

Full Length Research Paper

Brewer's residues and cocoa pod shells as a substrate for cultivation of *Pleurotus ostreatus* CCIBt 2339 and enzymes production

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This work investigated the best composition of a substrate for cultivation of *Pleurotus ostreatus* CCIBt 2339 using brewer's spent grain (SG) and cocoa pod shells (CP) complemented with hot trub (HT) and/or residual brewer's yeast (RY). The residue HT was detrimental for cultivation and a substrate composition (% w/w) of 58% SG + 40% CP + 2% RY – with a C/N ratio of 25.77 g/g – resulted in the best values of biological efficiency ($BE = 1.204.0$ g/kg) and productivity [$Pd = 32.5$ g/(kg.day)]. The crude multi-enzymatic extract, obtained as a result of the mycelial growth in this substrate was a good source for: laccases (7.644.4 U/g), xylanases (110.9 U/g) and amylases (277.4 U/g). The obtained results demonstrate the biotechnological potential for the proposed substrate for edible mushrooms production as much as for the obtaining of enzymes with industrial application.

Key words: Carbon/Nitrogen ratio, cellulases, pectinase, simplex-centroid design, tannase.

INTRODUCTION

Mushrooms are excellent natural agents for degradation of lignocellulosic compounds besides all of their nutritional and/or medicinal value (Stamets, 2005). A good example of versatility and efficiency, is the edible mushroom *Pleurotus ostreatus*, which is a basidiomycete highly appreciated in different countries and has been cultivated on a wide variety of vegetable-based substrates

(Ergun and Urek, 2017; Silva et al., 2019). Considering the current context when it is necessary to promote the valorization of different residues for different purposes, four agro-industrial residues generated from the production lines of beer [brewer's spent grain (SG), hot trub (HT) and residual brewer's yeast (RY)] and chocolate [cocoa pod husks (CP)] were selected as

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potential substrates for cultivation of edible mushrooms.

The two main residues selected, SG and CP, can be considered good sources of carbon. In general, SG contains around 530 g/kg of polysaccharides and 100 g/kg of lignin (Hassan et al., 2020; Rojas-Camorro et al., 2020), while CP can contain from 430 to 490 g/kg of carbon with about 210 g/kg of lignin (Adjin-Tetteh et al., 2018; Antwi et al., 2019). To balance the ratio between carbon and nitrogen, HT and RY were selected, basically due to their protein content. For HT, levels of 200 to 700 g/kg of protein were estimated (Mattioli et al., 2020) and, for RY, around 25.3 g/kg of nitrogen (Puligundla et al., 2020). Considering the available data for 2018, over 5 million tons of cocoa beans are produced worldwide (FAOSTAT, 2020) and CP can represent 70 to 75% of the total weight of the fruit (Shet et al., 2018). Additionally, China, United States and Brazil were the three largest beer producers reaching more than 730 million hectoliters in 2018 (STATISTA, 2020) and SG is estimated to be around 85% of the total by-products generated in the beer-brewing process (Hassan et al., 2020). Thus, the four residues proposed, which have a rich nutritional composition, are generated in significant quantities and can be potentially suggested (with low environmental impact) for the production of edible mushrooms. Therefore, this present work investigated the best composition between SG and CP supplemented with HT and/or RY as a substrate for *P. ostreatus* CCIBt 2339 cultivation, and, consequently, to obtain multi-enzymatic extracts.

MATERIALS AND METHODS

Pleurotus ostreatus CCIBt 2339

P. ostreatus CCIBt 2339, originally from the Instituto de Botânica de São Paulo (São Paulo, SP, Brazil), was made available by the Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC, Itabuna, BA, Brazil). The strain was preserved in penicillin tubes (Castellani, 1967) and maintained in potato-dextrose-agar (PDA). Periodically, cultivation in a bio-oxygen-demand incubator (BOD) (SL-200, SOLAB Científica) was conducted at 25°C in Petri dishes with PDA until complete coverage of the surface by the vegetative mycelium (around 20 days).

