Ilorin Journal of Science
Volume 4, Number 1, 2017, pp.27–41 (Printed in Nigeria)
ISSN: 2408 – 4840 © 2017 Faculty of Physical Sciences, University of Ilorin
https://doi.org/10.54908/iljs.2017.04.01.002

ILORIN
JOURNAL OF SCEINCE

ILJS-17-022

Fungal Production of Itaconic Acid From Palm Kernel Cake in Submerged Fermentation By *Aspergillus niger* and *Aspergillus terreus*

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Abstract

Agricultural wastes often accumulate in the environment resulting in pollution and loss of biomass which could serve as substrate for production of other value-added products. In this study, two fungi; *Aspergillus niger* (ATCC 16404) and *Aspergillus terreus* (ATCC 20542) were used to ferment palm kernel cake (PKC); an agro-industrial waste to produce itaconic acid (IA). The proximate composition was determined. PKC was pre-treated with alkali and steam and fermented in mineral salt medium with inocula of *A. niger* and *A. terreus.* Fermentation parameters were pH 5.4; 10% substrate concentration; 10% inocula at 29±1 °C for 5 days. Fermentation was optimized by varying fermentation parameters. Proximate analysis of PKC showed carbohydrates (45.5%), crude protein (4.50%) and lipids (9.21%). IA yield of 47.0 g/L and 42.27 g/L was produced by *A. niger* and *A. terreus* from PKC respectively. Optimization produced higher IA yields of 100.33 g/L and 105.00 g/L by *A. niger and A. terreus* respectively at pH 4.0; substrate concentration of 10g; inocula size of 10% at 29±1°C on Day 5 of the fermentation representing 53.15% (*A. niger*) and 59.74% (*A. terreus*) increase over the pre-optimization levels. The results support the potential use of PKC for the industrial production of IA.

Keywords: Palm kernel cake, fermentation, itaconic acid, *Aspergillus niger*, *Aspergillus terreus*

1. Introduction

Palm kernel cake (PKC) a by-product of the oil palm industry, is produced after the extraction of the oil, usually via the screw press technique (Nik *et al.*, 2011). Moderately rich in protein and amino acids (Ezieshi and Olomu, 2007; Ramachandran *et al*., 2007), it is mainly used as a feed ingredient for ruminants such as cows (Atasie and Akinhanmi, 2009;

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MPOB, 2010), pigs (Kim *et al.,* 2001) and rabbits (Aganga *et al.,* 1991). However, due to its relatively high fibre content, presence of anti-nutritional factors and indigestible non- starch polysaccharides such as mannan and xylan (Atasie and Akinhanmi, 2009; Mohammed *et al*., 2014), its usefulness as feed for other animals such as poultry and fish is severely limited (Jorgensen, 2010).

Various microorganisms possess the ability to convert carbohydrate to yield low molecular weight organic acids through fermentation. Filamentous fungi are well known for being able to degrade lignocellulosic biomass to produce organic acids (Liaud *et al*., 2014). Prominent among them are members of Ascomycota; *Aspergillus niger, A. brasiliensis, A. flavus, A. terreus, Hypocrea lixii, Nectria pseudocinnabarina* and Basidiomycota: *Pycnoporus coccineus, Stereum hirutum, Tinctoporellus epimiltinus* and *Formitiporea mediterranea* (Jones, 1998; Magnuson and Lasure, 2004; Plassard and Fransson, 2009). They are mostly used due to their ability to grow at low pH and moisture content, high tolerance for acid, ability to form hyphae which penetrate substrate and their ability to produce various enzymes for the conversion of different wastes to useful products (Alva *et al.,* 2007). Some of the organic acids produced by fungi that have been studied extensively include citric, oxalic, gibberellic, fumaric, gluconic, itaconic and kojic acids (Mondala, 2015).

Itaconic acid (IA) (IUPAC: 2-methylenebutanedioic acid) has the chemical formula $C_5H_6O_4$; has a melting point of 167-168^o C; density of 1.632; is stable at acidic, neutral and middle basic conditions at moderate temperature (Chandragiri and Sastry, 2011; Kuenz *et al.,* 2012). It is unsaturated dicarbonic acid with high potential as a chemical building block used for a variety of industrial products including resins, plastics, paints, carpet and book cover coatings, adhesives, high-strength enhanced fiberglass, artificial gems, synthetic glass with nonlinear characteristics (Hajian and Yusoff, 2015; Steiger *et al.,* 2013). The global market price of IA is about $2 per kg (Van der Straat *et al.,* 2014), which is deemed to be expensive, therefore alternative or cheaper substrates may make the production process more profitable. The global IA market was valued at $126.4m in 2014; however, driven by concerns over diminishing world stocks of fossil fuels, global warming issues and the need to step up manufacture of ‘green chemicals’, the IA market is projected to reach $204.6m by 2023 (TMR, 2015).

