Nutrient Enhancement of Ground Sago (*Metroxylon sagu* Rottboll) Pith by Solid State Fermentation With *Rhizopus oligosporus* for Poultry Feed

Azilah Ab Jalil[1], Norhani Abdullah[1,2], Abdul Razak Alimon[1,3] & Suraini Abd-Aziz[4]

[1] Laboratory of Animal Production, Institute of Tropical Agriculture, Universiti Putra Malaysia, Selangor, Malaysia

[2] Department of Biochemistry, Faculty of Biotechnology & Biomolecular Sciences, Universiti Putra Malaysia, Selangor, Malaysia

[3] Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Selangor, Malaysia

[4] Department of Bioprocess Technology, Faculty of Biotechnology & Biomolecular Sciences, Universiti Putra Malaysia

Correspondence: Azilah Ab Jalil, Laboratory of Animal Production, Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. Tel: 603-8947-1172. Fax: 603-8938-1612. E-mail: azilahj@upm.edu.my

Abstract

Utilization of ground sago pith (GSP) as poultry feed ingredient is limited due to its high fibre content and negligible amount of protein. Hence, to enhance its utilization in poultry feed formulations, proper treatment method is necessary. This study was aimed to improve the nutritive value of GSP through solid state fermentation (SSF) using *Rhizopus microspores* var. *oligosporus* isolated from fermented cooked soybean seeds (local name *tempeh*). Fermentation of GSP was first conducted in 500 ml Erlenmeyer flasks to determine the optimized conditions. The substrate was prepared by mixing 50 g of GSP with 50 ml mineral solution and sterilized by autoclaving. The autoclaved GSP was inoculated with 5 ml of *R. oligosporus* spore suspension (1×10^6 spore/mL). Four factors were investigated; moisture levels (35, 45, 55, 65 and 75 ml liquid/50 g substrate), pH values (4.5, 5, 5.5, 6, 6.5, 7 and 7.5), incubation temperatures [ambient temperature (28 ± 2), 30, 32.5, 35 and 37.5°C] and inorganic nitrogen (ammonium sulphate, ammonium phosphate, ammonium carbonate, ammonium nitrate, ammonium chloride and ammonium molybdate) sources at 1 % (w/v) concentrations. Fermentation was conducted for 3 days and the parameters measured were reducing sugar, soluble protein and hydrolytic enzyme activities. The optimized conditions in the flask system were moisture level of 55 ml liquid/50 g substrate, citrate buffer pH 6.0, incubation temperature 30°C and ammonium sulphate as the inorganic nitrogen source. A time course study for 7 days under optimized conditions showed that the reducing sugar reached maximum from 15.7 ± 0.5 to 183.8 ± 22.6 mg/g (dmb) at 1.5 days and the soluble protein increased from 9.5 ± 0.5 to 11.4 ± 0.7 μg/g (dmb) at the end of fermentation period. Amylase and CMCase activities were maximum at 3 days of fermentation; 3.58 0.73 ± and 4.44 ± 1.50 U/mg protein, respectively but FPase activities did not show apparent changes during the time course study. Using the optimized condition, scaling up in a horizontal drum laboratory fermenter was conducted using 3 kg of GSP in the presence of air at 4 L/min flow rate. The nutrient composition of fermented GSP produced in the fermenter was 89.1% dry matter, 3.4% crude protein, 5.5% crude fiber, 6.3% ash and 3495 kcal/kg gross energy as compared to 88.2% dry matter, 1.6% crude protein, 6.0% crude fiber, 5.3% ash and 3608 kcal/kg gross energy for untreated GSP. Solid state fermentation by *R. oligosporus* had increased the reducing sugar by 61%, glucose and fructose by more than 100%, soluble protein by 1% and amino acids by 1.5 to 38.2%. Cysteine which was undetected in the unfermented GSP was produced during the fermentation at a concentration of 222 ppm. The other amino acids were increased by 1.5% (arginine), 2.1% (glutamic acid), 16.2% (isoleucine), 16.5% (leucine), 6.2% (phenylalanine), 25.7% (proline), 38.2% (serine), 27.3% (threonine)and 5.7% (tyrosine). The results showed that the nutritive value of GSP was enhanced by solid state fermentation with *R. oligosporus*.

Key words: solid state fermentation, ground sago pith, *Rhizopus oligosporus*, nutrient enhancement

1.Introduction

The cost of feed ingredients contributed to a large proportion of the total cost in poultry industry. The increasing cost of some of the feed ingredients used in the formulations especially the cereal grains as a source of energy, has stimulated research in the use of alternative feed resources. Local feedstuffs such as palm kernel cake, rice bran, cassava meal and sago products have been identified as alternative feeds in Malaysia to partially substitute feeds like corn and soybean for poultry.

Sago palms, *Metroxylon sagu* (Rottboll) belongs to the Palmae family and is an important starch producing crop distributed in the South Pacific Islands, extending through Melanesia into Thailand, Malaysia, Indonesia and the Papua New Guinea (Karim et al., 2008). It grows well in humid tropical lowland areas and can tolerate wet growing conditions, including peat swamps. The biggest sago growing area in Malaysia is in the state of Sarawak with the export revenue of about 45,300 metric tons of food-grade sago starch earning about RM40.4 million in 2005 (Dept.of Statistics Malaysia).

The sago starch can be processed to various products like sago pearls, as a substitute for modified corn starch in the production of monosodium glutamate and fructose syrup for non-alcoholic drinks when combine with other starches. Other products derived from sago starch such as modified starch, cyclodextrins and ethanol have a significant commercial value (Singhal et al., 2008). Ground sago pith (GSP) is another products obtained from the pith of the sago palmsafter removal of the lignified bark, undergoing size reduction, dried and ground to fine particles.

Chemical analysis showed that dry sago pith contain 722 to 751 g/kg starch, 66 to 74 g/kg cellulose, 36 to 40 g/kg hemicelluloses, 11 to 12 g/kg crude protein and 35 to 66 g/kg ash (Yahya et al., 2011). The GSP may be grouped into an energy feed based on an international classification standard (Harris et al., 1968). The use of feed sources containing highly fermentable carbohydrate such as GSP is important in optimizing livestock production in Malaysia. However, utilization of GSP as a feedstuff is limited due to its high moisture content, fibrous components and negligible amount of protein.

In order to improve its utilization in poultry feed formulations, enhancement of the nutritive value through proper processing methods is necessary. The microbiological method through solid state fermentation (SSF) is one of the strategies to improve the nutritive value of animal feed as the fermentation enhances the bioavailability of nutrients (Hotz & Gibson, 2007). Conversion through microbial SSF has been reported in starch based substrate such as soybean meal (Teng et al., 2012; Chen et al., 2013), rice bran (Schmidt & Furlong, 2012) and starch extracts from soybean flour (Olanipekun et al.,2009). Filamentous fungi such as *R. oligosporus* is commonly used in SSF for the production of food and feed because they do not produce any toxic substances and has long history in the production of *tempeh*, an Asian fermented food made of cooked soybean (Nout & Kiers, 2005).

Hence, the objective of the present study was to enhance the nutritive value of GSP by SSF with *R. oligosporus* locally isolated from fermented cooked soybean seeds (*tempeh*).

