SOLID-STATE FERMENTATION OF BREWER'S SPENT GRAIN FOR XYLANOLYTIC ENZYMES PRODUCTION BY *Penicillium janczewskii* AND ANALYSES OF THE FERMENTED SUBSTRATE

FERMENTAÇÃO EM ESTADO SÓLIDO COM BAGAÇO DE CEVADA PARA PRODUÇÃO DE ENZIMAS XILANOLÍTICAS POR Penicillium janczewskii E ANÁLISE DO SUBSTRATO FERMENTADO

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ABSTRACT: In recent decades, increasing interest has been devoted to xylanolytic enzymes due to their potential use in many industrial processes. This study describes the production of xylanase, β-xylosidase and α-L-arabinofuranosidase, belonging to the xylanolytic complex, by *Penicillium janczewskii* using brewer's spent grain as substrate for solid-state fermentation. The optimized conditions for high levels of xylanase, β-xylosidase and α-L-arabinofuranosidase production were: 50% initial moisture, which was provided by Vogel's salt solution, seven days of cultivation at 20-30 °C. Fermentation enriched the bioproduct with some amino acids and did not add mycotoxins to it. The use of brewer's spent grain as substrate for fungal cultivation and enzyme production can both add value to this waste and reduce the production cost of xylanolytic enzymes.

KEYWORDS: Enzyme production. Xylanolytic enzymes. *Penicillium janczewskii.* Solid-state fermentation. Brewer's spent grain.

INTRODUCTION

Xylan is the most common hemicellulose and it is composed mainly of D-xylose, although other sugars may be present as substituents of the principal chain. Due to its complex structure, the complete breakdown of xylan requires several enzymes acting cooperatively, known as xylanolytic system. Endo-β-1,4-xylanase (4-β-D-xylan xylanohydrolase EC 3.2.1.8) is the main enzyme responsible for xylan depolymerization, breaking the main chain, releasing xylooligosaccharides, while β-xylosidase (4-β-D-xylan xylohydrolase, EC 3.2.1.37) removes xylose from non-reducing ends of xylobiose and other xylooligosaccharides. Other enzymes responsible for removing xylan substituents such as α-L-arabinofuranosidase (EC 3.2.1.55), α-glucuronidase, and acetyl xylan, feruloyl, and p-coumaroyl esterases have been referred as auxiliary or accessory xylanolytic enzymes. These enzymes are important for the complete enzymatic hydrolysis of the xylan since side chains can restrict enzyme action on the polymer main chain (BIELY, 1985; BIELY, 2003; BEG et al., 2001).

Complete xylanolytic systems have been found to be quite widespread among fungi, actinomycetes, and bacteria; and the production of xylanolytic enzymes by *Penicillium* species has been often related (POLIZELI et al., 2005; CHÁVEZ et al., 2006). Filamentous fungi usually secrete plant cell wall degrading enzymes into the medium, releasing energy and nutrients from plant biopolymers. This physiological characteristic is interesting from the industrial point of view because it eliminates the need for releasing the enzymes by cell disruption in the downstream process; moreover the levels of extracellular enzymes produced by fungi are higher than those from yeasts and bacteria (KNOB et al., 2010).

In many countries, agricultural or agro industrial wastes constitute a problem, mainly those generated in large quantities and those that do not receive proper disposal. Therefore, such residues can be considered excellent substrates for the growth of filamentous fungi especially by solid-state fermentation that allows the use of these wastes, available in large amounts and usually at low cost, turning the production process economically profitable. On the other hand, it solves the problem of waste disposal that would cause, otherwise, environmental pollution (SOCCOL; VANDENBERGH, 2003; SINGHANIA et al., 2009). Moreover, when used for animal feed, the fermented substrate is more easily digested due to the presence of the enzymes and also presents higher nutritional value, especially in relation to the protein content (GRAMINHA et al., 2008).

