International Scholars Journals

African Journal of Virology Research ISSN 2756-3413 Vol. 17 (9), pp. 001-008, September, 2023. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Fibrolytic potential of spent compost of *Agaricus bisporus* to degrade forages for ruminants

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Accepted 28 March, 2023

To evaluate the fibrolytic potential of spent compost of champignon mushroom (*Agaricus bisporus;* SCAB) on forage degradation, liquids from spent compost were extracted and then evaluated *in vitro*. Firstly, SCAB was recovered at 50, 60 and 90 days post-harvest and enzyme activity was evaluated. Celluloses activity was highest at 60 days; xylanases activity was not affected by post-harvest stage; laccases activity increased as post-harvest stage increased. Secondly, liquid extracts of SCAB obtained at 60 days post-harvest were refrigerated, frozen, lyophilized and refrigerated plus benzoic acid, frozen plus benzoic acid, frozen plus glycerol or frozen plus benzoic acid- glycerol and then, the fibrolytic activity was evaluated. Fibrolytic activity was highest in SCAB frozen plus glycerol, lyophilization and frozen plus benzoic acid. Finally, effects of SCAB were evaluated on degradation of alfalfa hay, Taiwan grass, barley straw and spent compost of *A. bisporus*. Dry matter soluble fraction of alfalfa and potential degradation of dry matter of Taiwan grass and barley straw were increased by SCAB. It was concluded that SCAB showed a high fibrolytic activity which would increase degradation of forages for ruminants.

Key words: *Agaricus bisporus*, degradation, fibrolytic enzymes, forages, ruminants.

INTRODUCTION

Agaricus bisporus (champignon mushroom; AB) is the most important mushroom in the world economic-wise (Chang and Miles, 2004). It is produced on a composted substrate consisting of fiber and nitrogen compounds from various raw materials including straw, hay, corncobs, cottonseed hulls, poultry litter, cottonseed meal, among others (Lankinen et al., 2005; Mamiro and Royse, 2008). After AB composting, the substrate contains two major components: a lignocellulose fraction and a microbial biomass. Cellulose, hemicellulose and lignin degrading activities (Durrant et al., 1991; Bonnen et al., 1994; De Groot et al., 1998) are associated with the spent compost of AB (Ball and Jackson, 1995). Aerobic thermoplilic bacteria dominate at the start of composting, but their number decrease in later stages with the concomitant increase of actinomycete populations (Ball and Jackson, 1995). Thus, cellulases concentration in AB culture is high between 30 and 60 days post-harvest (Wood and Goodenough, 1977), while xylanase and laccase are high at the final stage of the process (60 days; Bonen et al., 1994). Enzyme activity during those growing stages of AB has been evaluated in the fruit body (cap, gills, stem), but no information exist on spent compost. Various preservation methods for enzymes

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Abbreviations: AB, *Agaricus bisporus*; **ADF,** acid detergent fiber; **DM,** dry matter; **NDF,** neutral detergent fiber; **SCAB,** spent compost of *Agaricus bisporus*; **R,** refrigerated; **RB,** refrigerated plus benzoic acid; **F,** frozen; **FB,** frozen plus benzoic acid; **FG,** frozen plus glycerol; **FBG,** frozen plus glycerol benzoic acid; **L,** lyophilized.

have been evaluated, such as refrigeration (Valenzuela and Ortiz, 2007), frozen, frozen plus benzoic acid or glycerol (Chanasattru et al., 2008), but no studies on enzymes from spent compost of mushrooms exist. Therefore, the objective of this research was to determine the fibrolytic activity and potential of spent compost of *A. bisporus* (SCAB) on degradation of some forages for ruminants.

MATERIALS AND METHODS

Strain, substrates and cultivation method

Nine SCAB blocks (-25 kg fresh weight each) were obtained from a commercial operation in a tropical area of Mexico. These blocks consisted of a mixture of barley straw, poultry litter, urea, calcium carbonate and sorghum grain. These blocks were colonized with mycelium of *A. bisporus* in solid substrate fermentation. At 50, 60 and 90 days (second, third and last harvest, respectively, of commercial champignon mushroom), mushrooms were harvested. Blocks were used to obtain the enzymatic extracts.

