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Full Length Research Paper

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

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To evaluate the fibrolytic potential to degrade forages, extracts from spent compost of *Agaricus bisporus* (SCAB) were evaluated *in vitro*. Firstly, SCAB was cultivated for 50, 60 and 90 days (culture stage) and enzyme activity was evaluated. The highest cellulase activity was at 60 days; xylanase activity was not affected by culture stage and laccase activity increased as culture stage increased. Secondly, extracts of SCAB cultivated for 60 days were preserved by refrigeration, freezing, freeze-drying, refrigeration + benzoic acid, freezing + benzoic acid, freezing + glycerol, or freezing + benzoic acid-glycerol and then fibrolytic activity was evaluated. The highest fibrolytic activity was shown by SCAB frozen with addition of either glycerol or benzoic acid, or freeze- dried. Finally, effects of SCAB as an additive to enhance ruminal degradation of alfalfa hay, Taiwan grass, barley straw, and spent compost of *A. bisporus* were evaluated. Soluble fraction of dry matter of alfalfa and potential degradation of dry matter of Taiwan grass and barley straw were increased by SCAB. It is concluded that SCAB showed a noticeable fibrolytic activity which might increase degradation of forages in the rumen.

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

INTRODUCTION

Agaricus bisporus (AB) is economically the most important mushroom in the world (Chang and Miles, 2004). It is produced on a composted substrate consisting of fiber and nitrogen from various raw materials including straw, hay, corn cobs, cottonseed hulls, poultry litter, cottonseed meal and others (Lankinen et al., 2005; Mamiro and Royse, 2008). Thus, spent mushroom compost is the residual compost waste generated by the mushroom production industry. After AB composting, the substrate contains two major components, a lignocellulose fraction and a microbial biomass. A broad range of activities involved in the degradation of the fiber fraction including 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 and the actinomycete populations increase (Ball and Jackson, 1995). Thus, cellulase concentration in the body of AB culture is relatively high between 30 and 60 days (Wood and Goodenough, 1977), while xylanase and laccase are high at the final of the process, after 60 days (Bonen et al., 1994). Enzyme activity during those growing stages of AB has been evaluated in

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Abbreviation: SCAB, Spent compost of *Agaricus bisporus*; **AB** *Agaricus bisporus*; **ADF,** acid detergent fiber; **DM,** dry matter; **NDF,** neutral detergent fiber; **R,** refrigeration; **RB,** refrigeration with adding of benzoic acid; **F,** freezing; **FB,** freezing with adding of benzoic acid; **FG,** freezing with adding of glycerol, **FBG,** freezing with adding of glycerol and benzoic acid; **L,** freeze-drying.

the fruit body, but in the literature reviewed no information was found about spent mushroom compost. Various conservation methods for enzymes have been evaluated, such as refrigeration (Valenzuela and Ortiz, 2007), freezing or freezing with addition of chemical preservatives such as benzoic acid or glycerol (Chanasattru et al., 2008), but reports about enzymes from spent compost of mushrooms were not found. Therefore, the objectives of this research were to determine the fibrolytic activity and potential of spent compost of *A. bisporus* (SCAB) on degradation of forages for ruminants.

MATERIALS AND METHODS

Strain, substrates and cultivation method

Nine SCAB blocks (~25 kg fresh wt each) were obtained from El Riojal S.A. de C.V. (*Las Vigas, Veracruz, México*) and they were made up 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 break respectively of commercial mushroom production), mushrooms were harvested. Thus, three SCAB blocks for each harvested time were used.

Preparation of enzyme extract

To obtain the enzyme extract, 400 g (fresh weight) of each block were sampled and mixed with 600 mL of sodium citrate buffer (50 mM, pH 5.3). The mixture was mechanically shaken (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). Supernatant was measured for enzyme activity and soluble protein concentration as indirect indicator of total enzyme concentration.

Protein was determined according to Bradford (1976) using a standard bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) (0.15 M) dissolved in sodium citrate buffer (50 mM, pH 5.3). The xylanase and cellulase activities were estimated by determination of reducing sugar using the method of dinitrosalicylic acid (Miller, 1959) modified by Márquez et al. (2007). Substrates used in these determinations were a solution of oat xilan (Sigma X-0627) 0.5% dissolved in sodium citrate buffer (50 mM, pH 5.3) for xylanase activity, and a solution of 0.5% carboxymethyl cellulose sodium salt (CMC, medium viscosity; Sigma-Aldrich C4888), dissolved in a sodium citrate buffer (50 mM, pH 5.3) for cellulase. Laccase activity was 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 molar extinction coefficient of 36,000 M/cm (Bourbonnais et al., 1997) modified by Márquez et al. (2007). Values were expressed in katal units (kat), where 1 kat 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 basis (kat/g substrate dry matter).

