



## Compositional changes in nutritional content of soybean straw agro-wastes during the production cycle of *Pleurotus sajor-caju*

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### Abstract

*Pleurotus sajor-caju* was cultivated on soybean straw for analyzing the nutritional content of spent mushroom straw (SMS). Results showed an increase in protein (3.60 to 5.85%), ash (7.50 to 9.20%) and moisture (7.00 to 8.30%) content while a decrease in pH (7.20 to 5.50%), Crude fiber (41.50 to 22.00%), total carbohydrates (81.00 to 51.60%), lignin (29.00 to 12.20%), cellulose (39.10 to 28.50%), hemicellulose (21.30 to 08.50%) and tannin (38.80 to 23.33%) content in spent straw as compare to soybean straw. During vegetative growth of the fungus (spawn run period), lignin degradation was faster and during fructification, lignin degradation was slower while cellulose and hemicellulose degradation in straw was faster in rate during fructification. The individual amino acid concentration in spent straw was also found to be increased. The cultivation of *Pleurotus sajor-caju* on soybean straw increased the nutritional content of spent straw. The protein content of spent straw increased while the lingo cellulosic content was reduced, making it more digestible and thus improved the potential feeding value of the resultant substrates as feed resources for ruminants.

**Keywords:** soybean straw agro, *Pleurotus sajor-caju*

### Introduction

In agriculture, there is a huge production of biomass as straw is a potent source of energy for ruminant nutrition but the availability of this energy is very low. In order to improve the nutritional composition of straw, several chemical and physical methods have been extensively undertaken, but most of them did not have practical applicability (Leng, 1991; Zahedifar, 1997) [31, 57]. In recent past, de-lignification of straw by solid-state fermentation (SSF) has been explored owing its capacity to of preferential removal of lignin (Fazaeli *et al.*, 1999) [15]. The straw content primarily comprises of cellulose (40%–50%), hemicelluloses (25%–30%), and lignin (15%–20%) (Chaurasia, 2019) [9].

The bio-conversion of straw is circumscribed to the group of white-rot fungi, which are capable to colonize on cereal straw and liberate water soluble substrates from the polymers during solid-state fermentation (SSF) and thus improve the digestibility (Zadrazil, 1997; Fazaeli *et al.*, 2003) [57, 14]. Hemicellulose, cellulose, and lignin serve as an energy source for fungal growth because they contain carbon, hydrogen, and oxygen, clarifying their decrease along the cultivation cycle (Andrade *et al.* 2010) [3]. Among the edible white-rot fungi, the *Pleurotus* species have been shown to be more efficient lignin-degrading fungi (Taniguchi *et al.*, 2005) [52]. *Pleurotus* mushroom (*P. ostreatus*) can decompose the cellular wall components present in the raw lingo cellulosic material, like cellulose, lignin, and hemicellulose through the action of complex

oxidative and hydrolytic enzymatic systems (Fernández-Fueyo *et al.* 2016) [16]. During bioconversion of lignocellulosic wastes in to feed at least one of the three objectives must be reached: (i) An increase in the protein level (ii) An increase in digestibility (iii) An increase in the essential amino acids (Kamara and Zadrazil, 1988) [24]. Fazaeli, (2007) [13] showed that fungal treatments upgrade the nutritional content of wheat straw by increasing the crude protein and ash content but decreasing the OM, NDF, ADF, ADL, cellulose, and hemicellulose contents. *Pleurotus* fungi are known to degrade large insoluble components of lingo cellulosic materials and hence play an important role in their bioconversion to foods and dietary supplements (Bisaria *et al.*, 1987) [5]. Due to a large variety of non-specific lingo cellulosic enzymes produced by *Pleurotus* spp., they can be grown on a large number of agro wastes, besides the traditional rice and wheat straw (WS) substrates (Zhang, Li, & Fadel, 2002) [58]. These lingo cellulosic materials are generally low in protein content.

