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Protein improvement of banana peel through sequential solid state fermentation using mixed-culture of *Phanerochaete chrysosporium* and *Candida utilis*

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Abstract

Banana peel (BP) is a major waste produced by fruit processing industries. Pre-treatment of BP at different temperatures led to 40% reduction in saponin at 100 °C (from 9.5 to 5.7 mg/g). Sequential mixed culture of *Phanerochaete chrysosporium* (*P. chrysosporium*) and *Candida utilis* (*C. utilis*) gave highest protein enrichment (88.93 mg/g). There is 26% increase in protein synthesis (from 88.93 to 111.78 mg/g) after media screening. Inclusion of KH_2PO_4, $FeSO_4 \cdot 7H_2O$, wheat flour and sucrose in the media contributed positively to protein synthesis, while elevated concentration of urea, peptone, K_2HPO_4, KCl, $NH_4H_2PO_4$, and $MgSO_4 \cdot 7H_2O$ are required to reach optimum protein synthesis. Total soluble sugar (TSS), total reducing sugar (TRS) and total carbohydrate (CHO) consumption varied with respect to protein synthesis in all experimental runs. Optimum protein synthesis required 6 days and inclusion of 5% sucrose, 0.6% $NH_4H_2PO_4$, 0.4% KCl, and 0.5% $MgSO_4 \cdot 7H_2O$ as concentration media constituents to reach 140.95 mg/g protein synthesis equivalent to 300% increase over the raw banana peel protein content (35.0 mg/g).

Keywords Banana peel · *Candida utilis* · *Phanerochaete chrysosporium* · Solid state fermentation · Protein

Introduction

Fruits wastes generation is increasing in developing nations due to agricultural subsidies and supermarkets quality specifications. In fact, the Food and Agricultural Organization (FAO) reported over 6.5 million tons of banana wastes in India alone (Arumugam and Manikandan 2011). Similar trend is obvious in other developing economies of sub-Saharan Africa and Southeast Asia. The phenomenal increase in banana peels and other fruits wastes by hospitality industry alone is a concern to governments (Jayabalan et al. 2010; Saheed et al. 2016).

Fruit wastes from food processing industries could be abundant but contain some nitrogen, vitamins and minerals. This poor nutritional composition restricted the wastes as basal feed ingredient (Kasapidou et al. 2015). Banana peel (BP)—a common solid waste contains sugars, minerals (potassium, sodium, calcium and iron), lignin, cellulose and hemicellulose. The nutrient composition suggests that BP could support microbial growth if inherent saponin that inhibits fungus growth in fermentation processes is reduced. Therefore, pretreatment step is a necessary prior to protein enrichment via solid-state fermentation (SSF).

In SSF of agro-residues, the most commonly used method involves monoculture cultivation of edible fungi. A novel approach to ensure robust consumption of nutrients, improved microbial growth and product synthesis is sequential fungal-yeast co-culture with assumption of synergistic actions under SSF. Sequential SSF co-culture involves cultivation of two microbes such that the second microbe is inoculated days after the first. Interestingly, sequential cultivation of physiologically different fungi or bacteria cultures abound in literature but information concerning performance

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of fungal-yeast sequential culture requires further study especially for protein enrichment of BP under SSF (Ahmed et al. 2010; Rajoka et al. 2011).

Edible bacidiomycete fungi and yeast cells could function wholly as animal feed supplement due to their high content of vitamin B-complex and low nucleic acid (Zayed 2018). Lignin degrading microorganisms have attracted interests due to their profound lignin degradation under SSF. *Phanerochaete chrysosporium* (*P. chrysosporium*), is capable of producing biomass rich in most essential amino acids at acceptable standards (Nitayavardhana et al. 2013). The lignolytic fungi could synthesize lignin degrading extracellular enzymes such as lignin peroxidase, manganese peroxidase and polyoxal oxidase (Castoldi et al. 2014). These three industrially important lignolytic enzymes are effective in degrading cellulose, xylan and plant polymer lignin (Elisashvili et al. 2008). The mechanism of fungal lignin degradation entails the release of carbon dioxide (CO_2) and water (H_2O) after depolymerization in the presence of free oxygen molecules (Cameron et al. 2000).

Yeasts are widely consumed in fermented foods such as Tofu (the cheese of Asia from soy) and Tempe (soy product) (Bakar et al. 2011). Moreover, *Candida utilis* (*C. utilis*), a hydrolytic enzyme producing yeast, is extensively cultivated on agro-residues for its aromatic flavor, higher nutritive value and non-toxigenic properties. Its extracellular enzymes are capable of degrading mono-saccharides, disaccharides and lignolytic agro wastes materials (Ke et al. 2011; Ionuţ et al. 2017).

