AGRONOMY (AGRONOMIA)

Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.18, n.1, e2970, 2023
Recife, PE, UFRPE. www.agraria.pro.br
DOI: 10.5039/agraria.v18i1a2970 - Protocol 2970
Subm. 04/11/2022 • App. 06/02/2023 • Pub. 10/03/2023

Shiitake cultivation in axenic blocks containing brewery residue

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ABSTRACT: Shiitake (*Lentinula edodes*) edible mushroom growth in an axenic block is the most popular method worldwide allowing higher productivity and a shorter cultivation cycle, than the conventional in wood logs. Blocks might be composed of residues from several agro-industrial activities. Among them, the brewery industry generates abundant waste that can cause negative environmental impacts if its incorrect disposal of in the environment, but it has not been studied to shiitake. The goal of this study was to evaluate if it is possible to produce it on barley malt bagasse (BMB) as one of the raw materials on the block. Results show shiitake biological efficiency (BE), productivity (PR), and their features. Substrates with C:N ratios of 30:1 and 36:1 resulted in BE values of 20.18 and 16.41% and PR of 10.91 and 10.19% showing that it is possible to product shiitake on blocks containing BMB.

Key words: edible mushroom; fungi; mushroom production; substrate; waste

Cultivo de shiitake em blocos axênicos contendo resíduo de cervejaria

RESUMO: O cultivo do cogumelo comestível shiitake (*Lentinula edodes*) em bloco axênico é o método mais popular em todo o mundo, permitindo maiores produtividades e ciclo de cultivo mais curto, do que o convencional em toras de madeira. Os blocos podem ser compostos por resíduos de várias atividades agroindustriais. Entre eles, a indústria cervejeira gera resíduos abundantes que podem causar impactos ambientais negativos se o seu descarte no meio ambiente for incorreto, mas não tem sido estudado para shiitake. O objetivo deste trabalho foi avaliar se é possível produzi-lo em bagaço de malte de cevada (BMB) como uma das matérias-primas do bloco. Os resultados apresentados mostram a eficiência biológica (BE), a produtividade (PR) do shiitake e suas características. Substratos com relações C:N de 30:1 e 36:1 resultaram em valores de BE de 20,18 e 16,41% e RP de 10,91 e 10,19%, indicando que é possível produzir shiitake em blocos contendo BMB.

Palavras-chave: cogumelo comestível; fungos; produção de cogumelos; substrato; resíduos

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Associate Editor: Mário de Andrade Lira Júnior

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Introduction

Edible mushroom *Lentinula edodes* (shiitake) is the most cultivated worldwide (Royse et al., 2017) because of its nutritional, and medicinal characteristics (Kobayashi et al., 2020). Axenic blocks are the most applied method, with low environmental impact because they reuse agro-industrial residues, have higher biological efficiency (BE), productivity (PR), and shorter cultivation cycle compared with the conventional method in wood logs (Chen, 2005; Kobayashi et al., 2020). Raw materials applied are very heterogeneous because growers use the agricultural residue available in their region and additives to meet the fungus's nutritional needs (Zied et al., 2016; Bach et al., 2018; Atila, 2019).

According to Kirin Holdings Company (2022), 177.5 million kiloliters of beer were consumed globally in 2020. China represents the largest beer-consuming country (accounting for 20% of the global beer demand), followed by the United States, Brazil, Mexico, and Germany. It is known from every 100 liters of beer made the brewery industry generates 24 kg of waste; from those 85% is barley malt bagasse (BMB) (Massardi et al., 2020). Its destination is limited to animal feed or disposal in landfills. It contains malt husk and pulp remains and its composition depends on the beer style. It is composed of lignocellulosic compounds (cellulose, hemicelluloses, and lignin), proteins, lipids, amino acids, vitamins, phenolic compounds (Qin et al., 2018; Saraiva et al., 2018; Massardi et al., 2020), and polymerized sugars (Mussatto & Roberto, 2006). Its use as a raw material in the axenic blocks for the production of shiitake mushrooms has not yet been studied.

In the present study, different quantities of BMB were evaluated with the aim of use to grow shiitake in axenic blocks with BE and PR satisfactory.