Various types of agro-waste such as wheat straw (Ruhl and Kues, 2007) [49], hazelnut husk (Ozcelik and Peksen, 2007) [38], barley straw and vineyard prunings (Gaitán-Hernández *et al.*, 2011) and sunflower hulls (Curvetto *et al.*, 2005) [11], paddy straw, soybean straw, and wheat straw (Mane *et al.* 2007) [34] have been researched for use as growing substrates in *Pleurotus* cultivation. Many other types of substrates were also reported to be useful for the cultivation of various species of *Pleurotus* spp. (Table 1).

Table 1

S.N.	Substrate	Reference
1	Waste tea leaves	Bisht and Harsh, 1983 [6]
2	Rice straw + waste paper + woodchips and their combination	Mathew, CY,2018 [35]

3	Cotton seed, paper waste, wheat straw, and sawdust	Girmay <i>et al.</i> , 2016 [20].
4	Cotton waste, rice straw, cocoyam peels and sawdusts of <i>Khaya ivorensis</i> , <i>Mansonia altissima</i> and <i>Boscia angustifolia</i> .	Kuforiji and Fasidi, 2009 [29].
5	Sugarcane bagasse (ScB), rice straw (RS), wheat straw (WS) and the combination of these substrates in ratio of 1: 1.	Khalaphallah, R. <i>et al.</i> (2020) [26]
6	Cotton stalks, groundnut haulms, soybean straw, pigeon pea stalks and leaves and wheat straw.	Mane <i>et al.</i> (2007) [34]
7	Soybean straw, paddy straw, wheat straw, groundnut straw, Pigeon pea stalk and sunflower stalk.	Patil, S. S. (2012) [44]
8	Water weeds	Jain <i>et al.</i> , 1988 [23]
9	soybean straw, paddy straw, wheat straw and their combination in 1:1 proportion	Ahmed <i>et al.</i> (2009) [11]; Telang <i>et al.</i> , 2010 [53]
10	Mustard haulm	Mukherjee and Nandi, 2001 [36]
11	Soybean straw, paddy straw, groundnut straw, sorghum, Tur stalk and sunflower stalk.	Patil and Baig, 2020 [42].
12	Water hyacinth	Murugesan <i>et al.</i> , 1995 [37]
13	Saw dust	Gurjar and Doshi, 1995 [21]
14	Coffee pulp	Guzman and Martinez, 1986 [22]
15	Wood logs and trunks	Chang and Miles, 1993 [8]
16	Oil palm bunch refuse	Ramesh and Ansari, 1987 [47]
17	Coconut and anjali logs	Suharban and Nair, 1991 [57]
18	<i>Lantana camara</i> (Verbenaceae)	Vats <i>et al.</i> , 1994 [55]
19	Chopped branches of <i>Euphorbia royleana</i>	Kaul and Janardhan, 1970 [25]
20	Maize stalk, pea residue (tendrils) and banana leaves with and without mixture of rice bran and chicken manure.	Pokhrel <i>et al.</i> , 2013 [46].

In mushroom producing regions, the spent mushroom substrate (SMS), is generated in large quantity as 1 kg of fresh mushrooms utilizes nearly 5 kg of spent substrate (Finney *et al.* 2009) [17]. SMS are bulky products long considered a waste stream (Pardo-Giménez *et al.* 2012) [40]. The traditional methods of disposal or burning it without any important use are neither eco-friendly nor economic (Carrasco *et al.* 2018) [7] which could cause a series of environmental problems including air pollution. Therefore, it is necessary to adopt new methods for the beneficial use of SMS in improved applications. The spent mushroom substrates have potential benefits in many fields, like a soil-less growing medium (Ribas *et al.*, 2009) [48], soil and water bioremediation (García-Delgado *et al.*, 2013; Lau *et al.*, 2003; Li *et al.*, 2012) [19, 30], energy feedstocks (Finney *et al.*, 2009) [17], animal feeds (Li *et al.*, 2001), and organic amendments (Paula *et al.*, 2017) [45]. However, most of these applications are not viable, and are unable to completely solve the disposal problem of SMSs; only agricultural use is an economically and ecologically acceptable way (Paredes *et al.*, 2016) [41]. During last decade, the area under soybean crop has increased in this part of India and a large quantity of biomass is produced after the cultivation of soybean. This soybean straw following harvest is used as animal feed (Terashima *et al.* 2009) [54]. Many studies have shown use of the soybean straw for cultivation of *P. sajor-caju* (Dehariya and Vyas, 2020; Mane *et al.*, 2007; Patil *et al.*, 2010) [12, 34, 53].

