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Protein Enrichment through Synergistic Activities of Fruit Wastes using White Rot Fungi under Submerged State Bioconversion

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In order to measure the suitability of microbes and substrate composites for animal feed supplements, submerged state bioconversion (SmB)was performed. Chemical analysis of all selected substrates showed high fermentable sugar which can support microbial growth. Selected microbes *Phanerochaetechrysosporium (P. chrysosporium), Panustigrinus*(M6) and RO2 were grown on sole liquid and solid substrates and their respective composites. Protein content of substrate increased over 127 times on sole liquid substrates and 216 times on solid.Additively, all selected strain showed improved protein synthesis on solid sole substrate with RO2 leading with 49.23 mg/g. Protein content of biomass increased in composite substrates with *P. chrysosporium* synthesizing its highest protein (18.59 mg/g) on solid mixture of all selected substrate whereas banana peel and pineapple peel composite (BpPin) was best for M6 (22.97 mg/g) and RO2 (26.34 mg/g) respectively.

Key words: Papaya; Animal feed; Bioconversion; Microbes;Composites.

Surging prices of animal feed supplements remained one of the challenges facing the livestock industries all over the world. This could be attributed to persistent drought, less cultivation of arable lands and increased human population. However, increased awareness on the consumption of fruits and vegetables for healthy living makes more fruit processing industries spring up in several agricultural based economies. The effects of such industrial activities are noticeable in the huge biodegradable wastes being thrown in water bodies, dumping sites and other unwholesome places. This has further complicated environmental concerns of several developing countries where more fruits are produced in large quantities.

Effective handling of agro-allied industryresidues remained a huge environmental concern due to its fowling and contamination of the aquatic environment. These environmental challenges are caused by conversion of natural nutrients domiciled in the residues to obnoxious gases by microorganisms. Fruit wastes are major culprit in contributing enormously to such environmental disorders[1]. Banana, pineapple and papaya are major fruit grown in large quantity in several tropical regions of the world. These fruits are easy to grow, produce high yields and enjoy wide acceptability. These qualities make their wastes dominantamong agricultural wastes found in several tropical and sub-tropical areas[2]. The peel

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of these fruits forms large part of their wastes and contains carbohydrates, vitamins and minerals which are necessary for the growth of microorganisms. This qualifies peels from these fruits for use in protein enrichment through innovative biotechnological approaches[3]. This method involves cultivation of fungi, yeast, bacteria and algae on agricultural residues for biomass production which can be used for human or animal feeding[4]. However, fungiand yeast biomass are most acceptable due to their reduced nucleic acid, ease of growth and high essential amino acids in their protein[3, 5].

Among known fungi, bacidiomycetes are most researched and widely consumed for their protein, vitamins and indigestible polysaccharides. White rot fungi are capable of degrading branched chain of complex carbohydrates and can survive under submerged state bioconversion (SmB). This quality necessitates their use for bioconversion of fruit wastes to tackle impending environmental concerns surrounding banana waste (peel), pineapple waste (peel) and papaya waste (peel) generated in several tropical vegetation. Bacidiomycetes cultivated under SmBproduce biomass rich in essential amino acids that can improve animal nutrition[5]. Secretion of extracellular enzymes by these fungi culminates into effective biodegradation of the complex ligncellulosic materials found in the peels of banana, pineapple and papaya.

In this work, the potential of each substrate under different states (liquid and solid) to support the growth of selected microorganisms wasexplored. Synergistic behavior of different mixtures of such substrates in supporting and secretion of protein by the microorganisms was expounded. To the best of our knowledge, this is the first time such substrates were investigated together for their synergistic capacity in producing value added product for amelioration of environmental challenges faced by fruit processing industries.

MATERIALS AND METHODS

Proximate composition of substrates

The chemical compositions of the substrates were determined to ascertain their quality for the bioconversion process.

Protein content analysis

The initial protein content of substrates and product were determined according to method suggested by Lowry[6] (1951) using folin phenol reagent and bovine serum albumin as for standard curve generation. All absorbance were taken at 660nm and triplicate samples were prepared.

Total soluble sugar (TOS) and total carbohydrate (TC)

Total soluble sugar and total carbohydrate of samples were determined by Dubois[7] (1956)methodology using phenol sulphuric acid. For TC substrates were hydrolyzed with 2.5N HCl for 3 hours before Dubois[7] (1956) methodology was used to measure the amount of released sugar. All absorbance were measured at 490nm and glucose solution was used standard.