Preparation of enzyme extract

To obtain the enzyme extract, 400 g of each block were sampled and mixed with 600 ml of sodium citrate buffer (50 mM, pH 5.3). The mixture was mechanically shaken for 30 min and manually squeezed to obtain the fluids, which were centrifuged twice (4°C, 10,000 g, 25 min and 4°C, 10,000 g, 15 min) (Márquez et al., 2007). Enzyme activity and soluble protein concentration were measured in the supernatant.

Protein content was determined according to Bradford (1976) using as standard bovine serum albumin (Sigma) (0.15 M) dissolved in sodium citrate buffer (50 mM, pH 5.3). The xylanases and cellulases activities were estimated by determination of reducing sugar using the method of dinitrosalicylic acid (Miller, 1959) modified by Márquez et al. (2007). Substrates used were: for xylanases, a solution of oat xilan (Sigma X-0627) 0.5% dissolved in sodium citrate buffer (50 mM, pH 5.3) and for cellulases, a solution of 0.5% carboxymethyl cellulose salt of sodium (CMC, medium viscosity; Sigma-Aldrich C4888), dissolved in a sodium citrate buffer (50 mM, pH 5.3) . The laccases activities were determined by the oxidation of 2, 2 ' Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) dissolved in sodium citrate buffer (50 mM, pH 5.3) with a 36,000 M/cm coefficient of molar extinction (Bourbonnais et al., 1997) modified by Márquez et al. (2007). Values were expressed in international units (IU) and 1 IU of enzyme activity was defined as the amount of enzyme required to release 1 µmol of product per minute under the assay conditions described. Enzyme activities were referred to initial substrate in dry weight (IU/g dry matter basis).

Preservation methods

Sixty days post harvesting SCAB was used. Liquid extracts were obtained using the procedure previously described. Liquid fluids were conserved in sealed amber flasks during 180 days, using the following procedures: refrigerated at 8°C (R); refrigerated plus benzoic acid 10 mM dissolved in acetate buffer at 10% (RB); frozen at -20°C (F); frozen plus benzoic acid at 10% (FB); frozen plus glycerol at 10% (FG); frozen plus glycerol at 5% and benzoic acid at 5% (FBG); and lyophilized (L). Enzyme activities (xylanases, celluloses and laccases) were measured as described earlier.

In vitro degradation of enzymes and forages

The first phase of the *in vitro* degradation technique (Tilley and Terry, 1963) was performed collecting ruminal fluid from two Holstein steers (450 kg body weight) fitted with ruminal cannulas. Steers had free access to alfalfa hay and oat straw, mineral premix and water, plus 500 g/day concentrate (crude protein 16% DM basis). Ruminal fluid samples were collected 3 h after the morning meal and squeezed through two layers of cheesecloth into a 500 ml Erlenmeyer flask with an O_2-free CO_2 headspace. All incubations were carried out in a water bath at 39°C, with constant stirring.

Firstly, to evaluate the enzyme degradation of SCAB, 168 roll tubes each with 1 ml of liquid extract of SCAB and 20 ml McDougall saliva and 5 ml of strained ruminal fluid were incubated for 0, 3, 6, 9, 12, 24, 48 and 72 h. Four tubes per incubation period contained SCAB conserved by R, RB, F, FB, FG, FBG or L. Immediately after incubation, 1 ml of ruminal fluid was collected from each tube. The fluid samples were acidified with 3 M metaphosphoric acid (1:10 dilution), cooled at 4°C for 30 min and centrifuged (25,000 x *g*; 4°C; 20 min). Supernatants were removed and frozen. Ammonia-N concentrations (McCullough, 1967) were determined on supernatants using a UVVIS spectrophotometer (630 nm, CARY I-E, VARIAN).

