

RESEARCH ARTICLE

## Increasing Nutritional Contents of Cassava Starch Wastes Using *Pleurotus ostreatus* (Jacq.) P.Kumm. and *Lentinus squarrosulus* Mont.

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### Abstract

This research studied the increasing of nutritional content in cassava starch fermented with *P. ostreatus* (Jacq.) P.Kumm. and *L. squarrosulus* Mont. The cassava starch waste was supplemented with maize grain, paddy rice, rice bran, broken rice, and soybean meal at 0, 10, 20, and 30%. Solid state fermentation was carried out for 28 days. The results showed that highest reduced sugar at 86.09 g/L was found in cassava starch waste 70% + rice broken 30% fermented by *L. squarrosulus* Mont. The highest protein content at 127.92 g/L was found in cassava starch waste 90% + soybean meal 10% fermented with cassava starch waste 70% + rice broken 30% for 14 days. It is concluded from this experiment that *P. ostreatus* (Jacq.) P.Kumm. and *L. squarrosulus* Mont. can increase nutritional content in several feed mixtures.

**Keywords:** Cassava Starch Wastes, *Pleurotus ostreatus* (Jacq.) P.Kumm., nutritional content, *Lentinus squarrosulus* Mont.

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## INTRODUCTION

It is estimated that the world cassava production in the year 2016 can be as high as 288 million tons. This is produced mainly in the continents of Africa, Asia and South America. The production in Thailand is estimated to be 3.2 million tons<sup>1</sup> Thailand uses cassava for cassava flour (54%), cassava chip (46%) and ethanol production (2%). By product of the cassava flour production is 10 – 15% cassava starch wastes, and this was used for animal feed but with some limitation due to high fiber and low protein contents<sup>2</sup>. Several methods were used to increase nutritional contents of this by-product. Some yeast and fungi species were used to increase the nutritional contents by fermentation<sup>3,4</sup>. Mushroom classified in the white rot fungi group possess ability to digest lignin and able to increase protein as found in Jonathan *et al.*<sup>5</sup>. In these work, *P. pulmonarius* was used to cultivate with rice straw and sorghum stem. Protein content in rice straw was raised from 4.50% to 9.36% and 5.31% to 8.62% in the sorghum stem<sup>5</sup>, Bentil *et al.* also found that cocoa pulp protein content increased from 21% to 26% by using *P. ostreatus* for 6 weeks<sup>6</sup>. Darwish *et al.* also experiment by fermenting corn stem with *P. ostreatus* for 28 days and found that protein content was raised from 3.6% to 8.15%<sup>7</sup>. A work by Nasehi *et al.* using *P. florida* showed that protein content in rice straw was raised from 3.02% to 7.06% and that in wheat straw was raised from 3.71% to 7.38%<sup>8</sup>. This study was objected to determine the effect of a solid state fermentation involving *P. ostreatus* (Jacq.) P. Kumm. and *L. squarrosulus* Mont. on soluble protein and reducing sugars in cassava starch waste.

## MATERIALS AND METHODS

### Experimental Design

The experiment was laid out based on Completely Randomized Design (CRD) with 16 treatments and each treatment contain 3 replicates. This is as follow:

- treatment 1 : controls (cassava starch waste 100 %)
- treatment 2 : cassava starch waste 90 % + corn 10 %
- treatment 3 : cassava starch waste 80 % + corn 20 %
- treatment 4 : cassava starch waste 70 %

- + corn 30 %
- treatment 5 : cassava starch waste 90 % + paddy 10 %
- treatment 6 : cassava starch waste 80 % + paddy 20 %
- treatment 7 : cassava starch waste 70 % + paddy 30 %
- treatment 8 : cassava starch waste 90 % + rice bran 10 %
- treatment 9 : cassava starch waste 80 % + rice bran 20 %
- treatment 10 : cassava starch waste 70 % + rice bran 30 %
- treatment 11 : cassava starch waste 90 % + rice broken 10 %
- treatment 12 : cassava starch waste 80 % + rice broken 20 %
- treatment 13 : cassava starch waste 70 % + rice broken 30 %
- treatment 14 : cassava starch waste 90 % + soybean meal 10 %
- treatment 15 : cassava starch waste 80 % + soybean meal 20 %
- treatment 16 : cassava starch waste 70 % + soybean meal 30 %

The experiment is carried out in 15 x 100 mm petri dishes. Material from treatment 1 – 16 at 30 g was placed inside the dishes and autoclave at 121 degree Celsius for 60 minutes. The mycelia from *P. ostreatus* (Jacq.) P. Kumm. and *L. squarrosulus* Mont. was sub-cultured and stored at 27 and 35 degree Celsius, respectively for 14 and 28 days.

