

## Improving the quality of agro-wastes by solid-state fermentation: enhanced antioxidant activities and nutritional qualities

A. Lateef · J. K. Oloke · E. B. Gueguim Kana ·  
S. O. Oyeniyi · O. R. Onifade · A. O. Oyeleye ·  
O. C. Oladosu · A. O. Oyelami

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**Abstract** Solid substrate fermentations of some agro-wastes, namely cocoa pod husk (CPH), cassava peel (CP), and palm kernel cake (PKC) were carried out for the production of fructosyltransferase (FTase) by a newly isolated fungal strain *Rhizopus stolonifer* LAU 07. The fermented substrate were studied for improved nutritional quality by determining the crude protein, crude fibre, ash and lipid contents, and antioxidant activities. The cyanide content of cassava peels was also determined. Some levels of value-addition occurred as a result of the fermentation. The protein contents of the substrates increased by 33.3, 55.4, and 94.8%, while the crude fibre contents decreased by 44.5, 8.6, and 7.2% in PKC, CP, and CPH, respectively. The cyanide content of cassava peel was reduced by 90.6%. Generally, fermentation of the substrates by *R. stolonifer* LAU 07 increased the antioxidant activity in a DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. The  $IC_{50}$  (mg/ml) values of the methanolic extracts (fermented/unfermented) were obtained as 7.0/14.9, 4.4/10.6, and 5.5/14.7 mg/ml for PKC, CP, and CPH, respectively. Results herein reported showed that the nutritional qualities and antioxidant activities of all the investigated solid substrates were enhanced by fungal fermentation. Thus, scope exists for microbial upgrading of these low-quality agro-wastes and development of healthy animal feed supplements.

**Keywords** *Rhizopus* · Solid state fermentation · Fructosyltransferase · Antioxidant · Nutritional quality · Feed supplement

### Introduction

Solid-state fermentation (SSF) is defined as a process in which micro-organisms are grown on solid substrates in the absence of free water (Lagemaat and Pyle 2001). SSF resembles the natural microbiological processes like composting and ensiling. In industrial applications, it can be utilized in a controlled way to produce desired products. This process has been known from ancient times and different fungi have been cultivated in SSF for the production of food. These include the fermentation of rice by *Aspergillus oryzae* to initiate the koji process and *Penicillium roquefortii* for cheese production. Also, in China, SSF has been used extensively to produce brewed foods (such as Chinese wine, soy sauce, and vinegar) since ancient times (Chen 1992). In Japan, SSF is used commercially to produce industrial enzymes (Suryanarayan 2003). Since 1986 in Brazil, a series of research projects for the value-addition of tropical agricultural products and sub-products by SSF has been developed due to the generation of large amounts of agricultural residues (Soccol and Vandenberghe 2003).

In Nigeria, large quantities of different types of agro-wastes are dumped in the environment due to low nutritional qualities and presence of antinutritional factors. Nigeria is the largest producer of cassava in the World, and the fifth largest producer of cocoa. It produced some 760,000 metric tonnes of palm oil in 1999/2000, ranking the third largest producer in the World (USDA 2000). About 205,000 tonnes of cocoa was produced during 1999–2000 (Koekoek 2003),

A. Lateef (✉) · J. K. Oloke · E. B. Gueguim Kana ·  
S. O. Oyeniyi · O. R. Onifade · A. O. Oyeleye ·  
O. C. Oladosu · A. O. Oyelami  
Biotechnology Group, Microbiology Unit, Department of Pure  
and Applied Biology, Ladoko Akintola University  
of Technology, PMB 4000 Ogbomosho, Nigeria  
e-mail: agbaje72@yahoo.com; agbaje@alphabiotech.org

while approximately 34 million tonnes of cassava was produced in 2002 (FAO 2004).