2. Materials and Methods

2.1 Fungal Culture And Preparation of Inoculum

Fungal culture was isolated from *tempeh* and identified by morphological characteristics and molecular techniques. It was identified as *Rhizopus microspores* var. *oligosporus* in the phylum of Zygomycetes. The culture grown on potato-dextrose agar (PDA) plates at 30 °C for 6 to 7 days was used to prepare the inoculum. The spores were harvested by adding 10 ml of 0.01% Tween-80 solution to dislodge them by using a glass rod under aseptic conditions. The spores were transferred into a 500 ml sterilized bottles by filtering with glass wool. The glass wool was folded into seven layers to provide filters with pore size of approximately 0.5 mm. Inoculum density was determined by counting using a haemacytometer and adjusted to a concentration of 1×10^6 spore/ml. Five milliters of inoculum were used to inoculate each fermentation flask or 300 ml for fermentation in a horizontal drum laboratory fermenter.

2.2 Ground Sago Pith

Ground sago pith (GSP) was prepared by first removing the lignified bark of the sago logs and chopping the pith to small pieces of approximately 5 to 10 cm using a mechanical chopper at the factory (Batu Pahat, Johor, Malaysia). The sago pieces were dried in a solar house dryer (38 ± 5 °C) at the Malaysian Agricultural Research and Development Institute (MARDI) for 2 weeks. The dried sago pith with moisture content of 10 to 11%, were shred to reduce the size to around 0.5 to 1 cm. The dried pith was later ground to a fine powder (0.2 to 5.5 mm size) by using a hammer mill grinder and stored in air-tight container at room temperature. Five sago logs of approximately

100 cm (length) × 45 to 48 cm (diameters) were used to obtain dried GSP of about 40 kg. The bulk density of the GSP was 264.39 ± 9.02 g/L as measured using a modified method by Grossman and Reinsch (2002).

2.3 Fermentation in 500 ml Erlenmeyer Flask

Fermentation of GSP was first conducted in 500 ml flasks to determine the optimized conditions by the conventional method. In each set of fermentation, one factor was investigated by varying the levels while keeping the other factors at constant values. Four factors were investigated; moisture levels (35, 45, 55, 65 and 75 ml liquid/50 g substrate), pH values (4.5, 5, 5.5, 6, 6.5, 7 and 7.5), incubation temperatures [ambient temperature (28 ± 2), 30, 32.5, 35 and 37.5 °C] and inorganic nitrogen from various sources (ammonium sulphate $(NH_4)_2SO_4$, ammonium phosphate NH_4PO_4, ammonium carbonate NH_4CO_3, ammonium nitrate NH_4NO_3, ammonium chloride NH_4Cl and ammonium molybdate NH_4MO_3) at 1 % (w/v) concentrations.

The substrate was prepared by mixing 50 g of GSP with 50 ml mineral solution [0.01M citrate buffer pH 6.0 containing 0.01% (w/v) KH_2PO_4, 0.01% (w/v) $MgSO_4.7H_2O$ and 1% (w/v) of $(NH_4)_2SO_4$ as nitrogen source] in 500 ml Erlenmeyer flask. The flask was plugged with cotton wool wrapped with gauze, covered with aluminium foil and autoclaved at 121 °C for 20 minutes.Each flask was inoculated with 5 ml of 1×10^6 spore/ml spore of *R. oligosporus*. The fermentation was performed for 3 days at 30 °C in an incubator (Memmert INE 700, Germany) with interior dimensions of (1040 × 500 × 800) mm. The flasks containing GSP were prepared in triplicates for each fermentation conditions. Parameters measured were reducing sugar, soluble protein, amylase, filterpaperase (FPase) and carboxymethylcellulase (CMCase). The time course study for the fermentation at optimized conditions was conducted for 7 days. Samples were collected every 12 hours for measurement of pH, temperature, reducing sugar, soluble protein and hydrolytic enzyme activities.

2.4 Fermentation in Horizontal Drum Fermenter

Fermentation was performed in a stainless steel horizontal drum fermenter with inner dimensions of 210 mm × 178 mm (length × diameter). The total volume is approximately 5 L (Biosys SF-20 rotary-shaft SSF, Malaysia). The drum is equipped with water jacket, inlet for water supply, motor system, heater, air compressor, airflow meter, air filter, an opening on top of the drum, an opening at the rear side of the drum and related control valves. The system is connected to a controller to regulate functions of mixing, aeration and the fermenter temperature. The top opening is covered with glass for visual inspection during fermentation. Both openings at the top and the side of the drum have water-tight seals that prevent outside contamination or rapid moisture lost from the fermented material. Air was supplied through air filters to provide sterilised air blown across the top of the substrate bed. The excess gas will escape through the outlet valve. The fermenter temperature is controlled by circulation of cooling water through the outer jacket of the drum. The substrate humidity was measured by a relative humidity (RH) probe and the substrate temperature was measured by a temperature probe.

Substrate for the fermenter was prepared by mixing 3 kg of GSP with mineral solution as in the flask experiment. Then, the substrate was divided into approximately 200 g in bags, autoclaved and cooled to room temperature. The inner surface of the fermenter was disinfected by 70% alcohol before loading the substrate. Fermentation was conducted using optimized conditions previously determined from the flasks experiment. The fermentation conditions wereset at 30 °C in unmixed mode and the incoming airflow of 4 L/min for 44 hours fermentation.

Fungal growth during fermentation was monitored by visual inspection and the fermentation was stopped when the mycelia fully covered the substrate. At the end of fermentation, the product was removed through the rear side opening and manually mixed and dried at 50 °C in a hot air oven for 4 days. The product was analysed for reducing sugar, soluble protein, proximate compositions and hydrolytic enzyme activities.

2.5 Analytical Methods

Dried samples of untreated and fermented GSP were determined for dry matter, ash, crude protein, crude fiber and gross energy according to standard methods (AOAC, 2000). Analysis of reducing sugar and soluble protein was conducted using culture filtrate. The filtrate was prepared by mixing 5 g of fermented GSP with 50 ml of cold deionized water, homogenized in a Warring blender and samples shaken on a rotary shaker for 2 hours at a speed of 200 rotations per minute and 4 °C. The mixture was later centrifuged at 3000g for 10 min at 4 °C (Eppendorf 5810R with A-4-62 swing bucket rotor). The resulting suspension was filtered through Whatman No.1 filter paper. Reducing sugar was measured using dinitrosalycilic acid reagent (Miller, 1959) and soluble protein by Bradford assay (1976).

Monosaccharides and amino acids were determined by high performance liquid chromatography (Waters e2695, USA) using a refractive index detector (Waters 2998) and a fluorescent light detector (Waters 2475), respectively. Samples for monosaccharide measurement were prepared according to Dubois et al. (1956). The calibration

standards were mixture of glusoce, fructose, xylose and mannose. The column was COSMOSIL sugar-D and the mobile phase consisted of acetonitrile and water (80:20) with a flow rate of 0.8 ml/min.

Samples for amino acids measurement were prepared using AccQ Tag Amino Acid Analysis Kit (Waters Corporation, U.S.A.) according to the manufacturer's instruction. The internal standard was α-amino butyric acid (AABA) and the calibration standards were Standard-H (Thermo Scientific) containing the common mixture of amino acids. The column was WATERS AccQ and the mobile phase consisted of aqueous buffer and 60% acetonitrile in gradient concentrations with a flow rate of 1.0 ml/min.

2.6 Enzyme Assays

The culture filtrate prepared above was used for measuring amylase, FPase and CMCase activities according to Fogarty and Kelly (1980), Ghose (1987) and Mandels and Weber (1974), respectively. One unit of activity was defined as the enzyme required for producing one millimole of glucose per minute.

