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PHYSICOCHEMICAL CHARACTERIZATION OF COMPOSTS FOR THE CULTIVATION OF *Pleurotus ostreatus*

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ABSTRACT: *Pleurotus ostreatus* var. Florida is one of the main varieties of mushroom that is expanding in the Brazilian market. In Brazil, the most widely used method to obtain the cultivation substrate is the short composting with subsequent steam pasteurization and conditioning. Nevertheless, there is not a standard procedure related to the preparation of substrate adopted by Brazilian mushroom producers, therefore it becomes relevant to find out through research a more efficient method in which producers may back themselves up. Thus, tests were carried out in three different composting periods (T1= 6 days, T2= 4 days and T3=2 days) corresponding to three treatments. The experiment occurred between August and October 2013 by relating the physical and chemical characteristics of the composts with the mushroom production parameters. Treatments T1 and T2 presented the best results regarding biological efficiency (T1=108.91% and T2=102.49%, respectively) and productivity (T1=26.22% and T2=25.72 %), as well as a higher number of harvested mushrooms (T1=44.44 and T2=43.52). These treatments presented the best results mainly due to a lower C/N ratio (T1=47 and T2=46) and a higher amount of crude protein (6.61% and 6.52%) available for the compost at the end of Phase II. These two periods of composting also influenced the mushroom cellulose and hemicellulose consumption by the fungus. Therefore, composting influences the production of *P. ostreatus* var. Florida.

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KEY WORDS: Oyster mushroom; Biological efficiency; Shimeji; Composting; Sugar cane.

CARACTERIZAÇÃO FÍSICO-QUÍMICA DE COMPOSTOS PARA O CULTIVO DO *Pleurotus ostreatus*

RESUMO: O Pleurotus ostreatus var. Florida é um dos principais cogumelos em expansão no mercado brasileiro. No Brasil, o método mais utilizado no preparo do substrato de cultivo envolve uma compostagem curta com posterior pasteurização a vapor e condicionamento. Contudo, não há um protocolo padrão adotado pelos produtores de cogumelos no Brasil em relação à preparação do substrato. Portanto, torna-se relevante a pesquisa de um método mais eficiente no qual possam se basear. Assim, foram testados três períodos diferentes de compostagem (T1= 6 dias, T2= 4 dias e T3= 2 dias), correspondentes a três tratamentos. O experimento foi realizado no período de agosto a outubro de 2013, relacionado as características físico-químicas dos compostos com parâmetros da produção do cogumelo. Os tratamentos T1 e T2 apresentaram os melhores resultados de eficiência biológica (T1=108,91% e T2=102,49%) e produtividade (T1=26,22% e T2=25,72%), assim como maiores números de cachos colhidos (T=44,44 e T2=43,52). Estes tratamentos apresentaram esses resultados devidos principalmente à menor relação C/N (47 e 46) e à maior concentração de proteína bruta (6,61% e 6,52%) disponível ao cogumelo ao final da Fase II; além disso, estimularam o consumo de celulose e hemicelulose pelo fungo. Portanto, há influência do tempo de compostagem na produção do cogumelo P. ostreatus var. Florida.

PALAVRAS-CHAVE: Cogumelo ostra; Eficiência biológica; Shimeji; Compostagem; Cana-de-açúcar.

INTRODUCTION

P. ostreatus Florida is one the varieties of mushroom that is expanding in the Brazilian market due mainly to its cultivation characteristics; it resists temperatures between 25 and 30°C in rustic installations, with minimum control (humidity and insects). The best method for a non-sterile cultivation is the collection of the substrate by short composting, followed by steam pasteurization and a high C/N re-

lation (between 60 and 90/1) (DIAS, 2010, p. 799). Such methods gives the compost a better protection against damaging contaminants for the mushroom development due to the action of a desirable microbiota that is responsible for the transformation of the compost during the humidification, composting and pasteurization phases of the raw material.

The composting and pasteurization currently used in Brazil are based on procedures adopted in China, Europe and the United States, where the climate and raw materials are different. Thus, research showing better ways to conduct these steps of the mushroom production is necessary to reach satisfactory results that also involve tests with different strains, searching the ones that have the highest productivity and biological efficiency, showing that, among other factors, they are adapted to the national weather.