Brewer's spent grain (BSG) is the main byproduct of the brewing industry, and it is primarily composed of 28% arabinoxylan, 28% lignin and 17% cellulose. Despite of the large

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amounts generated annually, BSG has received little attention and its use on large scale is limited to animal feed (MUSSATTO; ROBERTO, 2006; MUSSATTO et al., 2006; ALIYU; BALA, 2011). Some studies have investigated BSG as substrate for xylanolytic enzymes production by different microorganisms (NASCIMENTO et al., 2002; PANAGIOTOU et al., 2006; 2007; MANDALARI et al., 2008; TERRASAN et al., 2010). However, there are no studies evaluating specifically the use of BSG for animal feed, after biological or enzymatic treatment. In this regard, there is only one publication about the effect of BSG supplemented with exogenous xylanases in the development of broilers (DENSTALDI et al., 2010). Thus, the conversion of renewable agricultural sources to bioproducts with improved nutritional quality to be used for feed supplementation can be considered a challenging area in biotechnology.

In this sense, the use of solid-state fermentation (SSF) as an alternative to decrease the costs of enzyme production, and also the possibility of subsequent use of the fermented substrate for animal feed turn this technique quite economical and environmentally advantageous. The objective of this work was to optimize the culture parameters one-variable-at-a-time for the production of xylanase, β-xylosidase and α-L-arabinofuranosidase by *P. janczewskii* in solid cultures with BSG and evaluate the properties of fermented material for animal feed.

MATERIAL AND METHODS

Microorganism: maintenance and inoculum production

Penicillium janczewskii strain (CRM 1348) is deposited in The Central of Microbial Resources, CMR-UNESP, Brazil. The microorganism was maintained in Vogel solid medium (VOGEL, 1956) with 1.5% (w/v) wheat bran at 4 °C and cultured periodically. Cultures were inoculated in the same medium with 1.5% (w/v) glucose and incubated for 7 days at 28 °C for conidia production. Conidia were harvested and suspended in distilled water, tap water or Vogel's salt solution (as described below), and the concentration was adjusted to 10^7 conidia mL^{-1}.

Cultivation on BSG for enzyme production

Solid-state fermentation was performed in triplicate in 250 mL Erlenmeyer flasks containing 5 g of dried BSG. BSG was provided by a local brewery (Baungartner, Ipeúna, São Paulo, Brazil) and exhaustively washed with distilled water and dried at 80 °C to constant weight.

Initial 20, 30, 40 and 50% (v/w) moistures were established with distilled water, tap water or Vogel's salt solution (VOGEL, 1956). Each medium was then inoculated with 1 mL of the conidia suspension completing the moisture content, according to the cultivation parameters. Cultivation was carried out for 7 days at 28 °C.

Production of xylanase, β-xylosidase and α-L-arabinofuranosidase was followed for 15 days at 28°C starting with 50% (v/w) moisture in medium established with Vogel's salt solution.

Production of xylanase, β-xylosidase and α-L-arabinofuranosidase was evaluated after 7 days incubation under different temperatures from 20 to 35 °C, starting with 50% (v/w) moisture in medium established with Vogel's salt solution.

Preparation of the crude extract

Extraction of the enzymes was performed by adding 50 mL of ice-cold distilled water to the fermentation medium followed by orbital shaking (200 rpm, 30 min.) under refrigeration. The resulting suspension was vacuum filtered and the filtrate was used as source of extracellular proteins.

Determination of protein and enzyme activities

The protein concentration was determined by the modified Bradford method (SEDMACK; GROSSBERG, 1977) using bovine serum albumin as standard.

Xylanase activity was determined, according to Bailey et al. (1992) using 1.0% (w/v) birchwood xylan (Sigma) in a 0.05 M sodium acetate pH 5.5 buffered reaction and appropriately diluted enzyme solution at 50 °C; the reducing sugars were quantified with dinitrosalicylic acid reagent (MILLER, 1959). β-xylosidase and α-L-arabinofuranosidase activities were determined using 0.25% (w/v) ρ-nitrophenyl β-D-xylopyranoside (Sigma) and ρ-nitrophenyl α-L-arabinofuranoside (Sigma), respectively, in McIlvaine pH 4.0 buffered reaction and appropriately diluted enzyme solution, at 50 °C. The reaction was stopped with a saturated sodium tetraborate solution, and the absorbance was read at 405 nm (KERSTERS-HILDERSON et al., 1982). One unit of enzyme activity was defined as the amount of enzyme required to release 1 μmol of product equivalent (reducing ends for xylanase assays or ρ-nitrophenol for β-xylosidase and α-L-arabinofuranosidase assays) per min in the assay conditions. Specific activities were expressed as enzyme units per milligram of protein.