2.7 Statistical Analysis

The effects of fermentation conditions on *R. oligosporus* activities were analysed by a one-way analysis of variance (ANOVA) and means comparison using the least significant differences (LSD), by using the GLM procedure of SAS (SAS, 2004). The results were presented as the mean ± standard deviation values of three replicates. Probability values less than 0.05 were considered significant.

3. Results

3.1 Optimised Fermentation Condition in 500 ml Erlenmeyer Flask

The highest concentration of reducing sugar was produced at 55 ml liquid/50 g GSP (Figure 1a). The soluble protein concentrations increased significantly ($P<0.05$) when additional moisture was available in the substrate. Amylase and CMCase activities were higher at lower moisture level but FPase activity was not affected by changes in the moisture levels (Figure 1b). Based on the results, moisture level of 55 ml liquid/50 g GSP was selected for the subsequent experiments.
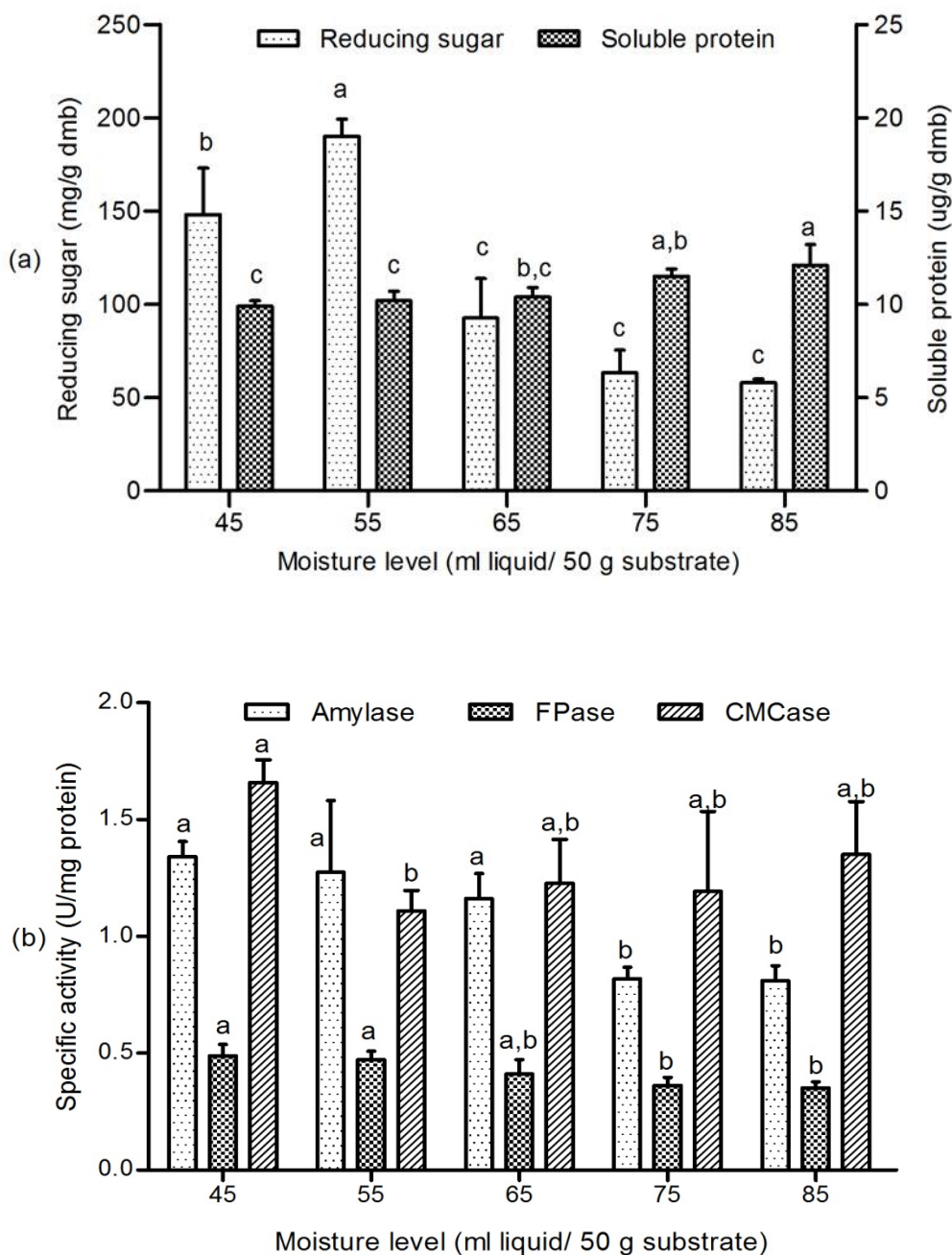
Figure 1. (a) Reducing sugar and soluble protein content (b) Amylase, FPase and CMCase activities in fermented GSP at different levels of initial moisture content

Error bar indicates standard deviation. Means with different letters are significantly different ($P<0.05$).

The effect of incubation temperatures showed the highest concentration of reducing sugar was produced at ambient temperature (28 ± 2 °C), but at this temperature, the soluble protein was lower than the other incubation temperatures (Figure 2a). Amylase, FPase and CMCase were maximum when fermentation was conducted at ambient temperature or 30 °C (Figure 2b) but differences between these two temperatures were insignificant ($P>0.05$). Hence, subsequent experiments were conducted at 30 °C.
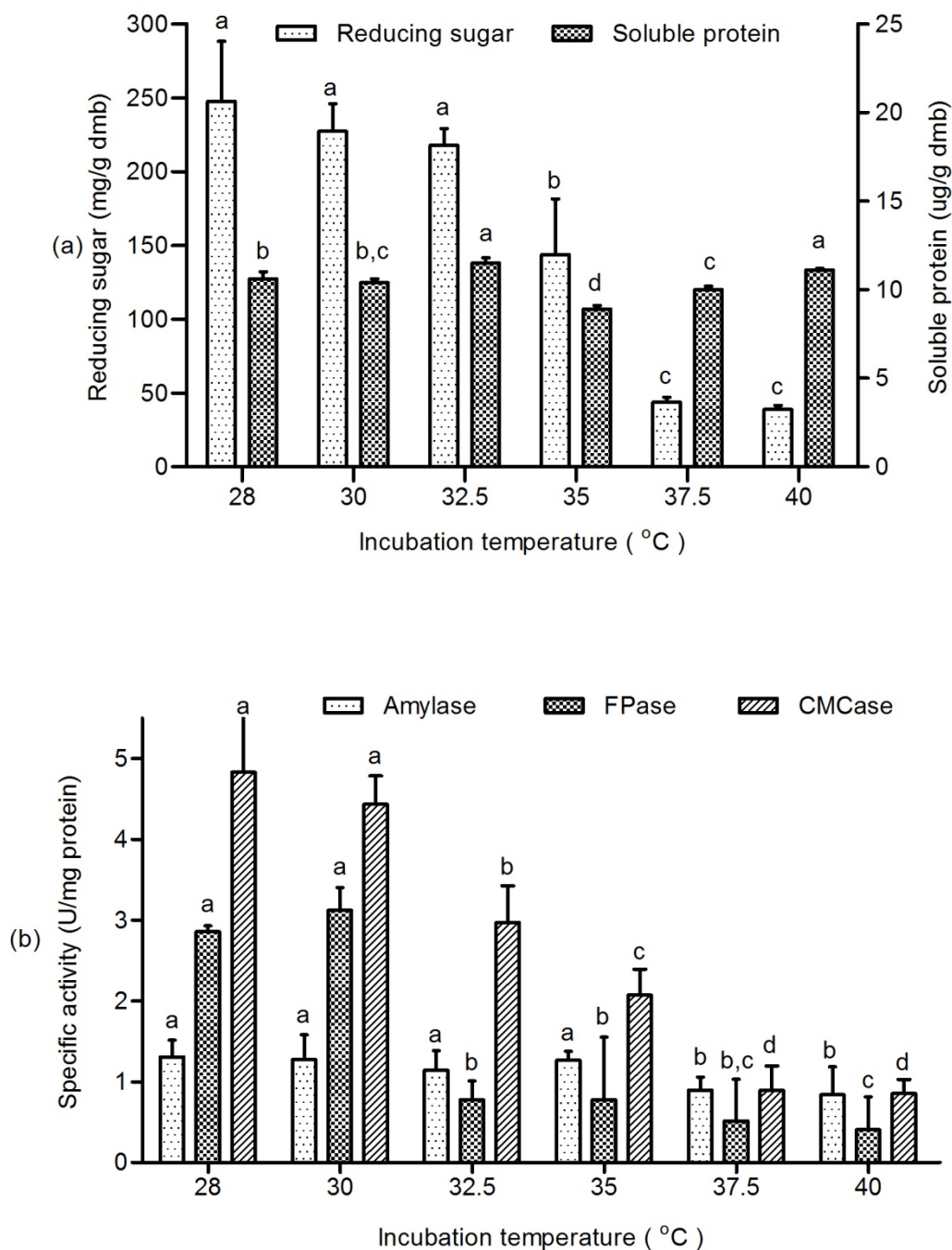
Figure 2. (a) Reducing sugar and soluble protein content (b) Amylase, FPase and CMCase activities in fermented GSP at different incubation temperatures and 55 ml liquid/50 gm substrate moisture