Secondly, to evaluate effects of SCAB on degradation of dry matter (DM) of alfalfa hay, barley straw, Taiwan grass and spent compost, 100 g (DM basis) of these forages were ground using a Wiley Mill fitted with a 1 mm screen (Arthur H. Thomas, Philadelphia, PA, USA). Samples (500 mg) of ground forages were weighed in 54 roll polypropylene tubes equipped with butyl runner stopper. A commercial fibrolytic enzyme product (Fibrozyme, Alltech Inc., Nicholasville, KY, USA) was compared with SCAB enzyme extract. A liquid extract from Fibrozyme was obtained according to the methodology described by Márquez et al. (2007), where each ml of the liquid extract provides an enzymatic activity equivalent to 160 IU xylanases. One h before incubation, 1 ml of the enzymatic solution (SCAB or Fibrozyme) were applied directly onto the substrate (500 mg DM) contained in 120 ml propylene tubes. Then, a mixture of 40 ml McDougall artificial saliva and 10 ml of strained ruminal fluid were added to the tubes with the substrate and incubated for 1, 6, 12, 24, 48 and 72 h. Substrate in the controls (non-enzyme treatment) was treated with 1 ml of distilled water solution. Residuals were recovered by filtration (Whatman 541), dried at 65°C for 24 h and weighed. Only for alfalfa hay, a sample of the residual (100 mg) was recovered to quantify NDF and ADF contents (Van Soest et al., 1991) and determine its degradation. Then DM *in vitro* degradation was determined from the DM remaining in the roll tubes after incubation. The filter and undigested residues were oven-dried at 105°C for 24 h to remove excess moisture and weighed. Three roll tubes per enzyme and incubation time were used. Kinetics of *in vitro* degradation were analyzed using a Gompertz model (Susmel et al., 1999) as:

$$\text{dis}(t) = a + b * \exp[(-C)\exp(-Dt)],$$

Where: dis is the disappearance of material (g/kg) from the bag or tube at time *t*; *a* is the DM (g/kg) soluble in the rumen at *t* = time (h); *b* is the insoluble, but potentially disappearing fraction (g/kg); *C* is the fractional disappearance rate of *a* + *b* and *D* is a parameter to measure the rate of disappearance.

According to the Gompertz model, the fractional rate of disappearance varies as a function of time and the average value

Table 1. Protein content and enzyme activity of liquid extracts from spent compost of *A. bisporus* harvested at 50, 60 and 90 days.

	Days post-harvest			SEM
	50	60	90	
Protein (mg/g DM) [L]	8.8	8.7	5.7	0.8
Cellulases (IU/g DM) [Q]	10.0	20.2	5.7	0.8
Xylanases (IU/g DM) [L]	1102.7	1024.4	825.9	89.2
Laccases (IU/g DM) [L]	3593.1	4035.7	5663.3	515.8

L, linear ($P < 0.01$); Q, quadratic ($P < 0.001$).

Table 2. Protein content and enzyme activity of liquid extracts from spent compost of *A. bisporus* harvested at 60 days and preserved by different methods.

	Preservation method [1]							SEM
	R	RB	F	FB	FG	FBG	L	
Protein (mg/g DM)	6.9^{a}	6.3^{bc}	6.7^{ab}	6.5^{b}	6.0^{bc}	5.6^{d}	6.1^{c}	0.09
Cellulases (IU/g DM)	3.9^{b}	3.7^{c}	4.9^{a}	3.9^{b}	3.9^{b}	3.4^{d}	3.7^{c}	0.05
Xylanases (IU/g DM)	258.6^{d}	312.6^{bc}	443.2^{a}	436.7^{a}	341.5^{b}	372.4^{b}	286.5^{d}	8.34
Laccases (IU/g DM)	560.2^{a}	299.2^{b}	622.6^{a}	308.9^{b}	359.6^{b}	327.9^{b}	197.2^{c}	20.6

[1] Refrigeration (R); refrigeration plus benzoic acid (RB); frozen (F); frozen plus benzoic acid (FB); frozen plus glycerol (FG); frozen plus glycerol and benzoic acid (FBG); and lyophilization (L). [a-d] Means in the same row with different superscripts differ ($P < 0.05$).