Chemical analysis

Samples of the fermented BSG were pre-dried and used for determination of dry matter, mineral matter, crude protein and ether extract according to AOAC (1990), and neutral detergent fiber (NDF) and acid detergent fiber (ADF), according to Van Soest (1994). Hemicellulose was calculated as the difference between NDF and ADF, and cellulose as the difference between ADF and ADL (acid detergent lignin). Analyses were performed by Biobell Technologies, Mirassol, SP, Brazil. Non-fermented BSG sample was prepared in the same conditions and used as control.

Amino acid composition

Amino acid determinations were carried out before and after acid hydrolysis. The crude material (5 mg) was hydrolyzed with a solution of 6 mol l^{-1} of HCl (1 mL) and 5% phenol/water solution (0.08 mL) for 72 h at 110 °C to guarantee quantitative hydrolysis of peptide bounds. Pyrex tubes with plastic Teflon-coated screw caps (13.0 x 1.0 cm) were used for hydrolysis. Samples were dried, diluted with 1.0 mL of citrate buffer pH 2.2 and filtered in a GV Millex Unity (Millipore). Amino acids analyses were performance by cation exchange chromatography using an automated amino acid analyzer Shimadzu LC-10A/C-47A, with sodium eluents and post-column derivatization with OPA (*o*-phthaldialdehyde) (FOUNTOULAKIS; LAHM, 1998). Amino acid identification and quantification was performed comparing each peak profile (retention time and area) with a standard amino acid mixture (17 amino acids, 100 nmol mL^{-1}). Non-fermented BSG was prepared in the same conditions and used as control.

Mycotoxin analysis

Analysis of mycotoxins was performed by Eurofins Food Analysis Ltd. Ochratoxin A was determined with an immunoaffinity column clean-up in HPLC with fluorescence detection, according to modified EN 14132 Method (ENTWISLE et al., 2001). Zearalenone and deoxynivalenol were extracted with acetonitrile:water (1:1, v:v), solid-phase extraction (SPE) clean-up, solvent-exchange, and HPLC-MS/MS analysis, according to internal method. Patulin was extracted with ethylacetate, followed by partitioning of the extract with aqueous sodium carbonate solution, and analyzed by HPLC with DAD detector, according to internal method. Aflatoxins were extracted, transferred to phosphate buffered saline, cleaned up with monoclonal antibody affinity column. After elution, the aflatoxins were post-derivatized using a cobra-cell-reactor and then analyzed by HPLC with fluorescence detection, according to internal method based on European Standard EN 14123. Non-fermented BSG was prepared in the same conditions and used as control.

RESULTS AND DISCUSSION

Production of xylanolytic enzymes by *P. janczewskii* in solid-state fermentation of BSG

The xylanase, β-xylosidase and α-L-arabinofuranosidase production by *P. janczewskii* increased with the moisture content, with the highest enzyme production observed in 40% (α-L-arabinofuranosidase) and 50% (xylanase and β-xylosidase) moisturized cultures. This effect can be assigned to the faster growth of the microorganism in the highly moisturized cultures, resulting in higher enzyme production (KALOGERIS et al., 1998). Strong reduction in enzyme yields was obtained at lower moisture contents (Table 1).

Many studies reported similar effects with intermediate or higher moisture contents favoring the production of xylanolytic enzymes by fungi as *Aspergillus niger* (MACIEL et al., 2008), *Fusarium oxysporum* (XIROS et al., 2008a), *Melanocarpus albomyces* (NARANG et al., 2001), *Neurospora crassa* (XIROS et al., 2008b), *Paecilomyces themophila* (YANG et al., 2006), *Penicillium brasilianum* (MAMMA et al., 2007) and *Penicillium canescens* (BAKRI et al., 2003). Furthermore, it is noteworthy that the xylanase and α-L-arabinofuranosidase productions were much higher when the moisture was provided by Vogel's salt solution than the production obtained in cultures moisturized with distilled or tap water. These results suggest that the presence of the different salts in this solution favor fungal growth, increasing enzyme production. However, for β-xylosidase production, similar activity yields were obtained using both Vogel's salt solution or distilled water. Higher values of specific activities were also observed in 50% moisturized cultures with distilled or tap water.

