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Protein enrichment of agroindustrial waste by semi-solid fermentation from *Lentinus tigrinus*

Enriquecimento proteico de resíduos agroindustriais por fermentação semissólida a partir de *Lentinus tigrinus*

Enriquecimiento protéico de residuos agroindustriales mediante ferme ntación semisólida a partir de *Lentinus tigrinus*

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Abstract
Selection different microorganisms and substrates are important challenges to enable potential waste for animal feed. Therefore, the aim of the study was to evaluate semisolid fermentation (SSF) effects from fungus Lentinus tigrinus on protein enrichment and fibrous fractions degradation in different agro-industrial residues for animal feed. The strains were subcultured for 7 days until visible mycelium growth and subsequently substrates inoculated. Five types of agro-industrial residues were used as substrate: sugarcane bagasse, sisal coproduct, sisal coproduct dry, dry brewery residue and wet brewery residue. Substrate samples without innocuous and after FSS were collected for bromatological analysis in triplicate. The Dry Matter contents did not show great loss rates during the 24-hour period, maintaining a very close value to the initial one in all residues. Protein enrichment was observed in the five types of agro-industrial residues studied using SSF by L. tigrinus, with emphasis on the wet brewery residue, 50%. It was also in this substrate that there was a significant lignin reduction (17%) under the established conditions. All residues from this study can be used as a substrate for protein enrichment and animal feed destined. However, the brewery residue and the sisal coproduct, in the different evaluated forms, stood out.
Keywords: Basidiomycetes; Ruminant; Lignin; Forage; Cellulose.

Resumo
Seleção de diferentes microrganismos e substratos são importantes desafios para viabilizar potenciais resíduos para alimentação animal. Diante disso, objetivou-se avaliar os efeitos da fermentação semissólida (FSS) com o fungo Lentinus tigrinus no enriquecimento proteico e degradação das frações fibrosas de diferentes resíduos agroindustriais para alimentação animal. As cepas foram repicadas por 7 dias até crescimento visível do micélio e posteriormente inoculadas nos substratos. Cinco tipos de resíduos agroindustriais foram usados como substrato: Bagaço de cana (BC), coproduto do desfibramento do sisal (CDS), feno do CDS, resíduo seco de cervejaria (RCS) e resíduo úmido de cervejaria (RUC). Amostras do substrato sem inócuo e após a FSS foram coletadas para análise bromatológica em triplicata. Os teores de MS não apresentaram grandes índices de perdas durante o período de 24 horas, mantendo um valor muito próximo ao inicial em todos os resíduos. Foi observado um enriquecimento proteico nos cinco tipos de resíduos agroindustriais estudados usando FSS por L. tigrinus com destaque para o resíduo úmido de cervejaria, 50%. Foi também neste substrato que houve significativa redução de lignina (17%) sob as condições estabelecidas. Todos os resíduos deste estudo podem ser usados como substrato para o enriquecimento protéico e destinados como alimentos para animais. Todavia, o resíduo de cervejaria e o coproduto do desfibramento do sisal, nas diferentes formas avaliadas, se destacaram.
Palavras-chave: Basidiomicetos; Ruminante; Lignina; Forragem; Celulose.

Resumen
La selección de diferentes microorganismos y sustratos son desafíos importantes para permitir residuos potenciales para la alimentación animal. Por tanto, el objetivo fue evaluar los efectos de la fermentación semisólida (FES) con el hongo Lentinus tigrinus sobre el enriquecimiento proteico y la degradación de fracciones fibrosas de diferentes residuos agroindustriales para la alimentación animal. Las cepas se subcultivaron durante 7 días hasta el crecimiento visible del micelio y posteriormente se inocularon en los sustratos. Se utilizaron como sustrato cinco tipos de residuos agroindustriales: bagazo de caña de azúcar (BC), coproducto de trituración de sisal (CTS), heno de CTS, residuo cervecero seco (RCS) y residuo cervecero húmedo (RCU). Se recolectaron muestras de sustrato sin inocuos y después de FSS para análisis bromatológicos por triplicado. Los contenidos de MS no presentaron grandes pérdidas durante el periodo de 24 horas, manteniéndose un valor muy cercano al inicial en todos los residuos. Se observó un enriquecimiento proteico en los cinco tipos de residuos agroindustriales estudiados mediante SSF por L. tigrinus, con énfasis en el residuo húmedo de cervecería, 50%. También fue en este sustrato donde hubo una reducción significativa de lignina (17%) en las condiciones establecidas. Todos los residuos de este estudio pueden ser utilizados como sustrato para el enriquecimiento proteico y destinados a la alimentación animal. Sin embargo, se destacaron el residuo de cervecería y el coproducto triturado de sisal, en las diferentes formas evaluadas.
Palabras clave: Basidiomicetos; Rumiante; Lignina; Forraje; Celulosa.