Preparation of residues and substrates

Brewer's spent grain (SG), hot trub (HT) and residual brewer's yeast (RY) were acquired at the Microbrewery of the Universidade Estadual de Santa Cruz (Ilhéus, BA, Brazil) after a production of a Witbier beer with: wheat and barley malts, cardamom, nutmeg and hop pellets (U.S. Golding Hops). SG was dried in an oven (MA-035, MARCONI) at 60°C until constant weight; HT and RY were autoclaved at 121°C/15 min (CS50, Primatec), frozen (-80°C/24 h) and dried in a lyophilizer (LS3000, TERRONI). Cocoa pod shells (CP) were obtained from local producers from Ilhéus (Bahia, Brazil) and were manually chopped before drying, as performed for SG, and then crushed to a particle size of 3 to 4 cm. The compositions (g/kg, dry base) of carbon (C) and nitrogen (N), for each residue, were estimated (IAL, 2008); for technical limitations, only humidity

(% w/w, dry base) was possible to be determined in triplicate (MB-120, OHAUS). Table 1 presents these compositions which were applied in the calculations of the C/N ratio (g/g) and the amount of water necessary to reach the substrate initial humidity of 70% (w/w).

The substrates were prepared, according to each composition to be evaluated, by weighing the pre-treated residues and mixing them with water. After 1 h, 100 g of substrate were transferred to polypropylene bags (50 x 30 cm), which were closed with an acrylic fabric and rubber bands (to allow aeration) and autoclaved (121°C/20 min) twice within 48 h (Marino and Abreu, 2009; Oliveira et al., 2007).

Spawn preparation

Based on Oliveira et al. (2007) and Shibata and Demiate (2003), with some modifications, the spawn (seeds) were produced using wheat grains purchased from local business (Ilhéus, BA, Brazil); the grains were cooked in boiling water [ratio of 1:2 (kg:L)] for 15 min; the excess water was drained and, after cooling down, 30 g/kg of a mixture [1:4 (w/w)] of CaCO₂ (for pH adjustment) and plaster (to prevent particle agglomeration) was added. The wheat grains were transferred to polypropylene bags (50 x 30 cm) until about 2/3 of its volume (200 g), the bags were closed with an acrylic fabric and rubber bands and then autoclaved (121°C/30 min). After cooling down, 1/4 of a Petri dish with complete mycelial growth of *P. ostreatus* CCIBt 2339 was inoculated on the surface of the wheat grains and, once closed, the bags were incubated at 25°C for 10 to 15 days (or until complete colonization). A tray with water was placed at the bottom of the BOD in order to maintain a high humidity (80 – 90% w/w) and it was constantly renewed.

Inoculation, mycelium running, fruiting and harvesting

The substrate inoculation proceeded with 10% (w/w) of spawn and incubation (mycelium running) occurred as described for spawn preparation but for 15 to 20 days (or until complete colonization). Only then, the bags were moved to a refrigerator (4°C/24 h) to promote a temperature shock to induce the appearance of the primordia. After that, the plastic bags were removed to expose the substrate blocks which were individually hung with a hook in boxes in the "fruiting room". The room conditions were maintained as 80 to 90% (w/w) of humidity and 23 to 25°C and a direct artificial light was maintained with fluorescent lamps (55 W, G-LIGHT PREMIUM). After a period of around 15 to 20 days, the fully developed fruiting bodies (first flush) were harvested with a slight twist and pull and were weighed (fresh weight) and measured (diameter).

Study of substrate composition

The best compositions for the residues: SP, HT, RY and CP were investigated based on a 3-component Simplex-Centroid design, totaling 10 different runs, with SP varying between 10 and 90% (w/w) and HT and RY, individually, from 0 to 5% (w/w); CP was used to complement the weight to 100 g. Each run from the matrix was performed in triplicate and the statistical analysis was performed considering the individual (and valid) responses. The main responses analyzed were: biological efficiency (*BE*, g/kg) and productivity [*Pd*, g/(kg day)], the secondary responses were: pileus diameter (*d_{pil}*, cm) and periods (days) of each phase: mycelium running (*t_{mr}*), appearance of primordia (*t_{ap}*) and colonization and harvesting (*t_{ch}*). Two compositions (% w/w) were selected and performed in triplicate for experimental validation: S5 (50% SG + 2.5% RY + 47.5% CP) and S11 (58% SG + 2% RY + 40% CP).