Error bar indicates standard deviation. Means with different letters are significantly different ($P<0.05$).

Different initial pH values did not affect significantly ($P>0.05$) the concentration of reducing sugar but soluble protein was affected (Figure 3a). Amylase activity was higher at alkaline pH but FPase and CMCase activities were not affected by pH variations (Figure 3b). pH 6.0 was then selected for the next set of experiment considering the soluble protein concentration in relation to other parameters measured and it is the median value between acidic and alkaline pH.
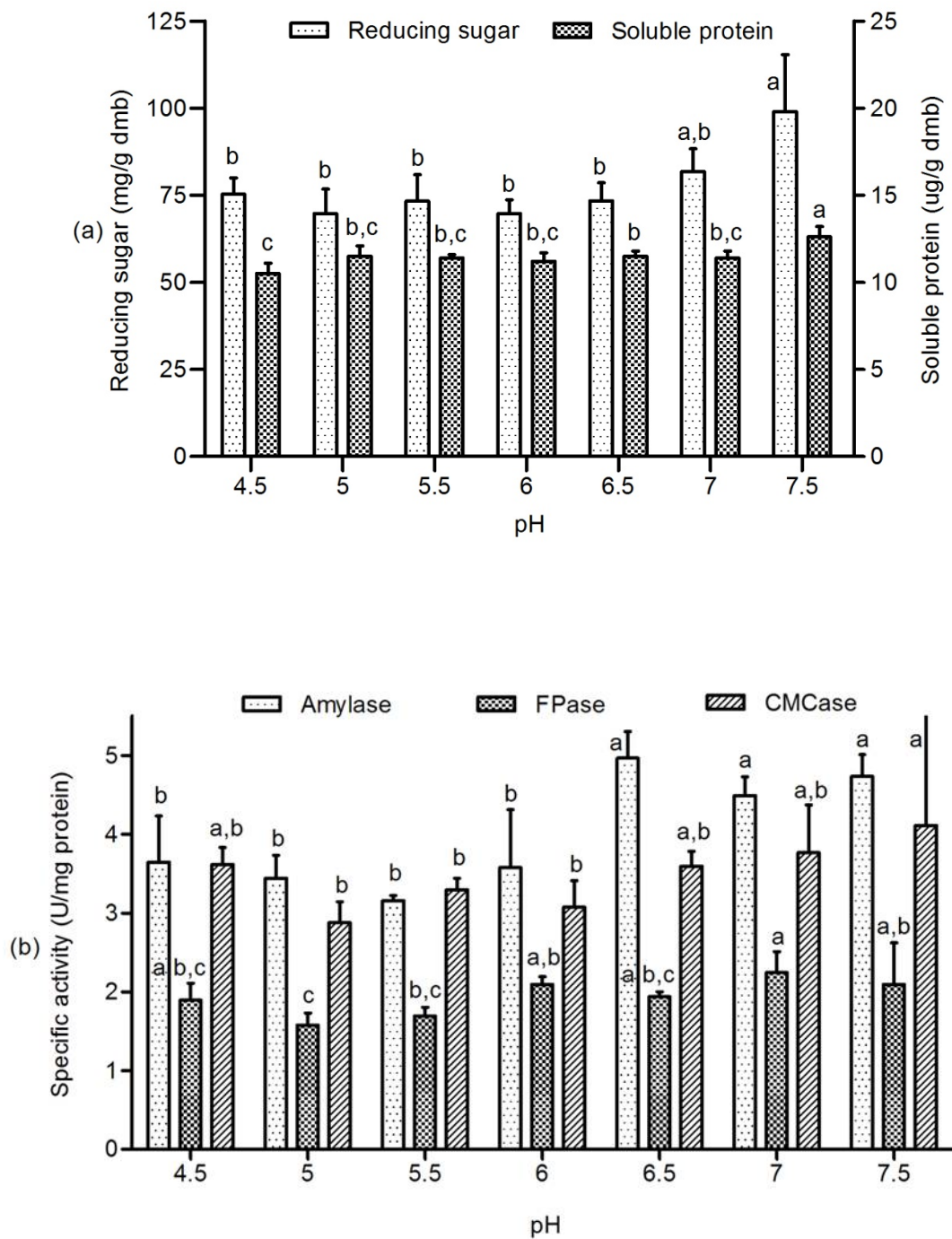
Figure 3. (a) Reducing sugar and soluble protein content (b) Amylase, FPase and CMCase activities in fermented GSP at different initial pH, 55 ml liquid/50 gm substrate moisture and 30 °C incubation temperature

Error bar indicates standard deviation. Means with different letters are significantly different ($P<0.05$).