(that is, a constant comparable to the exponential rate of disappearance) is derived as: $c = D/C$. The DM, NDF and ADF remaining at each incubation time were used to fit a nonlinear regression model using the NLIN option of SAS (2002).

Statistical analysis

In the first assay, nine SCAB blocks obtained at 50, 60 and 90 days post-harvest of the champignon mushroom were considered as the experimental units. Data were analyzed as a completely randomized design using the GLM procedure of SAS (SAS, 2002). The effect of time post-harvest (50, 60 and 90 days) was analyzed with orthogonal polynomials (linear and quadratic), calculating coefficients with the IML option of SAS (SAS, 2002). In the second assay, data of ammonia-N concentrations were analyzed as a completely randomized design with a factorial arrangement of treatments (7 x 7): storage period (1, 7, 14, 28, 56, 101 and 180 days) and preservation method (R, RB, F, FB, FG, FBG or L). Enzyme activities data were analyzed as a completely randomized design in which preservation methods was the source of variation. Finally, kinetic data of the *in vitro* assay were analyzed as a complete randomized design using the GLM procedure of SAS (SAS, 2002). In all assays differences among treatments were accepted at $P < 0.05$.

RESULTS

Enzyme activity and stage of culture

Protein concentration in spent compost of *A. bisporus* decreased ($P < 0.01$) linearly as time post- harvest increased (Table 1). There was a quadratic effect of time post-harvest on cellulases activity with the highest activity at 60 days. Xylanases and laccases activities decreased linearly as time post-harvest increased (Table 1).

Preservation method and storage time on enzyme activity

Protein content was higher ($P < 0.01$) in SCAB refrigerated and frozen, whereas fibrolytic activity of SCAB was affected by the preservation method and the storage time (Table 2; Figure 1) . Thus, cellulases activity was greater in SCAB frozen, xylanases activity was higher in SCAB frozen and frozen plus benzoic acid and laccases activity was greater in SCAB refrigerated and frozen. The lowest fibrolytic activity was found in SCAB lyophilized.

Cellulases, xylanases and laccases activities at 1 day storage period were greater ($P < 0.01$) in SCAB frozen and refrigerated, compared with the other preservation methods. There were quadratic and cubic effects of storage time on cellulases activity (Figure 1). Thus, for 28 days, storage period the cellulases activity was constant but higher, compared with 56 days; afterwards it remained constant until 180 days. Cellulases activity of SCAB frozen was higher ($P < 0.01$) at 1, 7, 14 and