### Chemical analysis

Reduced sugar: The amount of reduced sugar present in the samples was determined using DNS methodology. The absorbance at 530 nm was measured using a spectrophotometer<sup>9</sup>.

Soluble protein: The soluble protein concentrations were evaluated using a colorimetric method based on the standard curve of bovine serum albumin at 750 nm<sup>10</sup>.

Carbon content: The carbon content was determined following the rapid wet oxidation method<sup>11</sup>.

## RESULTS AND DISCUSSION

### Reduced sugar

Reduced sugar content in various substrates was found to be significantly different

( $P < 0.05$ ) after fermentation with *P. ostreatus* (Jacq.) P.Kumm. for 14 days (Table 1). Treatment 13 (cassava starch waste 70 % + rice broken 30 %) showed the highest reduced sugar reading at 16.99 g/L. The lowest reduced sugar content was found in treatment 15 (cassava starch waste 80 % + soybean meal 20 %).

Reduced sugar contents was also found to be significantly different ( $P < 0.05$ ) after fermentation with *P. ostreatus* (Jacq.) P.Kumm. for 28 days (Table 2). At this period, treatment 10 (cassava starch waste 70 % + rice bran 30 %) showed the highest reduced sugar content (20.28

g/L). Treatment 16 (cassava starch waste 70 % + soybean meal 30 %) was found to contain the lowest reduced sugar reading (0.69 g/L).

Reduced sugar content in all treatments was also found to be significantly different ( $P < 0.05$ ) after fermentation with *L. squarrosulus* Mont. for 14 days (Table 1). Treatment 12 (cassava starch waste 70 % + rice broken 30 %) showed the highest reduced sugar reading at 22.87 g/L. The lowest reduced sugar content was found in treatment 15 (cassava starch waste 80 % + soybean meal 20 %).

Reduced sugar contents was also found to be significantly different ( $P < 0.05$ ) after

**Table 1.** Effect of *P. ostreatus* (Jacq.) P.Kumm. and *L. squarrosulus* Mont. on reduced sugar content at 14 days (g/L)

Type of substrate	Control	<i>P. ostreatus</i> (Jacq.) P.Kumm.		<i>L. squarrosulus</i> Mont.	
	14 day	14day	Increasing	14 day	Increasing
	g/L	g/L	g/L	g/L	g/L
cassava starch waste 100%	0.75 <sup>defg</sup>	14 <sup>b</sup>	13.26 <sup>a</sup>	7.17 <sup>bc</sup>	6.42 <sup>cd</sup>
cassava starch waste 90% + corn 10%	1.25 <sup>cdef</sup>	15.61 <sup>ab</sup>	14.37 <sup>a</sup>	11.29 <sup>abc</sup>	10.04 <sup>abcd</sup>
cassava starch waste 80% + corn 20%	1.32 <sup>cde</sup>	14.63 <sup>b</sup>	13.31 <sup>a</sup>	15.65 <sup>abc</sup>	14.34 <sup>abcd</sup>
cassava starch waste 70% + corn 30%	1.53 <sup>bcd</sup>	15.82 <sup>ab</sup>	14.29 <sup>a</sup>	13.76 <sup>abc</sup>	12.23 <sup>abcd</sup>
cassava starch waste 90% + paddy 10%	0.69 <sup>defg</sup>	13.65 <sup>b</sup>	12.97 <sup>a</sup>	8.65 <sup>bc</sup>	7.97 <sup>bcd</sup>
cassava starch waste 80% + paddy 20%	0.39 <sup>fg</sup>	14.91 <sup>b</sup>	14.52 <sup>a</sup>	16.89 <sup>abc</sup>	16.50 <sup>abcd</sup>
cassava starch waste 70% + paddy 30%	0.21 <sup>g</sup>	16.93 <sup>ab</sup>	16.72 <sup>a</sup>	20.89 <sup>ab</sup>	20.67 <sup>abc</sup>
cassava starch waste 90% + rice bran 10%	3.19 <sup>a</sup>	16.48 <sup>ab</sup>	13.28 <sup>a</sup>	14.66 <sup>abc</sup>	11.48 <sup>abcd</sup>
cassava starch waste 80% + rice bran 20%	3.93 <sup>a</sup>	19.24 <sup>a</sup>	15.31 <sup>a</sup>	17.75 <sup>abc</sup>	13.83 <sup>abcd</sup>
cassava starch waste 70% + rice bran 30%	3.79 <sup>a</sup>	8.36 <sup>c</sup>	4.57 <sup>b</sup>	17.26 <sup>abc</sup>	13.47 <sup>abcd</sup>
cassava starch waste 90% + rice brokens 10%	0.56 <sup>efg</sup>	14.75 <sup>b</sup>	14.19 <sup>a</sup>	22.82 <sup>ab</sup>	22.27 <sup>abc</sup>
cassava starch waste 80% + rice brokens 20%	0.36 <sup>fg</sup>	15.53 <sup>ab</sup>	15.17 <sup>a</sup>	26.20 <sup>a</sup>	25.84 <sup>a</sup>
cassava starch waste 70% + rice brokens 30%	0.35 <sup>fg</sup>	17.34 <sup>ab</sup>	16.99 <sup>a</sup>	23.21 <sup>ab</sup>	22.87 <sup>ab</sup>
cassava starch waste 90% + soybean meal 10%	1.59 <sup>bcd</sup>	6.01 <sup>cd</sup>	4.43 <sup>b</sup>	11.82 <sup>abc</sup>	10.23 <sup>abcd</sup>
cassava starch waste 80% + soybean meal 20%	2.27 <sup>b</sup>	2.35 <sup>d</sup>	0.075 <sup>b</sup>	2.885 <sup>c</sup>	0.61 <sup>d</sup>
cassava starch waste 70% + soybean meal 30%	2.12 <sup>bc</sup>	2.66 <sup>d</sup>	0.53 <sup>b</sup>	3.785 <sup>c</sup>	1.67 <sup>d</sup>