Figure 4 shows that *R. oligosporus* responded differently to the various nitrogen sources added to the SSF system. GSP supplemented with $(NH_4)_2SO_4$, NH_4CO_3 and NH_4MO_3 showed higher reducing sugar compared to the other nitrogen sources (Figure 4a). GSP containing NH_4CO_3 also resulted in high soluble protein but activities of hydrolytic enzymes were maximum only in substrate supplemented with $(NH_4)_2SO_4$ (Figure 4b). It was concluded that $(NH_4)_2SO_4$ was the most suitable nitrogen source.
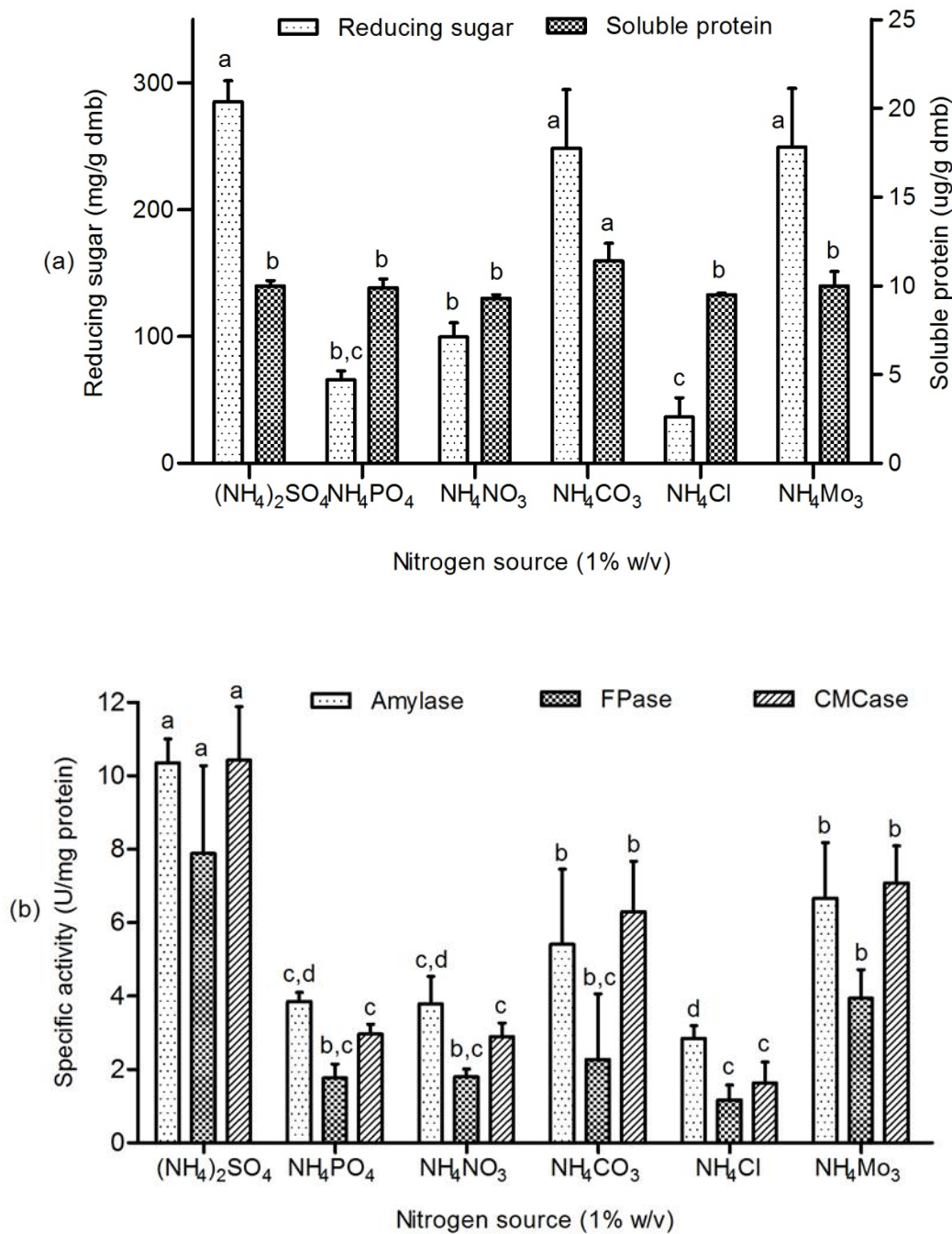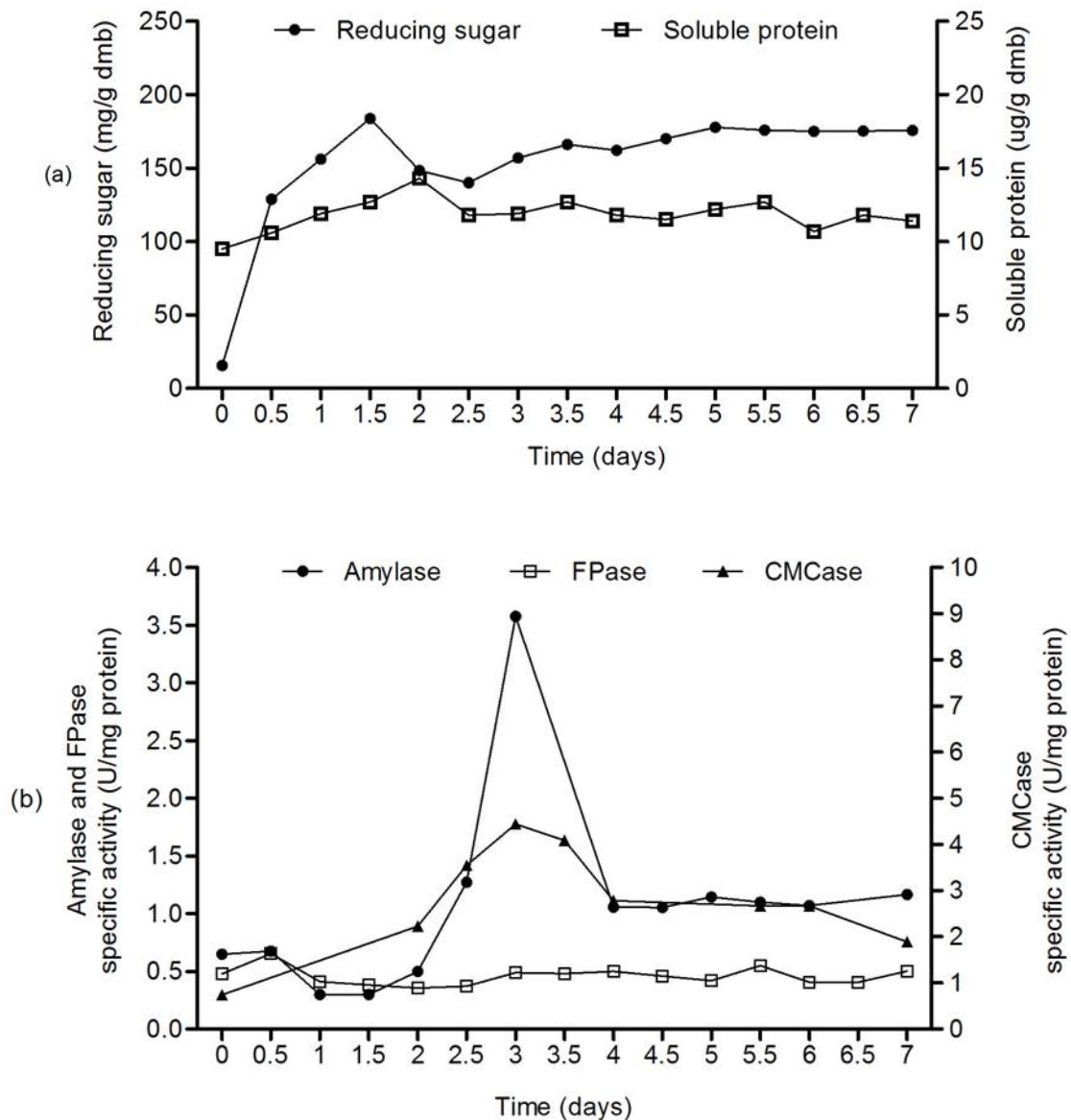
Figure 4. (a) Reducing sugar and soluble protein content (b) Amylase, FPase and CMCase activities in fermented GSP at different source of nitrogen, 55 ml liquid/50 gm substrate moisture, 30 °C incubation temperature and initial pH of 6.0

Error bar indicates standard deviation. Means with different letters are significantly different ($P<0.05$)

The above experiments demonstrated that optimum conditions for GSP solid state fermentation with *R. oligosporus* in 500 ml flask system were at initial moisture 55 ml/50 g substrate, incubation temperature of 30 °C, initial pH at pH 6.0 using 0.01M citrate buffer and inorganic nitrogen source of 1% (w/v) $(NH_4)_2SO_4$.