The objectives of this study were to determine the suitability of PKC as a cellulosic agro-industrial waste substrate for the fermentative production of itaconic acid; to determine the

ability of *Aspergillus niger* and *Aspergillus terreus* to utilize PKC as substrate for the production of IA and to determine the optimal conditions for IA production.

2. Materials and Methods

2.1 Collection and preparation of palm kernel cake

Palm kernel cake (PKC) was collected from a local mill factory in Omu-aran in Irepodun local government area of Kwara state Nigeria. It was allowed to air dry at room temperature (29 ±1°C), ground and kept in an air-tight container with some bags of silica gel in order to avoid moisture absorption until needed.

2.2 Collection of microoganisms and preparation of spore suspensions

The test organisms *Aspergillus niger* (ATCC 16404) and *Aspergillus terreus* (ATCC 20542) were collected from Federal Institute for Industrial Research Oshodi, Lagos, Nigeria. The organisms were sub-cultured on Potato Dextrose Agar (PDA) slants and incubated for a period at 25 °C for 5 days, after which they were stored at 4°C. For the preparation of the spore suspension, 10 ml of sterile water was added to 5-day old culture slants of the fungi, the surface of the culture was scratched with a sterilized loop and agitated thoroughly at 250 rpm on a shaker to suspend the spores (Omojasola and Jilani, 2009). The number of the spores were counted by using the improved Neubauer Haemocytometer (Weber England B.S 748) and adjusted to approximately 2.6x 10^8 CFU/ml and 3.7x10^8 CFU/ml of *Aspergillus niger* and *Aspergillus terreus* respectively (Petrucciolli *et al*., 1999) which were used as inocula throughout the study.

2.3 Proximate composition of PKC substrate

The proximate analysis of the PKC substrate was determined. The parameters assayed were; moisture content, ash, crude protein, total sugars, crude lipids, crude fibre (AOAC, 2002). Carbohydrate content was obtained by difference

2.4 Pre-treatment of substrate

Alkali hydrolysis was used for the pre-treatment of the PKC substrate. PKC and the CMC control were soaked separately in 5% (w/v) NaOH and autoclaved at 121°C for 30 minutes. The autoclaved materials was filtered through muslin cloth and neutralized with 1N HCl,

which was then washed with distilled water until no traces of alkaline was found (Omojasola and Jilani, 2009).

2.5 Submerged Fermentation

PKC (10% w/v) was mixed in 100 ml of distilled water in separate flasks. Mary Mandel's Mineral Salts Medium; 0.25 % (w/v) NH_4Cl, 0.095 % (w/v), $MgSO_4$, 0.0088 % (w/v), KH_2PO_4 and 0.0004 % (w/v) and $CuSO_4$ was used for fermentation. The media were sterilized by autoclaving at 121 °C for 15 min. The sterilized media were inoculated separately with inocula containing 2.6 x 10^8 and 3.7 x 10^8 CFU/ml of *Aspergillus niger* and *Aspergillus terreus*. Each flask was cultured on a rotary shaker (Gallenkamp, England) at 400 rpm; temperature 29±1 °C and samples were assayed for IA at 24 h intervals. Fermentation parameters were pH 5.4; 10% substrate concentration; 10% inocula at 29±1 °C for 5 days. The quantitative estimation of IA was determined by UV-VIS spectrophotometer (Jenway 6105) at 385 nm (Meena *et al*., 2010).

2.6 Optimization of Fermentation Parameters

The fermentation parameters were varied to increase the yield efficiency and determine optimal conditions for fermentative IA production.

i. Effect of varying substrate concentration

The concentration of the PKC substrate was varied at 2-10% in the fermenting media. The pH was held at 5.4 and 10% inocula size, temperature 29 ± 1°C for 5 days.

ii. Effect of varying pH

The pH of the fermentation media was adjusted to between 2.5-6.5 by the addition of 0.1N HCl and 0.1N NaOH. Other fermentation parameters were kept constant at 10% substrate concentration and inocula size of 10%, temperature 29 ± 1°C for 5 days.

iii. Effect of varying time

The fermentation period was varied between 1-10 days for IA production. Substrate concentration and inocula size were held at 10%, pH 5.4, temperature 29 ± 1°C.

iv. Effect of varying inocula size

The inocula size was varied from 1-11 ml. Spore suspension containing approximately 2.6 x 10^8 CFU/ ml and 3.7 x 10^8 CFU/ ml of *Aspergillus niger* and *Aspergillus terreus* suspensions were prepared according to the method of Petruccioli *et al.* (1999). These were used as inocula for the fermentation process. The pH was held at 5.0, substrate concentration of 10%, temperature 29 ± 1°C.

