Bioethanol Production from Bitter Yam (*Dioscorea dumetorum*) and Water Yam (*Dioscorea alata*) Peels

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Abstract: Bioethanol which is an alternative source of fuel to fossil fuels can be produced from renewable crops. However, some of these renewable feedstocks are food competitive. Hence, this study therefore investigated the production of bioethanol from bitter yam and water yam peels. Strains of *Aspergillus* spp and *Saccharomyces cerevisiae* were obtained from the Microbiology laboratory of the University and characterized using morphological characteristics. The spores of *Aspergillus tamarii* and colonies of *Saccharomyces cerevisiae* were cultured in bitter yam and water yam medium using the bitter and water yam peels as substrates for ethanol production at substrate concentrations of 5 – 30 %, temperature range of 25 - 45 ^{o}C, agitation speed of 60-160 rev/min and pH range of 4.0 - 8.0. Optimum bioethanol yield of 13 % was obtained with bitter yam peels at substrate concentration of 20 %, temperature of 35 ^{o}C, agitation of 100 rev/min and pH of 7.0. Similarly, optimum bioethanol yield of 11 % was obtained with water yam peels at substrate concentration of 20 %, temperature of 35 ^{o}C, agitation of 100 rev/min and pH of 5.0. Bioethanol yield from bitter yam peels was observed to be higher than that of water yam peels at substrate concentration of 20 %, temperature of 35 ^{o}C, agitation of 100 rev/min and pH of 7.0. This study shows the potential of bitter yam and water yam peels as substrates for the biosynthesis of ethanol which can serve as alternative source of fuel.

Keywords *Aspergillus tamarii*; Bioethanol; Bitter yam peels; *Saccharomyces cerevisiae*; Water yam peels.

INTRODUCTION

The demand for bioethanol as an alternative source of fuel is vast receiving attention globally. This liquid which is a product of sugar fermentation can be obtained from hydrolysis of starchy biomass (Ibeto *et al.,* 2011). It is the principal fuel used as a substitute for road transport vehicles (Tofighi *et al.,* 2010). This colourless liquid is biodegradable, low in toxicity and causes little environmental pollution by reducing air pollution (Ofoefule *et al.,* 2009). The main sources of sugar required to produce ethanol come from fuel or energy crops (Kim and Dele, 2005). These crops are grown specifically for energy use and they include; maize, corn, waste straw, willow, cassava, sorghum, cord grasses (Ibeto *et al.,* 2011). Several feed stocks have been employed in the production of bioethanol, however the peels of some tuber crops such as bitter yam (*Dioscorea dumetorum*) have been underutilized (Owuamanam *et. al.,* 2013).

Bitter yam (*Dioscorea dumetorum*) is a species of yam in the *Dioscorea* genus and Dioscoreacae family found in Africa (Fasaanu *et. al.,* 2013). In south-western Nigeria, bitter yam is used in the treatment of malaria (Dike *et al*., 2012), suggesting a widespread ethno-medicinal importance of bitter yam. Bitter yam is rarely consumed because of its unpalatable bitter taste and post-harvest hardening of the tubers (Medoua *et al*., 2005). Bitter yam is rich in phyto-nutrients, including proteins (Medoua *et al*., 2005; Alozie *et al*., 2009), yet it remains an underutilized tropical tuber (Owuamanam *et al.*, 2013). .

Water yam (*Dioscorea alata*) is valued for its starch content hence it has been processed for various uses. It has been processed as a flavor for ice cream, milk, tarts, Swiss rolls, cookies, etc. It is an essential ingredient in Undhiyu (Degras and Coste, 1993).

In folk medicine, it has been used for treating fever, gonorrhoea, leprosy, tumours and also inflamed hemorrhoids (Saklani *et al.*, 2013). *D. alata* has been reported to contain relatively high levels of oxalates (Wanasundera, 1994). *D. alata* has many culinary uses and some medical uses but its industrial uses still remain unexplored (Saklani *et al.*, 2013).

The microorganisms of choice for alcoholic fermentation are usually yeasts belonging to *Saccharomyces* genus. *Saccharomyces cerevisiae* is usually considered the typical yeast for bioethanol production (Caldwell *et al.*, 2010). To maximize the ethanol yield, yeast strains resistant to high temperatures and high ethanol concentrations are utilized to maximize cost (Alvira *et al.*, 2010).

