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Research Article
Soild-state Fermentation of Coconut Coir by *Pleurotus sajor-caju* Increases the Anti-oxidant Properties and Nutritional Value

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Abstract

Background and Objective: An enormous amount of lignocellulosic materials are produced every year throughout the world which can be converted to nutritionally enriched ruminant feed by combination of chemical and biological treatment. This study was undertaken to improve nutritional values of lime treated coconut coir, a lignocellulosic material, by solid-state fermentation with *Pleurotus sajor-caju*. **Methodology:** The $CaCO_3$ treated coconut coir was fermented with *P. sajor-caju* for 56 days at 30°C. Changes of crude protein, lipid, carbohydrate, ash, lignin, cellulose, hemicellulose, reducing sugar, ascorbic acid and carotinoid contents due to fermentation were detected using standard methods. **Results:** At the end of 56 days fermentation, crude protein, ash and reducing sugar contents were increased by 744.44, 55.61 and 113.80%, respectively. However, crude fiber, lipid, carbohydrate, lignin, cellulose and hemicelluloses contents were decreased by 33.04, 63.42, 20.94, 25.75, 18.57 and 24.42%, respectively. Ascorbic acid and carotenoid were increased by 63.18 and 580.49%, respectively. **Conclusion:** Thus, bioconversion of coconut coir by *P. sajor-caju* offers a promising way to convert the lignocellulosic wastes into nutritionally improved animal feed.

Key words: Solid-state fermentation, *Pleurotus sajor-caju*, coconut coir, protein, lignocellulose

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

A huge amount of lignocellulosic materials, i.e., agricultural wastes, forestry residues, grasses and woody materials are produced every year throughout the world. The materials are generally composed of about 10-25% lignin, 20-30% hemicellulose and 40-50% cellulose[1-3]. The largest reservoir of fermentable carbohydrates[4] is mostly wasted in the form of pre-harvest and post-harvest agricultural losses and wastes of food processing industries. However, due to their abundance and renewability, there has been a great deal of interest in utilizing lignocellulosic wastes for the production of protein rich food, fuel and other value-added products[5-7]. The barrier to the production of valuable materials from lignocellulosic wastes is the structure of lignocelluloses which has evolved to resist degradation due to crosslinking between the polysaccharides (cellulose and hemicellulose) and the lignin via ester and ether linkages[8,9]. The main target of lignocellulose degradation, therefore is to amend or eliminate structural and compositional hurdles for hydrolysis and subsequent degradation processes in order to improve digestibility, rate of enzymatic hydrolysis and product yields[10,11]. The degradation can be achieved by single or combined implementation of mechanical, physico-chemical or biological treatments.

Microbial conversion of lignocelloses to energy and nutritionally enriched ruminant feed is becoming popular day-by-day. Coconut coir, a natural fibre extracted from the husk of coconut, which is mainly used to prepare cheap floor mats, doormats, brushes and mattresses in Bangladesh has a promising possibility to convert as nutritionally improved animal feed after proper delignification and solid-state fermentation. White rot fungi, capable of degrading lignin, cellulose and hemicelluloses, have already been reported for efficient bioconversion of many lignocellulosic wastes[1,12,13].

Until now, there are only a few reports evaluating the nutritive value of coconut coir and even fewer reports endeavoring increase the value by biological conversion[14,15]. However, combination of chemical and biological treatment is expected to improve the nutritive value of this apparently useless lignocellulosic waste[16,17]. In the present study, $CaCO_3$-pretreated coconut coir was used for solid-state fermentation (SSF) by *P. sajor-caju* with an aim to use the fermented product as nutritionally enriched animal feed. The authors further checked the rise of anti-oxidant properties and enzyme activities of crude coconut coir extracts because of SSF.

MATERIALS AND METHODS

Preparation of substrates: Cellulosic materials collected from different sources were first cleaned off all dirt and unwanted materials. Then they were sun dried and cut into tiny pieces 2-3 cm.

Pretreatment of substrates: Five hundred grams of untreated coconut fiber were taken and then soaked with a calcium carbonate solution (2.67 g $CaCO_3$ L^{-1} DH_2O). The substrates were left in soaking condition overnight. Then the lime solution was drained out by tap water. Treated substrates were then spread over aluminum foils and allowed to dry overnight at 60°C.