3. Result and Discussion

Proximate composition of PKC

In this study, PKC an agro-based waste was used as substrate for the fermentative production of IA. The proximate composition of PKC revealed a high carbohydrate and soluble sugar content of 45.50% and 16.50% respectively (Table 1). These nutrients would serve as a good source of carbon and energy source for the fermenting microorganisms. The crude protein of 4.50% would provide a source of nitrogen for microbial metabolism.

Table 1: Proximate composition of palm kernel cake

Moisture Content (%)	Crude Protein (%)	Lipid Content (%)	Ash Content (%)	Crude Fibre (%)	Carbohydrate (%)	Soluble sugars (%)
8.50 ± 1.7	4.50 ± 1.2	9.56 ± 1.7	5.42 ± 1.3	10.02 ± 1.7	45.50 ± 3.9	16.50 ± 2.3

Values represented are means of triplicates ±SEM

The proximate composition of PKC has been reported by other researchers: dry matter 90%, 4.1% ash, 15.6%, fat 8%, crude protein 12.9% (Hutagulung, 1983); moisture content 8.26%, crude protein 14.50%, crude fibre 10%, crude fat 9.48%, ash 4.34%, carbohydrate (NFE) 53.42% (Ezieshi and Olomu, 2007); 63.06 g dietary fibre, 8.49 g crude fibre, 14.4 g crude protein, 4.43 g ash per 100 g of PKC (Nik *et* al., 2011). PKC has been reported to contain various proximate compositions depending on source, processing technique used and type of palm kernel (Ezieshi and Olomu, 2007). PKC is reported to be an energy feed, as it contains about 16-18% protein (Alimon, 2004). Its relatively high content of carbohydrates would serve as a valuable carbon and energy source for the fermenting fungi as well as substrate for IA production (Meena *et al*., 2010).

Pre-optimization Fermentation of PKC

The results of the pre-optimization fermentation of the PKC showed that the maximum IA yields of 47.0 ± 2.3 g/L and 42.27 ± 1.2 g/L was recorded *A. niger* and *A. terreus* respectively on Day 5 of fermentation (Table 2). This confirms that PKC is a suitable substrate for IA production.

Table 2: Fermentation of palm kernel cake by *A. niger* and *A. terreus* to produce itaconic acid

Fermentation (Days)	Itaconic acid produced (g/L)			
	Aspergillus niger		*Aspergillus terreus*	
	PKC	CMC	PKC	CMC
1	5.30±2.7[a]	2.38±0.9[a]	4.67±1.2[a]	3.44±0.6[a]
3	18.0±1.2[b]	7.0±0.08[a]	15.32±1.3[b]	6.67±0.5[a]
5	47.0±2.3[c]	15.67±1.2[b]	42.27±1.2[c]	10.67±0.43[b]

Fermentation parameters: pH 5.4; 10% substrate concentration; 10% inocula, time 5days at 29±1 °C

The results confirm that PKC is a good substrate for IA production and confirming the observations of Couto and Sanroman (2006) and Ncube *et al.* (2012) about the suitability of agro-industrial as good fermentable substrates. Different substrates have been employed in IA production using various different organisms. These include corn starch (Yahiro *et al.*, 1997); cane molasses (Meena *et al.* 2010); *Jatropha* seed cake (Rao *et al.*, 2007; El Imam *et al.*, 2013); sweet potato peel (Omojasola and Adeniran, 2014); rice bran, groundnut shell, orange pulp, groundnut oil cake and sugar cane bagasse (Rafi *et al.* 2014); sago starch hydrolysate (Dwiarti *et al.* 2007). It was also observed that the PKC substrate yielded significantly ($p<0.05$) higher amounts of IA than the CMC control (Table 2). This may also be due to the relatively richer chemical composition of the PKC as compared to CMC a cellulose derivative, an anionic polysaccharide devoid of the other growth stimulating nutrients (Togrul and Arslan, 2004).