Research addressing the aspects of mushroom production – such as the studies carried by Dias (2010), Siqueira (2012) and Vieira (2012), from raw material tests, a topic extensively addressed in research, to the metabolic processes of catalytic chemical reactions involved in the biodegradation of lignocellulose, as well as the selection of strains, among others, is extremely important to the fungi culture development.

In this context, the aim of this study was to analyze the physicochemical transformations that occur in the composts made of sugarcane straw and wheat bran, obtained in different periods of the Phase I of composting, and relate them with *P. ostreatus* var. Florida production parameters.

2 MATERIALS AND METHODS

The experiment was carried out at the Mushroom Module at the FCA/UNESP, Botucatu, a city located in the state of São Paulo - latitude 22.88° and longitude -48.44° (IBGE, 2016) in the period between August and October 2013, which is comprised of three treatments and composting times: 6, 4 and 2 days.

2.1 OBTAINING OF THE SPAWN

The strain POS 09/101, which was taken from the Mushroom Module bank of strains, was isolated from a basidioma obtained in the city of São Pedro/SP in 2009. The mycelium was maintained in PDA and the spawn was connected to the compost in wheat grain.

2.2 PREPARATION OF THE SUBSTRATE

A unique formulation of the compost was used for the three stacks of composting that were prepared. To calculate the amounts (in Kg) of sugarcane straw and wheat bran, the weights on dry basis for each material were used. The formulation was established to have an initial C/N ratio of approximately 66/1. The gypsum ($CaSO_4$) is responsible for controlling the humidity and the calcitic lime for ensuring a buffering effect in the control of acidity. Table 1 shows the amount of each ingredient used for each composting stack.

Table 1. Formulation of the compost

Ingredients	Wet mass	Dry mass	C	N	Humidity	C/N ratio (1)	Amount
	Kg	Kg	G kg $^{-1}$		%		kg
Sugarcane straw	190.5	180	480	5	5.5	96	86.5
Wheat bran	25.27	23	460	25	9	18	11
Calcitic lime	3	3	---	---	---	---	1.5
Gypsum	2	2	---	---	---	---	1
Initial carbon in the compost	---	96.98	86400	900	---	---	---
Initial nitrogen in the compost	---	1.48	10580	575	---	---	---
Initial C/N in the compost	---	---	----	----	---	65.75	---

(1) To perform the calculation, humidity, nitrogen (N) and carbon (C) concentrations of sugarcane straw and wheat bran were determined before the pre-moistening

First, the straw was pre-moistened for three days, and then turned every 24 hours. Next, the wheat bran was added with the calcitic lime and the gypsum. The measures were set following the approximate dimensions of 1.5 m high and 1.5 m wide and were turned every 48 h. During the turnings, water was added until there was a water flow in the ground, due to water excess.

At the end of Phase I, the composts were added manually in latticed polypropylene boxes and submitted to pasteurization and conditioning in Dalsem Mushroom equipment – a type "Reffer" Dutch container, with dimensions of 12 m long x 2.25 m high x 2 m wide. The process of pasteurization at 60 ± 2 °C during 8 hours and the conditioning at 48 ± 2 °C for 72 hours were programed on the Dalsem Mushroom Projects software system, version 20000301,001 and on the VEC 31 control and automation system. The mass of compost in each box was verified before and after the pasteurization stage followed by the conditioning.

After Phase II and the cooling of the substrate, the inoculation was performed manually by packing the substrate and the spawn (2% compared to the wet weight of the substrate) in dark polyethylene bags, previously perforated (24 holes of 7 mm of diameter) and containing 8 kg of each substrate. Each treatment consisted of 25 repetitions (*P. ostreatus* production packages), which were identified and incubated for 12 days in a climate chamber (25 °C) until the colonization was complete. Then, they were randomly housed in grow house with a semi-controlled environment, where the internal temperature (ranging from 17 ° C to 37 ° C) and humidity (ranging from 40% to 96%) were monitored.

2.3 ASSESSED PARAMETERS

The samples analyzed were made up of of three subsamples of 0.3kg each approximately; they were collected at different points of the raw material (sugarcane straw and wheat bran), compost (end of Phase I), cultivation substrate (end of Phase II) and each treatment from Phase III (end of cultivation). These samples were homogenized and used to determine the main parameters related to mushroom production and biological efficiency, which are: humidity (forced ventilation at 65 °C for 72h with subsequent drying in an oven during 6h at 105 °C), lignin levels,

cellulose and hemicellulose through the methodology suggested by Van Soest and Wine (1967, p. 51) and Van Soest et al. (1991, p. 3586), crude protein, CN ratio and PH Index [estimated by the Kjeldahl modified method and potentiometric measurement, according to Gomes and Oliveira (2011, p. 217)].