Processing of these crops into finished or semi-finished products generates large amounts of solid waste annually. For instance, about 10 million tonnes of cassava are processed into 'garri' alone in Nigeria (Oboh 2006), and the peels could make up to 10% of the wet weight of the roots which are discarded as wastes. Similarly, about 8 million tonnes of cocoa pod husk (CPH) are discarded as waste annually in Nigeria (Egunjobi 1975). With this, 64,000–94,000 tonnes of nutrients such as K, Ca, and P, and 6,000–9,000 tonnes of N are lost annually, due to the richness of CPH in these nutrients. In a well run palm oil mill, each 100 tonnes of fresh fruit bunches (FFB) yields 20–24 tonnes of crude palm oil and about 4 tonnes of palm kernels. Thus, 72–76% of the FFB comes out at various stages of the process as wastes, including the palm kernel cake (PKC) which is obtained as a by-product of extraction of palm kernel oil from palm kernel.

In this work, SSF was carried out using a locally isolated fungal strain *Rhizopus stolonifer* LAU 07 to produce fructosyltransferase (E.C. 2.4.1.9) on some abundant Nigerian agro-wastes, namely cocoa pod husk (CPH), cassava peel (CP), and palm kernel cake (PKC). Fructosyltransferase (FTase) catalyses the formation of fructooligosaccharides from sucrose, which have broad applications in the food and pharmaceutical industries due to their physiological and physico-chemical properties. At the end of fermentation, the substrates were then studied for value-addition, in terms of improved nutritional qualities and antioxidant activities. The value-addition, as a result of microbial upgrading of these low-quality lignocellulolytic agro-wastes may expand the scope of their utilization particularly as feed supplements in animal husbandry in Nigeria.

## Materials and methods

### Isolation of microorganism and preparation of inoculum

*Rhizopus stolonifer* LAU 07 was isolated from spoiled orange fruit using potato dextrose agar (PDA) that was supplemented with 20% sucrose. Dark patches were scrapped with sterile scapel to inoculate the medium and incubated at  $30 \pm 1^\circ\text{C}$  for 48 h. The pure culture was subsequently stored in PDA slants at  $4^\circ\text{C}$ . It was identified conventionally according to its macroscopic and microscopic features following the scheme of Domsch et al. (1980). Inoculum was developed by transferring a loopful of mycelium into the inoculum medium (1% sucrose, 0.2% yeast extract, pH 5.50). The flasks were incubated at  $30 \pm 1^\circ\text{C}$  on a shaker at 100 rev/min for 24 h.

### Preparation of solid substrates

Dried substrates (CPH, CP, and PKC) were sourced locally in Ogbomoso, Nigeria, and dried at  $60^\circ\text{C}$  for 8 h before pulverization. While CPH was obtained from a cocoa plantation, CP, and PKC were obtained from cottage industries. The moisture contents were determined by drying to constant weight at  $110^\circ\text{C}$  in a hot-air oven. SSF was carried out in 250 ml capacity bottles containing 25 g of the substrates, moistened to a final moisture contents of 65% for CP; 69% for CPH; and 72% for PKC, respectively. The bottles were sterilized at  $121^\circ\text{C}$  for 1 h, cooled, inoculated with the inoculum, and then fermented for 5 days at  $30 \pm 1^\circ\text{C}$ . The pH values of CP (6.7) and PKC (6.4) were not adjusted prior to fermentation, while the CPH (initial pH of 8.9) was adjusted with 0.1 N HCl to the final pH of 6.4 before fermentation. The substrates were supplemented with or without sucrose and yeast extract.

### Harvesting

At the end of fermentation, cultures were harvested to determine the pH and FTase activity. Contents of each bottle were mixed with 50 ml of water and agitated at 100 rpm for 1 h. The filtrates (crude FTase) were squeezed out in a muslin cloth, and then centrifuged at 5,000 rev/min at  $10^\circ\text{C}$  for 25 min. The fermented substrate with highest FTase activity in each case was selected for further work. The substrates were oven dried at  $60^\circ\text{C}$  overnight, and then used for analysis.

### Nutritional composition

Ash, fat, and crude fibre contents of both fermented and unfermented substrates were determined following the methods of Cordenunsi et al. (2004), while the crude protein contents were determined using micro-Kjeldhal method ( $N \times 6.25$ ). The cyanide content of cassava peel was determined by the silver nitrate method (Oboh et al. 2002).

### Antioxidant or free-radical scavenging activities

#### *Preparation of methanol extracts*

After drying at  $60^\circ\text{C}$  overnight, the fermented and unfermented substrates (CPH, CP, and PKC) were pulverized to pass 30-mesh screen. Samples were extracted with methanol (1:5 w/v) at  $55^\circ\text{C}$  for 2 h in a shaking water bath at 100 rev/min. After filtering through Whatman No. 1 filter paper, the extracts were concentrated to dryness.