a, b, c, d, e, f, g = Means in the same column with different superscripts are significantly different ( $P = 0.05$ )

**Table 2.** Effect of *P. ostreatus* (Jacq.) P.Kumm. and *L. squarrosulus* Mont. on reduced sugar content at 28 day (g/L)

Type of substrate	Control	<i>P. ostreatus</i> (Jacq.) P.Kumm.		<i>L. squarrosulus</i> Mont.	
	28 day	28day	Increasing	28 day	Increasing
	g/L	g/L	g/L	g/L	g/L
cassava starch waste 100 %	0.66 <sup>b</sup>	8.61 <sup>e</sup>	7.95 <sup>f</sup>	8.82 <sup>hi</sup>	8.16 <sup>ghi</sup>
cassava starch waste 90% + corn 10%	1.46 <sup>b</sup>	12.03 <sup>de</sup>	10.58 <sup>ef</sup>	31.6 <sup>e</sup>	30.14 <sup>d</sup>
cassava starch waste 80% + corn 20%	1.69 <sup>b</sup>	16.89 <sup>bc</sup>	15.2 <sup>bcd</sup>	52.09 <sup>c</sup>	50.41 <sup>c</sup>
cassava starch waste 70% + corn 30%	1.76 <sup>b</sup>	13.26 <sup>cd</sup>	11.5 <sup>def</sup>	62.76 <sup>b</sup>	61 <sup>b</sup>
cassava starch waste 90% + paddy 10%	0.39 <sup>b</sup>	13.9 <sup>cd</sup>	13.52 <sup>bcde</sup>	21.1 <sup>fg</sup>	20.71 <sup>def</sup>
cassava starch waste 80% + paddy 20%	0.44 <sup>b</sup>	11.86 <sup>de</sup>	11.42 <sup>def</sup>	42.86 <sup>d</sup>	42.42 <sup>c</sup>
cassava starch waste 70% + paddy 30%	0.26 <sup>b</sup>	13.82 <sup>cd</sup>	13.57 <sup>bcde</sup>	47.2 <sup>cd</sup>	46.95 <sup>c</sup>
cassava starch waste 90% + rice bran 10%	1.36 <sup>b</sup>	18.67 <sup>b</sup>	17.32 <sup>ab</sup>	16.52 <sup>gh</sup>	15.17 <sup>efgh</sup>
cassava starch waste 80% + rice bran 20%	1.79 <sup>b</sup>	18.82 <sup>b</sup>	17.03 <sup>abc</sup>	18.18 <sup>g</sup>	16.39 <sup>efg</sup>
cassava starch waste 70% + rice bran 30%	5.17 <sup>a</sup>	25.44 <sup>a</sup>	20.28 <sup>a</sup>	28.19 <sup>ef</sup>	23.02 <sup>de</sup>
cassava starch waste 90% + rice brokens 10%	0.67 <sup>b</sup>	15.97 <sup>bcd</sup>	15.3 <sup>bcd</sup>	63.22 <sup>b</sup>	62.56 <sup>b</sup>
cassava starch waste 80% + rice brokens 20%	0.33 <sup>b</sup>	16.08 <sup>bcd</sup>	15.76 <sup>bc</sup>	80.41 <sup>a</sup>	80.09 <sup>a</sup>
cassava starch waste 70% + rice brokens 30%	0.32 <sup>b</sup>	13.51 <sup>cd</sup>	13.19 <sup>cde</sup>	86.41 <sup>a</sup>	86.09 <sup>a</sup>
cassava starch waste 90% + soybean meal 10%	1.14 <sup>b</sup>	2.15 <sup>f</sup>	1.01 <sup>g</sup>	14.47 <sup>gh</sup>	13.33 <sup>ghi</sup>
cassava starch waste 80% + soybean meal 20%	1.3 <sup>b</sup>	2.72 <sup>f</sup>	1.42 <sup>g</sup>	5.13 <sup>i</sup>	3.84 <sup>i</sup>
cassava starch waste 70% + soybean meal 30%	1.84 <sup>b</sup>	2.53 <sup>f</sup>	0.69 <sup>g</sup>	8.57 <sup>hi</sup>	6.73 <sup>hi</sup>