Preservation methods

A composite sample of liquid extracts of the three blocks cultivated of spent mushroom compost (SCAB) cultivated for 60 days as previously described, were used. Extracts were conserved for 1, 7, 14, 28, 56, 101 and 180 days in sealed amber glass flasks using the following methods: refrigeration (R) at 8°C; refrigeration adding benzoic acid dissolved in acetate buffer at 10% (RB); freezing (F) at -20ºC; freezing with addition of benzoic acid at 10% (FB); freezing with addition of glycerol at 10% (FG); freezing with addition of glycerol at 5% and benzoic acid at 5% (FBG); and freeze-drying or lyophilisation (L). Enzyme activities (xylanase, cellulase and laccase) were measured as described above.

In vitro degradation of forage and enzyme deactivation

The first phase of the *in vitro* degradation technique (Tilley and Terry, 1963) was performed collecting ruminal fluid (2 L) from two Holstein steers (450 kg body weight) fitted with ruminal cannulas. Steers had free access to alfalfa hay (*Medicago sativa* L.) and oat straw *(Avena sativa)*, mineral premix and water, with 500 g/d of concentrate (crude protein 16% DM basis). Samples of ruminal fluid (about 1 L) were collected 3 h after the morning feeding 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 with agitation at 39°C.

Firstly, to evaluate the enzyme degradation of SCAB, 192 glass tubes (40 mL) each with 1 mL of liquid extract of SCAB and 20 mL McDougall (1948) saliva and 5 mL of strained ruminal fluid were incubated for 0, 3, 6, 9, 12, 24, 48 and 72 h. Three tubes per incubation period contained SCAB conserved by R, RB, F, FB, FG, FBG or L. Control was defined as tubes without SCAB. 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 × g; 4ºC; 20 min). Supernatants were sampled and frozen. On supernatants, ammonia-N concentrations (McCullough, 1967) were determined using a UVVIS spectrophotometer (630 nm, CARY I-E, VARIAN).

Secondly, to evaluate effects of SCAB on degradation of dry matter (DM) of alfalfa (*Medicago sativa* L.) hay, orchard grass (*Dactylis glomerata*), barley (*Hordeum vulgare* L.) straw, Taiwan grass (*Pennisetum purpureum*) 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 polypropylene tubes equipped with butyl rubber stoppers. A commercial fibrolytic enzyme product (Fibrozyme, Alltech Inc., Nicholasville, KY, USA) was compared to SCAB enzyme extract (cultivated for 60 days and frozen for 1 day). A liquid extract from Fibrozyme was obtained according to the methodology described by Márquez et al. (2007) who indicate that each mL of the liquid extract provides an enzymatic activity equivalent to 160 kat xylanase. A composite liquid extract of SCAB was obtained from the tree blocks of SCAB cultivated for 60 days; then it was frozen and conserved by 1 day period.

One hour before the incubation, 1 mL of the enzymatic solution (SCAB or Fibrozyme) was applied directly onto the substrate (500 mg DM) contained in 120 mL propylene tubes. Then, a mixture of 40 mL McDougall 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 (Van Soest et al., 1991) contents and determine its degradation. Then, DM *in vitro*

Table 1. Protein content and enzyme activity of liquid extracts from spent compost of *A. bisporus* cultivated at 50, 60 and 90 days (values expressed in dry wt basis).

	Culture stage (days)			
	50	**60**	**90**	**SEM**
Protein (mg/g) [Q]	8.8	8.7	5.7	0.8
Cellulase (kat/g) [Q]	10.0	20.2	5.7	0.8
Xylanase (kat/g) [L]	1102.7	1024.4	825.9	89.2
Laccase (kat/g) [L]	3593.1	4035.7	5663.3	515.8

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

degradation was determined from the DM remaining in the tubes after incubation. The filter and undigested residues were oven-dried at 105°C for 24 h to remove excess moisture and weighed. Kinetics data of *in vitro* degradation were analyzed using a Gompertz model according to Susmel et al. (1999) as: dis(t) = a + b exp[(−C) exp(−Dt)] where: dis is the disappearance of material (g/kg) from the 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 (*i.e.* , a constant comparable to the exponential rate of disappearance) is derived as: $c = D/C$.