The indiscriminate disposal of the peels of *D. dumetorum* and *D. alata* might constitute a form of environmental pollution. In recent times, there has been the use of citrus fruits, sugar cane, cassava (including the peels), and some other crops for the production of bioethanol. However, report on the use of bitter yam and water yam peels in bioethanol production has been scanty. Hence this study investigated the potentials of bitter yam and water yam peels in biosynthesis of ethanol which has applications in the transport industry as alternative source of fuel.

MATERIALS AND METHODS

Fungal source

Strains of *Aspergillus tamarii* and *Saccharomyces cerevisiae* were obtained from the Microbiology Laboratory of Wellspring University, Benin city, Nigeria and sub cultured on Sabouraud Dextrose Agar (SDA) to obtain pure colonies.

Sample collection and processing

Bitter yams (*Dioscorea dumetorum*) were purchased from Mowe market, Ogun State and Ekututu market, Anambra State. The water yams (*Dioscorea alata*) were purchased from Oba market, Benin City, Edo State. The yams were peeled and the peels oven dried (Gallenkamp, size 1 England) for 4 h at 140 ^{o}C according to the method of Obianwa *et al.* (2016). The dried peels were milled into powdery form using a waring blender. The powdered peels were sieved using 4.0 mm sieve to obtain a fine powder which was kept in an airtight container.

Screening and selection of amylolytic and cellulolytic moulds

Screening for amylolytic moulds

Screening of amylase producing moulds from the soil environment was carried out according to the method of Kareem *et al.* (2009). The mould isolates were inoculated on starch agar plates and incubated at 30 ^{o}C for 48 h. The plates were flooded with Lugol's iodine solution after 48 h and zones of clearance observed around the moulds.

Screening for cellulolytic moulds

This was carried out by the methods of Mahasneh and Stewart (1980) using Carboxyl methyl cellulose-Congo red (CMC-CR) medium. Pure mould isolates were inoculated on CMC-CR medium and incubated at 30 ^{o}C for 96 h.

Production and quantification of bioethanol by Submerged Fermentation under optimum conditions

Amylolytic and cellulolytic strains of *Aspergillus tamarii* identified using Carboxyl methyl cellulose-Congo red (CMC-CR) and Starch-agar media were grown aerobically to early stationary phase with shaking in separate 250 ml flasks containing 10 % Bitter yam and Water yam peels. The incubation was done at 30 ^{o}C for 96 h (Hashem *et al.*, 2013). The sugar produced was quantified with a refractometer at 12, 24, 36, 48, 60, 72, 84 and 96 h of fermentation. For ethanol production, the yeast strain was inoculated into the fermentation medium at the optimum fermentation time for sugar production and ethanol production monitored at 12, 24, 36, 48, 60, 72, 84 and 96 h of fermentation by fractional distillation using the method of James (1995).

Optimization of the fermentation conditions of ethanol production

Effects of substrate concentration on ethanol production

Ethanol production was carried out at 30° C and pH 5 using various substrate concentrations (5, 10, 15, 20, 25 and 30 %) of bitter yam and water yam peels. Ethanol production by yeast strains were determined as previously described.

Effects of temperature on ethanol production

The effect of temperature on ethanol production was investigated at 20 % substrate concentration and pH 5. The fermentation medium was incubated at different temperature (25, 30, 35, 40, 45 and 50 ^{o}C). Ethanol production was determined as previously described.

Effect of pH on ethanol production

The effect of pH on ethanol production was studied in the range 4.0 - 8.0 (4.0, 5.0, 6.0, 7.0 and 8.0) at 20 % substrate concentration and temperature of 35^{o} C. Ethanol production was determined as previously described.

Effect of agitation on ethanol production

The effect of agitation on ethanol production was studied in the range 60 - 160 (60, 80, 100, 120, 140 and 160 revolution per minute) at substrate concentration of 20 %, temperature of 35^{o} C, pH 5 for *D. alata* and pH 7 for *D. dumetorum.*

RESULTS

Screening for amylolytic and cellulolytic properties

The *Aspergillus tamarii* obtained from the laboratory tested positive for amylase and Cellulase production by showing zone of clearance on the starch-agar plate (Plate 1) and the CMC-CR plate (Plate 2).