Optimization of IA Production

Generally, changes in culture conditions greatly affect the production ability of a fermenting microorganism (Meena *et al*., 2010). The fermentation conditions were varied to determine the effects on IA yield.

Effect of Fermentation Time

The fermentation of PKC was allowed to proceed until IA yield had dropped consistently for at 5 days (Table 3). IA production increased steadily to a maximum of 62.67±3.2 g/L and 60.67±4.3 g/L by *A. terreus* and *A. niger* respectively after which no further increases were recorded (Table 3). Consistently, the PKC substrate yielded significantly ($p<0.05$) higher IA amounts during the fermentation.

Table 3: Effect of varying time on IA production by *A. niger* and *A. terreus* using PKC

Fermentation (Days)	Quantity of Itaconic acid produced (g/L)			
	Aspergillus niger		*Aspergillus terreus*	
	PKC	CMC	PKC	CMC
1	14.0±1.2^{a}	3.0±1.2^{a}	19.67±2.9^{ab}	2.67±1.2^{ab}
2	17.0±1.2^{a}	8.0±1.2^{ab}	30.33±0.9^{c}	8.67±1.5^{cd}
3	24.0 ±2.1^{b}	16.67±1.2^{e}	40.33±1.5^{d}	13.33±0.9^{ef}
4	36.33±2.3^{c}	22.67±1.8^{f}	50.0±2.7^{e}	21.0±0.6^{g}
5	60.67±4.3^{e}	30.0±0.6^{g}	62.67±3.2^{f}	29.0±1.7^{h}
6	51.33±2.0^{d}	24.0±1.5^{f}	50.67±1.5^{e}	20.0±0.6^{g}
7	35.0±2.1^{c}	14.67±0.9^{de}	44.0±1.2^{de}	15.33±1.5^{f}
8	26.0 ±2.1^{b}	11.33±0.9^{cd}	38.3±2.6^{d}	10.0±0.6^{de}
9	20.0±0.6^{ab}	7.0±0.6^{bc}	27.33±3.2^{bc}	5.67±1.8^{bc}
10	16.0±0.6^{a}	4.67±1.3^{ab}	18.33±4.3^{a}	1.67±0.6^{a}

Fermentation parameters: pH 5.4; 10% substrate concentration; 10% inocula, time 5days at 29±1 °C

These results are in agreement with some other studies that have reported maximum IA yields on Day 5 or 120 h of fermentation (Rao *et al*., 2007; Meena *et al*., 2010; Chandragiri and Sastry, 2011; El Imam *et al.*, 2013; Omojasola and Adeniran, 2014). Petruccioli *et al*. (1999) recorded peak IA yields on Day 6 (144 h) by *A. terreus* using various raw starchy substrates. Reduction in IA yield after Day 5 may be attributed to the gradual exhaustion of nutrients in the fermentation medium. Substrates are utilized quite rapidly in submerged fermentation and if not replaced, as in batch fermentation, the product yield will decline (Subramaniyam and Vimala, 2012).

Effect of Substrate Concentration

The substrate concentration of the medium was varied between 2-10%. It was observed that the IA yield increased in tandem with substrate concentration up to 10%. Highest IA yields recorded were 63.67 ±2.9 g/L and 67.67 ±2.8 g/L at 10% substrate concentration by *A. niger* and *A. terreus* respectively (Table 4). These yields were higher than the pre-optimized IA yield (Table 2).

Table 4: Effect of substrate concentration on itaconic acid production by *A. niger* and *A. terreus* using palm kernel cake

Substrate	Itaconic acid produced (g/L) Substrate concentration (%)				
	2	4	6	8	10
Aspergillus niger					
Palm Kernel Cake	26.67 ±0.9^{a}	33.33± 1.5^{b}	37.67 ±1.9^{c}	44.01 ±2.1^{d}	63.67 ±2.0^{d}
CMC (Control)	11.01 ±1.2^{a}	15.0 ±1.2^{b}	17.33 ±1.8^{c}	20.02 ±2.3^{cd}	23.33 ±3.3^{d}
Aspergillus terreus					
Palm Kernel Cake	38.67 ±2.7^{a}	51.03 ±2.7^{b}	55.10 ±2.1^{bc}	61.1 ±1.9^{cd}	67.67 ±2.8^{d}
CMC (Control)	16.33 ±1.2^{ab}	18.01 ±1.5^{b}	21.33 ±0.9^{c}	23.67 ±1.8^{d}	26.20 ±2.1^{d}