Statistical analysis

In the first assay, nine SCAB blocks cultivated at 50, 60 and 90 days were considered as the experimental units. Data were analyzed as a completely randomized design. The culture stage (50, 60 and 90 days) effect was analyzed with polynomial analysis (linear and quadratic), calculating coefficients with the 'IML' option of SAS (2002).

In the second assay, enzyme activity of SCAB was evaluated within 7-period conservation (1, 7, 14, 28, 56, 101 and 180 days) and 7- method conservation (R, RB, F, FB, FG, FBG and L) . A total of 147 glass flasks were used, of which three corresponded to each conservation method per conservation period. Flask was considered as the experimental unit. Data were analyzed as a completely randomized design with a 7 × 7 factorial arrangement of treatments. Enzymatic solutions of SCAB conserved with F, FB, FG, FBG, R, RB and L were incubated for 0, 3, 6, 9, 12, 24, 36, 48 and 72 h. Additionally, three tubes per time without SCAB were used as control. Therefore, in 216 glass tubes, the ammonia-N concentrations were measured. Data were analyzed as a completely randomized design with a 9 × 8 factorial arrangement of treatments (9 incubation times and 7 conservation methods plus control).

For the forage degradation, 18 polypropylene tubes per forage were used. Tube was considered the experimental unit, and three tubes per incubation time (1, 6, 12, 24, 48 and 72 h) were used. The data of DM (for all forages), NDF and ADF (for alfalfa hay) remaining at each incubation time were used to fit a nonlinear regression model using the "NLIN" option of SAS (2002). For the forages, the data of degradation kinetics (DM, NDF or ADF) were analyzed as a completely randomized design with three repetitions per treatment using.

All data were analyzed with GLM procedure (SAS, 2002), and means of main factors and interactions were compared with LSMEANS of SAS (2002). In all assays differences of 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) quadratically as the culture stage increased (Table 1). There was a quadratic effect of culture stage on cellulase activity and the highest activity was found at 60 days. Xylanase decrease and laccase activities increase linearly as culture stage increased (Table 1).

Preservation method and storage time on enzyme activity

Protein content was higher in SCAB refrigerated and frozen, whereas fibrolytic activity of SCAB was affected by the preservation method and the storage time (Table 1 and Figure 1). Thus, cellulase activity was greater in freezing SCAB, xylanase activity was higher in freezing and freezing with glycerol SCAB and laccase activity was greater in SCAB refrigerated and frozen. The lowest fibrolytic activity was found with freeze-drying SCAB.

Cellulase, xylanase and laccase activities in SCAB at 1 day storage period were higher for freezing and refrigeration as compared to the other preservation methods. There were quadratic and cubic effects of storage time on cellulase activity (Figure 1). Thus, for 28 days storage period the cellulase activity was constant but higher as compared to 56 days; afterwards it remained constant until 180 days. Cellulase activity of freezing SCAB was higher at 1, 7, 14 and 28 days, as compared to freeze-drying SCAB. Using the initial cellulase activity (day 1), freeze-drying SCAB were less affected between 1 to 180 days when 98% of its cellulase activity 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 cellulose activity during 180 days was L with 98.79% of residual activity (3.26 kat/g dry wt), 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 kat/g dry wt, respectively).