Materials and Methods

Two cultivations were carried out to evaluate the use of BMB as a raw material for axenic blocks. The BMB used was provided by Cervejaria Blumenau (Blumenau, SC, Brazil), obtained after the mashing stage of artisanal Pilsen beers. The preparation of the axenic blocks was carried out at Cogumelos do Vale Europeu (Gaspar, SC, Brazil). The FF50 lineage spawn utilized was provided by the company Funghi & Flora (Valinhos, SP, Brazil), and the mushroom cultivation was carried out at the Post-harvest Laboratory of Horticultural Products in the Crop Science Department at the Universidade Federal do Paraná (UFPR, Curitiba, PR, Brazil).

In the first cultivation, the raw materials utilized were sawdust as the sulk and BMB as the supplement. Five substrates were defined in proportions that allowed for reaching the estimated Carbon:Nitrogen (C:N) ratios of 30:1, 40:1, 50:1, 60:1, and 70:1 (Table 1). In the preparation of the mixture, it was considered that eucalyptus sawdust has an average C:N ratio of 500:1 and BMB has an average C:N ratio of 40:1 (Cooper et al., 2010).

In the second cultivation, a chemical analysis of the raw materials was made before preparing the substrates. Raw materials utilized were sawdust as the sulk with the addition of soy husk (SH), rice bran (RB), wheat bran (WB), and BMB as supplements (Table 2).

Chemical analysis realized provides information to reach exact the C:N ratios of 17:1, 20:1, 27:1, 31:1, and 36:1 (Table 3), according to the C:N ratio of the best substrate in the first cultivation.

Raw materials listed in Table 1 (first cultivation) and Table 3 (second cultivation) were homogenized, humidified, and packed in high-intensity polypropylene bags with a weight

Table 1. Estimated C:N ratios and proportions of each raw material (%) used to compose the axenic blocks in the first shiitake (*Lentinula edodes*) cultivation.

C:N ratios	Raw materials (%) *				
	ES	BMB	Limestone	Plaster	Charcoal
30:1	60.0	35.74	0.81	2.03	1.42
40:1	71.0	24.74	0.81	2.03	1.42
50:1	77.0	18.74	0.81	2.03	1.42
60:1	81.0	14.74	0.81	2.03	1.42
70:1	84.0	11.74	0.81	2.03	1.42

* Values in proportions of moist mass per axenic block; ES - Eucalyptus sawdust; BMB - Barley malt bagasse.

Table 2. Chemical characteristics of raw materials used to compose the axenic blocks, in the second shiitake (*Lentinula edodes*) cultivation.

Evaluated characteristics	Raw materials (%) *				
	ES	SH	RB	WB	BMB
Nitrogen	0.10	1.98	2.46	2.94	3.74
Cellulose	61.02	43.52	11.41	10.60	15.96
Hemicellulose	4.11	21.55	20.45	29.18	37.71
Lignin	18.23	2.06	4.55	2.44	3.33

* Values in percentage (%) of dry matter; ES - Eucalyptus sawdust; SH - Soy husk; RB - Rice bran; WB - Wheat bran; BMB - Narley malt bagasse.

Table 3. C:N ratios and proportions of each raw material (%) used to compose the axenic blocks in the second shiitake (*Lentinula edodes*) cultivation.

C:N ratio	Raw materials (%) *						
	ES	RB	WB	SH	BMB	Limestone	Plaster	Charcoal
17:1	25.2	21.50	15.00	13.50	20.80	2.11	0.78	1.11
20:1	32.2	17.95	12.25	11.25	22.35	2.11	0.78	1.11
27:1	39.2	14.33	10.00	9.00	23.47	2.11	0.78	1.11
31:1	46.2	10.75	7.50	6.75	24.80	2.11	0.78	1.11
36:1	51.8	9.46	6.58	5.96	22.20	2.11	0.78	1.11

* Values in proportions of moist mass per axenic block; ES - Eucalyptus sawdust; RB - Rice bran; WB - Wheat bran; SH - Soy husk; BMB - Barley malt bagasse.

Table 4. Chemical characteristics of substrates used in the second shiitake (*Lentinula edodes*) cultivation.

Evaluated characteristics	C:N ratio of substrates				
	17:1	20:1	27:1	31:1	36:1
Nitrogen	1.84	1.67	1.43	1.34	1.17
Cellulose	31.32	33.85	39.20	41.61	42.47
Hemicellulose	19.63	19.43	18.61	18.17	18.99
Lignin	9.47	11.12	12.10	14.28	15.10

* Values in percentage (%) of dry matter.

of 2.0 kg, containing a microbiological filter (microporous) of 4 cm^2 previously heat-welded to the bag. Bags were autoclaved for 4 hours at 121 °C and 1 atm (Montini, 2001), thus forming the axenic blocks. After 24 hours of cooling, they were inoculated in a laminar flow chamber, with 3% relative to the total weight with the FF50 lineage spawn. In the second experiment, a sample of each substrate was taken and sent for analysis of its chemical characteristics (Table 4).