Table 1. Compositions (g/kg) of carbon (C) and nitrogen (N) and humidity (% w/w) obtained for brewer's spent grain (SP), hot trub (HT), residual brewer's yeast (RY) and cocoa pod shells (CP).

Residue	C (g/kg)	N (g/kg)	C/N (g/g)*	Humidity (% w/w) [§]
SG	501.7	25.1	19.99	7.58 ± 2.50
HT	460.5	46.7	9.86	5.00 ± 3.42
RY	497.3	25.3	19.66	3.57 ± 4.88
CP	481.1	10.2	47.17	9.78 ± 3.42

*C/N ratio; [§]Mean value ± standard deviation, from triplicates.

Characterization of mushrooms and cultivation

Biological efficiency (BE , g/kg) expresses the relationship between the weight of fresh mushrooms obtained for each kilogram of initial dry substrate and productivity [Pd , g/(kg day)] expresses BE by the total time of cultivation until harvesting (t_{ch} , days) (Fonseca et al., 2015; Oliveira et al., 2007). The diameters (d_{dik} , cm) of the harvested fruiting bodies were measured using a (millimeter) ruler. Following the order of cultivation phases, the periods (days) of: mycelium running (t_{mr}), appearance of primordia (t_{ap}) and cultivation until harvesting (t_{ch}) were determined.

Obtaining and characterizing the crude multi-enzymatic extract

Four compositions (% w/w) were selected and prepared in Petri dishes (30 g, triplicate): S5, S11, S1 (90% SG + 10% CP) and S9 (23.6% SG + 0.85% HT + 3.35% RY + 72.2% CP). The mycelial phase was conducted as previously described and the myceliated substrates were macerated with distilled water [ratio of 1:10 (g:mL)] and stirred in a shaker for 1 h (25°C/200 rpm). The solids were separated by vacuum filtration followed by centrifugation at (4,000 g/15 min/5°C) and the supernatant was identified as the crude multi-enzymatic extract (CME) which was investigated for the enzymes: laccase, xylanase, CMCase, FPase, amylase, pectinase and tannase. The spectrophotometric methodologies applied for each enzyme were described previously by Ghose and Bisaria (1987), Lu et al. (2013), Sharma et al. (2000), Umsza-Guez et al. (2011) and Vasconcelos et al. (2013) and the enzymatic activities (U) were expressed per gram of initial dry substrate (U/g).

Statistical analysis

The best adjustment of the obtained responses to a mathematical model (linear, quadratic, special cubic) was selected based on the Analysis of Variance (ANOVA) performed with at least 90% of confidence and the Contour Curves were generated with a statistical software (STATISTICA v.8, StatSoft). Also, the Tukey test was applied to compare mean values of specific conditions with 95% of confidence.

RESULTS AND DISCUSSION

Study of the substrate composition

Table 2 presents the Simplex-Centroid matrix, the C/N ratios for each substrate and the mean responses obtained. Some of the replicates performed were disregarded since they did not result in fruiting bodies during the entire period of cultivation (30 - 45 days), while

the other replicates of the same condition did. According to the results, higher C/N (between 30 and 39 g/g) were obtained when CP > 60% w/w (S2, S3, S6, S8, S9 and S10) (Table 2). The two highest values of BE (> 550 g/kg) and Pd [> 16 g/(kg.day)] were obtained with the substrates S1 and S5 (Table 2), with S5 presenting the best performance and a more balanced composition between SG and CP in relation to S1 which, had the highest SG content and the lowest C/N among all the substrates.

Based on the ANOVA for the responses BE and Pd it was possible to adjust quadratic models with p -values < 0.05, however, both mathematical models will not be presented because they also resulted in R^2 and R_{adj}^2 not higher than 0.65 and a statistically significant Lack of Adjustment (p -value < 0.05). It should be noted that this does not invalidate the study and the Contour Curves were analyzed together with the experimental data in order to make decisions about the compositions, as described in the following.

As Pd is calculated from the BE value, only the Contour Curve for Pd (Figure 1a) will be presented and discussed, since both curves are remarkably similar. The analysis of Figure 1a indicates a narrow range area in which the best results were obtained close to zero for HT, above 0.25 for SG and below 0.75 for RY where S1 and S5 are located (C/N between 21 and 28 g/g).