As compared to cellulase, xylanolitic activities were

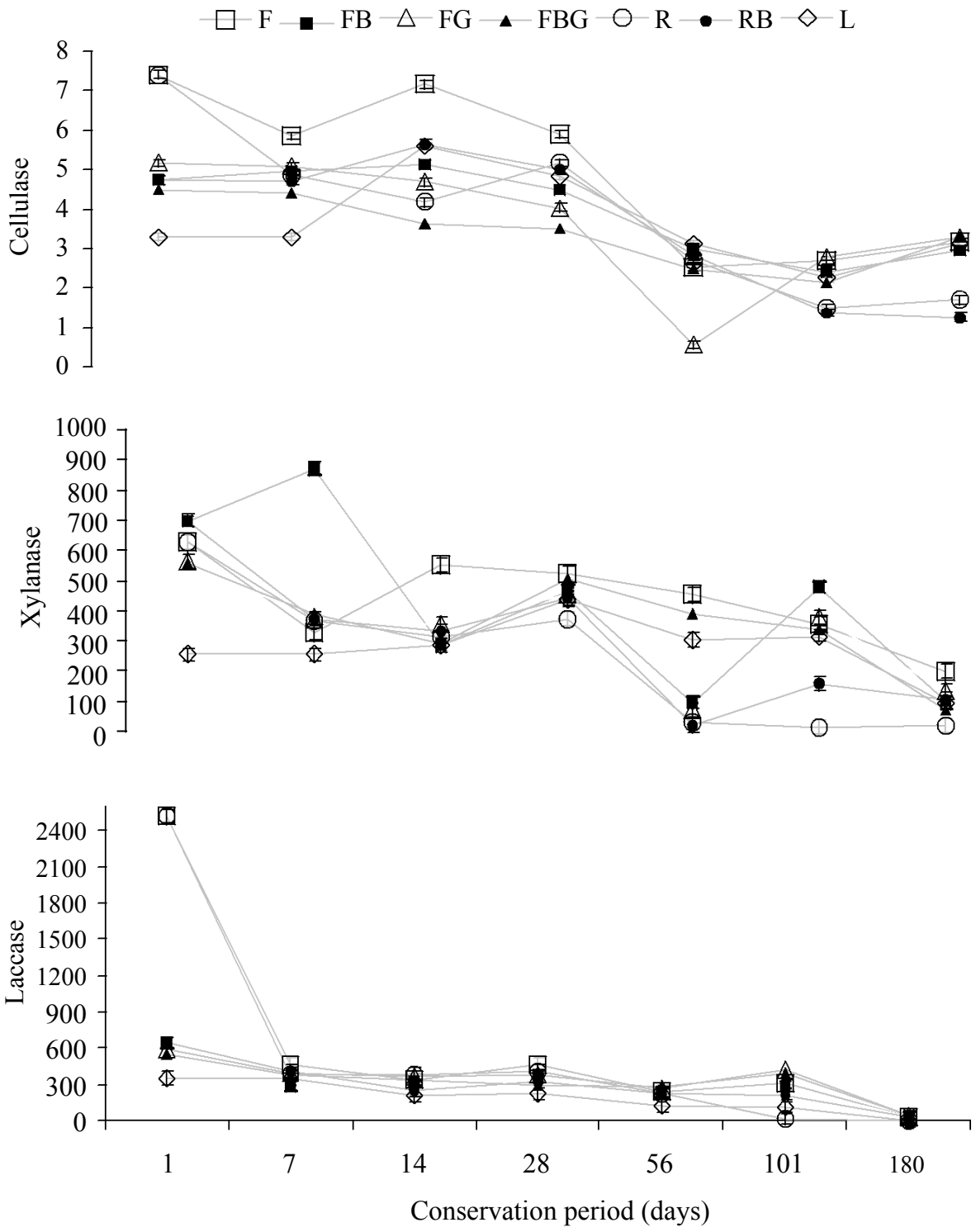

Figure 1. Influence of conservation method and storage time on cellulase, xylanase and laccase activity of SCAB. F, freezing; FB, freezing with adding of benzoic acid; FG, freezing with adding of glycerol; FBG, freezing with adding of benzoic acid and glycerol, R, refrigeration; RB, refrigeration with adding of benzoic acid; L, freeze-drying.

variable for all preservation methods during 180 days (Figure 1). Thus xylanase activity decreased linearly for L and FBG as time of storage decreased; however, as time increased there was a quadratic effect on xylanase activity for FB, FG, L, R and RB: it was highest at 1, 7 and 121 days of storage for FB, but it was lowest at 51,