The cultivations were carried out in cold rooms with control of photoperiod, humidity, and temperature in each phase, as proposed by Chen (2005) and Oei & Nieuwenhuijzen (2006) (Table 5).

The following evaluations were carried out: time for complete colonization (TCC), where mycelial development was evaluated in days between the spawn inoculation period until the axenic block presented 80% or most of its outer surface with uniform reddish-brown coloration; time for the first harvest (TFH) was evaluated in days from the spawn inoculation to the first mushroom harvested; time in production (TP) was evaluated in days, and it consisted of the interval between the first and the last mushrooms harvested; time until the end of the cultivation (TEC) was evaluated in days, and it consisted of the sum of the duration of all cultivation phases; the number of mushrooms harvested per axenic block (NMH); fresh mass of each mushroom harvested (g $unit^{-1}$); and, yield - weight of fresh mushrooms harvested (g per^{-1} axenic block).

Agronomic parameters such as biological efficiency: [BE = (weight of fresh mushrooms harvested) × 100/ weight of dry substrate]; productivity: [PR = (weight of fresh mushrooms harvested) × 100/ weight of fresh substrate]. In the second experiment, a visual evaluation of the pileus morphology was performed at the ideal mushroom harvesting point.

In both cultivations, the design used to distribute the blocks on the shelves of the cold chamber was completely randomized, with 12 repetitions of axenic blocks in each of the five treatments. For analysis of the results, the ActionStat program was used, and the mean tests performed were the non-parametric Kruskal-Wallis test, and when significant, the comparison of mean values was performed using the Bonferroni test at a 95% confidence level.

Results

Responses about the time duration of each cultivation phase are presented in Table 6. The different C:N ratios with the respective BMB proportions affected the cultivation phases; the substrates with C:N ratios of 30:1 and 40:1 were the best formulations examined resulting in production precocity.

The substrates inside the ideal C:N ratio range from 20:1 to 50:1 for growing shiitake in axenic blocks (Philippoussis et al., 2003), presented the best agronomic parameters, as shown in Table 7. It can notice that the C:N ratios 30:1 and 40:1 showed high values. These respective substrates contain more quantity of BMB in the formulation (35.74 and 24.74%) (Table 1), recycling more waste and getting favorable production of shiitake mushrooms.

The addition of BMB proportioned BE and PR is considered good for commercial production, as exhibited in Table 8. Where is possible to observe better values for the substrates with the C:N ratios of 30:1 and 40:1.

In the second experiment, substrates with C:N ratios of 17:1 and 20:1 were prepared to assess whether a lower C:N ratio would be more favorable for shiitake cultivation in substrates containing BMB. However, they did not complete any of the

Table 6. The effect at time duration of the shiitake (*Lentinula edodes*) cultivation phases in the first cultivation.

C:N ratio	Duration of cultivation phases (days)			
	TCC	TFH	TP	TEC
30:1	65 a	76 b	15 c	89 b
40:1	65 a	74 a	8 a	82 a
50:1	70 b	79 c	30 d	109 c
60:1	81 c	90 d	31 e	121 e
70:1	100 d	109 e	11 b	120 d
p-value	$p = 1.02^{-08}$	$p = 2.68^{-09}$	$p = 6.72^{-09}$	$p = 1.52^{-09}$

TCC - Time for complete colonization; TFH - Time for the first harvest; TP - Time in production; TEC - Time until the end of the cultivation; * Averages followed by the same letters in the column were determined by the Kruskal-Wallis test to not differ from each other ($p \geq 0.05$).

Table 7. The effect on agronomic parameters of shiitake (*Lentinula edodes*) production in the first cultivation.

C:N ratios	NMH (unit)	Yield (g per^{-1} axenic block)	Average mushroom weight (g)
30:1	11 a	218.19 a	24.69 a
40:1	7 a	161.28 ab	27.65 ab
50:1	5 a	122.04 b	26.25 b
60:1	2 b	64.46 c	48.19 b
70:1	1 b	46.27 c	35.80 b
p-value	$p = 7.14^{-07}$	$p = 5.98^{-07}$	$p = 9.09^{-03}$

NMH - Number of mushrooms harvested per axenic block. * Averages followed by the same letters in the column were determined by the Kruskal-Wallis test to not differ from each other ($p \geq 0.05$).