Table 1. Xylanase, β-xylosidase and α-L-arabinofuranosidase production by *P. janczewskii* in solid-state cultivation with brewer's spent grain at different initial moisture content.

	Initial moisture (%)	Enzyme activity					
		Xylanase		β-xylosidase		α-L-arabinofuranosidase	
		(U/g dry BSG)	(U/mg prot.)	(mU/g dry BSG)	(mU/mg prot.)	(mU/g dry BSG)	(mU/mg prot.)
Vogel's salt solution	20	22.8 ± 2.3	38.6 ± 2.1	23.8 ± 2.9	37.5 ± 8.4	29.7 ± 2.3	50.9 ± 8.9
	30	145.7 ± 13.1	102.8 ± 11.2	97.7 ± 10.9	70.7 ± 8.9	236.8 ± 33.0	165.4 ± 32.9
	40	209.2 ± 6.4	134.4 ± 4.0	169.6 ± 5.1	108.9 ± 2.8	674.8 ± 30.9	229.0 ± 32.8
	50	370.0 ± 30.1	159.3 ± 8.9	246.5 ± 14.7	106.2 ± 8.9	606.7 ± 19.5	291.3 ± 19.5
Distilled water	20	19.5 ± 1.9	2190.2 ± 75.9	21.5 ± 2.0	2586.4 ± 125.8	17.0 ± 1.8	2111.2 ± 521.4
	30	80.8 ± 2.1	4474.3 ± 318.6	102.0 ± 6.2	5602.7 ± 148.7	91.6 ± 4.3	4919.3 ± 454.2
	40	165.6 ± 7.4	5853.3 ± 382.7	188.2 ± 6.2	6642.2 ± 237.5	177.1 ± 13.7	6250.2 ± 467.4
	50	263.9 ± 7.0	7889.2 ± 407.1	245.6 ± 16.2	7341.0 ± 564.6	227.6 ± 15.1	6793.6 ± 297.2
Tap water	20	12.8 ± 0.4	1858.3 ± 112.9	14.7 ± 1.0	2136.4 ± 24.7	12.7 ± 1.0	1841.9 ± 164.7
	30	53.2 ± 2.7	3641.8 ± 138.5	54.6 ± 2.2	3736.9 ± 79.9	55.7 ± 3.7	3812.9 ± 161.1
	40	136.7 ± 10.4	5419.3 ± 337.0	134.0 ± 4.7	5466.3 ± 419.2	155.3 ± 13.7	6379.9 ± 365.0
	50	169.7 ± 4.8	4954.7 ± 149.4	181.2 ± 16.1	5283.0 ± 489.2	220.6 ± 20.9	6425.6 ± 549.2

Cultivation was carried in 250 mL Erlenmeyer flasks containing 5 g of dried BSG for 7 days at 28 °C. Data are mean and standard deviation of triplicates.

Xylanase production increased until the seventh day of cultivation, when it reached the maximal yield (371 U/g dry BSG), and decreased at the eighth day, from which remained stable until the 11th day, decreasing slowly in the following days (Figure 1a).