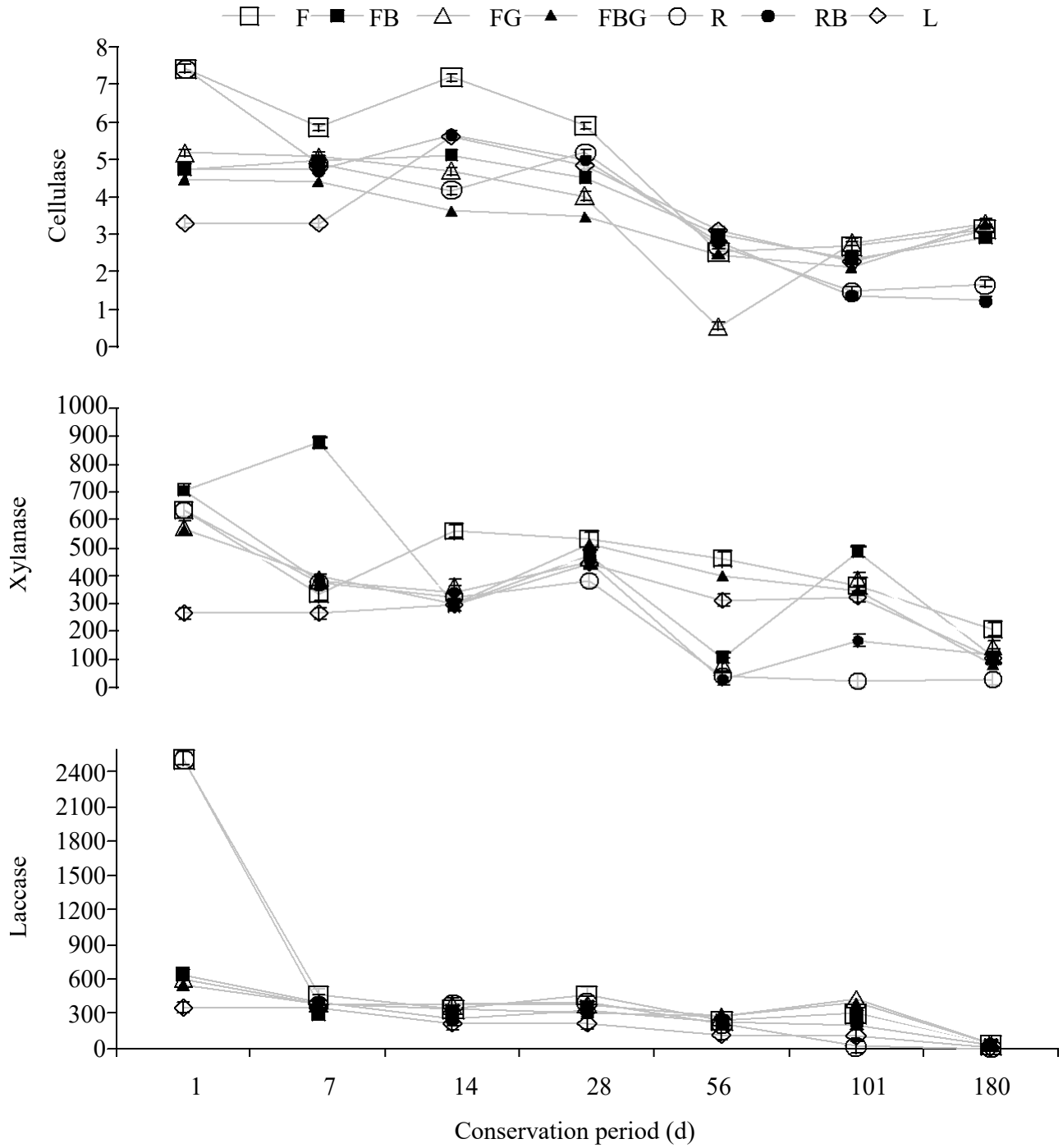

Figure 1. Influence of conservation method and storage time on celluloses, xylanases and laccases activity of SCAB. F, frozen; FB, frozen plus benzoic acid; FG, frozen plus glycerol; FBG, frozen plus benzoic acid-glycerol, R, refrigerated; RB, refrigerated plus benzoic acid; L, lyophilized.

28 days, when compared with SCAB lyophilized. Using the initial cellulases activity (day 1), lyophilized liquid extracts were less affected between 1 to 180 days, when 98% of cellulases activity of SCAB lyophilized was similar, followed by FBG (62.1%), FB (65.2%), FG (73.7%), F (42.5%), RB (26.4) and R (22.8%). The best method to maintain the cellulases during 180 days was L with 98.79% of residual activity (3.26 IU/g dry wt),

Table 3. Effects of the experimental enzymes on ruminal kinetics and dry matter fractions disappearance in orchard grass, Taiwan grass, barley straw, and spent compost.