The results also allowed to identify that the increase in HT was detrimental to Pd , probably due to astringent compounds (such as tannins) normally found in this residue (Mattioli et al., 2020), which could act as an anti-nutritional factor (Luz et al., 2013). This effect can be observed when comparing runs S4 and S5 (Table 2), both with the same values for SG and CP and similar C/N, however, S4 contained HT and none of its replicates presented primordia. Thus, considering S5, the relationship between the predicted values of Pd and the coded components values (Figure 1b) suggests maximum responses around SG = 0.7 and RY = 0.3 (which are indicated with arrows in Figure 1b). However, this theoretical condition is remarkably close to run S7 (Table 2) which did not present primordia. Consequently, a new codified composition was selected and identified as S11 (SG = 0.60 + RY = 0.40, C/N = 25.77 g/g).

Table 2. Substrate composition for *Pleurotus ostreatus* CCIBt 2339 according to a coded Simplex-Centroid matrix for the components: brewer's spent grain (SG), hot trub (HT) and residual brewer's yeast (RY) with cocoa pod shells (CP) as a complementary composition (to 100 g); real values (% w/w) for the components are presented in parentheses and the C/N ratio (g/g) of each substrate is also indicated. The responses were: biological efficiency (BE, g/kg), productivity [*Pd*, g/(kg.day)], diameter (*d_{pil}*, cm) and times (days) for the stages of: mycelium running (*t_{mr}*), appearance of primordia (*t_{ap}*) and cultivation and harvest (*t_{ch}*).

Run	Component			CP (%, w/w)	C/N (g/g)	Responses*					
	SG (% w/w)	HT (% w/w)	RY (% w/w)			EB (g/kg)	<i>Pd</i> [g/(kg.day)]	<i>d_{pil}</i> (cm)	<i>t_{mr}</i> (days)	<i>t_{ap}</i> (days)	<i>t_{ch}</i> (days)
S1 [#]	1 (90.00)	0 (0.00)	0 (0.00)	10.00	21.16	550.70 ± 169.14	16.81 ± 4.11	1.91 ± 0.40	19.0 ± 0.0	2.5 ± 2.1	32.5 ± 2.1
S2 [§]	0 (10.00)	1 (5.00)	0 (0.00)	85.00	35.67	227.23 ± 46.39	6.40 ± 1.80	2.63 ± 0.32	22.3 ± 2.3	7.7 ± 0.6	36.3 ± 7.5
S3	0 (10.00)	0 (0.00)	1 (5.00)	85.00	38.89	148.9	3.82	2.21	25	10	39
S4 [§]	½ (50.00)	½ (2.50)	0 (0.00)	47.50	26.44	0 ± 0	0 ± 0	0 ± 0	25.0 ± 3.5	0 ± 0	0 ± 0
S5 [#]	½ (50.00)	0 (0.00)	½ (2.50)	47.50	27.28	649.80 ± 8.06	19.40 ± 0.17	2.21 ± 0.04	17.0 ± 2.8	4.5 ± 2.1	33.5 ± 0.7
S6 [#]	0 (10.00)	½ (2.50)	½ (2.50)	85.00	37.21	211.90 ± 60.81	6.75 ± 2.08	2.19 ± 0.28	19.0 ± 0.0	9.0 ± 0.0	31.5 ± 0.7
S7 [§]	⅔ (63.60)	⅙ (0.85)	⅙ (0.85)	34.70	27.55	0 ± 0	0 ± 0	0 ± 0	19 ± 0	0 ± 0	0 ± 0
S8 [#]	⅙ (23.60)	⅓ (3.35)	⅙ (0.85)	72.20	32.24	253.35 ± 1.91	5.98 ± 0.54	2.17 ± 0.07	20.0 ± 1.4	7.0 ± 0.0	42.5 ± 3.5
S9 [§]	⅙ (23.60)	⅙ (0.85)	⅔ (3.35)	72.20	33.46	320.73 ± 91.65	8.62 ± 1.25	3.42 ± 1.44	21.0 ± 3.5	9.7 ± 3.1	36.7 ± 5.9
S10 [#]	⅓ (36.40)	⅓ (1.65)	⅓ (1.65)	60.30	29.65	307.15 ± 25.95	7.68 ± 0.65	2.73 ± 0.56	21.0 ± 0.0	8.5 ± 0.7	40.0 ± 0.0

*Mean values ± standard deviation, from [§]triplicates and [#]duplicates.