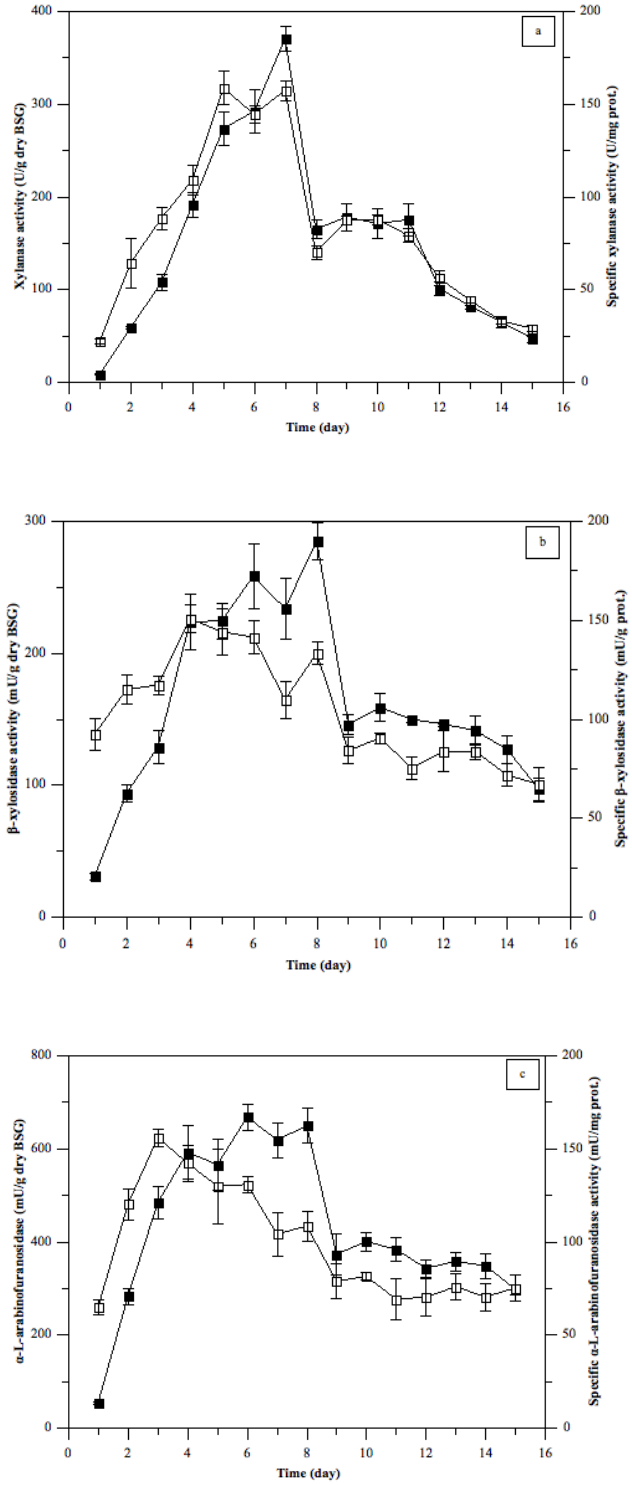

Figure 1. Time-course of xylanase **(a)**, β-xylosidase **(b)** and α-L-arabinofuranosidase **(c)** production by *P. janczewskii* in solid-state fermentation with brewer's spent grain. Growth conditions: 50% (v/w) initial moisture established with Vogel's salt solution at 28 °C. (■) Enzyme activity (U/g BSG or mU/g BSG); (□) Specific enzyme activity (U/mg prot. or mU/g prot.).

The highest values of specific activity were observed from the fifth to seventh day (≈150 U/mg prot.). Considering the experimental error, the β-xylosidase production increased until the eighth day (285 mU/g BSG), decaying from this. High values of specific activity were observed from the fourth to seventh day, corresponding to approximately 150 mU/mg prot. (Figure 1b). The α-L-arabinofuranosidase production increased until the sixth day (668 mU/g dry BSG), remained stable up to the eighth day decreasing from this. The highest specific α-L-arabinofuranosidase activity was observed at the third day of cultivation (Figure 1c).

Similar pattern of time-dependent enzyme production was reported for other fungal strains, *e.g*. *Paecilomyces themophila* after eight days of growth on solid medium with wheat bran (Yang et al., 2006); *Penicillium brasilianum* after 96, 108 and 196 h (4.0, 4.5 and 8.2 days) of growth on BSG produced the highest yields of xylanase, arabinofuranosidase and feruloyl esterase activities (Panagiotou et al., 2006; 2007); *Fusarium oxysporum* after 144 h (5 days) of growth in a reactor containing BSG and corncobs (FES) produced the highest yields of xylanolytic enzymes (Xiros et al., 2008a). These results are supported by the fact that β-xylosidases are only required after the initial action of xylanases on the substrate. The reduction of the enzyme activities may be associated to the production of proteases, a secondary and undesirable process that decreases the net productivity of the xylanolytic enzymes.