But even after harvest the nutritional status of the spent straw need to be investigated. Attempt were made to analyze the spent straw for use in animal feed with enhanced nutrition or application as soil conditioner following grinding of the spent straw. The present study evaluated important information about the chemical / nutritional content along with amino acid profile of the soybean spent straw during and after cultivation of *P. sajor-caju* at different growth stages.

## Material and Methods

### Strains of mushroom

*Pleurotus sajor-caju* strain was obtained from National Centre for Industrial Microbes, National Chemical Laboratory, Pune, India. The cultures were preserved on 2

% malt extract agar slants at 4° C. Sub-culturing were done after every 15 days interval.

### Spawn preparation

Spawn was prepared in polythene packets. Sorghum grains were boiled in water bath for 10-15 min in the ratio of 1:1 (Sorghum grains: water) and mixed with 4% (w/w) CaCO<sub>3</sub> and 2% (w/w) CaSO<sub>4</sub>. Sorghum grains were then packed (250g) in polythene bags (of 200x300 mm. size) and sterilized in an autoclave at 121°C for 30 min. After sterilization, the bags were inoculated with actively growing mycelium of the *P. sajor-caju* from malt extract slants and incubated (at 27±2 °C) for mycelial growth without any light for 10-15 days until the mycelium fully covered the grains.

### Experimental details

Experiment was conducted in Randomized block design with five replications.

### Cultivation of mushroom

Soybean straw was used as cultivation substrates following the method described earlier (Patil and Baig, 2020) [42].

### Chemical analysis

Analysis of moisture, protein, fat, crude fibre, total carbohydrates, ash, lignin, cellulose, hemicellulose and tannin of samples were done by standard methods (AOAC, 1995) [4].

### Amino acid analysis

Amino acid composition of dried samples of soybean straw before and after mushroom cultivation was determined by using a high performance liquid chromatography (HPLC) based amino acid analyser.

### Extraction

One gram of each dried and pulverised straw sample was soaked in 20 ml of distilled water for 24 h and the resulting

extract was filtered and kept in the refrigerator until when required for analysis.

### Amino acid analysis

The amino acid composition of each sample was determined using a high performance liquid chromatograph (HPLC)-based amino acid analyzer (Agilent 1120 Compact LC) with single binary pump; Variable Wavelength Detector and the column used was ZORBAX Eclipse C18 with a detection wavelength 450 nm with the Ezchrom elite compact compliance software for data processing. The standards for different amino acids was procured from Himedia Mumbai and calibration chromatogram was established for 22 known amino acids (L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-cystine, L-glutamic acid, L-glutamine, glycine, L-histidine, trans-4-hydroxy-L-proline, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine). Thus, a 0.05 mmol standard solution of each of the standard amino acids was prepared by dissolving the corresponding acid in distilled water and then a mixture was constituted by mixing 1ml of each of the 22 standard amino acid solutions and this was later used to establish the standard chromatogram. The mobile phase consisted of a 10 mM aqueous sodium phosphate (pH 6.8) solution (buffer solution A) mixed with acetonitrile, running in a gradient, starting with a mixture consisting of 5% acetonitrile in the buffer solution, and ending with acetonitrile alone. The free amino acids in the standards and in the mushroom species were automatically derivatized by reacting with o-phthalaldehyde under basic conditions to produce o-phthalaldehyde derivatives in the reaction columns of the amino acid analyser. Two derivatization reagent solutions were prepared as follows: to 10 ml of 0.01 M sodium borate (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> · 10H<sub>2</sub>O) buffer solution B (pH 9.1) were added 10 ml of b mercaptopropionic acid to make reagent solution I. Reagent solution II was prepared by mixing 10 ml of 0.01 M sodium borate (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> · 10H<sub>2</sub>O) buffer solution B (pH 9.1) with 10 mg of o-phthalaldehyde (OPA) dissolved in 3 ml of ethanol. Solutions I and II were filtered through 0.45 mm membrane filters before use. Following derivatization, the buffer solution A (mixed in acetonitrile in a 2:1 v/v ratio), containing the derivatized amino acids, was transferred into the HPLC for separation at a temperature of 45 °C, with 10 ml injection volume and a flow volume of 1.0 ml/min.