Collection and storage of *P. sajur-caju*: Stock culture of *Pleurotus sajur-caju* was obtained as Potato Dextrose Agar (PDA) slant from Microbiology and Industrial Irradiation Division of Bangladesh Atomic Energy Commission. The culture was maintained on PDA medium at 4°C and sub-cultured every 15 days.

Solid-state fermentation: *Pleurotus sajor-caju* was sub cultured from stock PDA slant to PDA plate. After 14 days of incubation at 30°C three pieces of mycelia growth (1 cm in diameter) were inoculated in 100 mL Erlenmeyer flask containing 50 mL PDA broth. The flask was incubated at 30°C in shaking condition in an orbital shaker for 7 days and then the inoculums was transferred in pre-sterilized soaked substrates (into 1000 mL Erlenmeyer flask) containing 20 g substrates and 50 mL distilled water and incubated at 30°C for 56 days.

Biochemical analyses: Coconut coir with different periods of fermentation were collected aseptically, oven dried at 60°C and used for biochemical analysis. The substrate without $CaCO_3$ treatment and SSF was used as control and also dried overnight at 60°C before biochemical analyses. Ash, fat, crude fiber and moisture contents were determined following the methods of AOAC[18], while the crude protein contents (N×6.25) were determined using micro Kjeldahl method (ISO 20483)[19]. The carbohydrate contents were determined by DuBois *et al.*[20]. Gravimetric determination of lignin, cellulose and hemicellulose of the substrates were estimated according to Sun *et al.*[21] and Adsul *et al.*[22]. The cellulose to lignin ratio was also determined. Reducing sugar contents in control and fermented substrates at their various stages of fermentation were determined by the dinitrosalicylic acid (DNS) method[23].

Determination of enzyme activity: The crude enzyme solution was obtained by soaking moldy substrate with considerable volume of 0.01 M acetate buffer (pH 5.5). The mixture was shacked for 2 h and centrifuged at 5000 rpm for 10 m to remove cells and residual substrate. The clarified extract representing crude enzyme was used for assaying endoglucanase (CMCase), exoglucanase (Avicilase), xylanase[24], pectinase[25], cellobiase[26] and amylase[5] activities. Enzyme assays were carried out in triplicate using three culture replicates at room temperature. The enzymatic activities are expressed as International Units (IU), defined as the amount of enzyme required producing 1 μmol product min^{-1} and are reported as IU g^{-1} substrate used in the SSF as described by Shrivastava *et al.*[27].

Quantification of anti-oxidants: Amount of ascorbic acid was quantified by spectrophotometric method after extraction with 3% HPO_3 as described in the Methods of Vitamin Assay[28]. Total carotenoid was extracted in 80% acetone and absorption was taken at 663, 645 and 480 nm. Finally the amount of carotenoid was calculated using the following equation as described by Hiscox and Israelstam[29]:

$$\text{Total carotenoid of sample (mg g}^{-1}) = A_{480}+(0.114\times A_{663}) -(0.638\times A_{645})\times V/1000\times W$$

Statistical analysis: Data from different biochemical analyses of non-fermented and fermented samples at different periods were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. Analyses were performed using statistical applications and differences and were considered significant at an alpha level of 0.05. The statistical program used was stat view^R 5.0 (Mind vision software, Abaccus, Concepts, Inc., Berkeley, CA, USA).