The production of xylanases, β-xylosidases and α-L-arabinofuranosidases by *P. janczewskii* was evaluated in cultivations carried out at 20, 25, 30 and 35 °C. As presented in Table 2, the highest xylanase production was verified at 25 °C, the highest the β-xylosidase production occurred at 20 °C, and the highest α-L-arabinofuranosidase production occurred at 30 °C. Similarly, Panagiotou et al. (2006; 2007) found the highest production of xylanase, arabinofuranosidase and feruloyl esterase by *P. brasilianum* at temperatures between 25 and 30 °C.

Table 2. Xylanase, β-xylosidase, and α-L-arabinofuranosidase production by *P. janczewskii* in solid-state cultivation with brewer's spent grain at different temperatures.

Temperature (°C)	Enzyme activity					
	Xylanase		β-xylosidase		α-L-arabinofuranosidase	
	U/g dry BSG	U/mg prot.	mU/g dry BSG	mU/mg prot.	mU/g dry BSG	mU/mg prot.
20	231.0 ± 5.8	115.1 ± 3.9	246.5 ± 11.4	124.5 ± 9.6	371.7 ± 19.0	192.9 ± 19.0
25	370.2 ± 10.7	160.4 ± 4.6	225.1 ± 5.7	97.4 ± 2.5	403.4 ± 21.8	181.5 ± 9.2
30	318.9 ± 6.6	140 ± 1.9	210.3 ± 8.9	92.5 ± 8.9	674.6 ± 24.7	296.5 ± 11.5
35	ND	ND	ND	ND	ND	ND

Cultivation was carried in 250 mL Erlenmeyer flasks containing 5 g of dried BSG with 50% (v/w) initial moisture established with Vogel's salt solution for 7 days. Data are mean and standard deviation of triplicates. ND: not detected

Analyses of the Fermented Material

Table 3 presents data of chemical composition of the BSG before and after *P. janczewskii* growth. Such data are important because they represent an initial analysis of the product that will essentially define its nutritional value. After fungal growth, the composition changed mainly in relation to the hemicellulose fraction, *i.e*., the reduction in the hemicellulose fraction can be associated with the action of the xylanolytic enzymes produced by the fungus. The cellulose fraction was not changed, suggesting, as previously observed in liquid cultures (TERRASAN et al., 2010), the low or the absence of cellulolytic activity. Increase in the lignin content in relation to total dry matter may be directly related to the reduction of the hemicellulose. Furthermore, it was observed an increase in the protein content that is associated with the colonization and growth of the microbial biomass in the substrate. The decrease of TDN during fungal growth is not advantageous for application of the fermented substrate as a component of animal feed. The increase in mineral matter may be attributed to the salts present in the Vogel's solution added prior to the cultivation.

Amino acid composition was determined (Table 4) instead of analysis of total nitrogen because the last includes the non-protein nitrogen content such as that from nucleic acids that do not have nutritional value. Thus, this analysis can be considered a more realistic analysis of the protein content. Statistically different changes in amino acid

composition were observed only for alanine, histidine, proline and lysine.

Table 3. Chemical composition of fermented and non-fermented brewer's spent grain.

Analysis	Control (%)	Fermented BSG (%)
Dry matter	96.44	95.89
Ether extract	2.79	3.23
Mineral matter	3.92	4.46
Total nitrogen	17.53	19.95
NFD	72.07	72.27
AFD	39.27	42.69
Cellulose	22.32	23.01
Hemicellulose	32.81	29.59
Lignin	16.94	19.68
TDN	43.83	40.80

The results are expressed as percentage of dry matter. NFD: Neutral detergent fiber; AFD: Acid detergent fiber; TDN: total digestible nutrient (WEISS et al., 1992).

Table 4. Amino acid composition of fermented and non-fermented BSG.