### Result and Discussion

Table 1 shows the variation in chemical composition of soybean straw during the mushroom growth. In this study we found that the gradual decrease in pH of substrate from 7.20 to 5.50 % while the increase in moisture content of substrate from 7.00 to 8.30 %. Earlier Funda Atila, (2019) [18] also reported the variation in pH values and moisture

content of substrate in the initial period than those of the colonized and spent substrates.

Protein content of substrate was 3.60 %, it is decreased up to 3.35 % after sterilization in autoclave. When the spawn of mushroom is allowed to grow on this substrate, the protein content of substrate was found to increase to 5.00 % after colonization of mushroom mycelia over substrate. The protein content of substrate was 5.30, 5.70 and 5.85 % after the successive three pickings. Increase in protein content of spent straw was reported earlier by Patil *et al.*, (2010a) [53]. This result is also in agreement with the findings of Akinyele and Akinbsoye, (2005) [2]. Increased values of crude protein may have been a result of presence of some fungal biomass with substrate (Chen *et al.* 1995) [10].

Ash content of substrate was 7.50 % and decreased after autoclaved to 6.20 %. After spawn run period ash content reduced to 6.60 % but maximum increase were found (from 7.90 to 9.20 %) during fructification. Zhang *et al.* (2002) [58] and Adamovic *et al.* (1998) also reported an increase in ash content of substrate during fruit fication. Khattab *et al.*, 2013 [27] also reported that increase in crude protein (3.4 to 11.7 %) and ash (16.1 to 29.1 %) in rice spent straw. Increased amount of ash in the spent straw may have been due to the decrease in the organic matter content (Singh, 2000) [50].

Crude fibre content of substrate was 41.50 % which were found to be reduced consistently up to 22.00 % during fungal growth. Decrease in crude fibre content was reported earlier by Kinfemi *et al.* 2009 [28].

Total carbohydrate content of substrate was 81.00 % which reduced to 75.50 % after spawn run was completed and after IIIrd picking it was 51.60 %.

The maximum reduction in cellulose (from 39.10 to 28.50 %), hemicellulose (21.30 to 08.50%), lignin (from 29.00 to 12.20 %) and tannin (from 38.80 to 23.33 %) content of substrate were reported. It was observed that cellulose degradation rate during fructification was faster than in spawn run period. Li *et al.* (2001) also noted that the faster rate of reduction of cellulose in substrate after spawn run period. Hemicellulose content of substrate increased slightly during spawn run period then reduced in faster rate. Earlier Pandey and Singh (2014) [39] also reported that during vegetative growth of the fungus, lignin degradation was faster and during fructification, lignin degradation was slower than cellulose and hemicellulose. The degradation of lignin was more during spawn run period then the rate of degradation slowed down during fructification. This finding was supported by Singh (2000) [50] and Li *et al.* (2001) who reported maximum lignin degradation during spawn run period.

