex polymer in the cassava peel media has been completely metabolized by *A. awamori* KT 11 into reduction sugars. Accordingly, upon heating, the reducing sugars were destructed, and thus undetected. This can be identified from the media containing cassava peel, the mycelial growth of *A. awamori* KT 11 much more abundant than those on other media (Figure 2).

Glucoamylase activities are also affected by drying techniques since temperatures play

important roles in determining the enzyme structure. Interactions between temperature and enzyme have impacts on catalytic rates, acclimation of enzymatic activities to compensate for temperature changes, and interspecific differences in protein thermal stability (Somero, 1995). Therefore, glucoamylase powder from freeze dry has a higher activity than the oven-dried.

Activities of Glucoamylase Crude Enzyme

Glucoamylase powder has better ability to hydrolyze biomass than glucoamylase extract. It is confirmed by the higher levels of reducing sugars produced from the breakdown of starch sweet potato (Figure 5 and Figure 6). These are possibly resulted from higher protein contents of enzyme powder compared to those of enzyme extract. Application of powdered enzymes in industrial processes is also more profitable in comparison to the enzyme extraction.

Conclusions

The alternative substrate in solid state fermentation for glucoamylase production have been presented. Cassava peel can substitute rice as carbon source. The highest activity of glucoamylase, as high as 452 U/mL, produced in cassava peel with minerals with freeze drying technique. Drying technique affect the end product. Freeze drying produces brighter color, higher protein concentration, and higher glucoamylase activity than oven drying.

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References

Alomar, D., Fuchslocher, R., & Stockebrand, S. (1999). Effects of oven- or freeze-drying on chemical composition and NIR spectra of pasture silage. *Animal Feed Science and Technology*, (80): 309-319.

Anindyawati, T., Ann, Y. G., Ito, K., Iizuka, M., & Minamura, N. (1998). Two kinds of Novel α -glucosidases from *Aspergillus awamori* KT-11: Their purifications, properties, and specificities. *J. Ferment. Bioeng.* 85 (5): 465-469.

Anindyawati, T., Melliawati, R., Ito, K., Iizuka, M., & Minamura, N. (1998). Three different types of α -amylases from *Aspergillus awamori* KT-11: Their purifications, properties, and specificities. *Biosci. Biotechnol. Biochem.* 62 (7): 1351-1357.

Anto, H., Trivedi, U. B., & Patel, K. C. (2006). Glucoamylase production by solid-state fermentation using rice flake manufacturing waste products as substrate. *Bioresource Technology*, 97: 1161-1166.

Arslan, D., & Özcan, M. M. (2010). Study the effect of sun, oven and microwave drying on quality of onion slices. *LWT - Food Science and Technology*. (43): 1121-1127.

Arasaratnam, V., Mylvaganam, K., Balasubramaniam, K.. (2001). Glucoamylase production by *Aspergillus niger* in solid state fermentation with paddy husk as support. *J. Food Sci. Technol.* 38: 334-338

Bertolin, T. E., Schmidell, W, Maiorano, A. E., Casara, J., & Costa, J. A. (2003). Influence of carbon, nitrogen and phosphorous sources on glucoamylase production by *Aspergillus awamori* in solid state fermentation. *Z Naturforsch*, 58 (9-10): 708-12.

Bhatti, H. N., Rashid, M. H., Nawaz, R., Asgher, M., Perveen, R., & Jabbar, A. (2007). Optimization of media for enhanced glucoamylase production in solid-state fermentation by *Fusarium solani*. *Food Technol. Biotechnol.* 45(1): 51-56.

De Castro, A. M., Castilho, L. R., Freire, D. M. (2015). Multivariate optimization and supplementation strategies for the simultaneous production of amylases, cellulases, xylanases, and proteases by *Aspergillus awamori* under solid-state fermentation conditions. *Appl Biochem Biotechnol.* 175: 1588-602

Delabona, P. d. S., Pirola, R. D. P. B., Codima, C. A., Tremacoldi, C. R., Rodrigues, A., & Farinas, C. S. (2013). Effect of initial moisture content on two Amazon rainforest *Aspergillus* strains cultivated on agro-industrial residues: Biomass-degrading enzymes production and characterization. *Industrial Crops and Products*, 42: 236-242.