Loss of fresh mass and loss of dry mass (%) were determined for Phase II based on the masses of the wet and dry composts, before and after the process.

Regarding the production of mushrooms, data was collected for each treatment in the incubation period (days between the inoculation and the beginning of the harvest), number and mass of the basidiomas (average number of harvested bunches and the average mass of each of them), productivity (fresh mass of harvested mushroom/ fresh mass of the initial substrate x 100) and biological efficiency (fresh mass of the harvested basidioma / dry mass of the initial substrate x 100) (SIQUEIRA et al, 2012. p. 11633).

2.4 STATISTICAL ANALYSIS

The results for the incubation periods (days), number of harvested bunches, biological efficiency, productivity and physicochemical analyses were submitted to the analysis of variance (ANOVA), adopting a completely randomized design. We used the Sisvar® 5.0 statistical software (FERREIRA, 2011, p. 1040) and Turkey's Test at 5% of statistical significance.

3 RESULTS AND DISCUSSION

Treatments T1 and T2, though having showed a longer time of incubation, provided the best results for biological efficiency (Table 2). The main factor of this performance may be the greater number of bunches harvested by these treatments during the mushroom cultivation, once the average masses of the harvested mushrooms showed no significant differences and that the longer incubation period (T1) was also the one that showed best biological efficiency and productivity.

Table 2. Mushroom production parameters

Treatment[1]	Incubation period (days)	Number of bunches	Average mass of harvested bunches(g)	Productivity	Biological efficiency (%)
T1	19.60 a	44.44 a	47.98 a	26.22 a	108.91 a
T2	16.60 b	43.52 a	48.31 a	25.71 ab	102.49 a
T3	15.30 c	37.36 b	48.88 a	23.81 b	88.81 b
Average[2]	17.16	41.77	48.37	25.28	100.38
VC (%)	6.77	18.51	20.32	11.75	11.72
MSD	0.79	5.23	6.75	2.04	8.08

[1] T1: 6 days of composting, T2: 4 days of composting.

[2] Averages (three repetitions) followed by the same lowercase letters within the same column do not differ among them (Tukey 5%).

Buah et al (2010, p. 902) found different results compared to this study, with a positive relationship among the incubation time, the number of harvested bunches and the biological efficiency in the cultivation of *P. ostreatus* sterilized composts made of sawdust and/or corncob. In other words, the treatment with the shortest incubation period (27 days) caused the greatest number of harvested bunches (28 bunches) and the highest biological efficiency (91.21%). In the present study, the largest number of harvested mushrooms might be a result of the physicochemical aspect of the substrate acquired during the preparation process.

The composts prepared with 6 and 4 days of composting - Phase I - (Table 3) lost less fresh mass during pasteurization and conditioning (Phase II). This mass loss is mainly due to the water evaporation of the compost. The lowest loss recorded in the compost submitted to the longest composting is due to the biggest material compression and the lowest content of free water (not retained by lignocellulose material). Both facts – compression and free water level - can be explained by the longer microbial activity the compost was exposed to, which ensured more flexibility to the straw, reducing the volume of the compost pile and retaining more water in the fibers.

Table 3. Loss of fresh and dry mass of the composts during pasteurization and conditioning

Treatment[1]	Loss of fresh mass (%)	Loss of dry mass (%)
T1	14.28 c[2]	7.08 a
T2	16.20 b	5.06 b
T3	18.26 a	1.50 c
Average	16.24	4.54
VC (%)	7.33	28.98
MSD	1.32	1.47

[1] T1: 6 days of composting, T2: 4 days of composting, T3: 2 days of composting

[2] Averages (25 repetitions) followed by the same lowercase letters within the same column do not differ among them (Tukey 5%).

Vieira (2012, p. 110) submitted the formulated composts with different lignocellulose materials, with or without supplementation bran, to 7 days of Phase I followed by 8 hours of steam pasteurization (59.5ºC) and conditioning of 4 days (45 ° C) for experimentation with *P. ostreatus* var. Florida. The results for the loss of fresh mass during Phase II indicated that there is an influence of the raw material used - resulting from the nutritional differences and different physical aspects these raw materials provide to the compost, such as the highest compression since a lower C/N ratio (compost richer in nitrogen) provides more microbial activity, with a higher heat generation and, consequently, a higher loss of fresh mass. Adding to these results, this study indicated that the largest composting time (Phase I) can decrease the loss of fresh mass of the compost during the heat treatment of Phase II (Table 3).