According to Kortei et al. (2018), mushrooms with larger pileus, besides having higher commercial value, will result in higher *BE* and *Pd* values; these researchers found a correlation of *d_{pil}* with *BE* when cultivating *P. ostreatus* in composted sawdust. The results obtained in this study indicated, however, a different behavior since the highest *d_{pil}* values were obtained with runs S6 and S9 (Table 2) in contrast to what was already discussed considering the best results for *Pd* (and *BE*, consequently). The adjustment of a quadratic model to the *d_{pil}* data was statistically significant (p -value < 0.10), however, the R^2 and R_{adj}^2 values were lower (< 0.45) and the Lack of Adjustment was statistically significant (p -value < 0.10), nevertheless, the analysis continued for the purpose of better understanding the effect of the residues over *d_{pil}*.

According to the Contour Curve obtained (Figure 1c), in order to increase *d_{pil}* it would be

necessary to work with lower *SG* and higher *HT* and *RY*. Inside this region are located the runs S3, S6 and S9 and a narrow band around S2 (C/N = 33 - 39 g/g). This result suggests that, for *P. ostreatus* CCIBt 2339, higher *HT* and *CP* may induce the development of larger pileus, but with lower *BE* and *Pd*, as it can be observed when comparing runs S9 and S5 (Table 2).

Regarding the fact that it is always desirable to reduce the total time of a process to increase its productivity and lower the risks for contamination, *t_{mr}*, *t_{ap}* and *t_{ch}*, were analyzed individually in a similar way as described so far, although, the obtained Contour Curves will not be presented and only the results will be discussed. Values of *t_{mr}* between 15 and 19 days (Table 2) indicated a shorter mycelial running phase, in comparison to what was obtained by Kumari and Achal (2008) with *P. ostreatus* (20 days) and this response was favored by the same range of compositions that

favored the responses *Pd* and *BE*. The substrates S2, S3 and S4 (Table 2) indicated to increase *t_{cm}* to more than 22 days which is more similar to what was obtained for *P. ostreatus* (22 - 26 days) by Sharma et al. (2013).

It was observed that an increase in *HT* indicated an undesirable increase in *t_{ap}* values (similar to the analysis of *d_{pil}*). Shorter *t_{ap}* values (1 - 5 days) were obtained with S1 and S5 (Table 3) which are around to the 3 days required by *Pleurotus sajor-caju* in onion juice waste (Pereira et al., 2017) but less than what was observed (9 to 17 days) with *P. ostreatus* in weeds (Das and Mukherjee, 2007). According to the results, *t_{ch}* varied from 30 to 45 days (Table 3) as a consequence of *t_{mr}* and *t_{ap}* values.

Ultimately, besides S11, S5 was also chosen for experimental validation, in triplicate, and the obtained responses are presented in Table 3. Considering that some operational difficulties