RESULTS AND DISCUSSION

The proximal composition of coconut coir was altered significantly after solid-state fermentation ($p<0.05$) compared to non-fermented one (Table 1). The crude fiber content was decreased 33.81% after 56 days fermentation indicating the excretion of cellulose/hemicellulose-degrading enzymes by the white rot fungus during solid-state fermentation[25]. The total protein content was increased by 744.44% in fermented coconut coir refereeing massive increase of the fermenting fungal growth on coconut coir (Fig. 1). The finding was in agreement with several published reports[30-33]. In addition to fungal growth, excretion of certain extracellular enzymes also contributed to the increase of protein[34,13]. Similar increase in protein content due to fungal growth on cassava byproducts, wheat straw, coffee husk, corn bran and rice bran has also been reported previously[13,35,36]. The ash content was also found to increase and a total of 55.61% increased at the end of fermentation. Since the ash content determination is a measure of mineral levels in the substrates, it can be inferred that SSF contributed to the elevation of mineral levels in the fermented products. Similar improvement of ash content, following fermentation of lignocelluloses has been reported by Shrivastava *et al.*[13]. In contrary, Akinyele *et al.*[37] reported that reduction of ash content as a result of SSF in agro-wastes. Generally, fermentation led to reduction in the crude fat content. Here, the reduction was 63.42% after 56 days SSF. In a similar study, the fat content of okara was reduced from 15-9% by fermentation with *N. intermedia*[31]. Previous studies have shown reduction in the lipid content of different substrates fermented with different microorganisms. During SSF, lipolytic strains assimilate lipid from substrates for biomass production and cellular activities leading to a general reduction of the overall lipid content[38,32]. The carbohydrate content of coconut coir was also decreased significantly because of the SSF. Carbohydrates are used

Table 1: Proximate composition (Percentage of dry substrate) of coconut coir at various period of fermentation

Period of incubation (days)	Crude fiber	Protein	Ash	Lipid	Carbohydrates
Control	5.81 ± 0.04^{h}	1.44 ± 0.12^{a}	3.92 ± 0.04^{a}	0.842 ± 0.01^{f}	83.26 ± 0.01^{e}
7	5.69 ± 0.02^{gh}	1.96 ± 0.11^{ab}	4.00 ± 1.41^{a}	0.726 ± 0.06^{e}	83.47 ± 1.57^{e}
14	5.62 ± 0.07^{g}	2.28 ± 0.33^{b}	4.33 ± 0.47^{ab}	0.694 ± 0.02^{e}	83.19 ± 0.30^{e}
21	5.32 ± 0.09^{f}	2.61 ± 0.15^{b}	4.45 ± 0.06^{ab}	0.550 ± 0.03^{d}	83.58 ± 0.39^{e}
28	4.78 ± 0.06^{e}	3.43 ± 0.32^{c}	4.65 ± 0.04^{ab}	0.475 ± 0.01^{c}	83.00 ± 0.19^{e}
35	4.57 ± 0.05^{d}	4.40 ± 0.45^{d}	4.87 ± 0.03^{abc}	0.388 ± 0.01^{b}	75.36 ± 0.25^{d}
42	4.35 ± 0.06^{c}	7.61 ± 0.50^{e}	4.90 ± 0.14^{abc}	0.357 ± 0.01^{ab}	72.33 ± 0.18^{c}
49	4.14 ± 0.08^{b}	9.60 ± 0.22^{f}	5.50 ± 0.71^{bc}	0.338 ± 0.01^{ab}	69.07 ± 0.33^{b}
56	3.89 ± 0.04^{a}	12.16 ± 0.74^{g}	6.10 ± 0.29^{c}	0.308 ± 0.003^{a}	65.82 ± 0.11^{a}

Results are expressed as Mean±SD (Standard deviation) of three independent experiments. Values in the same column with different superscripts are significantly different at $p<0.05$