This investigation focused on the conversion of BP to protein rich biomass by edible bacidiomycete fungi and yeast under sequential SSF after environmental friendly substrate pretreatment. Next step involves design and optimization of the nutritional need of the cultivated microorganisms for improved protein synthesis.

Materials and methods

Substrate collection, pre-treatment and preparation for solid state fermentation

Fresh banana peels were collected from local banana juice processors in Kuala Lumpur, Malaysia and was oven dried immediately at 60 °C for 48 h to arrest any form of decay by microbial enzymes or adverse chemical reactions. After drying, raw BP was placed in 500 ml beaker up to 70% v/v, filled with tap water before subjecting independent triplicate samples to temperature treatment from 20 to 100 °C at 20 °C interval for 60 min in temperature controlled water bath. The saponin content released was then determined according to method described (Hudson and Ei -Difrawi 1979). Dried (10–12% moisture content) pre-treated peels were

grinded into pre-optimized 2 mm pore size with a grinding machine (Model D-79219 Staufen, IKA-WERKE GMBH & Co KG Germany) and stored in airtight container for 60 days at most.

Inoculum preparation

The fungi culture (*Phanerochaete chrysosporium* ATCC 20696 and *Panus tigrinus M609RQY*) was grown on potato dextrose agar (PDA, Merck, Germany) plates at 32 °C for 7 days, washed with 25 ml sterilized distilled water, then filtered with Whatman No.1 filter paper and spore suspension was collected in 100 ml Erlenmeyer flask. A haemocytometer was used to maintain the spore density at 1.0×10^8 spores/ml. Excess inoculum was stored at 4 °C for further use. Yeast culture (*Candida utilis*) maintained on PDA plates for 4–5 days and was washed with 25 ml of pre-sterilized distilled water into 250 ml Erlenmeyer flask without prior filtration. One loop full of cells was grown for 24 h on yeast extract 1%, peptone 0.5% and glucose 1% (YEPG) solution and cell density corrected to 1.0×10^7 and stored at 4 °C in a chiller.

Solid state fermentation and media development

Solid state fermentation (SSF) process comprise 6.0 g substrate (30%) and 14.0 g total liquid (70%) containing inoculum (added after sterilization), mineral supplements (0.1% KH_2PO_4 and 0.25% $NH_4H_2PO_4$) to maintain 70% moisture content in 250 ml Erlenmeyer flasks of 20 g working volume. Sequential SSF of *P. chrysosporium* and each of the three yeast cells involves cultivation of *P. chrysosporium* for 3 days after which yeast cells (*C. cylindracea, S. cerevisae* and *C. utilis*) are inoculated and both allowed grow until the end of fermentation period. Media constituent was screened using Plackett Burman design to determine high and low values of each constituent (Plackett and Burman 1946). Eleven factors comprising nitrogen source (organic and inorganic), carbon source and mineral source were selected and the experiment carried out in triplicate was designed accordingly (Table 1).

Analytical methods

Protein content and total soluble sugar (TSS) content of the product were determined according to described methods (Lowry et al. 1951; Dubois et al. 1956). Total carbohydrate (CHO) content of the product was determined using phenol–sulphuric acid method. 100 mg of the sample was hydrolysed with 2.5 N HCl for 3 h the solution was neutralized with sodium carbonate. Phenol–sulphuric acid reagent were added and absorbance read at 490 nm (Dubois et al. 1956). Total reducing sugar (TRS) was determined as

Table 1 Plackett Burman design for selected media factors for improved protein production