	Control	Fibrozyme	*A. bisporus*	SEM
Orchard grass				
Soluble fraction (g/kg DM)	300[a]	294[a]	288[a]	15.9
Potential degradation (g/kg DM)	308[b]	367[a]	319[b]	16.3
Total degradation (g/kg DM)	608[b]	661[a]	597[b]	22.8
Degradation rate (g/h)	30[a]	33[a]	31[a]	2.1
Taiwan grass				
Soluble fraction (g/kg DM)	211[a]	203[a]	210[a]	10.3
Potential degradation (g/kg DM)	118[c]	230[b]	252[a]	10.6
Total degradation (g/kg DM)	329[c]	431[b]	462[a]	14.7
Degradation rate (g/h)	24[a]	25[a]	26[a]	2.6
Barley straw				
Soluble fraction (g/kg DM)	244[a]	246[a]	240[a]	12.9
Potential degradation (g/kg DM)	168[b]	244[a]	243[a]	12.0
Total degradation (g/kg DM)	412[b]	490[a]	483[a]	18.7
Degradation rate (g/h)	26[a]	29[a]	28[a]	2.3
Spent compost				
Soluble fraction (g/kg DM)	284[b]	280[b]	304[a]	13.5
Potential degradation (g/kg DM)	238[c]	302[b]	368[a]	13.9
Total degradation (g/kg DM)	522[c]	582[b]	672[a]	19.9
Degradation rate (g/h)	29[a]	30[a]	32[a]	3.1

[a-c] Means in the same row with different superscripts differ ($P < 0.05$).

followed by FBG, FG, FB, F, RB and R with 62.1, 65.2, 73.7, 42.5, 26.4 and 22.8% (3.0, 3.4, 3.3, 3.2, 1.3 and 1.7 IU/g dry wt, respectively).

Compared with cellulases, xylanolitic activities were very variable for all preservation methods during 180 days (Figure 1). Thus, xylanases activity decreased linearly for L and FBG as time of storage decreased, whereas as time increased there was a quadratic effect on xylanases activity for FB, FG, L, R and RB. Xylanolitic activity was highest at 1, 7 and 121 days of storage for FB, but it was lowest at 51, 101 and 180 days for R. Using the initial xylanolitic activity as a reference, maintenance of this activity at 180 days was as follows: 39 and 33% (101.6 and 209.6 IU/g dry wt) for L and F, followed by FG, RB, FB, FBG and R (25, 16, 15, 14 and 5%; 142.8, 114.2, 107.3, 78.2 and 29.8 IU/g dry wt, respectively).

As storage time increased there was a cubic decrease of laccases activity in the spent compost (Figure 1): on day 1 frozen and refrigerated liquid extract showed the highest activity, but afterwards a large and significant decrease was observed for all treatments. Using the initial laccases activity as a reference, maintenance of this activity was as follows: 72% for FBG and FG, followed by FB (45%), F (32%), RB (32%), L (12%) and R (9%). At 101 days laccases activity was as follows: FBG 72.3% (398.8 IU/g dry wt), FG 71.5% (426.1 IU/g dry wt), FB 44.8% (286.4 IU/g dry wt), RB 32.3% (206.6 IU/g dry wt), L 31.7% (112.1 IU/g dry wt), F 12.2% (303.4 UI/g dry wt) and R 8.7% (199.93 UI/g dry wt). At 180 days no laccases activity was observed.

Degradation kinetics of forages

Soluble fraction of DM of orchard grass, Taiwan grass and barley straw were not affected by SCAB or Fibrozyme compared with control. For all forages, the degradation rate was not affected by experimental enzymes (Tables 3 and 4). Soluble fraction of DM of spent compost and alfalfa hay were increased by SCAB, compared with control and Fibrozyme.

Potential degradation and total degradation of DM increased as follows: by Fibrozyme, when compared with control or SCAB, for orchard grass; by SCAB, followed by Fibrozyme and control, for Taiwan grass and spent compost; by Fibrozyme or SCAB, compared with control, for barley straw; by Fibrozyme but not by SCAB when compared with control, for alfalfa hay. For alfalfa hay, there were no changes in potential and total degradation of DM. Finally, total degradation of DM was increased by both Fibrozyme and SCAB when compared with control,

Table 4. Effects of the experimental enzymes on ruminal kinetics and disappearance of the dry matter and fiber fractions in alfalfa hay.