1. Introduction

Semi-solid Fermentation (FSS) is a biotechnology widely used to enrich agro-industrial residues used in animal feed. It is worth mentioning that, during the FSS, there is the degradation of lignocellulosic compounds of the substrates, thus allowing a better use of their fibrous fractions (Mahesh & Mohini, 2013). According to Conceição (2010) the main agents of degradation of these fractions are among the basidiomycete fungi, with *Lentinus tigrinus being* an option.

According to Tang et al. (2015), enabling the use of a greater number of agro-industrial residues as substrates for FSS not only offers economic benefits but also helps to protect the environment and can lead to benefits in animal production when intended for animal feed. ruminants (Mahesh & Mohini, 2013). Among the agro-industrial residues with high biomass production, sugarcane (*Saccharum sp)* stands out as the second largest bioenergy crop in the world (Yang, 2021). According to Brasil (2024) the national production of sugarcane was 691 million tons and that 27% of this mass represents the amount of sugarcane bagasse residue after the production process of the sugar and alcohol plants.

Sisal (*Agave sisalana*) is widely cultivated for fiber production in the semi-arid region of Bahia (Brandão et al., 2011) and according to the IBGE (2021) in 2020, Bahia produced 81,124 tons of sisal in an area corresponding to 93,376 hectares for harvesting, which makes this crop a great producer of reusable biomass. Another abundant source is brewery waste. Brazil is the third largest beer producer in the world according to the Sindicerv (2022), and in 2020 production was 14.1 billion liters. After the brewing of beer, in addition to the beverage, the wet beer residue is obtained (Geron et al., 2007) and this residue is already used in animal feed.

Several studies demonstrate the potential of FSS as an alternative in the protein enrichment of residues (Santos et al., 2015) and some authors have already reported more pronounced results in the first 24 h of FSS, under an average temperature of 35ºC, using different substrates and microorganisms. De França Silva (2020) found a protein increase of 30% for gherkin in 24 h of FSS as well as Araújo et al. (2008) who recorded a protein increase of 100% in the same period for forage cactus, both using *Saccharomyces cerevisiae.*

Campos et al. (2005), studying FSS in cashew peduncle bagasse, confirms that the fermentation time required for maximum conversion of soluble bagasse carbohydrates was around 24 hours. It was also with 24 h of fermentation with *Bacillus subtilis* that Seo and Cho (2016) found the best values for the protein profile of soybean meal.

It should also be noted that Thomas et al. (2013) states that the selection of the microorganism and the substrate are also challenges, since the substrate is the nutrient consumed by the microorganism in the fermentation process for protein synthesis (Araújo et al., 2017) emphasizing the importance of evaluating more substrates for this purpose. Allied to this,

knowing the potential of different residues after a short fermentation period, increases even more food alternatives in areas with little food supply.

The protein enrichment of residues through the FSS is well studied, however the evaluations of protein enrichment associated with the degradation of the fiber components are scarce. Thus, the objective was to evaluate the effects of FSS with the fungus *Lentinus tigrinus* on protein enrichment and degradation of fibrous fractions of different agro-industrial residues for animal feed.

2. Methodology

This study corresponds to quantitative laboratory research. The methodologies used in this research are consolidated and have already been described by several authors such as Araújo et al. (2017), Canedo et al. (2016), Fonseca et al. (2019).

The strains of Lentinus tigrinus CCMB553 used belongs to the culture collection of the Microbiology Research Laboratory of the Universidade Estadual de Feira de Santana - BA. The fungi were subcultured onto agar containing 8 g of agar agar I (HiMedia); 8 g of sugarcane bagasse obtained after extracting the juice in a cafeteria in the region; 2 g of ammonium sulfate and 400 ml of distilled water, and then incubated at 27° (± 2°C) for 7 days until visible growth of the mycelium to proceed with the FSS.