a, b, c, d, e, f, g, h, i = Means in the same column with different superscripts are significantly different (P=0.05)

fermentation with *P. ostreatus* (Jacq.) P.Kumm. for 28 days (Table 2). At this period, treatment 13 (cassava starch waste 70 % + rice broken 30 %) showed the highest reduced sugar content (86.09 g/L). Treatment 15 (cassava starch waste 80 % + soybean meal 20 %) was found to contain the lowest reduced sugar reading (3.84 g/L).

Reduced sugar content in every treatment increased as the fermentation time increased. Hossain *et al.* also found reduced sugar content increasing after using *P. sajor-caju* co-cultivated with bean shell, rice straw, wheat straw, and

sugarcane bagasse<sup>12</sup>. Adamafio *et al.* also found that cellulose was double after fermenting corn cob with *P. ostreatus*. Solid state fermentation with *P. ostreatus* decrease accumulated fiber by enzymes activities<sup>13</sup>. It is indicated that mushroom contains several enzymes that involves lignocellulose metabolism<sup>14</sup>. A similar finding was also found by Darwish *et al.* which used *P. ostreatus* and *Saccharomyces cerevisiae* with corn stem<sup>7</sup>. Benti *et al.* also found a similar result when experimented with cocoa pulp.

**Carbon content**

Carbon content in every treatment showed significantly different ( $P < 0.05$ ) after fermentation for 14 days (Table 3). It was found that cassava starch waste 70 % + corn 30 % showed the lowest reduction in carbon content (5.63%). It was also found that a substrate mixture of cassava starch waste 70 % + rice broken 20 % showed the lowest reduction in carbon content (0.11%). At day 28 after fermentation, the results were also significantly different ( $P < 0.05$ ) (Table 4). The

mixture of cassava starch waste 70 % + soybean meal 30 % found the highest reduction in carbon content (6.21%) and the mixture of cassava starch waste 90 % + rice broken 10 % resulted in the lowest reduction in carbon content (0.14%).

**Protein contents**

Protein content percentage in all treatments showed significantly different ( $P < 0.05$ ) after fermentation for 14 days (Table 5). It was found that protein content in cassava starch waste 70 % + soybean meal 30 % showed the highest

**Table 3.** Effect of *P. ostreatus* (Jacq.) P.Kumm. and *L. squarrosulus* Mont. on carbon percentage content at 14 day (g/L)