Run	Solid content (%)	Urea (%)	Peptone (%)	K$_2$HPO$_4$ (%)	KH$_2$PO$_4$ (%)	KCl (%)	NH$_4$H$_2$PO$_4$ (%)	FeSO$_4$·7H$_2$O (%)	MgSO$_4$·7H$_2$O (%)	WF (%)	SC (%)	Response (mg/g)
1	1 (72.5)	1 (0.6)	− 1 (0.3)	1 (0.15)	1 (0.9)	− 1 (0)	1 (0.5)	− 1 (0.3)	− 1 (0.3)	− 1 (0)	1 (6)	98.41
2	− 1 (62.5)	− 1 (0)	− 1 (0.3)	1 (0.15)	1 (0.9)	1 (0.6)	− 1 (0)	1 (0.7)	1 (0.9)	− 1 (0)	1 (6)	106.88
3	1 (72.5)	1 (0.6)	1 (0.7)	− 1 (0.05)	1 (0.9)	1 (0.6)	− 1 (0)	1 (0.7)	− 1 (0.3)	− 1 (0)	− 1 (0)	87.49
4	− 1 (62.5)	1 (0.6)	1 (0.7)	1 (0.15)	1 (0.9)	− 1 (0)	− 1 (0)	− 1 (0.3)	1 (0.9)	1 (4)	1 (6)	98.81
5	1 (72.5)	1 (0.6)	− 1 (0.3)	1 (0.15)	− 1 (0.5)	− 1 (0)	− 1 (0)	1 (0.7)	1 (0.9)	1 (4)	− 1 (0)	83.81
6	− 1 (62.5)	− 1 (0)	1 (0.7)	− 1 (0.05)	1 (0.9)	− 1 (0)	1 (0.5)	1 (0.7)	− 1 (0.3)	1 (4)	− 1 (0)	93.26
7	1 (72.5)	− 1 (0)	− 1 (0.3)	1 (0.15)	1 (0.9)	1 (0.6)	1 (0.5)	− 1 (0.3)	1 (0.9)	1 (4)	− 1 (0)	111.78
8	− 1 (62.5)	1 (0.6)	1 (0.7)	− 1 (0.05)	− 1 (0.5)	1 (0.6)	1 (0.5)	− 1 (0.3)	1 (0.9)	− 1 (0)	− 1 (0)	106.10
9	− 1 (62.5)	1 (0.6)	− 1 (0.3)	1 (0.15)	− 1 (0.5)	1 (0.6)	1 (0.5)	1 (0.7)	− 1 (0.3)	1 (4)	1 (6)	88.46
10	1 (72.5)	− 1 (0)	1 (0.7)	− 1 (0.05)	− 1 (0.5)	1 (0.6)	− 1 (0)	− 1 (0.3)	− 1 (0.3)	1 (4)	1 (6)	101.75
11	1 (72.5)	− 1 (0)	1 (0.7)	− 1 (0.05)	− 1 (0.5)	− 1 (0)	1 (0.5)	1 (0.7)	1 (0.9)	− 1 (0)	1 (6)	103.62
12	− 1 (62.5)	− 1 (0)	− 1 (0.3)	− 1 (0.05)	− 1 (0.5)	− 1 (0)	− 1 (0)	− 1 (0.3)	− 1 (0.3)	− 1 (0)	− 1 (0)	106.63

WF wheat flour, *SC* sucrose

Table 2 Pre-treatment of banana peels at different treatment and physical conditions

Treatment	Saponin concentration (mg/g)
Raw	9.50 ± 1.41
Normal washing	9.00 ± 0.21
Soaking	7.50 ± 0.07
After fermentation	1.65 ± 0.58
Treatment at 20 °C	7.90 ± 1.06
Treatment at 40 °C	6.50 ± 0.71
Treatment at 60 °C	6.50 ± 0.71
Treatment at 80 °C	6.17 ± 0.35
Treatment at 100 °C	5.70 ± 1.77

described (Miller 1959). 100 mg of sample was extracted with 80% hot ethanol. DNS reagent was added and the mixture was boiled (~ 100 °C) for 5 min on a temperature regulated water heater, samples were cooled before absorbance was measured at 510 nm. Ash content of BP and product was determined according to the suggested method (Poorter and Bergotte 1992) while moisture content (MC) was measured according to AOAC Official Method 950.46.

Results and discussion

Substrate pre-treatment

Pretreatment process is an important unit operation when converting lignocellulosic agro-residues to new products. Microbial growth inhibiting agent—saponin had profound removal as result showed increased saponin removal by aqueous pretreatment at elevated temperature (Table 2). Mere washing removed 5.3% saponin from BP while 20% reduction happened when soaked in tap water at room temperature for 60 min. Gradual but increased reduction was recorded in the plant glycoside as pretreatment temperature reached 100 °C at 20 °C interval. Saponin is temperature, salt concentration, and pH sensitive in aqueous phase (Güçlü-Üstündağ and Mazza 2007). The saponin reduction in BP could be caused by high glycoside solubility in water, concentration gradient and possible lignocellulosic fibers modification (Oberoi et al. 2011; Salihu et al. 2015).

Effect of saponin concentration on microbial growth

To ascertain the effect of saponin on the growth of selected microorganisms, the strains were inoculated on raw dried BP. Result showed that only *P. chrysosporium* successfully grow on the un-treated substrate (Table 3). The inability of other microbes to grow on raw BP could be attributed to the

Table 3 growth characteristics of selected microbial cells on the pretreated substrate

Microbial cell	Picture	Remarks
P. chrysosporium		Growth
Panus tigrinus M609RQY		No Growth
C. cylindracea		No Growth
S. cerevisae		No Growth
C. utilis		No Growth
Control		No Growth

presence of branched-chain tri-saccharide moiety without oxygen-containing groups at C_2 and C_{12} of the saponin molecules (Miyakoshi et al. 2000; Francis et al. 2002). However, *P. chrysosporium* demonstrated ability to metabolize recalcitrant chemicals via extracellular secretion of enzymes that can breakdown complex organics but not plant sterols (Zacchi et al. 2000; Shi et al. 2014). However, all selected strains grew well at 40% saponin reduction (from 9.5 to 5.70 mg/g) after boiling BP at 100 °C for 60 min.