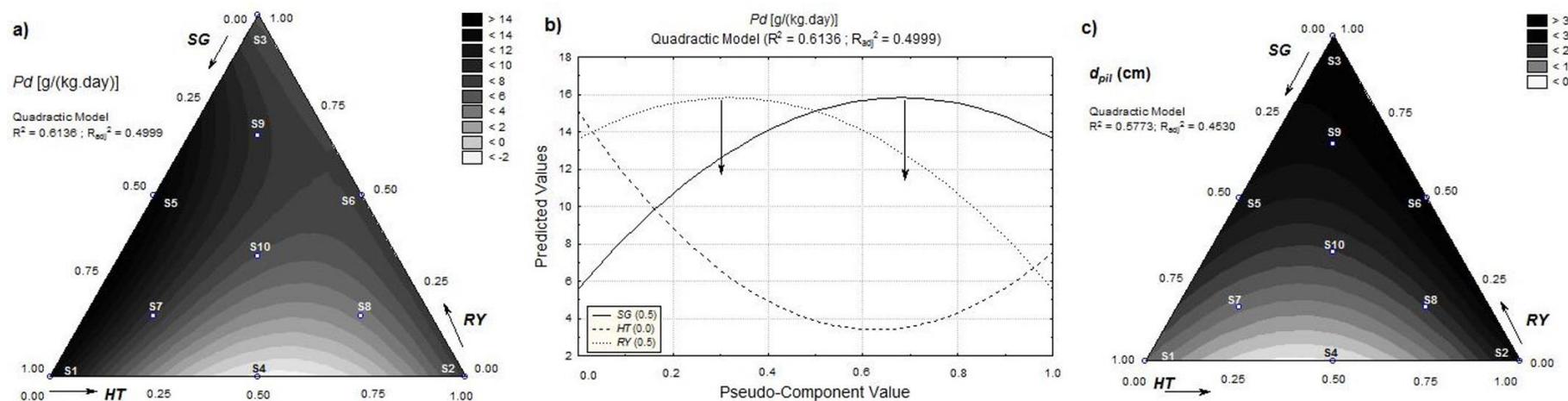


Figure 1. (a) Contour Curve obtained for productivity [Pd , g/(kg.day)] considering the coded composition of: brewer's spent grain (SG), hot trub (HT) and residual brewer's yeast (RY) and (b) the relation between the predicted values and the coded levels for a reference blend value of $SG = RY = 0.5$ and $HT = 0.0$ and (c) the Contour Curve obtained for pileus diameter (d_{pil} , cm).

Table 3. Biological efficiency (BE), productivity (Pd), pileus diameter (d_{pil}) and times for: mycelium running (t_{mr}), appearance of primordia (t_{ap}) and cultivation and harvest (t_{ch}) obtained for the cultivation of *Pleurotus ostreatus* CCIBt 2339 on substrates (S5 and S11) containing brewer's residues and cocoa pod shells.

Substrate	Responses*					
	EB (g/kg)	Pd [g/(kg.day)]	d_{pil} (cm)	t_{mr} (days)	t_{ap} (days)	t_{ch} (days)
S5	437.8 ± 142.5 ^a	11.8 ± 9.8 ^a	2.1 ± 0.5 ^a	27.0 ± 0.0 ^a	6.6 ± 1.0 ^a	37.0 ± 0.0 ^a
S11	1,204.4 ± 207.8 ^b	32.6 ± 18.7 ^b	3.2 ± 0.5 ^b	22.0 ± 0.0 ^b	3.5 ± 1.0 ^b	37.0 ± 0.0 ^a

*Mean values ± standard deviation, from triplicates. Values with different superscript letters, in the same column, are significantly different ($p < 0.05$).

were faced to maintain humidity inside the fruiting room for the first couple of days of cultivation and acknowledging the inherent variability of the experiments, substrate S11 (defined with the statistical analysis) was considered the best condition for *P. ostreatus* CCIBt 2339 cultivation. In comparison to S5, the increase in SG and the decrease in RY and CP resulted in a C/N ratio 6% lower and a Pd around 2.8 times higher (Table 3).

Enzymatic screening

The enzymatic screening for the obtained crude multi-enzymatic extracts (CMEs) is presented in Table 4, from which it is possible to identify the biotechnological potential of each CME, especially in relation to the activities of laccase, xylanase, pectinase and amylase. In general, the reduction of SG (% w/w) from 90% (S1) to 58% (S11)

associated with the increase of CP and RY (Table 2), indicated to be positive for the production of laccases and amylases. However, when considering to reduce SG even more, from 58% (S11) to 50% (S5) also associated with the increase of CP and RY, that indicated not to be beneficial for laccases, amylases and pectinases (Table 4). Regarding S9, the only condition with HT and the highest CP, the lowest laccase,

Table 4. Enzymatic screening of crude multi-enzymatic extracts obtained after the mycelial running of *Pleurotus ostreatus* CCIBT 2339 cultivated on different substrates (S1, S5, S9 and S11) containing brewer's residue and cocoa pod shells.