Fermentation parameters: pH 5.4; 10% inocula, time 5days at 29±1 °C; Values with different superscript are significantly different at $p<0.05$

These results show that the fermenting organisms were able to utilize the substrate for metabolism. *Aspergillus* species are reported to possess all the components of the cellulase enzyme system required to hydrolyze cellulosic biomass (Vries and Visser, 2001). Glucose, sucrose and xylose are reported to be the preferred substrates for IA fermentation, which are known to be utilized efficiently by most of the *Aspergillus* spp. (Meena *et al.*, 2010). Glucose is the dominant sugar produced on the hydrolysis of cellulose present in waste biomass with smaller quantities of xylose and arabinose (Torrado *et al.*, 2011). The results from this study largely correlate with those of El Imam *et al.* (2013) and Omojasola and Adeniran (2014) who observed maximum IA yield at 10% substrate concentration by *A. terreus* and *A. niger* respectively. Meena *et al.* (2010) reported maximum IA yield by different species of *Aspergillus* at substrate concentrations of 4.8 g/L and 8.1 g/L; Chandragiri and Sastry (2011) observed highest IA yields by *Ustilago maydis* at 35% substrate concentration.

Effect of pH

The pH of the fermentation medium was adjusted to between pH 2.5 and 6.5 to determine the influence of pH on IA production by the fermenting organisms. The optimum pH which gave the highest IA yield for both fermenting organisms was pH 4.0 (Table 5). It was observed that the IA yield increased up to pH 4.0 after which it decreased. The results indicated that *A.*

terreus yielded higher IA amounts 84.67±3.7 g/L than *A. niger* which yielded 77.67±5.8 g/L (Table 5).

Table 5: Effect of varying pH on itaconic acid production by *A. niger* and *A. terreus* using palm kernel cake

Substrate	Itaconic acid produced (g/L) pH						
	2.5	3.5	4.0	4.5	5.0	6.0	6.5
Aspergillus niger							
Palm Kernel Cake	43.0±2.9^{de}	51.3± 3.2^{e}	77.7±3.7^{f}	48.7±2.3^{de}	37.0±2.7^{c}	29.0±1.5^{b}	21.7±3.1^{a}
CMC (Control)	17.0±1.2^{c}	19.3±1.2^{d}	24.3±3.3^{e}	21.0±2.3^{cd}	15.0±1.8^{d}	13.0±1.5^{b}	11.0±1.2^{a}
Aspergillus terreus							
Palm Kernel Cake	47.3±2.3^{b}	63.0±4.1^{c}	87.7±5.8^{d}	61.7±2.6^{c}	41.0±2.1^{b}	43.0±1.7^{b}	30.0±2.3^{a}
CMC (Control)	18.1±1.2^{b}	22.3±2.1^{c}	26.3±4.1^{d}	21.3±1.8^{c}	18.0±2.1^{b}	14.0±1.5^{b}	10.3±1.2^{a}

Fermentation parameters: 10% substrate concentration; 10% inocula, time 5days at 29±1 °C; Values with different superscript are significantly different at $p<0.05$

An initial low pH has been suggested to enable the cells develop the biochemical machinery for IA production (Boruta and Bizukojc, 2017). It is well documented that both internal and external pH play important roles in the growth and metabolism of a microorganism (Chandragiri and Sastry, 2011). While microorganisms have mechanisms to maintain their internal pH at relatively constant levels, the external pH is a function of the external environment. When pH differs from the optimum, there is increase in maintenance energy requirement which may negatively affect growth, product formation and other physiological activities (Meena *et al.*, 2010). These findings correlate with those of other workers who put the range of optimum pH for IA production between pH 3.0-4.0 (Chandragiri and Sastry, 2011; El Imam *et al*., 2013; Meena *et al*., 2010; Omojasola and Adeniran, 2014; Rafi *et al*., 2014; Rao *et al*., 2007; Sudarkodi *et al*., 2012).

Effect of Inocula Size

Maximum IA yield was recorded with 5% inoculum of *A. niger* producing 74.0 g/L and *A. terreus* which yielded 87.0 g/L (Table 6). These yields were significantly higher ($p<0.05$) from yields of other inocula sizes.