Effects of microbial grouping on protein synthesis

Protein synthesis increased in all treatments as the fermentation period increased but sequential SSF of *P. chrysosporium* and *C. utilis* gave the highest (88.93 mg/g) protein synthesis. Synthesis by *P. chrysosporium* and *Saccharomyces cerevisiae* (*S. cerevisiae*) followed, while combination of *P. chrysosporium* and *Candida cylindrisae* (*C. cylindrisae*) produced least protein (Table 4). *P. chrysosporium* and *C. utilis* protein enrichment reached 150% increase compared with control. This performance suggested synergistic metabolism of sugars present in BP. Similar trend was reported when *Archnioutus sp.* and *Candida utilis* were grown by sequential SSF on corn stover (Ahmed et al. 2010). The high protein synthesis could be influenced by extracellular production of lignocellulosic enzymes (lignin peroxidase and manganese peroxidase) by *P. chrysosporium* leading to the conversion of complex sugar to forms that are easily consumed by yeast cells (Villas-Bôas et al. 2003; Ke et al. 2011). The was no measurement of protein synthesis profile of *C. utili* alone, *S. cerevisiae* alone and *C. cylindricea* alone since no growth was recorded for them even after pretreatment to remove saponin.

Table 4 Screening of Potential Microbes for improved protein synthesis

Organisms	Protein content (mg/g) dry weight			
	Day 0	Day 3	Day 6	Day 10
P. chrysosporium	19.68 ± 3.58	40.17 ± 5.59	40.17 ± 0.14	78.27 ± 0.44
P. chrysosporium /C. utilis	8.23 ± 0.32	18.82 ± 0.15	18.82 ± 0.6	88.93 ± 3.38
P. chrysosporium /S. cerevisiae	7.24 ± 1.27	25.29 ± 0.67	25.29 ± 4.52	85.43 ± 4.53
P. chrysosporium/C. cylindricea	13.58 ± 1.60	27.70 ± 0.77	68.41 ± 0.22	84.16 ± 1.35
Control	2.35 ± 0.08	5,4 ± 0.03	8.3 ± 0.08	35.0 ± 1.27

Selection of media parameters

To identify required media constituents, eleven media parameters that cover carbon source, nitrogen and mineral salt undergo twelve experimental runs according to Plackett Burman design to determine individual contribution to protein synthesis. Highest protein synthesis (111.78 mg/g) corresponded to low concentration of five (KH_2PO_4, solid content, $FeSO_4 \cdot 7H_2O$, WF and sucrose) media parameters and high concentration of another five (peptone, K_2HPO_4, KCl, $NH_4H_2PO_4$ and $MgSO_4 \cdot 7H_2O$) in run 7 (Table 1). Microbial protein synthesis could depend on the concentration and elemental nature of media components of the growing media (Ruqayyah et al. 2011).

Effects and significance of media concentrations on protein synthesis

High concentrations of KH_2PO_4, KCl, $NH_4H_2PO_4$, $MgSO_4 \cdot 7H_2O$ and sucrose favoured protein synthesis while low levels of $FeSO_4 \cdot 7H_2O$, wheat flour (WF), solid content, urea, peptone and K_2HPO_4 are required (Fig. 1). Easy metabolism of potassium ion (K^+) ammonium ion (NH_4^+) and phosphate ion (PO_4^+) in $NH_4H_2PO_4$ and KH_2PO_4 could trigger growth dependent product synthesis via profound hyphae growth, spore formation and hydrolytic yeast cells processes (Rajendran et al. 2007; Jamal et al. 2008; Ruqayyah et al. 2011). Positive influence of $MgSO_4 \cdot 7H_2O$ and $FeSO_4 \cdot 7H_2O$ could be caused by increased mycelia branching of *P. chrysosporium* resulting from actions of Mg^+, Fe^{3+} and SO_4^{2-} that fovoured secretion of enzymes and co-factors (Ikram-ul et al. 2004). Similarly, the positive contribution of sucrose to protein synthesis by sequential culture of *P. chrysosporium* and *Candida utilis* on BP was expected since the microbes required the sugar to trigger growth and product formation. Similar observation has been reported by other researchers (Molla et al. 2004).

Urea, peptone, wheat flour, $FeSO_4 \cdot 7H_2O$ and solid content contributed negatively to protein synthesis. This observation suggested that *P. chrysosporium* and *Candida utilis* preferred inorganic nitrogen source ($NH_4H_2PO_4$) to organic sources though other white rot fungus could respond differently (Ruqayyah et al. 2011). The microbes performance was independent of $FeSO_4 \cdot 7H_2O$, K_2HPO_4 and solid content. This response of microbes to certain micronutrients and substrate moisture could be case dependent (Rosma and Cheong 2007; Gad et al. 2010; Hu et al. 2012).