Unlike the fresh mass, the composts with a higher composting time were observed to lose more dry mass during the pasteurization and conditioning stage (Table 3). This may be attributed to the longer biological processes of degradation over the ingredients, which also caused physical processes (heat) and, thus, can have made the compost with 6 days of composting, at the end of Phase I, more suitable for microorganism development, beneficial for mushroom growth, which are selected and prevailing during Phase II, such as, for example, the actinobacteria, as explained by Silva (2010, p. 13).

Regarding the chemical characteristics of the compost and due to the highest microbial action time on the ingredients, treatments T1 and T2 had, as results, a greater tightening of the C/N ratio (Figure 1) and a greater increase in the concentration of crude protein level (Table 4).

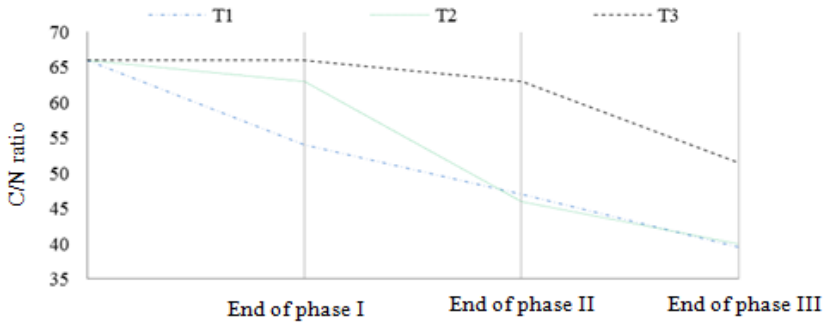

Figure 1. C / N ratio of the composts at the end of composting (Phase I), at the end of the pasteurization and conditioning (Phase II) and at the end of cultivation (Phase III): T1 - 6 days of composting, T2 - 4 days of composting, T3 - 2 days of composting.

Table 4. Results of physical and chemical analyses: humidity, pH index, crude protein and ash (Continua)

Treatment[1]	STAGE[3]		
	Final Phase I	Final Phase II	Final Phase III
	Humidity (%)		
T1	77.65 b(2)	75.92 a	57.68 a
T2	77.41 b	74.64 a	53.37 b
T3	78.92 a	73.42 a	52.97 b
Average %	77.98	74.66	54.67
VC %	0.53	1.52	1.95
MSD	1.03	2.83	2.66
	pH Index		
T1	6.80 b	6.46 a	5.00 a
T2	6.76 b	6.50 a	5.13 a
T3	6.90 a	6.50 a	5.06 a
Average %	6.82	6.48	5.06
VC %	0.49	0.51	1.47
MSD	0.08	0.08	0.18
	Crude Protein (%)		
T1	5.54 a	6.52 a	6.15 a
T2	5.18 b	6.61 a	5.67 a
T3	4.02 c	4.59 b	4.82 b
Average %	4.91	5.91	5.55

(Conclusão)

Treatment[1]	STAGE[3]		
	Final Phase I	Final Phase II	Final Phase III
	Crude Protein (%)		
VC %	1.91	1.19	4.76
MSD	0.23	0.17	0.66
	Ashes (%)		
T1	18.44 a	20.50 b	30.33 a
T2	18.42 a	22.93 a	32.88 a
T3	16.16 a	16.64 c	21.73 b
Average %	17.67	20.02	5.59
VC %	7.73	3.55	28.31
MSD	3.42	1.78	3.96

(1) T1: 6 days of composting, T2: 4 days of composting, T3: 2 days of composting.
(2) Averages (three repetitions) followed by the same lowercase letters within the same column do not differ among themselves (Tukey 5%)
(3) Phase I = composting; Phase II = Pasteurization and conditioning; Phase III = cultivation.