Total reducing sugar (TRS)

Aqueous extraction of reducing sugar from banana peel, pineapple peel and papaya peel was performed in 50 ml stoppered conical ûask containing air dried peels for solid sample and slurry for liquid sample. Ten (10) ml of 0.2 (mol/L) of disodium hydrogen phosphate and 0.1 (mol/L) of citrate buffer (pH 4.8) was added before centrifugation. Total reducing sugar of the supernatant was determined by Miller method[8]using dinitrosylsalicylic acid reagent (DNS).

Ash content

Ash content represents the amount of trace and major minerals in the biomass. The ash content of all substrates wasdetermined[9] by weighing 1g of the sample into a crucible and converting to ash at 550°C.

Moisture content

Moisture content of the biomass was determined according to AOAC Official Method 950.46. One gramme of the sample was weighed and dried in a hot air oven for 16-18hrs until the mass becameuniform after 30 minutes interval.

Organic matter

Organic matter was calculated by difference between initial and final mass of the samples after drying and ashing.

Substrate and preparation

Fresh banana peels, pineapple peels and papaya peels were collected from juice processors in various restaurants within Kuala Lumpur, Malaysia and were Oven dried immediately at 60°C

for 48hrs; samples were sieved with 002 mesh size and kept in a cool dry place. Liquid substrates were prepared by blending fresh pre-washed peels and sieved through 002 mesh size. Samples were frozen immediately at -20°C for subsequent use.

Inoculums Preparation

Innoculum were prepared by using 25ml of sterilized distilled water to wash a petri dish of 7days old fungi mycelium. For spore forming fungi, whatmanNo 1 filter paper was used to filter out suspended spores[5]. Laboratory stock of *P. chrysosporium* was used while M6 (locally isolated and identified by CABI identification services) and RO2 strains were isolated locally and their laboratory stocks were used.

Submerged state bioconversion

Submerged state bioconversion was carried out in 250 ml Erlenmeyer flasks using 2% substrate and 2% innoculum. Volume was adjusted to 50ml with mineral solutions. The flasks were kept at ambient temperature and 150rpm for 7 days.

RESULTS AND DISCUSSION

Chemical analysis of banana, pineapple and papaya peels (liquid and solid) showed that they are low in protein but contained reducing sugar, soluble sugar and acid soluble carbohydrates (Table 1 and Table 2) which are carbon sources capable of supporting growth and development of selected microorganisms for protein enrichment. However, there are differences in sugar (soluble, reducing and carbohydrate) composition between solid and liquid substrates. This discrepancy could be attributed to moisture distribution within the matrixes of the solid and liquid which affects leaching of such sugar during extraction (soluble and reducing sugar) and hydrolysis (carbohydrate).Sugar composition of substrates for bioconversion processes has been reported by other investigators with results showing presence of same type of sugars[4, 10]. Similarly, available information supported their

Table 1. Proximate composition of liquid substrates

Sample (peel)	Soluble sugar (mg/g)	Carbohydrate (mg/g)	Reducing sugar (mg/g)	Protein content (mg/g)	Ash content (%)	Organic matter (%)	Moisture content (%)
Banana	36.71	22.83	1.30	0.54	0.86	2.98	96.16
Pineapple	75.45	40.22	1.80	0.50	0.32	3.38	96.30
Papaya	52.35	47.51	4.54	0.61	0.60	3.63	95.77

Table 2. Proximate composition of solid substrates

Sample (peel)	Soluble sugar (mg/g)	Carbohydrate (mg/g)	Reducing sugar (mg/g)	Protein content (mg/g)	Ash content (%)	Organic matter (%)	Moisture content (%)
Banana	32.84	20.23	1.29	0.83	18.07	42.70	39.23
Pineapple	40.74	36.84	1.70	0.80	5.33	54.17	40.50
Papaya	24.94	18.92	0.86	0.73	12.21	46.72	41.07

Table 3. Protein synthesis of *P. chrysosporium*, M6 and RO2 on the substrates

Microorganism	Protein content (mg/g)					
	Liquid substrate			Solid substrate		
	Bp	Pin	Pw	Bp	Pin	Pw
P. chrysosporium	13.56	6.85	10.21	15.09	12.27	8.16
M6	8.07	12.61	10.09	13.24	12.32	9.23
RO2	14.08	12.79	12.94	15.88	17.71	15.64

ability as sole carbon source for protein enrichment through the cultivation of microorganisms[3, 1]. Therefore, the substrates are appropriate for bioconversion processes to further enrich them with protein through synthesis process by the microorganisms.