3.2 Time Course Study at Optimized Fermentation Conditions

A time course study for 7 days at these optimized conditions showed the reducing sugar increased from 15.7 ± 0.5 mg/g (dmb) and reached maximum value of 183.8 ± 22.6 mg/g (dmb) at 1.5 days (Figure 5a). At longer

fermentation period, the concentration of reducing sugar decreased. Soluble protein increased gradually with fermentation time up to 7 days. Amylase and CMCase activities were highest at 3 days of fermentation; 3.58 0.73 ± and 4.44 ± 1.50 U/mg protein, respectively. However, FPase activities did not show any apparent changes during the fermentation (Figure 5b). The temperature showed a rapid increase between 0.5 days and 1.5 days (Figure 5c). At this point, the temperature was maximum (34 °C) but later decreased to 30 °C and remained constant until the end of fermentation. pH of the fermented substrate dropped by more than 2 pH units at the end of fermentation from the initial pH of 6.09 ± 0.22 to 3.97 ± 0.09.
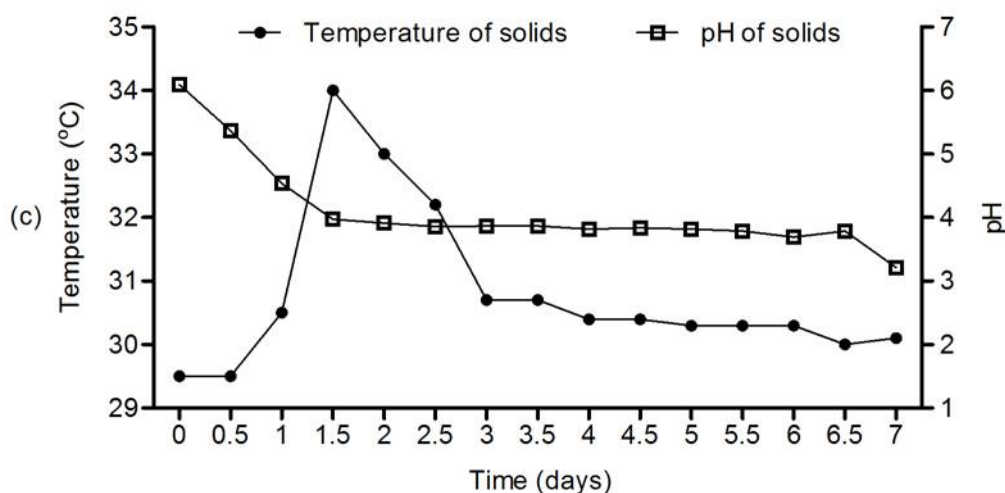
Figure 5. (a) Reducing sugar and soluble protein content (b) Amylase, FPase and CMCase activities (c) Internal temperature and pH of fermented GSP at optimized conditions for 7 days

3.3 Nutrient Compositions of Untreated and Fermented GSP

The nutrient compositions of untreated (UGSP) and fermented GSP (FGSP) are presented in Table 1. The dry matter, crude protein and ash in FGSP were increased but crude fibre and gross energy were decreased. Reducing sugar and soluble protein in the aqueous extract of FGSP were increased by more than 100 percent and the pH was reduced by more than 2 pH units from 5.42 ± 0.23 to 3.02 ± 0.02.

Table 1. Proximate composition, reducing sugar and soluble protein of untreated and fermented GSP produced in laboratory fermenter

Composition	UGSP	FGSP	% increament or reduction	Corn meal*
Proximate analysis:				
Dry matter, %	88.2	89.1	increased by 1.0%	89
Crude protein, %	1.6	3.4	increased by >100%	8.5
Crude fibre, %	6	5.5	reduced by 9.1%	2.2
Ash, %	5.3	6.3	increased by 18.9%	4
Gross energy, cal/kg	3608	3495	reduced by 3.2%	3350
Aquaeous extract:				
Reducing sugar, mg/g (dmb)	285.4 ± 3.35	736.4 ± 23.52	increased by >100%	
Soluble protein, µg/g (dmb)	1.4 ± 0.17	9.1 ± 0.11	increased by >100%	
pH of wet solids	5.42 ± 0.23	3.02 ± 0.02	reduced by 79%	

UGSP untreated ground sago pith; FGSP fermented ground sago pith.

g (dmb) = gram (dry matter basis). Values are mean ± standard deviation.

*Corn meal as comparison (NRC, 1994).

Monosaccharides detected in both UGSP and FGSP were xylose, fructose, mannose and glucose but the percentage of fructose and glucose in FGSP had increased remarkably (Table 2). Amino acids profiles are presented in Table 3. Cysteine which was undetected in the unfermented GSP was produced during the fermentation at a concentration of 222 ppm. The other amino acids were increased by 1.5% (arginine), 2.1%

(glutamic acid), 16.2% (isoleucine), 16.5% (leucine), 6.2% (phenylalanine), 25.7% (proline), 38.2% (serine), 27.3% (threonine)and 5.7% (tyrosine).

Table 2. Monosaccharides concentration of untreated and fermented GSP produced in laboratory fermenter

Monosaccharides	UGSP [mg/g (dmb)]	FGSP [mg/g (dmb]	% increament
Xylose	0.02	0.1	80
Fructose	0.38	5.32	> 100
Mannose	0.12	0.14	< 1
Glucose	0.47	5.77	> 100

UGSP, untreated ground sago pith; FGSP, fermented ground sago pith.

Table 3. Amino acids concentration of untreated and fermented GSP produced in laboratory fermenter.

Amino acids	UGSP (ppm)	FGSP (ppm)	% increament
Essential amino acids			
Cysteine	n.d.	222	-
Threonine	1143	1572	27.3
Isoleucine	31	37	16.2
Leucine	637	764	16.5
Phenylalanine	30	32	6.2
Tyrosine	727	771	5.7
Lysine	n.d.	n.d.	-
Methionine	n.d.	n.d.	-
Valine	n.d.	n.d.	-
Histidine	n.d	n.d.	-
Non essential amino acids			
Serine	147	238	38.2
Proline	133	179	25.7
Glutamic acid	3199	3269	2.1
Arginine	3231	3279	1.5
Alanine	n.d.	n.d.	-
Asparagine	n.d.	n.d.	-
Aspartic acid	n.d.	n.d.	-
Glycine	n.d.	n.d.	-
Hydroxyproline	n.d.	n.d.	-
Tryptophane	n.d.	n.d.	-

UGSP, untreated ground sago pith; FGSP, fermented ground sago pith.

n.d.= not detected i.e. the values were lower than the detection limit (< 10 ppm).

4. Discussion

4.1 Optimization of SSF Conditions

The main factors that influenced the behavior of microorganisms in solid state conditions include water activity and moisture, temperature, pH, aeration, bed properties and nature of solid substrates employed (Singhania et al., 2009). In the current study, GSP moistened with 55 ml liquid/50 g substrate provided an optimum level for fungal growth and enzyme activities. In general, fungal cultures required low moisture level (20 to 70%) compared to the requirement of a bacterial culture, which is higher than 70% (Gowthaman et al., 2001). Very high moisture caused substrate compaction thus limiting nutrient transfer and gas diffusion between the substrate particles. An appropriate combination of the substrate moisture and the incubation temperature is necessary in fungal SSF. Results of this study showed the moisture level of 55 ml liquid/50 g GSP and incubation temperature of 30 °C produced maximum growth.