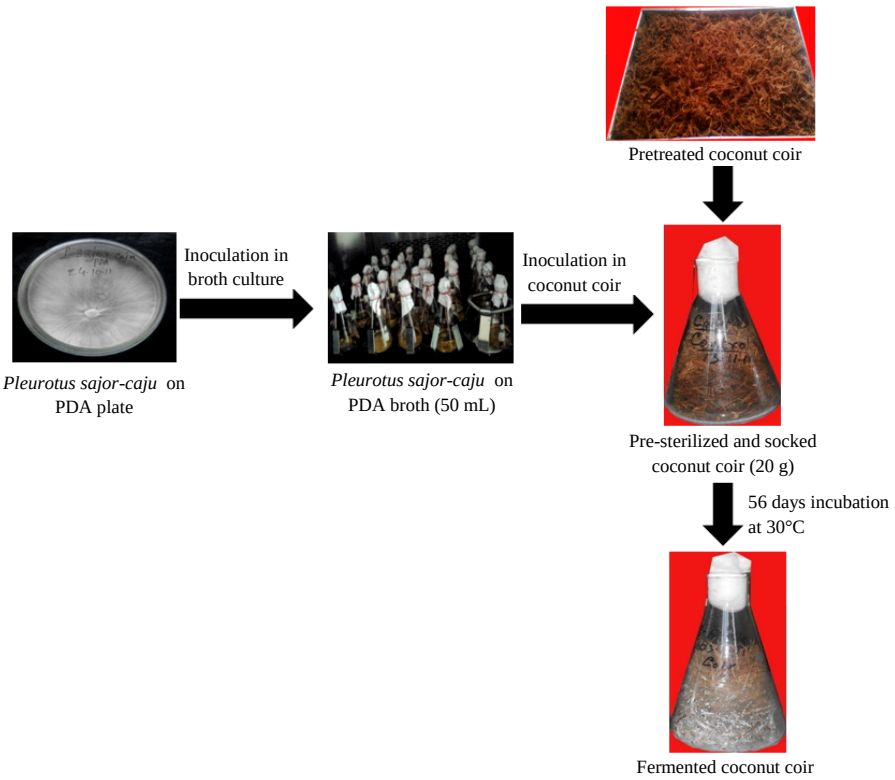

Fig. 1: Bioconversion of coconut coir to nutritionally improved animal feed. The *P. sajor-caju* was sub cultured from PDA plate to 50 mL PDA broth and incubated at 30°C in shaking condition for 7 days. The inoculum was then transferred in pre-sterilized soaked substrates containing 20 g substrates and 50 mL distilled water and incubated at 30°C for 56 days

Table 2: Lignin, cellulose, hemicelluloses, C/L and reducing sugar contents (Percentage of dry substrate) of coconut coir at different period of SSF by *P. sajor-caju*

Period of incubation (days)	Lignin	Hemicelluloses	Celluloses	Cellulose and lignin ratio (C/L)	Reducing sugar
Control	50.87±0.66^{f}	15.56±0.62^{b}	43.66±0.93^{f}	0.94±0.028^{c}	0.29±0.01^{a}
7	45.82±0.72^{e}	14.72±0.86^{b}	40.87±0.30^{e}	0.89±0.007^{ab}	0.40±0.02^{b}
14	44.94±1.03^{de}	14.55±0.63^{b}	40.00±0.46^{de}	0.88±0.014^{ab}	0.42±0.01^{bc}
21	44.88±1.11^{de}	14.55±0.64^{b}	39.83±0.70^{de}	0.89±0.007^{ab}	0.43±0.01^{cd}
28	43.55±0.64^{cd}	14.55±0.64^{b}	38.87±0.76^{cd}	0.91±0.007^{ab}	0.46±0.03^{d}
35	42.70±0.88^{bc}	14.00±0.95^{b}	38.67±0.95^{cd}	0.88±0.007^{b}	0.47±0.02^{e}
42	42.73±0.85^{bc}	14.00±0.94^{b}	37.87±0.76^{bc}	0.86±0.014^{a}	0.52±0.01^{f}
49	41.50±0.71^{b}	13.77±0.79^{b}	36.71±0.54^{ab}	0.94±0.007^{ab}	0.56±0.01^{g}
56	37.77±0.80^{a}	11.76±0.80^{a}	35.55±0.78^{a}	0.94±0.007^{c}	0.62±0.01^{h}

Results are expressed as Mean±SD (Standard deviation) of three independent experiments. Values in the same column with different superscripts are significantly different at $p<0.05$

through different biochemical processes by microorganisms to produce simple sugars during bioconversion of lignocelluloses[37,39].

The reducing sugar content of coconut coir was increased intensely and correlated directly with increase of biomass and decrease of carbohydrates during 56 days fermentation period (Table 2). The reducing sugar content of coconut coir was found to increase up to 56 days of fermentation indicating enzymatic degradation of cellulose, hemicelluloses and pectin fractions of the substrate[40].