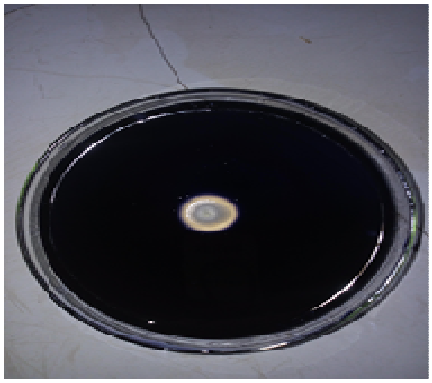

Plate 1: Amylase producing mould on Starch-Agar plate

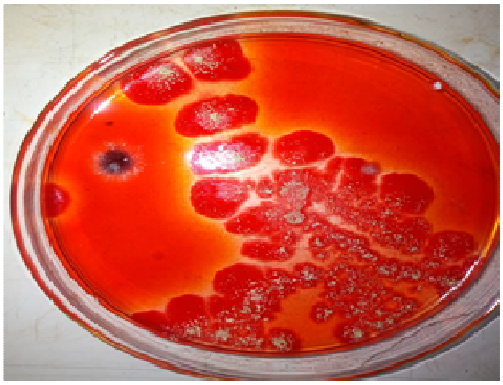

Plate 2: Cellulose producing mould on CMC-CR plates

Production and quantification of bioethanol by submerged fermentation under optimum conditions

The optimum sugar production at 72 h of fermentation was 18 % and 16 % for *D. dumetorum* and *D. alata* respectively. However, there was a decline in sugar production after this optimum fermentation time for sugar production (Table 1). Fermentation was initiated by the introduction of *Saccharomyces cerevisiae* into the fermentation medium at the optimum fermentation time of 72 h for sugar production. Optimum time for bioethanol production was observed to be 48 h. Bitter yam peels had higher ethanol yield of 6 % than water yam peels which had 5 % ethanol yield (Table 2).

Table 1: Sugar production from *Dioscorea dumetorum* and *Dioscorea alata* peels by *Aspergillus tamarii*

SUBSTRATE (PEELS)		SUGAR YIELD							
	TIME (HOURS)	12	24	36	48	60	72	84	96
D. dumetorum		0.0	0.0	5.0	8.0	11.0	18.0	10.0	10.0
D. alata	PERCENTAGE (%)	0.0	0.0	4.0	6.0	9.0	16.0	7.0	7.0

Table 2: Biosynthesis of ethanol from *Dioscorea dumetorum* and *Dioscorea alata* peels by *Aspergillus tamarii* and *S. cerevisiae*

SUBSTRATE (PEELS)		BIOETHANOL YIELD							
	TIME (HOURS)	12	24	36	48	60	72	84	96
D. dumetorum		0.0	1.5	2.0	6.0	3.0	2.0	1.0	0.5
D. alata	PERCENTAGE (%)	0.0	0.5	1.5	5.0	1.5	1.0	0.5	0

Optimization of the fermentation conditions of ethanol production

Effect of substrate concentration on ethanol production

Effect of substrate concentration on ethanol production is presented in Figure 1. The concentration ranged from 5 % to 30 %. Substrate concentration of 20 % resulted in an optimum ethanol production of 7 % and 5 % for *D. dumetorum* and *D. alata* respectively. There was a reduction in ethanol production beyond the optimum substrate concentration.