#### *Measurement of DPPH radical-scavenging activity*

The modified methods of Shimada et al. (1992) and Mensor et al. (2001) were used to study the free radical-scavenging

activities of the extracts using 1,1-diphenyl-2-picrylhydrazyl, DPPH (Fluka Chemika). Graded concentrations of extracts in 2.0 ml methanolic solution was added to 1.0 ml methanolic solution of 0.3 mM DPPH. The mixture was shaken and left in a dark box to stand for 30 min at room temperature ( $30 \pm 1^\circ\text{C}$ ). The blank was prepared with sample solution, 2.0 ml with 1.0 ml of methanol instead of DPPH, while 1.0 ml of methanolic DPPH plus 2.0 ml of methanol served as the control. The absorbance of the resulting solution was measured at 517 nm on a UV/Vis spectrophotometer model 6405 (Jenway Ltd. Essex, UK). The inhibitory percentage of DPPH was calculated accordingly: Scavenging/antioxidant activity (%) =  $\{1 - (\text{absorbance}_{\text{sample}}/\text{absorbance}_{\text{control}})\} \times 100\%$ .

The  $\text{IC}_{50}$ , the efficient concentration of methanolic extracts decreasing the initial concentration of DPPH radical by 50% was obtained by interpolation from linear regression analysis (Lin et al. 2006).

## Results

Nutritional quality of substrates: CPH, PKC, and CP

The fermentation of substrates with *R. stolonifer* LAU 07 improved the nutritional qualities of the substrates, however, these varied from one substrate to another (Table 1). The crude fibre contents of the substrates were reduced by 44.5, 8.6, and 7.2% in PKC, CP, and CPH, respectively. The protein contents of the fermented substrates were increased by 33.2, 55.4, and 94.8% in PKC, CP, and CPH, respectively. Generally, fermentation of the substrates led to reduction in the crude fat contents. The reductions were 15.4, 36.5, and 10.6% in PKC, CP, and CPH, respectively (Table 1). The ash contents of all the fermented substrates were improved at varying degrees (Table 1). Since the ash content determination is a measure of mineral levels in the substrates, it can be inferred that fermentation contributed to the higher levels of the minerals obtained. Similar improved levels of ash content, following fermentation have been reported by various authors (Oboh 2006). The fermentation of cassava peel with *R. stolonifer* LAU 07 reduced the cyanide content by 90.6%.

**Table 1** Approximate composition (%) of the substrates

Substrate	Crude fibre	Protein	Crude fat	Ash	Cyanide (ppm)
Palm kernel cake (PKC) 1	22.5	19.7	8.3	5.0	NA
Palm kernel cake (PKC) 2	12.5	26.3	7.1	8.6	NA
% change	−44.5	33.3	−15.4	72.6	NA
Cassava peel (CP) 1	14.7	12.3	5.8	9.4	14.7
Cassava peel (CP) 2	13.5	19.0	3.7	11.8	1.4
% change	−8.6	55.4	−36.5	26.0	90.6
Cocoa pod husk (CPH) 1	18.3	8.2	4.7	11.3	NA
Cocoa pod husk (CPH) 2	16.9	16.0	4.2	20.8	NA
% change	−7.2	94.8	−10.6	83.1	NA

1, unfermented; 2, fermented; −, % reduction; NA, not applicable

**Table 2** The  $\text{IC}_{50}$  of methanolic extracts of substrates in DPPH radical scavenging ability

Substrates	$\text{IC}_{50}$ (mg/ml)	% improvement
Unfermented palm kernel cake	14.9	NA
Fermented palm kernel cake	7.0	53.1
Unfermented cassava peel	10.6	NA
Fermented cassava peel	4.4	58.4
Unfermented cocoa pod husk	14.7	NA
Fermented cocoa pod husk	5.5	62.2

NA, not applicable

Antioxidant activity (DPPH-scavenging capacity)

The antioxidant capacities of fermented substrates were generally increased. The half-inhibition concentration ( $\text{IC}_{50}$ ), is shown in Table 2. The  $\text{IC}_{50}$  of the substrates (fermented/unfermented) were obtained as 7.0/14.9, 4.4/10.6, and 5.5/14.7 mg/ml for PKC, CP, and CPH, respectively. Amongst the unfermented substrates, PKC had the highest  $\text{IC}_{50}$ , while fermented cassava peel was the most potent DPPH-radical scavenging substrate, having the lowest  $\text{IC}_{50}$  of 4.4 mg/ml.