Analysis of variance (ANOVA) showed significant contribution of $FeSO_4 \cdot 7H_2O$, $MgSO_4 \cdot 7H_2O$ and wheat flour to protein synthesis (Table 4). Profound influence contribution of $FeSO_4 \cdot 7H_2O$ and $MgSO_4 \cdot 7H_2O$ to protein synthesis suggested the importance of micronutrients to growth and performance of *P. chrysosporium* and *C. utilis* (Jamal et al. 2008). $NH_4H_2PO_4$ though contributed to protein synthesis, the effect was not significant.

Impact of media screening on chemical analysis of fermentation product

Total carbohydrate (CHO), total reducing sugar (TRS), total soluble sugar (TSS) and ash content of the product varied across experimental runs (Table 5). Sugar concentrations varied greatly across experimental runs suggesting a direct relationship between the microbes and the substrate sugar under sequential SSF (Saheed et al. 2013, 2016). CHO and other sugars generally reduced with corresponding protein synthesis. However, the reduction pattern differ and this suggested that the fungal-yeast system utilization is media design dependent (Yin et al. 2012). The ash content varied between 8.17 and 10.57% in the product; thus it falls within the acceptable level (AboSiada et al. 2017).

Fig. 1 Main effects of media parameters for optimal protein production by Placket-Burmann experimental design
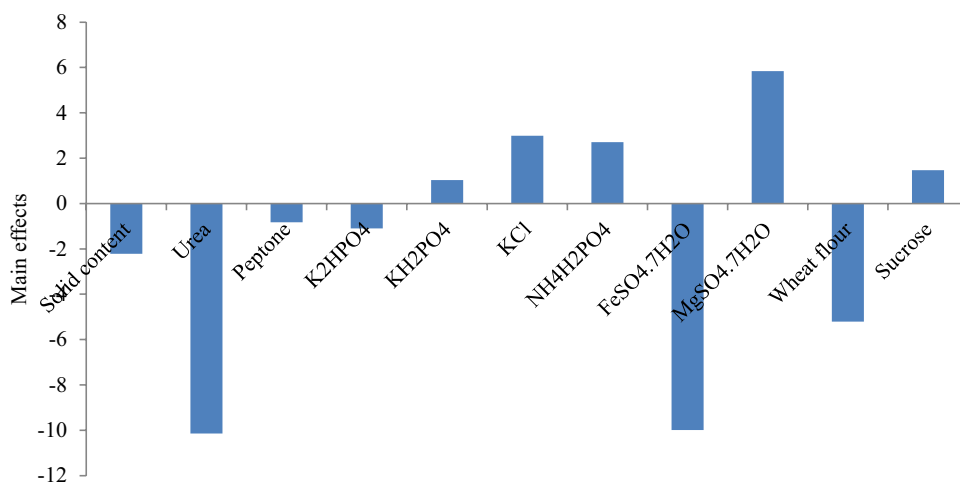
Table 5 Result of nutritional parameters based on experimental design

Run	Protein content (mg/g) DW	Ash content (%)	TSS (mg/g)	TRS (mg/g)	CHO (mg/g)
1	98.41 ± 2.47	9.51 ± 0.16	41.44 ± 10.61	161.47 ± 0.97	249.84 ± 10.83
2	106.89 ± 18.00	8.83 ± 0.27	29.56 ± 0.00	168.00 ± 1.88	238.44 ± 2.21
3	87.49 ± 9.45	9.43 ± 0.25	22.34 ± 1.24	167.13 ± 2.92	235.31 ± 0.00
4	98.81 ± 14.40	9.37 ± 0.52	19.28 ± 0.22	167.25 ± 2.74	250.00 ± 12.37
5	83.41 ± 8.32	8.80 ± 0.30	12.53 ± 1.72	163.04 ± 0.09	251.09 ± 3.31
6	93.26 ± 2.70	8.17 ± 0.60	33.53 ± 1.90	194.90 ± 5.97	242.50 ± 7.95
7	111.78 ± 9.90	10.01 ± 0.76	33.53 ± 3.49	186.92 ± 0.84	258.44 ± 5.30
8	106.10 ± 9.22	9.77 ± 0.17	38.88 ± 4.60	185.41 ± 1.06	234.84 ± 13.04
9	88.46 ± 2.47	10.57 ± 0.07	32.63 ± 0.88	174.86 ± 0.63	272.03 ± 13.04
10	101.75 ± 14.85	9.00 ± 0.10	39.31 ± 0.53	181.81 ± 3.49	204.06 ± 16.79
11	103.62 ± 0.22	9.93 ± 0.57	35.13 ± 0.35	179.01 ± 0.09	235.78 ± 0.66
12	106.63 ± 5.62	9.86 ± 0.34	50.38 ± 3.80	171.16 ± 0.27	235.00 ± 0.00