This study also indicated that *R. oligosporus* growth was not significantly affected by acidic or alkaline pH. The results were in agreement with previous studies by Nagel et al. (1999) and Sparinga et al. (2002) on the effect of pH using several buffering agent on the growth of *R. oligosporus* in SSF system. There were no significant difference in biomass produced between pH 4 and pH 7 but the optimum growth occurred at acidic pH below 6, provided by the citric acid buffer. A slightly acidic pH and lower moisture ratio offer an advantage in reducing the risk of bacterial contamination, since bacteria are unable to grow at low moisture level and acidic pH (Gowthaman et al., 2001).

Exogenous nitrogen added to SSF substrate balanced the carbon and nitrogen ratio to support growth and hydrolytic activities of filamentous fungi. The results showed that growth of *R. oligosporus* began on the first day of incubation and maximum enzyme activities were observed in GSP containing ammonium sulphate. Sulphur ions are essentials in fungal physiology and sulphate, the oxidized state of inorganic sulphur is the most common source supplied to the culture medium for fungi. Ammonium sulphate was reported to influence the level of protein and biomass production in SSF aimed for protein enrichment of rice bran using *R. oryzae* (Schmidt & Furlong, 2012) and food processing waste using *R. arrhizus* and *R. oryzae* (Jin et al.,2005).

4.2 SSF at Optimized Condition

Substrate utilization and the growth related activities of *R. oligosporus* in GSP at optimized conditions were represented by the production of reducing sugar, soluble protein, hydrolytic activities and changes in the internal temperature and pH of the solid substrate. An optimum fermentation rate could have occurred between 1.5 to 3 days of incubation as indicated by the maximum amount of reducing sugar and the related amylase and CMCase activities. The enzyme activities increased with time during fermentation and reached maximum values at 3 days. Similar observation was reported by Rashid et al. (2003) on filamentous fungi cultivation in palm oil mill effluent as a basal medium. The highest CMCase activities were detected after 3 to 5 days of fermentation.

The rapid temperature rise at the fermenting substrate was the result of metabolic heat generated from fungal growth. However, inefficient heat removal after reaching the maximum temperature may result in declining metabolic activity. Although the soluble protein increased, the fermentation slowed down with declining pH values. The lowering of pH was the result of organic acids production. *Rhizopus* sp. is known to produce organic acids such as lactic acids (Jin et al., 2005; Zhang et al., 2007) and fumaric acids (Liao et al., 2007) from the starch components in the substrate. Bioconversion into organic acids occur simultaneously from saccharification of starch that produce sugar and fermentation of sugar to lactic or fumaric acids.

4.3 NutrientComposition of Untreated and Fermented GSP

Fermentation is characterized by the conditions offered by a particular substrate for microbial growth and the changes in the substrate condition as a result of interactions between hydrolysis and products formed. Proximate analysis of FGSP showed higher percentage of dry matter, crude protein and ash. The dry matter and ash of FGSP had increased by 1% and 18.9%, respectively. This is in accordance with a study by Oduguwa et al. (2008). An increase up to 30% in ash content was observed after 120 hours of *R. oligosporus* SSF on corn cob, rice bran and cowpea husk. The crude protein of GSP showed an increase of more than 100 percent, from 1.6% in UGSP to 3.4% in FGSP. Crude protein increment has been demonstrated in *Rhizopus* SSF aimed for protein enrichment. Schmidt and Furlong (2012) reported a protein level of 20 g/100 g of dry weightin the presence of 8 g/L ammonium sulphate. Meanwhile, the crude fibre and gross energy had decreased, indicating some degree of degradation had taken place. The decrease of crude fibre was also reported by Pal Vig and Walia (2001) on fermented rapeseed meal using *R. oligosporus*. Fungal SSF generally reduced crude fibre content of agricultural feedstuffs because of the fungal ability to produce cellulase enzymes and the hyphael mode of growth.

High percentage of reducing sugar and monosaccharides in FGSP indicated that *R. oligosporus* was actively utilizing starch as this is the major component in GSP readily available to the fungus. The changes were associated with hydrolytic activities especially amylase and CMCase that were considerably high when GSP was supplemented with 1% (w/v) of ammonium sulphate (Figure 4b). Glucose and fructose amount in FGSP produced in fermenter increased by more than 100 percent. A study by Olanipekun et al. (2009) showed that starch and amylose content in extracts from soybean flour were reduced after 72 hours of SSF by *R. oligosporus*. A mixture of high and low molecular weight carbohydrates; fructose, glucose, xylose, inositol, cellobiose, galacturonic acid oligomers and polysaccharides, was produced in *R. nigricans* SSF on lemon peel as a result of hydrolysis (Hellín et al., 2001).

A small amount of xylose was produced (Table 2) as a result of hemicellulose depolymerization. The production was related to CMCase activity as demonstrated from the optimized conditions of fermentation in flask system (Figure 4b). The effect of CMCase could also be detected as indicated by the reduction of crude fiber in FGSP produced in the fermenter. A synergy amongst starch and fibre degrading enzymes were necessary to degrade GSP into digestible components. Meng and Slominski (2005) reported that treatment of corn, soybean meal, canola meal, and peas aimed for broiler feed using multicarbohydrase enzymes affected the nutritive values through degradation of the cell wall components and increasing starch availability.

The amino acids profiles indicated that SSF of GSP by *R. oligosporus* produced cysteine and increased threonine, isoleucine, leucine, phenylalanine, tyrosine, serine, proline, glutamic acid and arginine contents (Table 3) by 1.5 to 38.2%. The changes could be related to increment of soluble protein produced by the fungal activities (Table 1). Amino acids are metabolites produced during fungal SSF as a consequence of protein hydrolysis and synthesis related to growth. In SSF process of cactus pulp using *Aspergillus niger,* the principal amino acids produced were aspartic acids, threonine, glutamic acid, valine, serine, arginine and phenylalanine (Oliveira et al., 2001).

5. Conclusion

Solid state fermentation using *R. oligosporus* improved the nutritive value of GSP by increasing the reducing sugar by 61%, glucose and fructose by more than 100%, soluble protein by 1%, amino acids by 1.5 to 38.2% and producing cysteine at 222 ppm.

References

Association of Analytical Chemists, AOAC. (1990). *Official Methods of Analysis* (15th ed.), AOAC, Washington, DC, USA.

Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Annals of Biochemistry, 72*, 248-254. http://dx.doi.org/10.1016/0003-2697 (76)90527-3

Chen, L., Madl, R., & Vadlani, P. V. (2013). Nutritional enhancement of soy meal via *Aspergillus oryzae* solid state fermentation. *Cereal Chemistry, 90*(6), 529-534. http://dx.doi.org/10.1094/CCHEM-01-13-0007-R

Department of Statistics Malaysia. (2005). Sarawak Export of Sago Starch, SITC Code:592-150-100. Department of Statistics, Kuala Lumpur, Malaysia.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 350-356. http://dx.doi.org/10.1021/ac60111a017

Fogarty, W. M., & Kelly, C. (1980). Amylase, amyloglucosidase and related glucanases. In A. H. Rose (Ed.) *Microbial Enzymes and Bioconversions Economic Microbiology. Volume 5*. Academic Press, London, UK.