Protein synthesis by each strain on the substrates

The performance of selected strains on both liquid and solid substrates under submerged phase bioconversion was explored. Table 3 showed that the microbes were capable of using the substrates as carbon source for their protein enrichment. Result obtained after 7 days of SmB showed that more protein was produced by solid substrates comprising of banana peel (Bp), pineapple peel (Pin) and papaya peel (Pw) solely as carbon source. When selected microorganisms were tested on Bp (solid and liquid), RO2 synthesized highest amount of protein on solid matrix, followed by *P. crysosporium* while M6 produced the least. The high protein synthesis by solid substrates could be attributed to high lignocellulosic materials present in the dried peels. This result was consistent with that of other researchers working on protein enrichment of wastes[10, 11, 12].

On Pin,RO2 produced highest amount of protein followed by M6 while *P. crysosporium* produced least protein when cultivated on liquid substrate. The same trend was recorded when all the fungal strains were grown on solid Pin. However, the difference between *P. crysosporium* and M6 was minor showing congruence in their biochemical behavior on the substrate. Results recorded for Bp (solid) by same strains showed similarities. Furthermore, M6 and RO2 behaviour on liquid Pin was similar showing that white rot fungus could exhibit same metabolic pathways in sugar breakdown for protein synthesis. On Pw, RO2 produced highest protein on solid Pw while *P. crysosporium* and M6 protein synthesis were very close; signaling consistent SmB progression. Several investigators also reported different growth and product synthesis pattern for different fungi strains cultivated on same substrate[3, 4, 13].

Effects of composite substrate combinations on protein synthesis

Bacidiomycetesare fastidious organisms capable of synthesizing protein during their growth and development on lignocelullosic residues. Therefore, to further elucidate the performance of selected strains on the substrates, they were cultivated on their composites which include BpPinPw, BpPw, BpPin and PinPw. Figure 1 presents the protein synthesis of *P. chrysosporium* on the substrate composites (solid and liquid). The microorganism produced highest protein when the three substrates (solid) were mixed together at equal ratio and this was followed by BpPin while PinPw and BpPwproduced lowest protein after 7 days bioconversion. The fungi yielded more protein when cultivated on solid substrate when compared to liquid ones. The trend observed on the liquid composites showed that PinPwhad highest protein synthesis followed closely by BpPin whereas BpPinPw was third and BpPw produced least protein synthesis. Incidentally, the numerical increase in protein synthesis from cumulative 1.62 mg/g in unconverted substrate (solid control BpPinPw) to 18.59 mg/g after bioconversion demonstrates good protein enrichment. Similarly, an increment from 0.75 mg/g protein in control sample to 15.66 mg/g in bio-converted PinPw was recorded for liquid substrate.

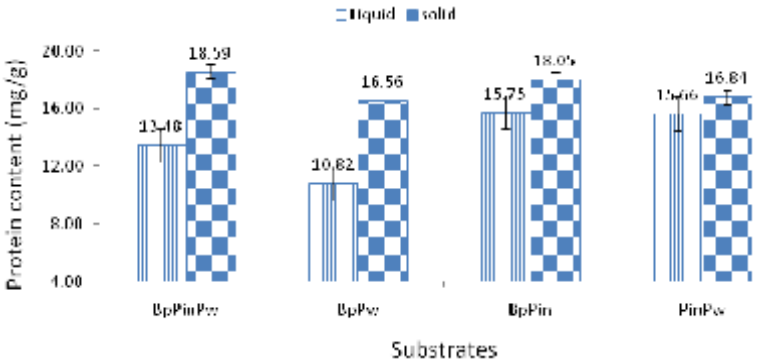

Fig. 1. Protein synthesis of *P. chrysosporium* on composite substrates

This biochemical bahaviour may have happened due to synergistic reactions within the substrates since protein synthesis increased generally when compared to their sole substrates performance. Similarly, closer trend within same state (solid and liquid) of all the substrate mixtures trend could be attributed to related sugar compositions. The result above is consistent with investigation conducted on performance of *P. chrysosporium* on agricultural residues as carbon sources for protein enrichment[3, 14].