	Control	Fibrozyme	*A. bisporus*	SEM
Dry matter				
Soluble fraction (g/ DM)	418[b]	412[b]	464[a]	20.2
Potential degradation (g/ DM)	268[b]	332[a]	261[b]	11.6
Total degradation (g/ DM)	686[b]	744[a]	725[a]	31.1
Degradation rate (g/h)	36	38	39	3.2
Neutral detergent fiber				
Potential degradation (g/kg)	447	467	472	31.9
Degradation rate (g/h)	25	28	28	3.1
Acid detergent fiber				
Potential degradation (g/kg)	284[c]	381[b]	414[a]	25.7
Degradation rate (g/h)	16	19	19	3.3

[a-c] Means in the same row with different superscripts differ ($P < 0.05$).

whereas potential degradation of ADF was increased by SCAB, followed by Fibrozyme and by control.

DISCUSSION

Enzyme activity and stage of culture

Marked degradation of cellulose, xylanes and phenolic compounds by enzymes at the cell wall was detected in the compost extracts analyzed. All fiber-degrading activities found in our study were higher than those reported for other fungi (Membrillo et al., 2008). Indeed, Ball and Jackson (1995) recovered a range of fiber-degrading activities such as peroxidases, the xylan-debraching enzymes acetylesterase and arabinofuranosidase, as well as the cellulose-degrading activities endoglucanase, cellobiohydrase and ß-glucosidase from spent compost of *A. bisporus* . Besides, Li et al. (2001) reported the following values for *Pleurotus ostreatus*: (1) 90% cellulose, 65% hemicellulose and 75% lignin, from cultivation to the first break (30 days culture); (2) 45% cellulose, 30% xylanase and 40% lignin and 35% cellulose, 20% hemicellulose and 30% lignin for the second break (45 days culture). Thus, cell wall components decrease when increasing post-harvest period, which seems to be directly related to the enzymatic activity.

As expected, celluloses and xylanases dominated the composting process in the first 60 days, but afterwards lignin-degradation activity increased. This succession of enzyme activities is due to a succession of mixed microbial populations; aerobic thermophilic bacteria prevail at the start of composting, but their numbers decrease in later stages, when the actinomycete and thermophilic fungi populations increase and remain constant throughout the fermentation process (Ball and Jackson, 1995). Therefore, following mushroom harvest, the substrate consists of two major components: a lignocellulose fraction and a microbial biomass (De Groot et al., 1998). A broad range of activities involved in the degradation of the lignocellulose fraction including celluloses, hemicelluloses and lignin degrading activities (Durrant et al., 1991; Bonnen et al., 1994) was found to be associated to *A. bisporus,* but, to the best of our knowledge, enzyme production in spent compost by post-harvest stage has not been reported before.

The enzymatic activity of *A. bisporus* has been related mainly to the production stages, without evaluating its potential for synthesizing fibrolytic enzymes. The cellulases activity (10 IU/dry wt g) observed in our study for enzymatic extracts from residuals at 50 days post-harvest, is in agreement with that reported by Arce et al. (2007), who found an activity of 11.7 IU/g dry wt in a 48 days post-harvest compost. Wood and Goodenough (1977) point out that the increase in cellulase as bodies appear is related to the production level, which would explain the larger cellulases activity (20.0 IU/g dry wt) 60 days post-harvest (the third crop), when compared with that of 50 days post-harvest. The xylanases activity was not different ($P > 0.01$) for the extracts from compost of 50, 60 and 90 days post-harvest, because it is not related to the production level, culture age or number of crops, but rather to the substrate colonization which took place between day 30 and 60 of growth and afterwards, it was exhausted. If there is no change in the substrate colonization during the productive stage of champignon mushroom, no difference will be observed in xylanases activity. The laccases activity was larger in the compost at 90 days post-harvest because it is directly related with the quantity of phenolic substrate present in the compost (Wood and Goodenough, 1977). Considering that in 60 days of growth the mushroom has consumed most of the forage cell wall components, a high proportion of phenolic

compounds would be available for the 90 days crop which, therefore, would end up with a larger concentration of laccases.