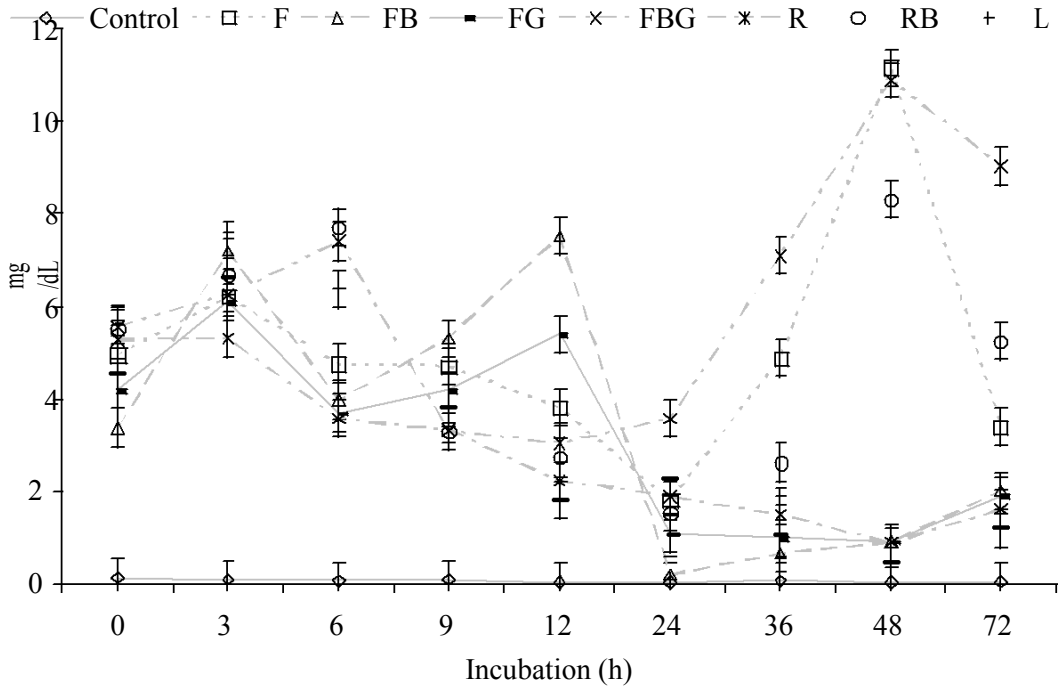

Figure 2. Ammonia-N released from incubation of ruminal fluid and SCAB. F, freezing; FB, freezing with adding of benzoic acid; FG, freezing with adding of glycerol; FBG, freezing with adding of benzoic acid and glycerol, R, refrigeration; RB, refrigeration with adding of benzoic acid; L, freeze-drying.

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 kat/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 kat/g dry wt, respectively).

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

Ammonia-N concentrations were variable with conservations methods, but the highest concentrations were found at 36, 48 and 72 h incubation of SCAB conserved by R, RB and F (Figure 2).

Degradation kinetics of forages

Soluble fraction of DM of orchard grass, Taiwan grass and barley straw were not affected by SCAB or Fibrozyme, as compared to control. For all forages, the degradation rate was not affected by experimental enzymes (Tables 2 and 3). Soluble fraction of DM of spent compost and alfalfa hay were increased by SCAB, as compared to control and Fibrozyme.

In Taiwan grass and spent compost, potential and total degradation of DM (Table 4) were increased with SCAB as compared to those with no enzyme (control) . In orchard grass, SCAB did not affect degradation of DM fractions. As compared to SCAB, Fibrozyme increased degradation of DM fractions of all those forages. Both SCAB and Fibrozyme did not affect degradation rate in forages. In alfalfa hay, SCAB and Fibrozyme increased total degradation of DM and potential degradation of ADF as compared to control. Both, SCAB and Fibrozyme did not affect NDF degradation fractions and any degradation rate of DM, NDF and ADF (Table 3).

DISCUSSION

Enzyme activity and stage of culture

Enzymes actively degrading cellulose, xylanes and phenolic compounds at the cell wall were detected at a significant level in the compost extracts analyzed. All fiber-degrading activities found in our study were higher than those reported for other fungi (Membrillo et al.,

Table 2. Protein content and enzyme activity of liquid extracts from spent compost of *A. bisporus* cultivated at 60 days and preserved by different methods (values expressed in dry wt basis).

	Preservation method [1]							
	R	**RB**	**F**	**FB**	**FG**	**FBG**	**L**	**SEM**
Protein (mg/g)	6.9[a]	6.3[bc]	6.7[ab]	6.5[b]	6.0[bc]	5.6[d]	6.1[c]	0.09
Cellulase (kat/g)	3.9[b]	3.7[c]	4.9[a]	3.9[b]	3.9[b]	3.4[d]	3.7[c]	0.05
Xylanase (kat/g)	258.6[d]	312.6[bc]	443.2[a]	436.7[a]	341.5[b]	372.4[b]	286.5[d]	8.34
Laccases (kat/g)	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 adding benzoic acid (RB); freezing (F); freezing with addition of benzoic acid (FB); freezing with glycerol (FG); freezing with adding of glycerol and benzoic acid (FBG); and freeze-drying (L). [a days]Means in the same row with different superscripts differ (P 0.05).