Five types of agro-industrial residues were used as substrate: sugarcane bagasse (BC), sisal shredding co-product (CDS), CDS hay, dry brewery residue (RCS) and wet brewery residue (RUC). BC was obtained after extracting the juice in a cafeteria in the region. CDS and CDS hay were collected on rural properties in the municipality of Valente - BA. The brewery residues, dry and wet, were obtained in producing industries in the city of Alagoinhas - BA. The collected materials were packed in sterile bags and taken to the laboratory to undergo the fermentation process.

Fermentations were carried out in triplicate in a batch system using rectangular plastic bioreactors, with dimensions of 10 x 27 x 9 cm. For every 1000 g of substrate, a concentration of 30 g (3%) of fungi, on a wet basis, was added. After inoculation, the bioreactors were properly identified for each type of substrate and subsequently placed in a forced air circulation oven at a temperature of 35 ± 2 ºC, during a 24-h fermentation period.

Substrate samples without innocuous and after FSS were collected in hermetically sealed plastic containers and sent to the nutrition laboratory for bromatological analysis in triplicate. The determinations of dry matter (DM), Crude Protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Cellulose (CEL) and Lignin (LIG) were performed according to Silva & Queiroz (2002). Hemicellulose (HEM) contents were obtained by difference (NDF – FDA) as determined by Silva & Queiroz (2002). Non-fibrous carbohydrate (NFC) contents were calculated using the equation: NFC = 100 – (PB% + EE% + MM% + NDF%), according to Sniffen et al. (1992). The chemical composition of the substrates before FSS are shown in Table 1.

Table 1 - Bromatological composition in natura agro-industrial residues in % DM

	BC	CDS	HAY CDS	RUC	CSR
MS	44.3	13.2	88.6	22.7	89.8
PB	2.6	9.3	8.6	21.8	22.1
CNF	12.4	50.3	49.1	17.2	15.7
NDF	59.8	28.1	31.7	63.1	65.2
FDA	40.2	20.1	23.3	25.2	26.5
HEM	19.6	8.0	8.4	37.9	38.7
ON	10.4	8.4	8.3	14.1	14.7

Source: Self elaboration (2023).

The difference in percentage between the values was then obtained, before and after fermentation, in order to quantitatively evaluate the influence of the use of L. tigrinus on different substrates.

$$AP\ ou\ DEF\ (\%) = \frac{\text{Valor após FSS } (\%) - \text{Valor } in\ natura\ (\%) x100}{\text{Valor } in\ natura\ (\%)}$$

3. Results and Discussion

The DM contents did not show great loss rates during the 24-hour period, maintaining a value very close to the initial one in all residues, demonstrating that the water content remained very close to the in natura material after the fermentation period, as shown by the Table 2.

Table 2 - Dry matter (DM), crude protein (CP) and protein increase (AP) values in %DM agro-industrial residues *in natura* and after FSS by *L. tigrinus.*

	BC		**CDS**		**HAY CDS**		**RUC**		**CSR**	
	in natura	**FSS**	**in natura**	**FSS**	**in natura**	**FSS**	**in natura**	**FSS**	**in natura**	**FSS**
MS	44.3	45.3	13.2	16.7	88.6	89.1	22.7	23.8	89.8	90.1
PB	2.6	3.4	9.3	12.4	8.6	12.4	21.8	32.7	22.1	31.8
AP	31%		34%		45%		50%		44%	

Source: Self elaboration (2023).

Moisture loss generally occurs more sharply after longer fermentation times, as described by Santana Neto et al. (2017) and França Silva et al. (2020) evaluating FSS in pineapple, guava and bravo maxixe residues, respectively. These authors observed a water loss of up to 60% after 72 h of fermentation and attributed this to evaporation, the use of an oven with air circulation and heating of the material, as well as the consumption of water to carry out the metabolic activities of the medium and synthesis of new cells of the microorganism as described by Fonseca et al. (2019). In the same studies cited, the DM levels did not change significantly in the first 24 h, as observed in the present work.

It is important to emphasize that in the FSS an amount of liquid is used that can guarantee the growth and metabolism of microorganisms, however, the maximum binding capacity of water with the solid matrix should not be exceeded (Pandey, 2003). According to Pontes (2009) the minimum limit for DM content would be around 12%, below which microorganisms do not develop and the maximum limit would be 80%. The author emphasizes, however, that it is important to take into account the type of material being used. The DM contents of the residues used are within this recommended range and can allow a good fermentation process.