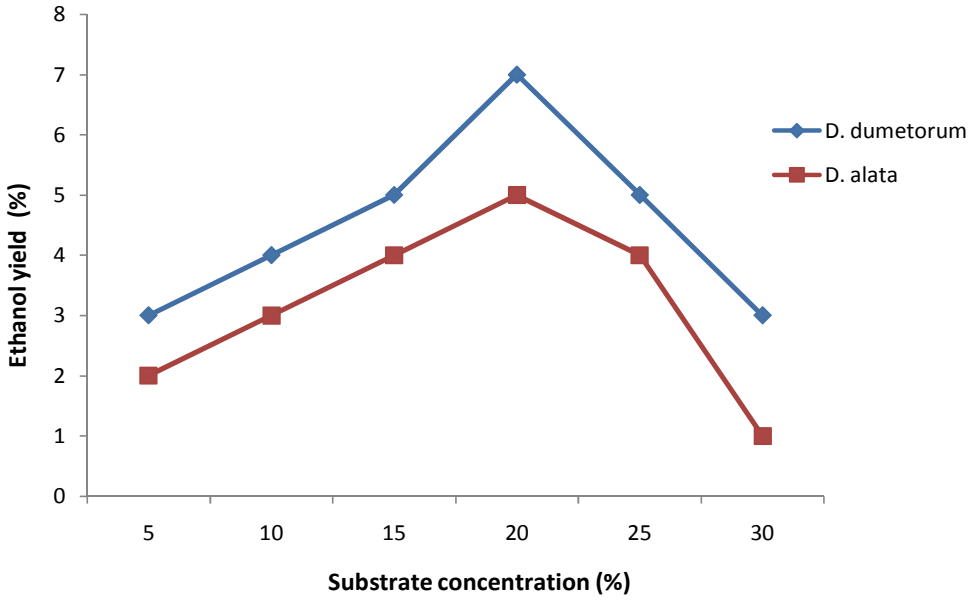

Figure 1: Effect of substrate concentration on ethanol production from *Dioscorea dumetorum* and *D. alata* peels by *Aspergillus tamari* and *Saccharomyces cerevisiae*

Effect of incubation temperature on ethanol production

The result shows optimum production of bioethanol of 8 % and 6 % at 35 ^{o}C for *D. dumetorum* and *D. alata* respectively (Fig.2).

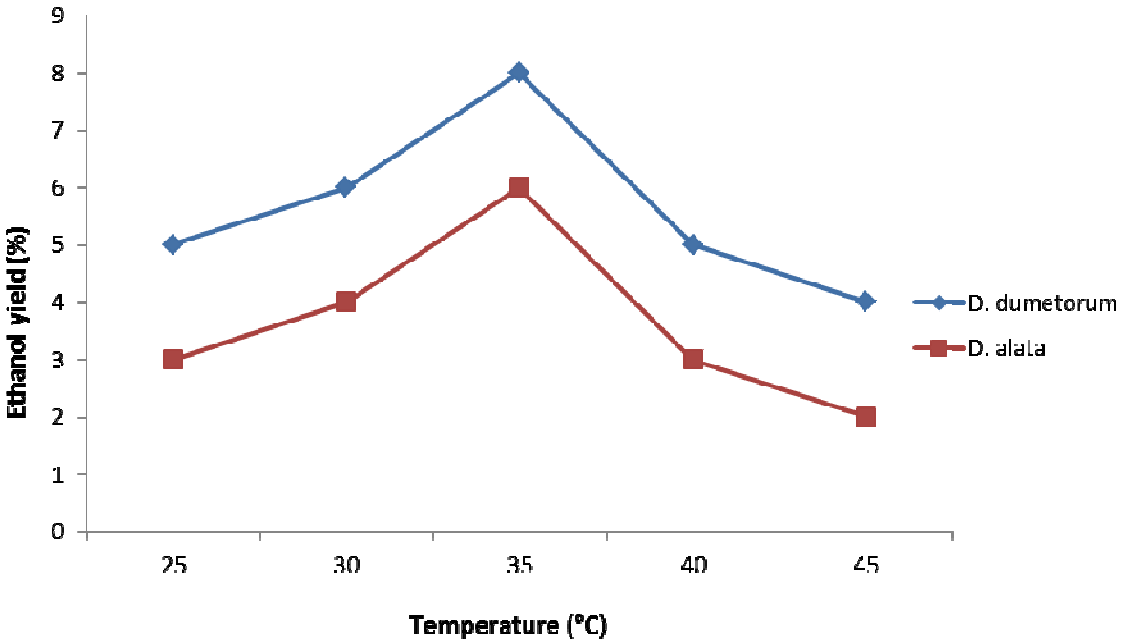

Figure 2: Effect of temperature on ethanol production from *Dioscorea dumetorum* and *D. alata* peels by *Aspergillus tamari* and *Saccharomyces cerevisiae*

Effect of pH on Ethanol Production

Effect of varying pH (4.0 to 8.0) as shown in Figure 3 revealed that the optimum pH for ethanol production was pH 7 for *D. dumetorum* and pH 5 for *D. alata*. This result shows optimum bioethanol production of 10 % at pH 7.0 and 8 % at pH 5.0 for *D. dumetorum* and *D. alata* respectively. Further increase in the pH of the fermentation medium resulted in decreased ethanol production.