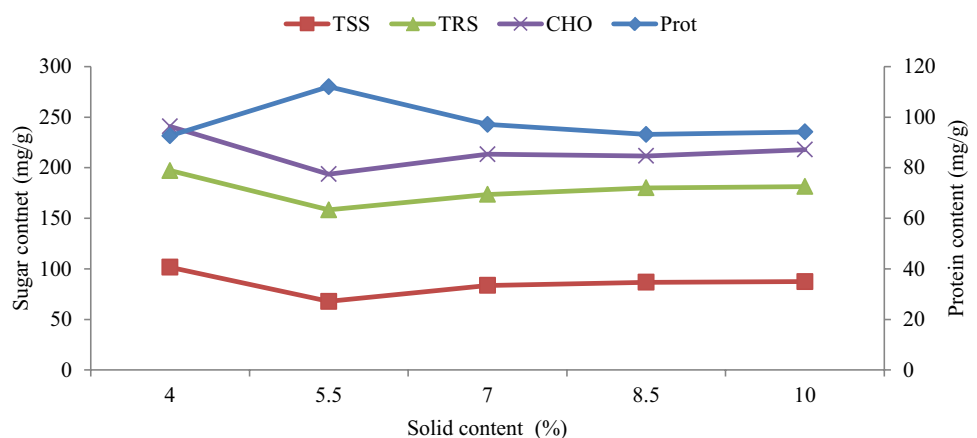*TSS* total soluble sugar, *TRS* total reducing sugar, *CHO* total carbohydrate, *DW* dry weight

Effect of fermentation period and solid content on protein synthesis

There was gradual increase in protein synthesis from day 2 and climaxed at day 6. This trend is typical of a batch process and has been reported during sequential SSF of *Arachniotus sp.* and *Candida utilis* on corn stover (Ahmed et al. 2010). The reduced protein synthesis between day 8 and 10 was consistent with other investigations and could be caused by substrate depletion, exhaustion of limiting nutrients and cell death (Jamal et al. 2012; Salihu et al. 2015). Protein enrichment increased sharply between 20 and 27.5% solid content; then declined (Fig. 2). Similar pattern was reported for white rot fungi possibly due to the ligninolytic activities of *P. chrysosporium* and *Schzophyllum commune* grown on fruit wastes (Saheed et al. 2013; Jamal et al. 2014).

Effects of micronutrients and sucrose on protein synthesis

KCl, $NH_4H_2PO_4$ and $MgSO_4 \cdot 7H_2O$ were selected to determine individual optimum concentration for improved protein synthesis using one-factor-at-a-time (OFAT) method. Gradual increase in KCl concentration resulted in elevated protein synthesis until 0.5%; albeit, synthesis dropped sharply with further increase in KCl concentration (Fig. 3). $NH_4H_2PO_4$ salt concentration of 0.6% supported increased protein synthesis to 112.92 mg/g—the highest value recorded among the optimized factors (Fig. 4). The presence of ammonium and phosphate (NH_4^+, PO_4^{2-}) ions in the salt could trigger the high synthesis. NH_4^+ and PO_4^{2-} have been reported to enhance microbial growth and product synthesis (Rosma and Cheong 2007). Optimum protein synthesis due to $MgSO_4 \cdot 7H_2O$ was recorded at 0.4% inclusion but declined at higher concentration (Fig. 5). Increased concentration of $MgSO_4 \cdot 7H_2O$ under solid state fermentation has been
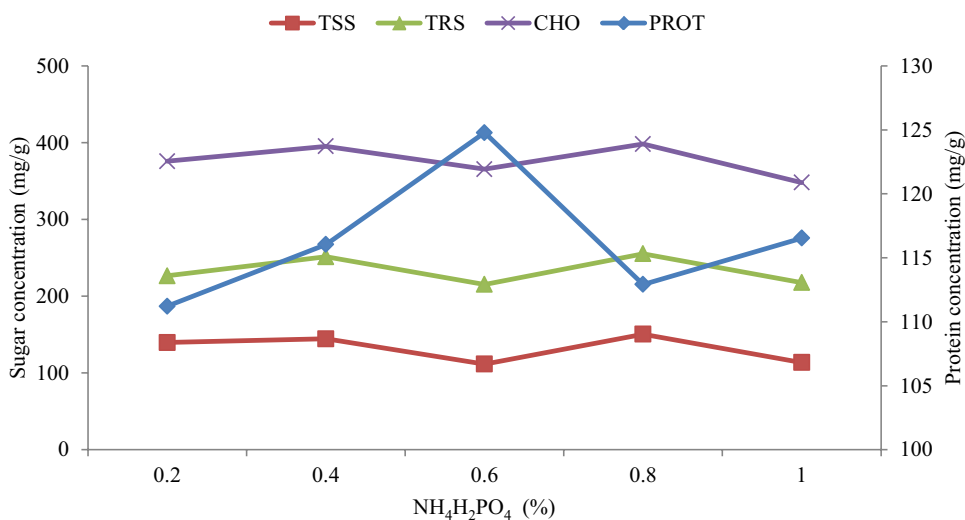
Fig. 2 Optimization of solid content for protein production (diamond) and relationship with total soluble sugar (square), total reducing sugar (triangle) and total carbohydrate (×)