The maintenance of DM levels after fermentation is an important aspect regarding the use of these residues in the feeding of ruminants in the semiarid region. According to Ferreira et al. (2009), foods with higher moisture contents are used during periods of prolonged drought as a food support for ruminants because, in addition to supplying part of the nutritional demand of animals, they can also supply part of the water requirements. With the exception of CDS Hay and RSC, which are foods that undergo previous drying processes to facilitate their storage, the maintenance of DM levels in the other residues studied ensured their characteristics in terms of water storage and possible use in dry periods.

The CP values before and after the FSS as well as the protein increase (AP) in the residues, presented in Table 2, demonstrate an AP above 30% in all evaluated foods. The lowest AP observed was for BC and the highest for RUC, being 31 and 50%, respectively, with the AP of CDS (45%), Hay of CDS (45%) and RSC (44%) close to the highest values found in this work.

The protein increase in BC infers that there was a lower development of the microorganism with this substrate, which may be a consequence of the lower supply of nutrients and soluble carbohydrates in this residue compared to the others. According to Lima (2009), when the substrate limits the metabolic activity, the microorganism can trigger processes such as sporulation, reduced growth or even cell death. Suhet & Fioreze (2011) showed that sporulation in the FSS occurs shortly after depletion of glucose in the medium. These authors corroborate the data observed in the present study in view of the low levels of carbohydrates available in BC (Table 1), which confirms the importance of the substrate in the process.

The protein increase observed after FSS in CDS, as well as in BC, was also not accentuated as in the other substrates. Although CDS offers higher amounts of NFC than BC (Table 1), the DM value of the sisal co-product, 13.2%, may have compromised the fermentation process, as it is close to the recommended minimum, as already mentioned. This possibility can also be raised with the 45% AP in CDS Hay (Table 2). Considering that both CDS and CDS Hay were submitted to the same fermentation conditions and both have similar amounts of nutrients, the divergent levels in the AP can be attributed to the DM contents of CDS hay that contributed substantially to the synthesis. cell in the middle.

According to Taragano & Pilosof (1999), in FSS the high moisture content can influence the physical state of the substrate, the availability and diffusion of nutrients and the exchange of oxygen and CO_2 in the medium. The high initial moisture content in the FSS can affect the growth of the microorganism, as the porosity of the medium and the diffusion of oxygen are reduced, hindering the formation of the product. However, Araújo et al. (2005), evaluating different moisture contents for protein enrichment of forage cactus, reported that the moisture of this cactus must be above 90%. However, the authors used the yeast *Saccharomyces cerevisiae*, a microorganism with different characteristics to the one used in the present study.

The RUC obtained the highest AP, 50% among the substrates. These data are consistent with Canedo et al. (2016) who evaluated the protein enrichment of brewery residue with the fungus *Rhizopus oligosporus*, found an AP of 50% when there was no supplementation in the FSS. In the same study, when the fermentation was supplemented with Ammonium Sulfate and urea, the gains exceeded 75% of AP. The authors attributed these gains to the good level of nutrients in the substrate and humidity around 70%. Also working with brewery residue, Ogunjobi et al. (2011) obtained AP of up to 55%

when the fungus *Aspergillus atyzae* was submitted to the best fermentation conditions for this microorganism. The results found in the present study confirm the potential of RUC for protein enrichment through FSS with *L. tigrinus* under the conditions evaluated here.

Some authors like Joshi & Sandhu (1996) and Alexandre et al. (2013) report even higher AP for other residues with FSS. In the case of the former, a threefold increase in the crude protein content of apple pomace was observed using three yeasts, *S, Candida utilise and Torula utilis.* The second, using *S. cerevisiae* to enrich the pineapple peel residue, found that the enriched residue had a high protein content (20.21%) in relation to the in natura residue (7.61%) with an AP of approximately 180%. It is also worth mentioning the protein increase found by Santos et al., (2015) of 78% when using the fungus *Aspergillus niger* in the FSS of forage cactus.

These authors also observed a reduction in cellulose, HEM and LIG contents of approximately 40%, 36% and 28%, respectively, and attributed these results to the fungus' ability to produce the enzymes that catalyze the hydrolysis of these polymers. Among the microorganisms that produce extracellular fibrolytic enzymes, fungi are the most studied due to their ability to produce abundant amounts. This corroborates the values in Table 3 when the FSS using the fungus *L. tigrinus* allowed the reduction of up to 22% of NDF and up to 17% of lignin in the residues studied.