Type of substrate	Control <i>P. ostreatus</i> (Jacq.) P.Kumm. <i>L. squarrosulus</i> Mont.				
	14 day	14day	reduction	14 day	reduction
	%	%	%	%	%
cassava starch waste 90 % + corn 10 %	47.74 <sup>ab</sup>	42.68 <sup>abc</sup>	5.06 <sup>ab</sup>	47.11 <sup>ab</sup>	0.63 <sup>a</sup>
cassava starch waste 80 % + corn 20 %	47.62 <sup>abc</sup>	43.27 <sup>abc</sup>	4.36 <sup>abc</sup>	47.42 <sup>a</sup>	0.20 <sup>a</sup>
cassava starch waste 70 % + corn 30 %	48.33 <sup>ab</sup>	42.70 <sup>abc</sup>	5.63 <sup>a</sup>	46.34 <sup>ab</sup>	2.00 <sup>a</sup>
cassava starch waste 90 % + paddy 10 %	47.06 <sup>abc</sup>	46.48 <sup>a</sup>	0.58 <sup>bc</sup>	45.99 <sup>ab</sup>	1.06 <sup>a</sup>
cassava starch waste 80 % + paddy 20 %	45.4 <sup>bcd</sup>	44.84 <sup>abc</sup>	0.57 <sup>bc</sup>	43.45 <sup>abcd</sup>	1.95 <sup>a</sup>
cassava starch waste 70 % + paddy 30 %	46.42 <sup>abc</sup>	43.92 <sup>abc</sup>	2.50 <sup>abc</sup>	43.91 <sup>abcd</sup>	2.51 <sup>a</sup>
cassava starch waste 90 % + rice bran 10 %	48.25 <sup>ab</sup>	45.34 <sup>abc</sup>	2.91 <sup>abc</sup>	44.44 <sup>abc</sup>	3.81 <sup>a</sup>
cassava starch waste 80 % + rice bran 20 %	46.62 <sup>abc</sup>	46.11 <sup>ab</sup>	0.51 <sup>bc</sup>	42.83 <sup>abcd</sup>	3.78 <sup>a</sup>
cassava starch waste 70 % + rice bran 30 %	48.50 <sup>a</sup>	46.11 <sup>ab</sup>	2.39 <sup>abc</sup>	44.62 <sup>ab</sup>	3.88 <sup>a</sup>
cassava starch waste 90 % + rice broken 10 %	47.63 <sup>abc</sup>	46.32 <sup>a</sup>	1.31 <sup>abc</sup>	46.96 <sup>ab</sup>	0.67 <sup>a</sup>
cassava starch waste 80 % + rice broken 20 %	46.25 <sup>abcd</sup>	46.15 <sup>ab</sup>	0.11 <sup>c</sup>	45.73 <sup>ab</sup>	0.53 <sup>a</sup>
cassava starch waste 70 % + rice broken 30 %	48.4 <sup>a</sup>	45.58 <sup>ab</sup>	2.84 <sup>abc</sup>	44.64 <sup>ab</sup>	3.77 <sup>a</sup>
cassava starch waste 90 % + soybean meal 10 %	44.65 <sup>cd</sup>	44.18 <sup>abc</sup>	0.48 <sup>bc</sup>	42 <sup>bcd</sup>	2.65 <sup>a</sup>
cassava starch waste 80 % + soybean meal 20 %	43.33 <sup>de</sup>	41.19 <sup>bc</sup>	2.15 <sup>abc</sup>	39.36 <sup>cd</sup>	3.98 <sup>a</sup>
cassava starch waste 70 % + soybean meal 30 %	41.03 <sup>e</sup>	40.29 <sup>c</sup>	0.75 <sup>bc</sup>	38.82 <sup>d</sup>	2.21 <sup>a</sup>

a, b, c, d = Means in the same column with different superscripts are significantly different ( $P = 0.05$ )

**Table 4.** Effect of *P. ostreatus* (Jacq.) P.Kumm. and *L. squarrosulus* Mont. on carbon percentage content at 28 day (g/L)