**Table 2:** Chemical composition of soybean straw and soybean spent straw

Substrates	Un-autoclaved	Autoclaved	Spawn run	I st picking	II nd picking	IIIrd picking
pH	7.20	6.90	6.70	6.25	5.80	5.50
Moisture	7.00	7.60	7.85	8.00	8.20	8.30
Protein	3.60	3.35	5.00	5.30	5.70	5.85
Ash	7.50	6.20	6.60	7.90	8.75	9.20
Crude fibre	41.50	39.00	29.20	28.80	24.50	22.00
Total carbohydrates	81.00	80.10	75.50	69.80	55.80	51.60
Lignin	29.00	27.10	18.30	15.00	13.50	12.20
Cellulose	39.10	38.30	38.50	35.20	30.80	28.50
Hemi-cellulose	21.30	19.80	20.10	16.20	11.10	08.50
Tannin	38.80	37.00	30.60	27.30	25.10	23.33

(The above values are in %)

**Table 3:** Amino acid profile of soybean straw and soybean spent straw

Amino acid	Un-autoclaved	Autoclaved	Spawn run	I <sup>st</sup> picking	II <sup>nd</sup> picking	III <sup>rd</sup> picking
Alanine	31.2 ± 1.8	26.5	34.2	39.8	48.3	51.6
Arginine	28.5 ± 1.4	22.6	33.6	43.5	55.4	59.2
Aspartic acid	52.1 ± 1.9	41.5	64.2	68.5	89.7	90.2
Cystine	8.8 ± 0.2	5.5	9.2	9.6	15.4	15.0
Glutamic acid	72.2 ± 1.6	59.8	81.6	87.5	126.2	128.5
Glycine	15.2 ± 1.4	12.8	16.5	19.6	19.8	18.2
Histidine	19.9 ± 0.6	11.8	20.6	24.4	22.6	21.8
Lysine	42.6 ± 2.2	35.6	59.8	68.5	85.2	83.5
Methionine	8.8 ± 0.2	4.8	6.8	7.2	7.6	09.2
Phenyl alanine	52.2 ± 2.6	40.9	56.5	55.2	52.5	46.2
Proline	31.2 ± 1.3	17.6	28.5	32.8	58.5	64.6
Serine	18.0 ± 0.4	15.2	28.4	31.2	42.8	45.0
Threonine	30.5 ± 1.2	19.6	34.8	35.2	38.5	38.8
Tryptophan	9.2 ± 0.6	5.6	11.8	12.6	18.2	16.0
Tyrosine	48.5 ± 0.8	40.2	47.8	47.2	43.8	41.5
Valine	35.4 ± 1.2	30.0	38.2	38.5	42.5	43.2
Leucine	83.0 ± 2.6	62.6	88.2	95.2	96.5	95.5
Isoleucine	25.5 ± 0.2	19.2	28.5	32.6	39.2	37.5
Glutamine	9.6 ± 0.8	4.7	10.8	11.2	15.5	15.9

(The above values are in mg/100g of dry straw)

Amino acid analysis revealed that fungal treatment at different growth stages has considerably improved the individual amino acid content of soybean straw. Only two individual amino acids were lowered in spent straw i.e. phenyl alanine and tyrosine than the original straw used while the most dominant amino acid was glutamic acid then leucine and aspartic acid (Table 2). However, increase in the quantity of amino acid was not uniform for all the amino acids. Glutamic acid concentration (72.2 mg/100gm) and leucine (83.0 mg/100gm) of soybean straw were increased to 128.5 and 95.5 mg/100gm respectively, which were found to be higher among all the amino acid content of spent straw. The maximum increase in amino acid content of spent straw were observed in case of serine (from 18.0 to 45.0 mg/100gm), proline (from 31.2 to 64.6 mg/100gm), while slight increase was observed in case of methionine (from 08.8 to 09.2 mg/100gm). The phenyl alanine and tyrosine content of soybean straw were found to reduced (from 52.2 to 46.2 and 48.5 to 41.5 mg/100gm respectively) in spent straw. Khattab *et al.*, (2013) also reported the variation in amino acid content of spent straw but slight increase in case of methionine.

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