Ghose, T. K. (1987). Measurement of cellulase activities. *Pure and Applied Chemistry, 59*(2), 257-268. http://dx.doi.org/10.1351/pac198759020257

Gowthaman, N. K., Krishna, C., & Mou-Young, M. (2001). Fungal solid state fermentation – an overview. In G. G. Khachatourians & D. K. Arora (Eds.). *Applied Mycology and Biotechnology. Volume I. Agriculture and Food Production*. Elsevier Science, B.V.

Grossman, R. B., & Reinsch, T. G. (2002). SSSA Book Series: 5.*Methods of Soil Analysis*. In J. H. Dane, Clarke Topp, G. (Eds). Soil Science Society of America, Inc. Madison, Wisconsin.

Harris, L. E., Asplund, J., & Crampton, E. W. (1968). An international feed nomenclature and methods for summarizing and using feed data to calculate diets. *Utah State University Agricultural Experiment Station Bulletin* No. 479.

Hellín, P., Ros, J. M., & Laencina, J. (2001). Changes in high and low molecular weight carbohydrates during *Rhizopusnigricans* cultivation on lemon peel. *Carbohydrate Polymers, 45*, 169-174. http://dx.doi.org/10.1016/S0144-8617(00)00317-9

Hotz, C., & Gibson, R. S. (2007). Traditional food-processing and preparation practices to enhancing the bioavailability of micronutrients in plant-based diets. *Journal of Nutrition, 137*, 1097-1100.

Jin, B., Yin, P., Ma, Y., & Zhao, L. (2005). Production of lactic acid and fungal biomass by *Rhizopus* fungi from food processing waste stream. *Journal of Industrial Microbiology & Biotechnology, 3*, 678-686. http://dx.doi.org/10.1007/s10295-005-0045-4

Karim, A., Pei-Lang, A., Manan, D., & Zaidul, I. (2008). Starch from the Sago (*Metroxylon sagu*) palm trees – Properties, prospects and challenges as a new industrial source for food and other sources. *Comprehensive Review in Food Science and Food Safety, 7*, 215-227. http://dx.doi.org/10.1111/j.1541-4337.2008.00042.x

Liao, W., Liu, Y., & Chen, S. (2007). Studying pellet formation of a filamentous fungus *Rhizopus oryzae* to enhance organic acid production. *Applied Biochemistry and Biotechnology, 137*, 689-701. http://dx.doi.org/10.1007/ s12010-007-9089-4

Mandels, M., & Weber, J. (1974). Measurement of saccharifying cellulose. *Journal of Advances in Chemical Series, 95*, 391. http://dx.doi.org/10.1021/ba-1969-0095.ch023

Meng, X., & Slominski, B. A. (2005). Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a multicarbohydrase preparation of cell wall degrading enzymes. *Poultry Science, 84*, 1242-1251. http://dx.doi.org/10.1093/ps/84.8.1242

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry 31*(3), 426-428. http://dx.doi.org/10.1021/ac60147a030

Nagel, F., Oostra, J., Tramper, J., & Rinzema, A. (1999). Improved model system for solid-substrate fermentation: effects of pH, nutrients and buffer on fungal growth rate. *Process Biochemistry, 35*, 69-75. http://dx.doi.org/10.1016/S0032-9592(99)00034-5

National Research Council, NRC. (1994). *Nutrient Requirements of Poultry. Ninth revised edition.* Washington, DC, USA: National Academy Press.

Nout, M. J. R., & Kiers, J. L. (2005). Tempe fermentation, innovation and functionality: update into the third millenium. *Journal of Applied Microbiology, 98*, 789-805. http://dx.doi.org/10.1111/j.1365-2672.2004.02471.x

Oduguwa, O. O., Edema, M. O., & Ayeni, A. O. (2008). Physico-chemical and microbiological analyses of fermented corn cob, rice bran and cowpea husk for use in composite rabbit feed. *Bioresource Technology, 99*, 1816-1820. http://dx.doi.org/10.1016/j.biortech.2007.03.036

Olanipekun, B. F., Otunola, E. T., Adelakun, O. E., & Oyelade, O. J. (2009). Effect of fermentation with *Rhizopusoligosporus* on some physico-chemical properties of starch extracts from soybean flour. *Food and Chemical Toxicology, 47*, 1401-1405.http://dx.doi.org/10.1016/j.fct.2009.02.023

Oliveira, M. A., Rodrigues, C., dos Reis, E. M., & Nozaki, J. (2001). Production of fungal protein by solid substrate fermentation of cactus *Cereus peruvianus* and *Opuntia ficus indica*. *Quimica Nova, 24*(3), 307-310. http://dx.doi.org/10.1590/s0100-40422001000300004

Pal Vig, A., & Walia, A. (2001). Beneficial effects of *Rhizopus oligosporus* fermentation on reduction of glucosinolates, fibre and phytic acid in rapeseed (*Brassica napus*) meal. *Bioresource Technology, 78*, 309-312. http://dx.doi.org/10.1016/S0960-8524(01)00030-X

Schmidt, C. G., & Furlong, E. B. (2012). Effect of particle size and ammonium sulfate concentration on rice bran by fermentation with the fungus *Rhizopus oryzae*. *Bioresource Technology, 123*, 36-41. http://dx.doi.org/10.1016/j.biortech.2012.07.081

Rashid, S. S., Alam, M. Z., Karim, M. I. A., & Salleh, M. H. (2009). Management of palm oil mill effluent through production of cellulases by filamentous fungi. *World Journal of Microbiology and Biotechnology*, *25*, 2219-2226. http://dx.doi:10.1007/s11274-009-0129-9

Singhal, R., Kennedy, J., Gopalakrishnan, S., Kaczmarek, A., Knill, C., & Akmar, P. (2008). Industrial production, processing, and utilization of sago palm-derived products. *Carbohydrate Polymers, 72*, 1-20. http://dx.doi.org/10.1016/j.carbpol.2007.07.043

Singhania, R., Patel, A., Soccol, C., & Pandey, A. (2009). Recent advances in solid state fermentation. *Biochemical Engineering Journal, 44*, 13-18. http://dx.doi.org/10.1016/j.bej.2008.10.019.

Sparinga, R. A., Kendall, M., Westby, A., & Owens, J. D. (2002). Effects of temperature, pH, water activity and CO_2 concentration on growth of *Rhizopus oligosporus* NRRL 2710. *Journal of Applied Microbiology, 92*, 329-337. http://dx.doi.org/10.1046/j.1365-2672.2002.01534.x

Statistical Analytical System. (2002). *SAS user's guide: statistics*. SAS Inst. Inc., Cary, NC, USA.

Teng, D., Gao, M., Yang, Y., Liu, B., Tian, Z., & Wang, J. (2012). Bio-modification of soybean meal with *Bacillus subtilis* or *Aspergillus oryzae. Biocatalysis and Agricultural Biotechnology, 1*, 32-38. http://dx.doi.org/10.1016/j.bcab.2011.08.005

Yahya, M., Mahyuddin, M., Alimon, A. R., Abdullah, N., & Ivan, M. (2011). Sago pith meal based diets in sheep containing different sources of nitrogen: Feed preparation, growth performance, digestibility and carcass quality. *Animal Feed Science and Technology, 170*, 45-52. http://dx.doi.org/10.1016/j.anifeedsci.2011.08.009

Zhang, Z. Y., Jin, B., & Kelly, J. M. (2007). Production of lactic acid from renewable materials by *Rhizopus* fungi: Mini review. *Biochemical Engineering Journal, 35*, 251-263. http://dx.doi.org/ 10.1016/j.bej.2007.01.028