Type of Substrate	Control		<i>P. ostreatus</i> (Jacq.) P.Kumm. <i>L. squarrosulus</i> Mont.		
	24 day	28day	reduction	28 day	reduction
	%	%	%	%	%
cassava starch waste	49.02 <sup>a</sup>	45.38 <sup>bc</sup>	3.64 <sup>ab</sup>	47.82 <sup>a</sup>	1.20 <sup>bc</sup>
90% + corn 10%					
cassava starch waste	48.83 <sup>ab</sup>	47.36 <sup>ab</sup>	1.47 <sup>bc</sup>	46.68 <sup>ab</sup>	2.15 <sup>abc</sup>
80% + corn 20%					
cassava starch waste	49.29 <sup>a</sup>	47.68 <sup>a</sup>	1.61 <sup>bc</sup>	45.98 <sup>abc</sup>	3.32 <sup>abc</sup>
70% + corn 30%					
cassava starch waste	46.04 <sup>cde</sup>	45.67 <sup>abc</sup>	0.37 <sup>c</sup>	45.2 <sup>abcd</sup>	0.84 <sup>c</sup>
90% + paddy 10%					
cassava starch waste	45.39 <sup>de</sup>	44.49 <sup>c</sup>	0.90 <sup>c</sup>	43.84 <sup>cd</sup>	1.55 <sup>bc</sup>
80% + paddy 20%					
cassava starch waste	44.81 <sup>ef</sup>	44.04 <sup>c</sup>	0.77 <sup>c</sup>	40.47 <sup>f</sup>	4.35 <sup>ab</sup>
70% + paddy 30%					
cassava starch waste	47.99 <sup>abcd</sup>	46.76 <sup>ab</sup>	1.23 <sup>bc</sup>	45.10 <sup>bcd</sup>	2.89 <sup>abc</sup>
90% + rice bran 10%					
cassava starch waste	48.02 <sup>abcd</sup>	45.87 <sup>abc</sup>	2.15 <sup>bc</sup>	43.52 <sup>cd</sup>	4.50 <sup>ab</sup>
80% + rice bran 20%					
cassava starch waste	48.17 <sup>abc</sup>	45.95 <sup>abc</sup>	2.22 <sup>bc</sup>	43.19 <sup>de</sup>	4.98 <sup>a</sup>
70% + rice bran 30%					
cassava starch waste	47.62 <sup>abcd</sup>	47.47 <sup>ab</sup>	0.15 <sup>c</sup>	46.68 <sup>ab</sup>	0.94 <sup>c</sup>
90% + rice broken 10%					
cassava starch waste	46.3 <sup>bcde</sup>	45.42 <sup>bc</sup>	0.88 <sup>c</sup>	46.07 <sup>abc</sup>	0.23 <sup>c</sup>
80% + rice broken 20%					
cassava starch waste	48.16 <sup>abc</sup>	47.23 <sup>ab</sup>	0.93 <sup>c</sup>	43.65 <sup>cd</sup>	4.51 <sup>ab</sup>
70% + rice broken 30%					
cassava starch waste	42.26 <sup>bcd</sup>	40.88 <sup>d</sup>	1.38 <sup>bc</sup>	40.74 <sup>ef</sup>	1.51 <sup>bc</sup>
90% + soybean meal 10%					
cassava starch waste	41.99 <sup>g</sup>	36.52 <sup>e</sup>	5.47 <sup>a</sup>	39.21 <sup>f</sup>	2.78 <sup>abc</sup>
80% + soybean meal 20%					
cassava starch waste	40.80 <sup>g</sup>	34.60 <sup>e</sup>	6.21 <sup>a</sup>	39.37 <sup>f</sup>	1.43 <sup>bc</sup>
70% + soybean meal 30%					

a, b, c, d, e, f, g = Means in the same column with different superscripts are significantly different (P=0.05)

protein content (69.92 mg/g). The mixture of cassava starch waste 70% + rice bran 30% showed the lowest protein content (3.93 mg/g). It was also showed significantly different (P<0.05) after fermentation for 28 days (Table 6). The highest protein content (51.43) were found in cassava starch waste 70% + soybean meal 30% and the lowest protein content were found in cassava starch waste 80% + paddy 20% (8.31 mg/g).

The experiment with *L. squarrosulus* Mont. for 14 days also showed significantly different (P<0.05) after fermentation for 14 days (Table 5). The highest protein content (127.92

mg/g) was found in cassava starch waste 90% + soybean meal 10%. The protein content in cassava starch waste 90% + paddy 10% showed the lowest percentage (3.40 mg/g). At day 28 after fermentation the result also showed significantly different (P<0.05) (Table 6). Cassava starch waste 80% + soybean meal 20% gave the highest protein content (100.52 mg/g). The lowest protein content (16.65 mg/g) was found in cassava starch 100%.

The result in this experiment clearly showed that both species of white rot fungi can increase protein content and other nutrients in cassava starch waste. An experiment by Akinifemi

et al<sup>15</sup> using *P. ostreatus* and *P. pulmonarius* co-cultivate with sorghum for 21 days showed that protein content increased from 2.54 to 4.54 and 4.59 respectively, the cocoa pulp nutrient increased when this was fermented with *P. ostreatus* for 6 weeks<sup>6,16</sup>. Bentil et al. study the influence of white rot fungi (*P. ostreatus*) on chemical compositions in agro-industrial residues and found that protein content sawdust and bagasse increased from 0.91% to 2.49% and 0.39%

and 2.99%, respectively<sup>16</sup>. Shrivastava et al. using *P. ostreatus* on sorghum straw and found protein content increased from 3.37% to 5.08%<sup>17</sup>. It was also found that carbon to nitrogen ratio decreased from 77.6 to 40.45 when compared to control without fermentation<sup>7</sup>. Experiment on corn stem using *P. ostreatus* by fermentation for 28 days and found that total protein was increased from 3.6% to 8.15%. Protein was increased from mycelium and released enzymes<sup>12</sup>.