Table 5. Environmental conditions used in each stage of shiitake (*Lentinula edodes*) cultivation in axenic blocks.

Stage of cultivation	Temperature (°C)	Luminosity (Lux)	Relative humidity (%)
Mycelial development	20 ± 2 °C	0	80 ± 10%
Development of the primordials	14 ± 2 °C	500 to 1000	95%
Mushroom production	16 ± 2 °C	500 to 1000	70 ± 10%

Table 8. The effect on biological efficiency and productivity of shiitake (*Lentinula edodes*) production in the first cultivation.

C:N ratio	BE (%)	PR (%)
30:1	20.18 a	10.91 a
40:1	14.25 ab	8.07 ab
50:1	10.68 b	6.10 b
60:1	5.16 c	2.95 c
70:1	3.71 c	1.93 c
p-value	p = 4.55^{-07}	p = 5.98^{-07}

BE - Biological efficiency; PR - Productivity. * Averages followed by the same letters in the column were determined by the Kruskal-Wallis test to not differ from each other (p ≥ 0.05).

Table 9. The effect at time duration of the shiitake (*Lentinula edodes*) cultivation phases in the second cultivation.

C:N ratio	Duration of cultivation phases (days)			
	TCC	TFH	TP	TEC
17:1	-	-	-	-
20:1	-	-	-	-
27:1	67 a	75 a	2 b	77 a
31:1	67 a	74 a	4 a	78 a
36:1	66 a	72 a	6 a	78 a
p-value	p = 5.65^{-01}	p = 1.72^{-01}	p = 1.64^{-02}	p = 9.98^{-01}

TCC - Time for complete colonization; TFH - Time for the first harvest; TP - Time in production; TEC - Time until the end of the cultivation; * Averages followed by the same letters in the column were determined by the Kruskal-Wallis test to not differ from each other (p ≥ 0.05).

evaluated phases as shown in Table 9. The substrates with the C:N ratios near to ideal range for shiitake cultivation tend to precocity cultivation compared to the first experiment.

As the quantity of BMB increased and the C:N ratio respectively NMH and yield presented in Table 10 tended to increase also. The tendency of higher NMH lighter was the average mushroom weight (g) (Table 10).

BE and PR for the second experiment are presented in Table 11. The addition of other supplement raw materials causes a

Table 10. The effect on agronomic parameters of shiitake (*Lentinula edodes*) production in the second cultivation.

C:N ratio	NMH (unit)	Yield (g per^{-1} axenic block)	Average mushroom weight (g)
17:1	-	-	-
20:1	-	-	-
27:1	7 b	61.55 b	7.57 a
31:1	31 a	155.98 a	5.34 a
36:1	38 a	204.36 a	5.54 a
p-value	p = 1.86^{-03}	p = 4.97^{-04}	p = 8.05^{-02}

NMH - Number of mushrooms harvested per axenic block. * Averages followed by the same letters in the column were determined by the Kruskal-Wallis test to not differ from each other (p ≥ 0.05).

Table 11. The effect on biological efficiency and productivity of shiitake (*Lentinula edodes*) production in the second cultivation.

C:N ratio	BE (%)	PR (%)
17:1	-	-
20:1	-	-
27:1	4.94 b	3.07 b
31:1	12.52 a	7.78 a
36:1	16.41 a	10.19 a
p-value	p = 4.96^{-04}	p = 4.97^{-04}

BE - Biological efficiency; PR - Productivity. * Averages followed by the same letters in the column were determined by the Kruskal-Wallis test to not differ from each other (p ≥ 0.05).

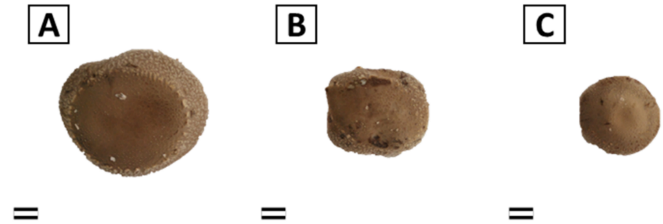

Figure 1. Morphology of the pileus shiitake mushrooms (*Lentinula edodes*) produced in axenic blocks containing barley malt bagasse in different C:N ratios in the second experiment; A: C:N 36:1, B: C:N 31:1, and C: C:N 27:1; bar: 1 cm.

decrease in the values of BE and PR, however, respond with more production precocity.