Fujio, Y., & Morita, H. (1996). Improved glucoamylase production by *Rhizopus* sp. A-11 using metal ion supplemented liquid medium. *Juornal of Fermentation and Bioengineering.* 87(6): 554-557.

- Hanson, R.S and J.A.Philips. (1981). Manual of methods for general bacteriology. American Society for Microbiol. Washington DC. 359-360
- Kahlon, T. S. (2009). Rice bran: production, composition, functionality and food applications, physiological benefits. In: Choo SS, Samuel P, eds. Fiber ingredients: food applications and health benefits. Boca Raton, Fla: Taylor and Francis Group LLC, 305–321.
- Krishna, C. (2005). Solid-state fermentation systems—An overview. *Critical Reviews in Biotechnology*. 25: 1–30.
- Lam, W. C., Pleissner, D., & Lin, C. S. K. (2013). Production of fungal glucoamylase for glucose production from food waste. *Biomolecules*. 3: 651-66.
- Lawal, A. K., Banjoko, A. M., Olatope, S. O., Alebiosu, F. A., Orji, F. A., Suberu, Y. L., Itoandon, E. E., Shittu, K. A., Adelaja, O. D., Ojo, E., Dike, E. N., & Elemo, G.N. (2014). Production and partial purification of amylase by *Aspergillus niger* isolated from cassava peel. *Journal of Basic & Applied Sciences*. 10: 287-291.
- Matsubara, T., Ammar, Y. B., Anindyawati, T., Yamamoto, S., Ito K., Iizuka, M., & Minamura, M. (2004). Degradation of Raw Starch Granules by α -Amylase Purified from Culture of *Aspergillus awamori* KT-11. *Journal of Biochemistry and Molecular Biology*, 37 (4): 422-428.
- Okpako, C. E., V. O. Ntui, A. N. Osuagwu & F. I. Obasi. (2008). Proximate composition and cyanide content of cassava peels fermented with *Aspergillus niger* and *Lactobacillus rhamnosus*. *Journal of Food, Agriculture & Environment*. 6 (2): 251-255.
- Pandey, A. (2003). Solid-state fermentation. *Biochemical Engineering Journal*. 13: 81–84.
- Pandey, A., Ashakumary, L., Selvakumar, P., & Vijayalakshmi, K. S. (1994). Influence of water activity on growth and activity of *Aspergillus niger* for glycoamylase production in solid-state fermentation. *World Journal of Microbiology & Biotechnology*. 10: 485-486.
- Pandey, A., Nigam, P., Soccol, C., Soccol, V., Singh, D., & Mohan, R., (2000). Advances in microbial amylases. *Biotechnol. Appl. Biochem.* 31: 135–152.
- Pirota, R. D. P. B., Miotto, L. S., Delabona, P. S., & Farinas, C. S. (2013). Improving the extraction conditions of endoglucanase produced by *Aspergillus niger* under solid-state fermentation. *Brazilian Journal of Chemical Engineering*. 30 (1): 117-123.
- Sani, A, Awe, F. A., & Akinyanju, J. A.. (1992). Amylase synthesis in *Aspergillus flavus* and *Aspergillus niger* grown on cassava peel. *Journal of Industrial Microbiology*. 10: 55-59
- Simate, G. S., & Ndlovu, S. (2015). The removal of heavy metals in a packed bed column using immobilized cassava peel waste biomass. *Journal of Industrial and Engineering Chemistry*. 21: 635–643.
- Somero, G.N., (1995). Protein and temperature. *Annu. Rev. Physiol.* 57: 43-68.
- Somogy, M., (1952). Notes on sugar determination. *J. Biol. Chem.* 195: 19-23.
- Verma, D. K., and P. P. Srivastav. (2017). Proximate composition, mineral content and Fatty acids analyses of aromatic and non-aromatic Indian rice. *Rice science*. 24(1): 21-31.
- Viniegra-Gonzalez, G., Favela-Torres, E., Aguilar, C. N., Romero-Gomez, S. dJ., Diaz-Godinez, G., & Augur, C. (2003). Advantages of fungal enzyme production in solid state over liquid fermentation systems. *Biochemical Engineering Journal*. 13:157–167
- Wang, Q., Wang, X., Wang, X., & Ma. H. (2008). Glucoamylase production from food waste by *Aspergillus niger* under submerged fermentation. *Process Biochemistry*. 43: 280–286.