**Table 5.** Effect of *P. ostreatus* (Jacq.) P.Kumm. and *L. squarrosulus* Mont. on protein content at 14 day (g/L)

Type of substrate	Control 14 day g/L	<i>P. ostreatus</i> (Jacq.) P.Kumm. 14day g/L	Increasing g/L	<i>L. squarrosulus</i> Mont. 14 day g/L	Increasing g/L
cassava starch waste 100%	3.75 <sup>d</sup>	9.51 <sup>f</sup>	5.91 <sup>cd</sup>	11.14 <sup>d</sup>	7.39 <sup>d</sup>
cassava starch waste 90% + corn 10%	4.63 <sup>d</sup>	19.28 <sup>de</sup>	10.88 <sup>cd</sup>	20.03 <sup>d</sup>	15.41 <sup>d</sup>
cassava starch waste 80% + corn 20%	6.88 <sup>d</sup>	21.21 <sup>cde</sup>	11.23 <sup>cd</sup>	22.16 <sup>d</sup>	15.28 <sup>d</sup>
cassava starch waste 70% + corn 30%	5.98 <sup>d</sup>	21.83 <sup>cde</sup>	11.51 <sup>cd</sup>	27.23 <sup>d</sup>	21.25 <sup>d</sup>
cassava starch waste 90% + paddy 10%	7.48 <sup>d</sup>	14.29 <sup>ef</sup>	7.83 <sup>cd</sup>	10.88 <sup>d</sup>	3.40 <sup>d</sup>
cassava starch waste 80% + paddy 20%	5.40 <sup>d</sup>	15.36 <sup>ef</sup>	7.69 <sup>cd</sup>	17.91 <sup>d</sup>	12.51 <sup>d</sup>
cassava starch waste 70% + paddy 30%	4.98 <sup>d</sup>	13.54 <sup>ef</sup>	5.68 <sup>cd</sup>	21.82 <sup>d</sup>	16.84 <sup>d</sup>
cassava starch waste 90% + rice bran 10%	13.13 <sup>cd</sup>	26.28 <sup>cd</sup>	9.48 <sup>cd</sup>	32.76 <sup>d</sup>	19.64 <sup>d</sup>
cassava starch waste 80% + rice bran 20%	21.69 <sup>c</sup>	28.59 <sup>c</sup>	7.25 <sup>cd</sup>	98.96 <sup>c</sup>	77.27 <sup>d</sup>
cassava starch waste 70% + rice bran 30%	19.85 <sup>c</sup>	28.54 <sup>c</sup>	3.94 <sup>d</sup>	114.17 <sup>c</sup>	94.31 <sup>bc</sup>
cassava starch waste 90% + rice broken 10%	6.21 <sup>d</sup>	14.93 <sup>ef</sup>	9.47 <sup>cd</sup>	21.70 <sup>d</sup>	15.49 <sup>d</sup>
cassava starch waste 80% + rice broken 20%	3.96 <sup>d</sup>	19.22 <sup>de</sup>	13.22 <sup>c</sup>	23.35 <sup>d</sup>	19.39 <sup>d</sup>
cassava starch waste 70% + rice broken 30%	3.92 <sup>d</sup>	19.30 <sup>de</sup>	12.80 <sup>cd</sup>	22.93 <sup>d</sup>	19.02 <sup>d</sup>
cassava starch waste 90% + soybean meal 10%	42.50 <sup>ab</sup>	83.13 <sup>b</sup>	51.05 <sup>b</sup>	170.42 <sup>a</sup>	127.92 <sup>a</sup>
cassava starch waste 80% + soybean meal 20%	37.08 <sup>b</sup>	108.03 <sup>a</sup>	61.36 <sup>a</sup>	146.04 <sup>b</sup>	108.96 <sup>ab</sup>
cassava starch waste 70% + soybean meal 30%	53.13 <sup>a</sup>	116.88 <sup>a</sup>	62.92 <sup>a</sup>	153.33 <sup>ab</sup>	100.21 <sup>b</sup>

<sup>a, b, c, d, e, f</sup> = Means in the same column with different superscripts are significantly different (P=0.05)