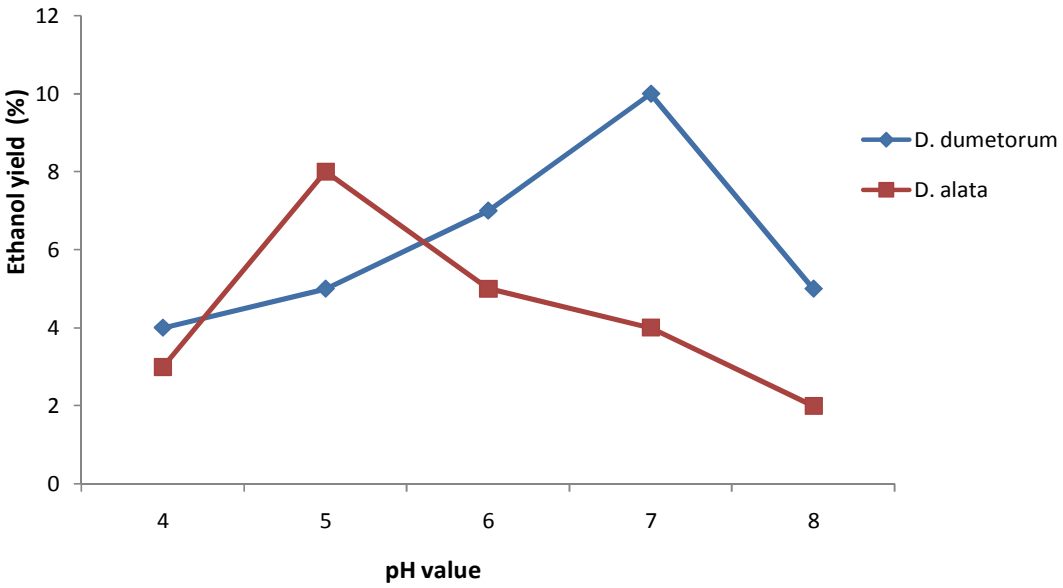

Figure 3: Effect of pH on ethanol production from *Dioscorea dumetorum* and *D. alata* peels by *Aspergillus tamari* and *Saccharomyces cerevisiae*

Effect of agitation on ethanol production

The effect of agitation speed on ethanol production revealed that optimum ethanol yields of 13 % and 11 % were produced at an agitation speed of 100 revolution per minute from the bitter yam and water yam peels respectively (Figure 4). Further increase in agitation speed resulted in reduction in ethanol yield.

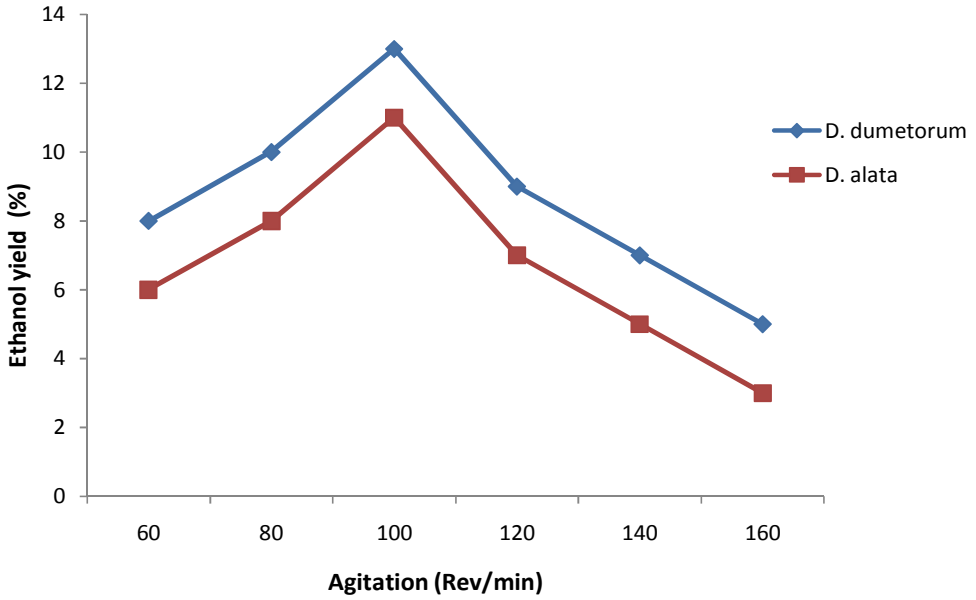

Figure 4: Effect of agitation on ethanol production from *Dioscorea dumetorum* and *D. alata* peels by *Aspergillus tamari* and *Saccharomyces cerevisiae*

DISCUSSION

The ethanol yield increased until 48 h after which it began to decline. This increase may be due to the gradual breaking down of complex sugars to simple sugars during fermentation (Oyeleke and Jibrin, 2009). Mazmanci (2011) reported similar value in the fermentation of fruits of *Washingtonia robusta* by *Saccharomyces sp.* where bioethanol production from fermentation of sweet sorghum juice by *S. cerevisiae* increased throughout 50 h of fermentation during ten-day fermentation.