## Discussion

The reduction in the crude fibre contents of the fermented substrates is an indication of secretion of cellulose/hemicellulose-degrading enzymes by the fungus during fermentation. Several fungi including *Aspergillus niger*, *Trichoderma longibrachiatum*, *Trichoderma* spp., and *Trichoderma reesei* have been reported to degrade cellulose/hemicellulose in similar manner (Gritzali and Brown 1979; Sheperd and Kung 1996; Nsereko et al. 2000; Iluyemi et al. 2006). Iluyemi et al. (2006) reported reductions in the hemicellulose content of palm kernel cake to the tune of 2.23, 27.80, 36.98, 49.32, and 57.24% when fermented with *Sclerotium rolfsii*, *T. harzianum*, *T. koningii*, *T. longibrachiatum*, and *A. niger*, respectively. Fermentation of

cassava peels with a mixed culture of *Saccharomyces cerevisiae* and *Lactobacillus* spp. also decreased both the crude fibre and carbohydrate contents of the peels by 6.4 and 20.9%, respectively (Oboh 2006). The results herein reported show that the fungal strain can effectively lower the crude fibre contents of the substrates, particularly PKC, and this will improve its digestibility by animals.

The rapid increase in growth of the fungus on all the substrates may account for the increase in the protein contents of fermented substrates, with the fungal hyphae serving as single cell protein. In a related study, Ofuya and Nwajiuba (1990) reported an increase of 185% (5.6–16%) in the protein content of cassava peels when fermented with *Rhizopus* spp. Ilyemi et al. (2006) also reported real increase in the protein content of PKC in the range of 29.4–54.5% when fermented with different fungal isolates. The protein content of CPH increased from 8.2 to 16.0%, and this report to the best of our knowledge is the first such investigation on the solid state fermentation of cocoa pod husk to increase its protein content. Typically, CPH is low in protein but very rich in crude fibre, a combination of factors which ultimately limits its utilization as feed in animal husbandry (Donkoh et al. 1991; Sobamiwa and Longe 1994). The most common usage of CPH in Nigeria and other West African countries, is in the production of black soap (Taiwo and Osinowo 2001). Therefore, the present study may serve as value-addition to CPH, CP, and PKC.

The reduction in lipid content as obtained in this study may be attributed to the accumulation of lipids by *R. stolonifer* LAU 07. It has been reported that during fungal processing, some lipolytic strains assimilate lipids from substrates for biomass production leading to a general reduction of the overall lipid content of the substrate (Das and Weeks 1979). These fungi are generally able to secrete lipases for lipid hydrolysis. In a similar study, Ilyemi et al. (2006) reported reduction in the lipid content of PKC fermented with *A. niger*, *S. rolfii*, and *T. harzianum*.

Cassava peels usually have higher concentration of cyanogenic glucosides than the parenchyma (pulp), which makes the peel unsuitable as animal feed (Oboh 2006). It has been reported that fungal solid state fermentation can effectively lower the cyanide content of cassava (Ofuya and Obilor 1994; Essers et al. 1995; Oboh 2006). The concentration of cyanide in the cassava peels fermented with *R. stolonifer* LAU 07 was low (1.39%), when compared with the usual cyanide content of cassava products in Nigeria [19.0 mg/kg (gari); 25 mg/kg (fufu)], and that of the cyanide content of some micro-fungi-fermented cassava products (9.1–17.2 mg/kg) (Oboh et al. 2002; Oboh and Akindahunsi 2003).