Were observed in the second experiment, at the ideal harvest point, where the pileus of the mushrooms presented morphological differences in terms of pileus size (Figure 1).

Discussion

Substrates are studied based on their C:N ratio and ability to supply N to the fungus (Bach et al., 2018; Gaitán-Hernández et al., 2020). The C:N ratio from 20:1 to 50:1 is the ideal range for growing shiitake in axenic blocks. However, if it were only necessary to meet the C:N ratio range, it would be assumed that all substrates within the range containing BMB would be satisfactory for cultivation. However, formulation responses showed that the substrate composition provides a complex nutritional content that influences all fungus responses.

For successful cultivation, the substrate must allow mycelial development in a short period and with vigor, because it is the phase with a high risk of contamination (Montini, 2001). In the studied substrates, the time to complete colonization ranged between 65 and 100 days (Tables 6 and 9), faster results than those obtained by Sousa et al. (2019) out of 126 days and slower than those obtained by Atila (2019) from 32 to 46 days and Gao et al. (2020) from 49 to 50 days; however, there are differences in formulations, compositions, raw materials, and lineages.

It is known that the duration of the mycelial development phase is a direct response to the sources, forms, and amounts of N present in the substrates. The fast mycelial development is favored at low C:N ratios because in them there is a greater bioavailability of N with easy and fast assimilation by the fungus (Philippoussis et al., 2007; Bach et al., 2018; Walker & White, 2018). In this research, substrates containing BMB in the C:N ratios of 30:1, 40:1 (Table 6), and 36:1 (Table 9) had the lowest time for full substrate colonization, but they are not the lowest. So, appear to be more responsive to the addition of BMB in a C:N ratio range of 30:1 to 40:1 to lead a vigorous and fast mycelial colonization. Another important factor that impacts colonization is the initial N content of the substrate, which should not exceed 1.5%, as it also makes the substrate more prone to the occurrence of contamination (Özçelik & Pekşen, 2007; Atila, 2019). Lower C:N ratios of C:N 17:1 and 20:1 containing BMB had initial content of N higher than 1.5% favoring the occurrence of contamination as well as the possibility of toxicity by nitrogen, and not even complete mycelial full mycelial colonization and neither the other phases (Table 9). Mycelial development is an efficient way to reveal the efficacy of the mixture and the raw materials utilized since there is a direct influence on the time needed to complete this phase, fact is that the addition of BMB and the range of C:N ratio results on better duration responses of the cultivation phases.

The C:N ratio and the N content enhance the fungus ability to degrade the lignocellulosic compounds present in the substrate (Philippoussis et al., 2011). Raw materials must be chosen with the main purpose to provide cellulose, hemicelluloses, and lignin, which is utilized during the cultivation phases. Carbon is readily available from cellulose, hemicelluloses, and lignin, but nitrogen mainly presents in bound forms which are not available until it is enzymatically released. According to Gaitán-Hernández et al. (2020), the concentration of hemicellulose in the initial stages of cultivation favors the biodegradation of other compounds and, consequently, the achievement of short periods of mycelial colonization. Substrates in C:N ratios of 30:1 and 40:1 (Table 6) as well as C:N ratios of 27:1, 31:1, and 36:1 (Table 9) had hemicellulose contents (Table 4) sufficient to enhance the enzymatic activities of the fungus in degrading lignocellulosic compounds and promote rapid mycelial development.

After the colonization, the next important phase to observe is the precocity of the first harvest. According to Özçelik & Pekşen (2007), there is a positive relationship between the precocity of the first harvest and the C:N ratio of the substrates, where the results obtained in this study ranged from 72 to 109 days (Tables 6 and 9), similar to the values reported by Özçelik & Pekşen (2007) from 77 to 129 days, and by Atila (2019) from 58 to 76 days. Substrates containing BMB with C:N ratios of 27:1, 30:1, 31:1, 36:1, and 40:1 had the shortest periods until the first harvest (Tables 6 and 9). Although the C:N ratio it is important to observe cellulose content, according to Atila (2019) in this phase the fungus consumes the most cellulose present in the substrate, it plays an important role in being the main carbon and energy source for the fungus (Walker & White, 2018). Providing harvest precocity in production where just one flush is conducted and achieving favorable BE and PR as shown ahead, more productions during the year the producer will be able to complete with success.