Enzyme activity, preservative method and storage time

The residual enzymatic activities during 180 days for cellulases, xylanases and laccases were better for lyophilization (98, 38 and 1.6%, respectively) due to lack of water which avoids contamination (Valenzuela and Ortiz, 2007), contrary to the refrigeration process (Grazyna et al., 2008; Mohapatra et al, 2008) which showed 23, 4.67 and 0% of activity in our study. Besides, lyophilization is not an aggressive process as freezing, which maintained 43, 33 and 1.2% of activity for cellulases, xylanases and laccases. Although, freezing diminishes contamination and most of the nutrients are retained during four months of storage, protein components are crystallized which leads to fractures or denaturalization of proteins (Grazyna et al., 2008) . To avoid this problem, glycerol was used in our study and 65, 25 and 7.8% of activity was maintained for cellulases, xylanases and laccases, because glycerol acts as cryoprotectant avoiding freezing damage to the proteins (Van and Therelein, 2003). Benzoic acid, which acts as bacteriostatic (Bonen et al., 1994), was also used in our study, resulting in 62, 15 and 4.5% activity for cellulases, xylanases and laccases.

Enzyme activity of *A. bisporus* can be considered as potential producer of exogenous enzymes when compared with other mushrooms, such as *Trametes* spp*., P. ostreatus* and *Aspergillus niger* (Márquez et al., 2007). For cultivation in substrate with *Aspergillus* spp. (40 days) activity (IU/mg protein) was 0.7 for laccases, 0.7 for xylanases and 1.0 for cellulases (Shah et al., 2005); with the same organism cultivated with coffee residue and corn stover, activity for xylanases was 268.7 IU/ml (Lagunas et al., 2006). In our study (60 days cultivation), the values (IU/mg protein) were 18.84 for xylanases, 0.47 for cellulases and 0.05 for laccases, which means that *A. bisporus* contains a significant concentration of xylanases. However, Sungurtas et al. (2004), for barley straw, found that xylanases activity was 2400 IU/ g dry wt, which is higher than the value in our study with *A. bisporus* and barley straw.

***In vitro* disappearance of forages by exogenous enzymes**

The effects of experimental enzymes on degradation of forages were variable. Thus, with the exception of orchard grass, potential DM disappearance was increased by SCAB when compared with control; this increment was due sometimes to SCAB and sometimes to Fibrozyme. The analysis of these results suggests that differences of forages species and enzyme profiles of Fibrozyme and SCAB could influence their effects on DM disappearance. With regard to this subject, Jalilvand et al. (2008) point out that exogenous fibrolytic enzymes were more effective as content of fiber content increased. Indeed, our results indicate that DM disappearance with SCAB was greater in Taiwan grass, barley straw and spent compost, than in orchard grass and alfalfa. The positive results of exogenous enzymes on *in vitro* disappearance of forages are consistent with various other studies (Colombatto et al., 2007; Elwakeel et al., 2007).

The mechanism by which enzymes improve digestion of forages is still subject to speculation, but Hristov et al. (2000) suggested that exogenous enzymes induce increments on rumen enzyme activity. That change would be due to higher amounts of soluble carbohydrate released from undigested feed particles which provide additional energy for improving microbial growth thus shortening the lag time for microbial colonization (Yang et al., 1999; Wang et al., 2001). Exogenous enzymes increased (34% Fibrozyme, 46% SCAB) the potentially disappearance fractions of ADF. The higher activity shown by SCAB on the ADF fraction when compared to Fibrozyme was due to the laccases activity of SCAB.

These results show that it was possible to obtain and maintain enzymatic extracts of the spent compost of *A. bisporus*, with a significant amount of fibrolytic enzymes, as well as an additional activity of laccases. The best preservation methods were lyophilized and frozen plus benzoic acid and glycerol. *In vitro* disappearance indicated that SCAB increased the degradation of forages DM and therefore, a potential to improve forage utilization for ruminants. It seems that SCAB can be included as an ingredient in diets for ruminants.

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