Amino acid	Control		Fermented BSG	
	g/100g dry BSG	% Total mass	g/100g dry BSG	% Total mass
Aspartic Acid	1.44	8.93	1.10	6.43
Threonine	0.73	4.57	0.80	4.72
Serine	0.66	4.13	0.70	4.09
Glutamic Acid	2.59	16.10	2.91	17.09
Proline*	0.81	5.04	0.58	3.42
Glycine	0.93	5.78	1.03	6.06
Alanine*	1.00	6.21	1.22	7.17
Cystein	0.00	0.00	0.00	0.00
Valine	1.15	7.17	1.23	7.20
Metionine	0.25	1.55	0.29	1.69
Isoleucine	0.82	5.07	0.85	5.00
Leucine	1.52	9.43	1.70	9.97
Tyrosine	0.65	4.06	0.61	3.60
Phenylalanine	0.94	5.83	1.01	5.94
Histidine*	0.79	4.89	1.64	9.63
Lysine*	0.82	5.13	0.42	2.44
Arginine	0.98	6.12	0.95	5.56
Total	16.09	-	17.05	-

*Means statistically different by Mann-Whitney Test ($p \leq 0,05$).

Alanine and histidine concentrations were significantly higher in fermented BSG while proline and lysine concentrations significantly decreased after fungal growth. These results are important for amino acids balance in formulating a bio-product intended to animal nutrition, considering that histidine is an important amino acid to monogastrics and ruminants. However, the concentration of lysine, also an important amino acid for nutrition, decreased after cultivation. In this sense, the evaluation of other additional micronutrients in medium composition is required for balancing the final product. Ultimately, processing this material by with *Penicillium janczewskii* increased the overall amino acid content.

Mycotoxins analysis revealed the fungus did not produce mycotoxins during cultivation on BSG. In fact, reduction of deoxynivalenol and zearalenone found in non-fermented BSG (at lower concentrations than those established by Brazilian, UE and EUA legislations for feedstuffs or animal feed) was observed after fungal growth (Table 5).

Table 5. Mycotoxin analysis of the fermented and non-fermented BSG.

Mycotoxin		Control (μg/kg)	Fermented BSG (μg/kg)
Aflatoxin	B1	< 0,1*	< 0,1*
	B2	< 0,1*	< 0,1*
	G1	< 0,1*	< 0,1*
	G2	< 0,1*	< 0,1*
Ochratoxin A		< 0,2*	< 0,2*
Deoxynivalenol		95	71
Zearalenone		125	48
Patulin		< 5*	< 5*

*Lower than detection limit.

CONCLUSIONS

Brewer's spent grain can be considered a very suitable substrate for the production of xylanolytic enzymes by *P. janczewskii* in solid-state cultivation, especially in order to replace wheat bran, widely used in the production of food and feed, which cannot be considered as an inexpensive substrate anymore.

Cultivation of the fungus *P. janczewskii* on this substrate is an interesting alternative to improve the nutritional quality and add value to this byproduct of the brewing industry.

The fermented BSG might be used to compose the diet of animals as a supplement with higher protein content, which, moreover, contains xylanolytic enzymes that may improve its digestibility with no health risks, once mycotoxins are absent. In this sense, the conditions selected for the production of high levels of xylanase, β-xylosidase and α-L-arabinofuranosidase by this fungal strain when cultured with this solid residue was 50% initial moisture provided by Vogel's salt solution and the cultivation carried out for seven days at 20-30 °C.

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RESUMO: Nas últimas décadas, há interesse crescente nas enzimas xilanolíticas devido à sua potencial utilização em muitos processos industriais. Este estudo descreve a produção de xilanase, β-xilosidase e α-L-arabinofuranosidase, três enzimas do complexo xilanolítico, por *Penicillium janczewski* utilizando bagaço de cevada como substrato para fermentação em estado sólido. As condições selecionadas para a produção de elevados níveis de xilanase, β-xilosidase e α-L-arabinofuranosidase por esta linhagem fúngica foram 50% de umidade inicial, sendo esta fornecida por uma solução de sais de Vogel e cultivo por sete dias a 20-30 °C. O bioproduto fermentado foi enriquecido com alguns aminoácidos e se apresentou livre de micotoxinas. O uso do bagaço de cerveja como substrato para o cultivo de fungos e produção de enzimas não só pode agregar valor a esses resíduos, mas também reduzir o custo de produção de enzimas xilanolíticas.

PALAVRAS-CHAVE: Produção de enzimas. Enzimas xilanolíticas. *Penicillium janczewskii.* Fermentação em estado sólido. Bagaço de cevada.

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