**Table 6.** Effect of *P. ostreatus* (Jacq.) P.Kumm. and *L. squarrosulus* Mont. on protein content at 28 day (g/L)

Type of Substrate	Control 28 day g/L	<i>P. ostreatus</i> (Jacq.) 28day g/L	<i>P. Kumm.</i> Increasing g/L	<i>L. squarrosulus</i> Mont. 28 day g/L	Increasing g/L
cassava starch waste 100%	3.04 <sup>h</sup>	13.08 <sup>d</sup>	8.55 <sup>d</sup>	19.69 <sup>g</sup>	16.65 <sup>g</sup>
cassava starch waste 90% + corn 10%	6.54 <sup>ef</sup>	20.11 <sup>d</sup>	12.00 <sup>cd</sup>	45.59 <sup>ef</sup>	39.04 <sup>efg</sup>
cassava starch waste 80% + corn 20%	7.21 <sup>e</sup>	19.49 <sup>d</sup>	11.24 <sup>cd</sup>	68.66 <sup>de</sup>	61.45 <sup>cde</sup>
cassava starch waste 70% + corn 30%	7.31 <sup>e</sup>	23.11 <sup>d</sup>	14.13 <sup>bcd</sup>	103.21 <sup>ab</sup>	95.90 <sup>ab</sup>
cassava starch waste 90% + paddy 10%	4.44 <sup>fgh</sup>	17.75 <sup>d</sup>	9.90 <sup>d</sup>	23.18 <sup>fg</sup>	18.74 <sup>g</sup>
cassava starch waste 80% + paddy 20%	5.46 <sup>efg</sup>	16.56 <sup>d</sup>	8.89 <sup>d</sup>	27.49 <sup>fg</sup>	22.03 <sup>g</sup>
cassava starch waste 70% + paddy 30%	4.69 <sup>fgh</sup>	18.03 <sup>d</sup>	11.57 <sup>cd</sup>	36.79 <sup>fg</sup>	32.10 <sup>fg</sup>
cassava starch waste 90% + rice bran 10%	12.88 <sup>d</sup>	26.02 <sup>d</sup>	13.52 <sup>cd</sup>	62.87 <sup>de</sup>	49.99 <sup>def</sup>
cassava starch waste 80% + rice bran 20%	15.50 <sup>c</sup>	51.34 <sup>d</sup>	32.15 <sup>abc</sup>	66.05 <sup>de</sup>	50.55 <sup>def</sup>
cassava starch waste 70% + rice bran 30%	18.52 <sup>b</sup>	60.46 <sup>bc</sup>	35.69 <sup>ab</sup>	95.53 <sup>abc</sup>	77.01 <sup>abc</sup>
cassava starch waste 90% + rice broken 10%	5.35 <sup>efgh</sup>	20.74 <sup>d</sup>	16.55 <sup>bcd</sup>	44.18 <sup>efg</sup>	38.82 <sup>efd</sup>
cassava starch waste 80% + rice broken 20%	3.98 <sup>gh</sup>	23.24 <sup>d</sup>	17.57 <sup>bcd</sup>	77.49 <sup>cd</sup>	73.51 <sup>bcd</sup>
cassava starch waste 70% + rice broken 30%	3.06 <sup>h</sup>	23.50 <sup>d</sup>	16.38 <sup>bcd</sup>	79.01 <sup>bcd</sup>	75.94 <sup>abc</sup>
cassava starch waste 90% + soybean meal 10%	18.00 <sup>b</sup>	54.38 <sup>c</sup>	29.17 <sup>bcd</sup>	82.09 <sup>bcd</sup>	64.09 <sup>cd</sup>
cassava starch waste 80% + soybean meal 20%	19.23 <sup>b</sup>	81.41 <sup>ab</sup>	11.20 <sup>cd</sup>	119.75 <sup>a</sup>	100.52 <sup>a</sup>
cassava starch waste 70% + soybean meal 30%	23.88 <sup>a</sup>	98.10 <sup>a</sup>	51.43 <sup>a</sup>	116.31 <sup>a</sup>	92.43 <sup>ab</sup>

a, b, c, d, e, f, g, h = Means in the same column with different superscripts are significantly different (P=0.05)

## CONCLUSION

We conclude that *Pleurotus ostreatus* (Jacq.) P.Kumm. and *Lentinus squarrosulus* Mont. can increase protein content, and reduced sugar in the substrates with significantly different (P<0.05). Carbon percentage was decreased after fermented with both species. The substrates were able to be used in livestock feed.

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## CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

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