Table 3 - Values of neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HEM), lignin (LIG) and their degradation (DEG) in %MS of agro-industrial residues after SSF by L. tigrinus.

	BC		CDS		HAY CDS		RUC		CSR	
	FSS	DEG	FSS	DEG	FSS	DEG	FSS	DEG	FSS	DEG
NDF	47.2	21%	23.6	16%	27.2	14%	49.2	22%	51.5	21%
FDA	32.9	18%	17.2	14%	20.2	13%	20.0	20%	21.2	20%
HEM	15.6	20%	6.8	14%	7.4	11%	32.6	14%	34.4	11%
ON	8.6	17%	7.56	10%	7.5	9%	12.5	11%	13.0	11%

Source: Self elaboration (2023).

The greatest degradations of the fibrous fractions observed were for BC, RCU and RSC. In these substrates, the NDF degradation reached 21, 22 and 21%, respectively. The degradation of lignin (LIG) was also more pronounced for these substrates with greater attention to BC which reached 17%. In CDS Hay, the values were the lowest observed in the study, being 14% for NDF and 9% for LIG degradation. These values did not differ considerably for the CDS and allow us to infer that the use of available nutrients in the sisal co-product was sufficient for the growth of the microorganism without the need for fiber degradation. According to Brandão et al. (2011) the content of non-fibrous carbohydrates is approximately 45% in the CDS, a value close to that found in this work (Table 1) and represents levels above the other substrates.

Fernández-Fueyo et al. (2014) state that the secretion of fibrolytic enzymes depends on environmental factors and is strongly regulated by the availability of nutrients. Thus, the lower supply of soluble sugars in BC, RUC and RSC probably enabled a greater degradation of the cell wall, including LIG, in these substrates.

Some of the enzymes synthesized during FSS are Laccase, Manganese Peroxidase and Lignin Peroxidase and these, in turn, are responsible for the degradation of lignin in substrates (Silva et al., 2014). In addition to these enzymes, Santos et al. (2013) report the xylanase that is responsible for the degradation of xylan, an important component of hemicellulose. It should be noted that fungi are potentially more useful for the production of xylanase as they secrete enzymes into the medium and their levels are generally higher than those of yeasts and bacteria.

Mukhopadhyay et al. (2011) achieved high levels, above 80%, of degradation of *Ricinus comunis LIG by* applying commercial laccase. Silva et al. (2014), working with the crude enzymatic extract of *T. villosa* after FSS, observed that it was able to reduce the lignin content by 35.0% for BC and 63.1% for CDS after 4 hours of treatment. fermentation and that no improvement was detected for longer reaction times. Zavarzina et al. (2018) evaluating ligninolytic enzymes in strains of *L. tigrinus* identified activities of laccases and MnP in the early development of the microorganism. Thus, the degradation of fibrous fractions, including HEM and LIG, as well as the protein increase observed in this study can be explained by the action and production of enzymes by *L. tigrinus* and by the proliferation of the microorganism in the medium.

Studies show that foods with higher levels of protein and lower levels of fiber favor consumption and weight gain in ruminants. Silva et al. (2002) reported thahenen they provided diets with 14 and 17% CP in DM to steers in the growing phase, they observed that consumption increased with the increase of CP in the diet. Santos et al. (2015) describe an increase in rumen degradability studying forage cactus enriched by FSS. Mertens (1987) places fiber in food as a limiting factor for consumption by ruminants, as it is inversely related to energy content and better represents the food's property in occupying space in the gastrointestinal tract. In view of the gains in residues in the present study, it is worth proposing that the improvement in their nutritional quality makes them foods, possibly, more favorable to greater consumption, greater degradability and, consequently, greater animal weight gain.

4. Conclusion

Protein enrichment was observed in the five types of agro-industrial residues studied using FSS by L. tigrinus. A protein increase of 50% of the wet brewery residue and of 17% of lignin was observed under the established conditions.

All residues from this study can be used as a substrate for the enrichment of animal feed in view of their AP and reduction of fibrous fractions. However, the brewery residue and the sisal shredding co-product, in the different evaluated forms, stood out.

Recommend FFS studies with L. tigrinus under different conditions of pH and temperature in order to possibly optimize the enzyme synthesis by the fungus. Consumption, digestibility and weight gain assays in ruminants fed with these enriched residues will be important for their use.

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