A wide variety of oxygen free radicals and other reactive oxygen species can be formed in the human body

and food system. In addition to inducing lipid peroxidation that causes the deterioration of foods (Duthie 1993), these radicals may also cause oxidative damage by oxidizing biomolecules leading to cell death and tissue damage (Kehrer 1993). Indeed, many common maladies, such as atherosclerosis, cancer, emphysema, cirrhosis, and arthritis are correlated with oxidative damage (Kehrer 1993; Jacob 1994). While oxidative damage no doubt plays a significant pathological role in disease, it has been suggested that the intake of food-derived antioxidants may reduce oxidative damage and have a corresponding beneficial effect on human health (Lin and Yen 1999). These antioxidants may be in the form of phenolic substances that are widely present in plants. However, it has been shown that fermentation can increase the concentrations of such substances. For example, the antioxidative activity of fermented soybean products such as miso, tempeh, and natto, inoculated with *A. oryzae*, *R. oligosporum*, and *Bacillus natto*, respectively, was significantly higher than in non-fermented steamed soybean (Santiago et al. 1992; Berghofer et al. 1998). More recently, Lin et al. (2006) reported a work on soybean koji fermented with various GRAS filamentous fungi, including *Aspergillus sojae* BCRC 30103, *Aspergillus oryzae* BCRC 30222, *Aspergillus awamori*, *Actinomucor taiwanensis*, and *Rhizopus* sp, where total phenolic content increased in soybean after fermentation. The DPPH-free radical scavenging activity ( $IC_{50}$ ) of the fermented soybean ranged from 2.4 to 17.8 mg/ml, while the unfermented control had  $IC_{50}$  of 12.6 mg/ml.

As far as we know, there is no report on the evaluation of antioxidant activities of extracts obtained from the substrates used in this work, namely: cocoa pod husk, palm kernel cake, and cassava peel. Related studies were only reported by Azizah et al. (1999), Lecumberri et al. (2007) and Othman et al. (2007). Azizah et al. (1999) evaluated antioxidant activities of extracts obtained from cocoa by-products, namely: cocoa powder, natural cocoa powder, cocoa nib, and cocoa shell. Othman et al. (2007) evaluated the antioxidant activities of extracts obtained from cocoa beans, and reported  $EC_{50}$  (DPPH assay) of 1.3, 1.3, and 1.5 mg/ml for Malaysian, Ghanaian, and Ivory coast cocoa beans, respectively. Lecumberri et al. (2007) reported a low antioxidant activity ( $7.73 \pm 0.47$   $\mu$ mol Trolox equivalents/g of dry matter) for a fibre-rich product obtained from cocoa bean husk by determining the free radical (ABTS, 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid, diammonium salt)-scavenging capacity using TEAC assay.

In the case of cassava, the only related study is that of Nassar et al. (2007), which reported the rare occurrence of lycopene (an important antioxidant) in one of the indigenous clones of cassava in Brazil. Lycopene occurs in

tomato, guava, watermelon, and pink grapefruit and its consumption appears to be associated with reduced degenerative diseases.

Red palm oil is a rich source of  $\beta$ -carotene,  $\alpha$ -tocopherol, and tocotrienols (Ong and Goh 2002). Similarly, recovered fibre from pressed palm fruits, which is burned as fuel to provide energy for the palm oil mills, has been found to be a rich source of carotenoids, vitamin E, and sterols (Choo et al. 1996). However, there is paucity of report on the evaluation of the antioxidant potential of palm kernel cake itself. The improved antioxidant activity of the fermented substrates investigated in this study to the tune of 53–62% (Table 2) is a clear indication that the fungal strain may be helpful in the creation of healthy and functional feeds that will not only meet a large section of the nutritional requirements of animals, but also prevents the development of degenerative diseases that are associated with the deleterious effects of free radicals.

The present study has shown the potential of a new fungal isolate, *Rhizopus stolonifer* LAU 07 in improving the nutritional qualities of some agro-wastes that are abundant and of low utilization in Nigeria. The strain improved the protein contents and the antioxidant activities of all the substrates. It gave a good performance in the elimination of cyanide in the cassava peel, while reducing the crude fibre contents of the substrates to varying degrees. It can be concluded that the strain will be useful in value-addition of the agro-wastes, towards their utilization as healthy feed supplements in animal husbandry. Furthermore, the enrichment of the substrates, particularly in protein content and antioxidant activities may reduce the level of fortification in the preparation of animal feeds as it is done at present, thereby reducing the cost of producing the feeds.

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