Fig. 3 Optimization of potassium chloride concentration (KCl) (diamond) and relationship with total soluble sugar (square), Total reducing sugar (triangle) and total carbohydrate (×)
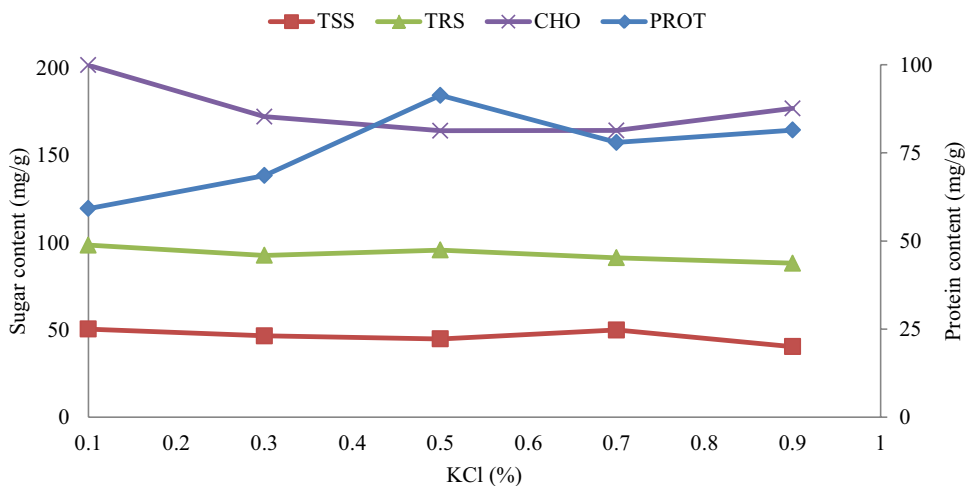
Fig. 4 Optimization of ammonium Di-hydrogen phosphate (NH$_4$H$_2$PO$_4$) (diamond) and relationship with total soluble sugar (square), Total reducing sugar (triangle) and total carbohydrate (×)

Fig. 5 Optimization of magnesium sulphate concentration (MgSO$_4$·7H$_2$O) (diamond) and relationship with total soluble sugar (square), Total reducing sugar (triangle) and total carbohydrate (×)
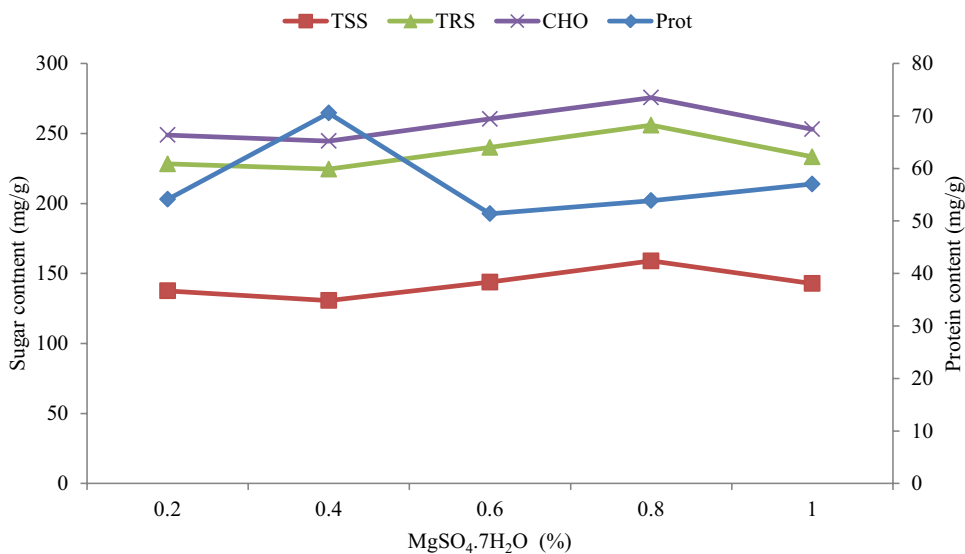
reported to hamper protein synthesis of microorganisms (Ruqayyah et al. 2011).