The yeast was capable of synthesizing bioethanol from solution containing up to 30 % of bitter yam and water yam peels with 20 % having the optimum ethanol yield. An increase in the substrate concentration beyond 20 % resulted in a decrease in ethanol concentration in the medium. This could have been due to high concentration of complex sugars in the fermentation medium which could have inhibited the growth of the yeast and its ability to produce ethanol (Iqbal *et al.*, 2010). *D. dumetorum* had more ethanol yield than *D. alata* (4 % and 3 % respectively). However, 30 % concentration of yam peels had the lowest ethanol yield of 0.5 % for *D. dumetorum* and 0.2 % for *D. alata* respectively). This agrees with the report of Rai *et al.* (2012) who reported that high sugar concentration may exert high toxicity on yeast and the nutrient may be deficient at the final stage of fermentation.

Increase in the substrate concentration beyond 20 % resulted in reduction of ethanol concentration in the medium. This could have been due to high concentration of complex sugars in the fermentation medium which could have inhibited the growth of the yeast and its ability to produce ethanol (Iqbal *et al.*, 2010). Furthermore, decrease in ethanol production during fermentation could also be caused by the composition of the substrate, reduction of the enzyme's active sides, and the inefficiency of mass transfer (Aliberti *et al.*, 2017).

The optimum temperature for bioethanol production from *D. dumetorum* and *D. alata* was 35 ^{o}C. Further increase in temperature resulted in decrease in bioethanol production.

Higher temperature might disrupt membrane function and enzyme activity, thus resulting in decrease in ethanol production (Yan *et al*., 2012). Shankar *et al.* (2015) also reported that an optimum temperature of 35 °C was responsible for maximal ethanol yield.

Optimum ethanol production in submerged fermentation by *A. tamarii* and *S. cerevisiae* was obtained at pH 7.0 and 5.0 for *D. dumetorum* and *D. alata* respectively. At pH 7.0, the optimum ethanol yield for *D. dumetorum* was 10 % and at pH 5.0, the optimum ethanol yield was 8 % for *D. alata*. The variation in the pH of the two *Dioscorea* species might be due to their different properties and composition. Limtong *et al.* (2007) reported that optimum ethanol production by *Kluveromyces marxianus* in sugar cane juice medium was obtained at pH 5.0. Narendranath, (2001) reported that the optimal pH range for the growth of yeast vary from 4 to 6. This agrees with the present study with optimum ethanol production at pH 5. The present study also correlates with the findings of Darvishi and Moghaddami (2019), who reported an optimum production of 10 % ethanol at pH 5.

In the present study, the highest ethanol production was obtained at optimum agitation speed of 100 rev/min. However, further increase in the agitation speed resulted in reduced ethanol production. The highest ethanol production was achieved at the optimum agitation speed because of the efficient distribution and transportation of air and nutrients to the cells. This is in agreement with the report of Kempf *et al.* (1997) who reported that dissolved oxygen concentration influenced the growth and production of secondary metabolites by microorganisms. Furthermore, Rodmui *et al.* (2008) reported that agitation speed creates turbulence and shear force in the cultivation process which will influence both cell growth and product formation. The reduction in ethanol production at higher agitation speeds might be due to the harmful effect of the shear forces on the fungal mycelium as a result of increase in agitation speed beyond the optimum speed (Techapun *et al*., 2003). At lower agitation speeds below the optimum, the decrease in ethanol production might be due to improper mixing of the medium (Jimenez *et al*., 2005).

CONCLUSION

In conclusion, this study has shown the potentials of *Aspergillus tamarii* and *Saccharomyces cerevisiae* in the production of bioethanol from bitter yam and water yam peels. This study revealed the potentials of bitter yam peels as a better substrate for ethanol production when compared to the water yam peels. No external enzyme was required as the appropriate hydrolytic enzymes were provided by both the mould and the yeast. This study established the production of bioethanol from agricultural wastes, thus turning waste to wealth.

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