Substrate	Enzymatic activities (U/g)*						
	Laccase	Xylanase	FPase	CMCase	Pectinase	Amylase	Tannase
S1	2,073.3 ± 0.03 ^a	110.4 ± 0.01 ^a	< 0.5	6.7 ± 0.05 ^a	370.7 ± 0.0 ^a	4.5 ± 0.01 ^a	< 0.5
S5	3,977.7 ± 0.04 ^b	100.8 ± 0.06 ^a	< 0.5	5.3 ± 0.03 ^b	96.8 ± 0.00 ^b	2.0 ± 0.01 ^b	< 0.5
S9	1,086.6 ± 0.01 ^c	1.6 ± 0.00 ^b	< 0.5	5.1 ± 0.01 ^b	2.2 ± 0.03 ^c	3.1 ± 0.02 ^c	< 0.5
S11	7,644.4 ± 0.04 ^d	110.9 ± 0.02 ^a	< 0.5	4.6 ± 0.02 ^c	126.1 ± 0.05 ^d	277.4 ± 0.06 ^d	< 0.5

*Mean values ± standard deviation, from triplicates. Values with different superscript letters, in the same column, are significantly different ($p < 0.05$).

xylanase and pectinase activities were obtained (Table 4). Between S1, S5 and S11, there were no significant changes in xylanase activities and, for all conditions evaluated, a similar CMCase production was obtained (Table 4).

Laccases, xylanases, FPases and CMCases are enzymes investigated for the degradation of lignocellulosic substrates. It was reported for *P. ostreatus*, for instance, that nitrogen supplementation of soybean hulls could result in an increase in laccase production, in this case, the highest activities (60 - 80 U/g) were reached at a much lower C/N (5.0 g/g) (D'Agostini et al., 2011). In another example, a co-cultivation of *P. ostreatus* MTCC 180 and *Penicillium oxalicum* SAUE-3.510 in sugarcane bagasse and bean husk resulted in a higher yield of xylanase (8,205.31 U/g) (Dwivedi et al., 2011). With cultivation in sesame straw and wheat bran (C/N = 27 g/g), it was possible to obtain a lower activity of CMCase (1.75 U/g) with a substrate with a very similar C/N to S5 (Kurt and Buyukalaca, 2010). Pectinases and amylases are enzymes capable to hydrolyze, for example, mucilages and starches that are naturally found in different vegetable parts. In the spent substrate of *P. sajor-caju* in onion residues (with a much higher C/N of 266.22 g/g) a pectinase activity of around 96 U/g was obtained (Pereira et al., 2017), close to what was obtained with S5 (Table 4). Considering amylases from *Pleurotus*, there are only a few reports in literature, for example, around 9 U/g was reported in a spent substrate of undeclared composition (Nakajima et al., 2018).

All four compositions investigated presented FPase and tannase activities close to the control conditions of the enzymatic methodologies ($0.43 < \text{U/g} < 0.01$), indicating that the extracts obtained did not present expressive activities for these two enzymes. In order to obtain a better quantification, it could be suggested a methodology of greater sensitivity (such as liquid chromatography). However, FPase (22 U/g) and tannase (1 U/g) activities have been reported for *P. ostreatus* when cultivated, respectively, in banana pseudostem supplemented with Tween 80 (Silva et al., 2019) and jatropha biodiesel residues (Luz et al., 2013).

When considering the world production of mushrooms and truffles, which was almost 9 million tons in 2018

(FAOSTAT, 2020), it is possible to understand that the spent substrate produced, accumulated or sub-utilized still has a great biotechnological value that needs to be better explored. For that reason, the enzymatic profiles obtained (Table 4) can indicate the potential for the spent substrates as a source of important enzymes (Nakajima et al., 2018; Pereira et al., 2017).

Conclusions

When working with different residues to compose a substrate for mushroom cultivation, it is important to investigate the best composition since it can modulate different responses related to growth. In this study it was possible, with the help of a statistical tool, to detect the advantages of balancing the compositions of brewer's spent grain and cocoa pod shells and it also permitted to choose the residual brewer's yeast over the hot trub in order to improve, for example, productivity and laccase activity. Thus, it is reinforced that the proper use of agro-industrial residues to produce edible mushrooms is a viable, necessary and a low-impact practice since it is possible to produce a nutritional food and obtain important enzymes from the spent substrate.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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