Table 6: Effect of varying inocula size on itaconic acid production by *A. niger* and *A. terreus* using palm kernel cake

Substrate	Itaconic acid produced (g/L) Inocula size (%)			
	1	3	5	11
Aspergillus niger				
Palm Kernel Cake	32.67 ±2.9^{b}	52.67± 3.2^{c}	74.0 ±2.1^{d}	49.0 ±1.7^{c}
CMC (Control)	15.01 ±1.2^{b}	18.0 ±1.5^{c}	23.33 ±2.1^{d}	20.67 ±1.2^{c}
Aspergillus terreus				
Palm Kernel Cake	59.0 ±1.2^{b}	71.33 ±2.9^{c}	87.0 ±2.9^{d}	61.1 ±1.9^{b}
CMC (Control)	17.67 ±1.5^{b}	20.0 ±2.1^{c}	25.33 ±2.1^{d}	23.67 ±1.8^{d}

Fermentation parameters: pH 5.4; 10% substrate concentration; time 5days at 29±1 °C; Values with different superscript are significantly different at $p<0.05$

Inocula size is important because low amounts may be inadequate for the biomass and lead to a reduction in the IA yield, while excessive inocula may lead to competition for nutrients (Chandragiri and Sastry, 2011; Karthikeyan and Sivakumar, 2010). El Imam *et al.* (2013) and Omojasola and Adeniran (2014) reported optimum inocula size of 5 ml using *U. maydis*; *A. terreus* and *A. niger* respectively. However, Meena *et al.* (2010) reported a much higher inoculum size of 10% using different species of *Aspergillus.*

Optimized Production of IA

The IA yield under optimized conditions recorded 100.33 g/L by *A. niger* and 105.0 g/L by *A. terreus* both on Day 5 of fermentation (Table 7). These were significantly higher ($p<0.05$) than the peak yields obtained from the CMC control under the same optimized conditions. These yields were higher than those from the pre-optimized fermentations which were 47.0 g/L and 42.27 g/L by *A. niger* and *A. terreus* respectively (Table 2). In addition, *A. terreus* produced higher amounts of IA, although not significant ($p<0.05$) than *A. niger* under optimized conditions.

Table 7: Optimized production of itaconic acid by *A. niger* and *A terreus* using palm kernel cake

Substrate	Itaconic acid produced (g/L)	
	Days	
	2	5
Aspergillus niger		
Palm Kernel Cake	46.67 ±2.1^{c}	100.33 ±4.6^{e}
CMC (Control)	15.0 ±1.2^{a}	27.00 ±1.7^{b}
Aspergillus terreus		
Palm Kernel Cake	67.33 ±1.5^{d}	105.0 ±4.7^{e}
CMC (Control)	29.67 ±1.5^{b}	38.33 ±2.1^{c}

Fermentation parameters: pH 4.0; 10% substrate concentration; 5% inocula size; time 5days at 29±1 °C; Values with different superscript are significantly different at $p<0.05$

The yields under optimized conditions represent a 53.15% and 59.74% increase in IA yield by *A. niger* and *A. terreus* respectively. While the IA yield by *A. terreus* was higher, it was not significantly different ($p<0.05$) from the yield of *A. niger* (Table 7). These results compare favourably with the IA yields of 24.45 g/L and 48.70 g/L from *Jatropha* seed cake (Rao *et al*., 2007; El Imam *et al*., 2013); 68.36 g/L by *Ustilago maydis* (Chandragiri and Sastry, 2011); 8.76 g/L by *A. flavus* on Czapek Dox media (Sudarkodi *et al.*, 2011) but lower than 112.67 g/L and 115.67 g/L by *A. niger* and *A. terreus* (Omojasola and Adeniran, 2014). The high yields in this study may be attributed to the suitability of the PKC substrate and fermenting organisms. *A. niger* is highly synthetic in nature (Liaud *et al.* 2014) while *A. terreus* is reported to be the most frequently used commercial producer of IA (Rao *et al.* 2007).

4. Conclusion

In conclusion, the results of this study have demonstrated the potential use of PKC as substrate for the fermentative production of IA using *Aspergillus niger* and *Aspergillus terreus.* The yield from PKC was higher when compared with the CMC (control). The factors which supported high IA production from the PKC substrate during submerged fermentation were duration, pH, inocula size and substrate concentration. Higher yields of the IA were recorded when the conditions of fermentation were optimized recording 53.15% and 59.74% IA yield increase by *A. niger* and *A. terreus* respectively. These are among the high yields documented in literature. This therefore demonstrates the dual importance of PKC as a suitable substrate for commercial production of IA and assisting in waste management by reducing environmental pollution.

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