M6 protein synthesis over 7 days SmB is presented in Figure 2. The protein synthesis on composite substrate was higher than individual substrates (solid and liquid). M6synthesized more protein with solid substrates while other liquid substrates were lower than their solid counterparts except BpPin where liquid substrate was 2.15 mg/g of protein more than solid substrate. Among solid substrates, BpPin led the pack; PinPw and BpPinPw were very close while BpPw recorded lowest protein content. BpPin recorded highest protein values among the liquid substrates; BpPw, PinPw and BpPinPw followed in this order. The efficacy of M6 on carbon source from agricultural residue was investigated by Tijani et al., 2011 and their results were similar to those recorded here. This further demonstrates that M6 is a viable strain for protein enrichment of unwanted agricultural residues.

RO2 produced more protein enrichment withcomposite substrates when compared with

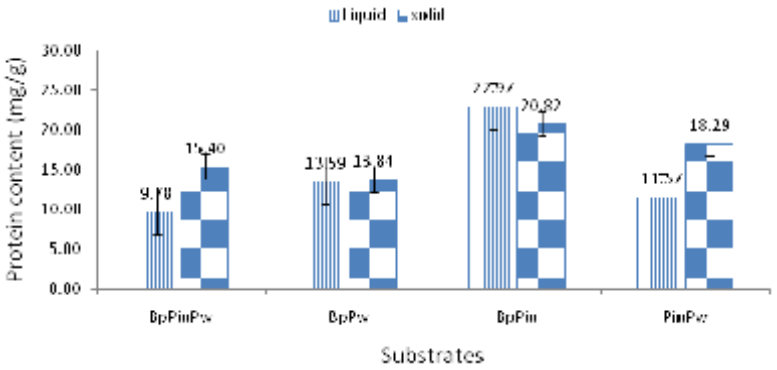

Fig. 2. Protein synthesis of M6 on composite substrates

sole substrates. BpPin produced highest protein quantity while PinPw differed with 1.00 mg/g when compared with BpPinPw; BpPw was thereforethe least in protein enrichment among solid substrates. Equally, among liquid composite substrates, BpPinPw provide highest protein enrichment followed by PinPw and BpPin while BpPw had the lowest protein enrichment. The general increase in the protein enrichment of composite substrates could be attributed to their synergistic effects in providing adequate carbon support for the microorganism which synthesize protein upon its growth and development over the bioconversion period. The use of RO2 by researchers for protein enrichment has not been deeply investigated; however, its performance deeply followed the

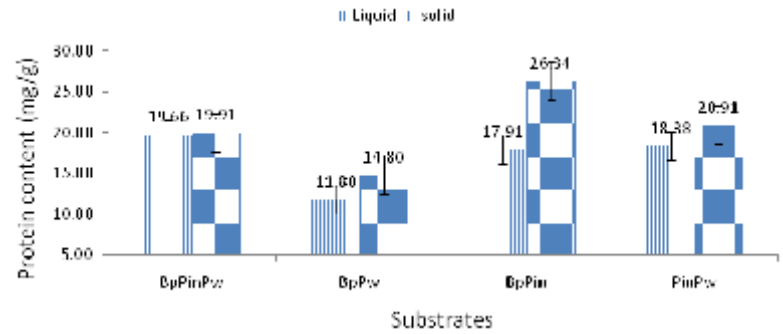

Fig. 3. Protein synthesis of RO2 on composite substrates

pattern of other white rot fungi that are well established for protein synthesis. This result therefore further strengthens the findings of other researchers concerning the biochemical behavior of bacidiomycetes as efficient degrader of lignocellulosic residues for protein synthesis[10, 15,16].

CONCLUSION

From the results presented above, it could be concluded that all selected substrates successfully supported the growth and development of microbes which further enriched the synthesis of protein. Composite substrates further supported microbial growth with more protein production over the bioconversion period. The synergistic behaviour of the mixed substrates led to robust protein enrichment which was most evident in the solid substrates as it looks promising in efficiently supporting microbial growth over the bioconversion period.

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