Protein synthesis increase slowly as the concentration of sucrose increased between 1% and 3% but climaxed at 5% before decline with further increase (Fig. 6). Overall, the amount of protein synthesized as effect of sucrose was the highest among all the media factors investigated. This profound effect could be due to ease of sucrose metabolism by microbes (especially yeast and fungi imperfecti) leading to growth and product synthesis (Castillo et al. 1994; Dhanasekaran et al. 2011).

There is a direct relationship between microbial growth and sugar consumptions since in all cases, the sugar content of the SSF product decreased either greatly or marginally throughout the fermentation period (Figs. 2, 3, 4, 5, 6). Corresponding dip in sugar at optimum protein synthesis signaled peak of microbial activity and product synthesis. Interestingly, cultivated microbes generally consumed maximum sugar at optimum sucrose content followed by KCl and solid content while least sugar was consumed at optimum $MgSO_4 \cdot 7H_2O$. TSS and CHO are the most susceptible to microbial attack and could be responsible for the corresponding increased protein synthesis. TRS metabolism was highest at optimum $NH_4H_2PO_4$ (Table 6). High consumption

of CHO and TSS at optimal sucrose content suggested ease of attack that could trigger cellular growth with corresponding product synthesis (Okamoto et al. 2011). Action of the white rot fungi—*P. chrysosporium* and simple sugar consuming yeast (*C. utilis*) could trigger elevated CHO and TSS consumption due to the secretion of lignocellulosic and hydrolytic enzymes needed to convert lignin and cellulose to simple sugars for microbial growth and product synthesis (Ahmed et al. 2010; Shi et al. 2014). Bioethanol and protein enriched cassava waste production in a batch SSF process involving fungi and yeast cells showed increased sugar consumption as a function of product synthesis (Dhillon 2011; Rajoka et al. 2011; Ruqayyah et al. 2013).

Conclusion

The results of this research indicated that bioling of dried banana peel under aqueous condition removed sufficient amount of saponin that previously hinder fungal-yeast growth. Cultivated microbes required micronutrients and sucrose to further increase protein enrichment of the substrate in many folds; simple and complex sugars in the substrate serve as carbon source consumed for enhanced product

Fig. 6 Optimization of sucrose (diamond) and relationship with total soluble sugar (square), Total reducing sugar (triangle) and total carbohydrate (×)
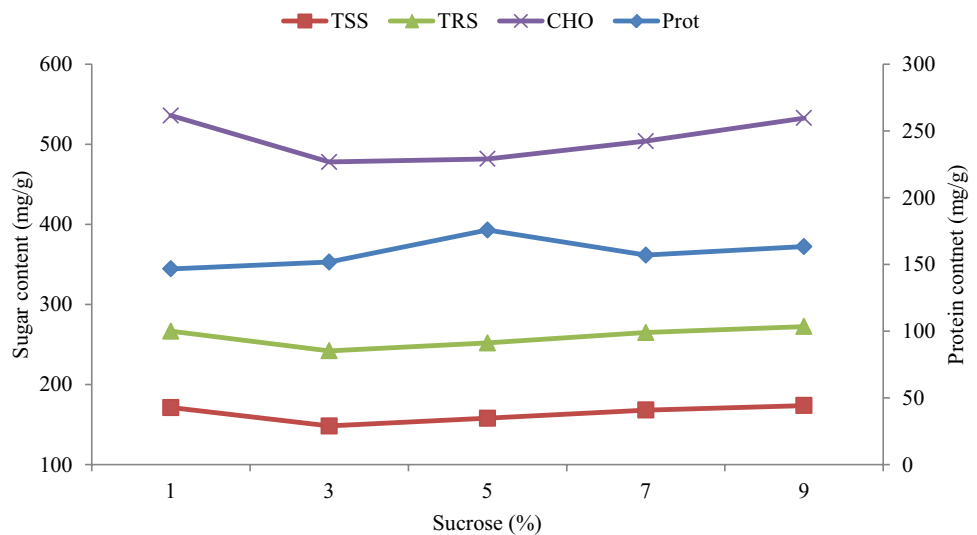
Table 6 Effect of microbial protein production on as function of sugar consumption

	Media constituent				
	Solid content	KCl	$NH_4H_2PO_4$	$MgSO_4 \cdot 7H_2O$	Sucrose
Protein difference	16.48	32.13	39.03	16.43	33.11
Sugars consumed					
CHO (mg/g)	8.25	37.52	24.52	0.52	39.44
TRS (mg/g)	5.10	2.85	14.87	3.12	1.52
TSS (mg/g)	33.75	5.62	28.15	6.87	13.20

TSS total soluble sugar, *CHO* carbohydrate, *TSS* total soluble sugar

formation at optimum media constituent concentrations. Sequential SSF proved efficacious in improving convertion of banana peel to high proetein product capabe of use as animal feed protein supplement.

Compliance with ethical standards

Conflict of interest There are no financial/commercial or any other conflicts of interest among authors.

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