The composting process made components rich in nitrogen concentrate due to reductions in the amount of nutrients rich in carbon, such as cellulose and hemicellulose, caused by the loss of this element in the form of CO_2, in accordance with Conceição (2010, p. 5) and Varnero, et al. (2010, p. 17). Moreover, many authors found that the supplementation of the compost and the consequent lower initial C/N ratio, with a higher protein level, increase the microbial activity and the carbon losses, as previously observed in this study about the highest composting time. Some authors – such as Vieira (2012, p. 110), Valente et al (2009, p. 63), Alananbeh, Bouqellah, Al Kaff (2014, p. 622), Fanadzo et al (2010, p. 2760) – also associate these factors with a high productivity and biological efficiency of *P. ostreatus*.

Though the treatments practically presented the same humidity at the end of Phase II, T1 obtained superior humidity (57.68%) at the end of Phase III. This reinforces the results obtained for loss of fresh mass during Phase II, indicating that the compost with a longer composting time is less susceptible to the evaporation of the retained water and that, therefore, it has more water available to the mushroom compared to the other treatments.

The pH index, despite being higher in T3 at the end of the composting, did not show statistical differences at the end of Phase II (ready substrate) and Phase III, so it did not interfere in the results. However, the ash level was lower in T3 at the end of Phase II and Phase III (Table 4) due to the shorter exposure of the raw materials to the biodegradation process in Phase I, thus evidencing, proportionally, the fungi increased consumption of other substrate components. These results are in agreement with the results obtained by Sales-Campos et al (2010, p. 73) who also observed an increase in the ash level at the end of the *P. ostreatus* cultivation in substrates obtained by different formulations.

Isikhuemhen e Mikiashvilli (2009, p. 1353), in a study on enzyme activity and degradation of lignin, cellulose and hemicellulose by *P. ostreatus* in sterilized composts based on wheat straw and biodigesters waste, found a positive correlation between the degradation of cellulose and hemicellulose with the biological efficiency, wherein this correlation was negative for lignin. Figueiró e Graciolli (2011, p. 929) concluded that lower levels of hemicellulose in the substrate, among other chemical factors, favored better production results to *P. ostreatus*. In this study, we observed the greatest reduction of cellulose and hemicellulose levels at the end of Phase III occurred in the treatments that presented the best biological efficiencies, as occurred for lignin, but with significant differences only between treatments obtained by 6 (T1) and 2 (T3) days of composting (Table 5). Nevertheless, there is not yet a definition about the influence of different levels of lignocellulose nutrients, caused by the biodegradation process in the preparation of the substrate, on the production of mushrooms. In this experiment, however, the longer periods of composting (6 and 4 days) encourage the consumption of lignocellulose nutrients by the *P. ostreatus*. Hypothetically, it may be explained by the possibility that the longer period of composting would have caused greater damages to the leaf structure present in the substrate. This factor would facilitate the entrance of the hyphae inside the sugarcane straw and, consequently, would lead to a better exploitation of these nutrients available to the fungus.

Table 5. Results of the physical and chemical analyses: lignin, cellulose and hemicellulose of the composts

Treatment[1]	STAGE[3]		
	Final Phase I	Final Phase II	Final Phase III
	Lignin (%)		
T1	8.40 a	8.88 a	4.32 b
T2	8.17 a	8.45 b	4.90 ab
T3	7.87 a	8.94 a	5.67 a
Average %	8.14	8.75	4.96
VC %	4.18	1.35	7.49
MSD	0.85	0.29	0.93
	Cellulose (%)		
T1	34.60 ab	31.77 b	21.16 b
T2	34.53 b	33.05 b	20.41 b
T3	36.77 a	36.83 a	28.04 a
Average %	35.30	33.88	23.20
VC %	2.53	3.31	6.41
MSD	2.23	2.81	3.72
	Hemicellulose (%)		
T1	27.98 b	27.62 a	13.82 b
T2	28.03 b	26.96 a	14.38 b
T3	30.57 a	28.69 a	18.94 a
Average %	28.86	27.76	15.71
VC %	2.77	5.52	3.94
MSD	2	1.75	1.55

(1) T1: 6 days of composting, T2: 4 days of composting, T3: 2 days of composting.
(2) Averages (three repetitions) followed by the same lowercase letters within the same column do not differ (Tukey 5%) among themselves.
(3) Phase I = composting; Phase II = Pasteurization and conditioning; Phase III = cultivation.

4 CONCLUSIONS

Different composting times cause varied physical and chemical changes in the compost, which influence the production of *P. ostreatus* var. Florida.

Treatments with 4 and 6 days of composting showed a better biological

efficiency than the treatment with 2 days of composting.

The treatment with 6 days of composting showed a better yield than the treatment with 2 days of composting.

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