Degradation of lignin, cellulose and hemicelluloses: The chemical pretreatment of coconut coir with $CaCO_3$ prior to SSF enhanced the delignification and resulted in a decrease of lignin content from 50.87% of total dry weight to 46.62% (8.35% loss). While comparing the contents of lignin, hemicelluloses and cellulose during SSF, a significant degrease ($p<0.05$) of all these contents were observed. However, cellulose and lignin ratio (C/L ratio) of fermented agro-industrial wastes was significantly increased ($p<0.05$) compared with their unfermented samples. The percentage of lignin content was decreased by 25.75% (Table 2) for SSF indicating the ability of *P. sajor-caju* to bulk of ligninases production such as laccases and peroxidases[41,42] while fermenting coconut coir. The finding was in agreement with the previous studies of Lechner and Papinutti[43] and

Table 3: Cellulolytic enzymatic activities (IU g^{-1}) of *P. sajor-caju* at 49 and 56 days SSF with coconut coir

Incubation period (days)	Cellobiase	Avicilase	CMCase	Pectinase	Xylanase	Amylase
49	0.11±0.001[a]	0.53±0.03[a]	0.50±0.16[a]	0.030±0.003[a]	0.062±0.01[a]	0.40±0.03[a]
56	0.13±0.01[a]	0.66±0.03[a]	0.65±0.08[a]	0.033±0.002[a]	0.017±0.002[a]	0.65±0.07[a]

Results are expressed as Mean±SD (Standard deviation) of three independent experiments. Values in the same column with different superscripts are significantly different at $p<0.05$

Table 4: Amounts of total dry weight, ascorbic acid, total carotinoid in coconut coir before and after SSF

Type of sample	Total dry weight (g)	Ascorbic acid (mg g^{-1})	Total carotinoid (mg g^{-1})
Control	20.00±0.92[a]	0.0402±0.001[a]	0.041±0.016[a]
Fermented substrates	21.86±0.88[b]	0.0656±0.014[a]	0.279±0.048[b]

Results are expressed as Mean±SD (Standard deviation) of three independent experiments. Values in the same column with different superscripts are significantly different at $p<0.05$

Sherief *et al.*[40] where lignolytic activities of fermenting microorganisms were found during biodegradation of rice straw, saw dust, wheat straw, coffee pulp and banana leaves. The percentage of cellulose was found to reach 35.55% of the total dry weight at the end of 56 days fermentation (Table 2) after a reduction of 18.57% from the initial cellulose content that indicates the increased production of cellulases. Cellulose degradation is a usual phenomenon during SSF of lignocelluloses as reported by Sherief *et al.*[40] and Jahromi *et al.*[44]. Hemicellulose degradation was found higher than that of cellulose and at the end the reduction was 24.42% compared to non-fermented one.

Cellulolytic enzyme activities: Edible mushrooms (*P. sajor-caju* and *P. pulmunarium*) are able to convert a wide variety of lignocellulose materials due to the secretion of extracellular enzymes[32]. Increase of free sugar (Table 2) and decrease of cellulose and hemicellulos (Table 2) during SSF indicated the presence of degradation cellulolytic enzyme activities of *P. sajor-caju* while growing on coconut coir. Therefore, crude enzymatic activities of *P. sajor-caju* were measured at the period of 49 and 56 days SSF (Table 3). CMCase, avicelase and cellobiase activities directly correlate with the degradation of cellulose. The fungus also showed high amylase activity and low xylanase and pectinase activity. Very low xylanase activity was also reported by Kumaran *et al.*[45] during SSF of sago hampus.

Improvement of anti-oxidative nature: The changes of dry matter and anti-oxidative properties of coconut coir as a consequence of SSF were also checked (Table 4). Increase of dry matter by 9.3% was because of increased biomass as a mycelial growth of the fungus. Ascorbic acid was improved by 63.18%. Growth of *P. sajor-caju* also contributed to improve significant level of total carotenoid by 580.48%.

CONCLUSION

Solid-state fermentation of $CaCO_3$ treated coconut coir augmented nutritional values such as protein and available polysaccharide fractions as energy source for ruminants as well as anti-oxidative properties. Therefore, the fermented product can be used as nutritionally enriched animal feed after *in vivo* feeding trail and toxicity tests.

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