The C:N 31:1 and 36:1 ratios contained the highest levels of initial cellulose in the substrate at 41.61 and 42.47%, sufficient to promote early harvests at 74 and 72 days. Furthermore, it is important to know that the substrate C:N ratio does not remain stable during the cultivation cycle, with a decrease in its value due to the consumption of organic compounds by the fungus. This consumption leads to a decrease in the amounts of C and an increase in the amounts of N in the substrate (Atila, 2019), and because of that the next phases had less time duration than the initial ones.

The phase where the axenic blocks stayed producing ranged from 2 to 31 days (Tables 6 and 9). Shortest producing periods were favored at low C:N ratios and this phase of production are where the greatest degradation of lignin occurs (Atila, 2019). Lignin is the most complex and difficult-to-degrade lignocellulosic compound (Walker & White, 2018). Substrates in C:N ratios of 50:1, 60:1, and 70:1 that had the highest proportions of ES (Table 1), a raw material that has the highest lignin content among those used (Table 2), thus resulting in a substrate with greater difficulty for degradation. These substrates were the ones that obtained the worst results. The use of BMB directly influenced the total period of cultivation, which ranged from 77 to 120 days (Tables 6 and 9), periods longer than those reached by Atila (2019) from 58 to 76 days and by Valenzuela-Cobos et al. (2019) from 35 and 65 days. However, these results corroborate with Philippoussis et al. (2007) that the C:N ratio is a nutritional factor that directly influences the duration of the phases, affecting the precocity of cultivation.

It was possible to observe differences in the quantity, mass (Tables 7 and 10), and morphological quality (Figure 1). These productive characteristics differed among the substrates, in which the C:N 30:1 and 36:1 ratios had the largest quantities and the slight mushrooms (Tables 7 and 10) these results were close to those obtained by Azman et al. (2019) average unit masses of 7.69 g, in the C:N ratios of 50:1, 60:1, and 70:1, were obtained the smallest amounts of mushrooms harvested (Table 7) and with larger average unit masses, in addition to these C:N ratios having the longest periods in production (Table 6), that is, long periods in which the axenic block keeps producing does not favor the attainment of higher yields. This information facilitates the decision-making of producers regarding the scheduling of production.

The BE values of the experiments (Tables 8 and 11) were lower than those obtained by Xu et al. (2020) at 56.21%, by Gao et al. (2020) at 42.19%, by Gaitán-Hernández et al. (2020) at 95.51%, by Atila (2019) of 37.92 %, and Valenzuela-Cobos et al. (2019) of 91.80%. However, the BE was close to those obtained by Mata et al. (2016) at 23.08% and higher than that obtained by Zied et al. (2016) at 12.48. The compositions of the substrates are not limited to the C:N ratio; they provide a broad and complex nutritional complex (Ranjbar & Olfati, 2017; Dayani et al., 2018), and according to Xu et al. (2020), to reach high BE, the C:N ratio must be as stable as possible, that is, no big difference from the initial C:N ratio of the substrate until the end of cultivation must occur. This is proven by Bach et al. (2018), in which the difference of 2% from the initial C:N ratio to the end of cultivation in axenic blocks is beneficial to obtain the best productive responses.

The PR obtained in the experiments (Tables 8 and 11) was higher than the PR achieved by Sousa et al. (2019) from 0.0 to 16.2%, they related to the higher yields obtained in substrates that had less cellulose degradation. Values are obtained in different formulations, compositions, and raw materials. The PR is a response of the fungus to the degradation of cellulose during its production phase.

Moreover, when BMB was utilized to produce other species of mushrooms, also showed good responses. Oliveira et al. (2022) showed that this brewery residue is efficient for the cultivation of King Oyster (*Pleurotus eryngii*) results demonstrated favor values of BE and PR indicating the efficacy of the BMB as raw material inserts in the substrate formulation for edible mushrooms production.

Conclusion

The agro-industrial residue of barley malt bagasse (BMB) has the potential to be used in the composition of substrates as a supplement raw material for the cultivation of shiitake (*Lentinula edodes*) in axenic blocks at a commercial production level. It seems particularly promising and can provide diversification of the mushroom industry worldwide.

Acknowledgments

The authors want to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Cogumelos do Vale Europeu farm, Cervejaria Blumenau, and Funghi and Flora® for contributing with the necessary inputs and infrastructure for the development of this research.

Compliance with Ethical Standards

Author contributions: Conceptualization: GDO, FLC; Formal analysis: GDO, FLC; Methodology: GDO, FLC; Writing-original draft: GDO, FLC; Writing-review & editing: GDO, FLC.

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Financing source: The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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