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	294	288	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	33	31	2.1
Taiwan grass				
Soluble fraction (g/kg DM)	211	203	210	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	25	26	2.6
Barley straw				
Soluble fraction (g/kg DM)	244	246	240	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	29	28	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	30	32	3.1

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

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 that for *Pleurotus ostreatus* cell wall components decrease when increasing the days of cultivation, which would be directly related to the enzymatic activity.

As expected, cellulase and xylanase dominated the composting process in the first 60 days, but afterwards lignin-degradation activity increased. These changing enzyme activities may be due to a succession of mixed microbial populations; aerobic thermophilic bacteria prevail at start of composting but their number decrease in later stages, when the actinomycete and thermophilic fungi populations increase and remain constant throughout (Ball and Jackson, 1995). Therefore, following mushroom harvest, the substrate consists of two major

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 ($P < 0.05$).

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 cellulose, hemicellulose and lignin degrading activities (Durrant et al., 1991; Bonnen et al., 1994) were found to be associated to *A. bisporus*. Interestingly, our results indicated high concentrations of laccase and xylanase after the composting was finished.

The enzymatic activity of *A. bisporus* has been related mainly to the production stages, without evaluating its potential for synthesizing fibrolytic enzymes. The cellulase activity (10 kat/g dry wt) observed in our study for enzymatic extracts with 50 days culture is in agreement with cellulase activity of 11.7 kat/g dry wt found in a 48 days culture compost by Arce et al. (2007). The higher cellulase activity in SCAB with 60 days as compared to 50 days could be due to a larger mushroom production level in the older stage (Wood and Goodenough, 1977). Contrarily, xylanase activity decreased with culture stage, probably because the substrate colonization does not change during the productive stage of the mushroom. The laccase activity was greater in the compost after 90 days of cultivation because it is directly related to the amount of phenolic substrate present in the compost (Wood and Goodenough, 1977). If we consider that in 60 days, culturing the mushroom has consumed most of the forage cell wall components, a high proportion of phenolic compounds would be available for the cultivation of 90 days culture which, therefore, would end up with a larger concentration of laccases.

Enzyme activity, preservative method and storage time

The residual enzymatic activities in extracts from 1 to 180 days decreased with all conservation methods. Sometimes, lyophilization elicited a high enzyme activity in the extracts which may be due to the fact that water is quickly eliminated avoiding contamination (Valenzuela and Ortiz, 2007), contrary to the refrigeration process (Grazyna et al., 2008; Mohapatra et al., 2008). 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 cellulase, xylanase and laccase, because glycerol acts as cryoprotect avoiding freezing damage to the proteins as well as ice formation (Van and Therelein, 2003) . In our study benzoic acid, which acts as bacteriostatic (Bonen et al., 1994), allowed 62, 15 and 4.5% residual activity for cellulase, xylanase and laccase, respectively.

Enzyme activity of *A. bisporus* can be considered as potential producer of exogenous enzymes if compared to that of other mushrooms, such as *Trametes* spp*., P. ostreatus* and *Aspergillus niger* (Márquez et al., 2007; Shah et al., 2005; Lagunas et al., 2006). The analysis of our results suggests that *A. bisporus* contains a significant concentration of xylanase, which is in agreement with the study reported by Sungurtas et al. (2004).

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; as compared to 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) pointed out that exogenous fibrolytic enzymes were more effective as fiber content increased. Indeed, our results indicate that DM disappearance was more effectively increased by SCAB 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 other studies (Colombatto et al., 2007; Elwakeel et al., 2007).

The mode of action by which enzymes can improve digestion is still subject to speculation, but most authors agree that exogenous enzymes favour rumen enzyme activity (Hristov et al., 2000). This 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 disappearing fractions of ADF. The higher activity shown by SCAB on the ADF fraction, as compared to Fibrozyme, is due to the laccase activity of SCAB.

The analysis of the results of our study 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 freeze-drying and freezing with benzoic acid and glycerol. *In vitro* disappearance indicated that SCAB increased the degradation of forages DM and, therefore, forage utilization for ruminants might be improved. The inclusion of SCAB as an ingredient in diets for ruminants needs further studies.

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