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ORIGINAL ARTICLE

Biological efficiency and nutritional value of the culinary-medicinal mushroom *Auricularia* cultivated on a sawdust basal substrate supplement with different proportions of grass plants

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Abstract *Auricularia polytricha* was cultivated on a sawdust basal substrate supplemented with different proportions (30%, 45%, and 60%, respectively) of stalks of three grass plants, i.e., *Panicum repens* (PRS), *Pennisetum purpureum* (PPS), and *Zea mays* (ZMS), to determine the most effective substrate. The mycelial growth rate, total colonization time, days to primordial formation, biological efficiency and chemical composition of fruiting bodies were evaluated. The results indicated that 30PPS was the best substrate for mycelial growth of *A. polytricha*, with a corresponding total colonization period of 32.0 days. With the exception of 30PPS, the total biological efficiency of all of the substrates containing *P. repens* stalk, *P. purpureum* stalk and *Z. mays* stalk was higher ($P < 0.05$) than that of the control. The most suitable substrate with a high biological efficiency was 60PRS (148.12%), followed by 30ZMS (145.05%), 45ZMS (144.15%) and 30PRS (136.68%). The nutrient values of fruiting bodies were affected by different substrates. The ash contents of *A. polytricha* cultivated on a substrate containing *Z. mays* stalk were higher than that of the control; meanwhile, the protein contents of mushroom cultivated on a substrate containing

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P. repens stalk (except substrate 45PRS) were higher than that of the control. The biological efficiency of the substrates was tested, and according to the results, it is feasible to use the stalks of *P. repens* and *Z. mays* on partially replaced sawdust to cultivate *A. polytricha*.

1. Introduction

Auricularia polytricha also known as wood ear or Jew's ear, is frequently consumed as a culinary-medicinal mushroom. This mushroom has several known biological activities, including antioxidant activity (Mau et al., 2001), immunomodulatory (Sheu et al., 2004), antitumor activities (Yu et al., 2009; Song and Du, 2012), anti-dementia properties (Bennett et al., 2013), attenuation of the inflammatory response, oxidative stress and lipid deposition (Chiu et al., 2014), and hypocholesterolemic effects (Zhao et al., 2015). Because of its delicacy and biological activities, consumer demand for *A. polytricha* has increased yearly. It is among the top four most cultivated mushrooms in Taiwan, with an annual production of 13,000 tons.

A. polytricha occurs as a saprophytic fungus, that is normally found on dead wood, or on dead parts of living trees in the wild. In Taiwan, sawdust is the main substrate for the cultivation of this mushroom. However, there has been a shortage of sawdust due to limit-cutting of the forest. Therefore, it is necessary to investigate appropriate materials to partially substitute for sawdust in the cultivation of *A. polytricha*.

In recent years not only agricultural wastes (Pant et al., 2006; Moonmoon et al., 2010, 2011; Abd Razak et al., 2012; Yang et al., 2013; Koutrotsios et al., 2014; Xu et al., 2016), but also certain plants have been reported to be utilized as mushroom cultivation substrates. These include water hyacinth (Murugesan et al., 1995), plantain peeling (Osemwegie et al., 2002), umbrella plant (Ohga and Royse, 2004), weed plant (Das and Mukherjee, 2007), and perilla (Li et al., 2016). Recently, *Panicum repens*, *Pennisetum purpureum* and *Zea mays* stalks have been used to cultivate mushrooms, such as *Pleurotus citrinopileatus* (Liang et al., 2009), and *P. pulmonarius* (Liang et al., 2011) in our laboratory. *P. repens* and *P. purpureum* are traditional grass plants that are widely available at altitude below 1500 m in Taiwan. *Z. mays* is widely cultivated throughout Taiwan, and its stalk of *Z. mays* can be used as feed for cattle. However, only a portion of the *Z. mays* stalk is used as feed. The rest is expended through burning or incorporation into the soil as organic fertilizer.

In this study, sawdust was used as the main ingredient, and stalks of *P. repens*, *P. purpureum* and *Z. mays* were used to supplement the substrate for cultivation of *A. polytricha*. Because both mycelial growth and fruiting body formation of mushrooms were affected by different substrates (Ohga, 2000), it was unknown whether the nutrient values of fruiting bodies were also affected. The aim of this study was to investigate the feasibility of using stalks of these three grass plants as alternative substrate for *A. polytricha* sawdust-based cultivation. The mycelial growth rate, total colonization time, days to primordial formation, biological efficiency and chemical composition of fruiting bodies were evaluated.

2. Materials and methods

2.1. Microorganism and spawn preparation

A. polytricha was obtained from Da-Nan Mushroom Farm (Puli, Nantou, Taiwan), was grown on a potato dextrose agar (PDA, 200 g/l diced potatoes; 20 g/l glucose; 15 g/l agar) medium at 25 °C for regular subculture, and was maintained on PDA slants at 4 °C for a maximum of 3 months. Sawdust spawn was prepared in 850-ml polypropylene plastic bottles filled with 250 g of sawdust, supplemented with 10% rice bran and 1% calcium carbonate (w/w, in terms of dry weight). The water content of the mixture was adjusted to approximately 60% and then sterilized at 121 °C for 60 min. After cooling to room temperature, the sterilized sawdust mixture of every bottle was inoculated with 9 cm^2 mycelial agar discs. The spawn was incubated at 25 °C until the substrate was fully colonized.

2.2. Substrate preparation and inoculation

The grass plants tested in this study were *P. repens*, *P. purpureum* and *Z. mays*. The stalks of *P. repens* (PRS) and *P. purpureum* (PPS) were obtained from two hillside fields (Da-Tsuen, Changhua, Taiwan), and the stalks of *Z. mays* (ZMS) were obtained from a local farm (Nantou, Taiwan). *P. repens* and *Z. mays* grew to eared condition and *P. purpureum* grew to over 2 meters; their stalks were then gathered. The stalks of these grass plants were kept in room temperature until air-dried and were chopped into pellets with a length of 0.5–1.5 cm and soaked in water overnight before substrate preparation. After draining the excess water in the stalk, the plants were used as a substrate to replace partially composted hardwood sawdust. To determine suitable substrates and ratios for the cultivation of *A. polytricha*, various materials and combination substrates were tested. The control substrate formulation (all ingredients based on dry substrate weight, w/w) consisted of 90% sawdust, 9% rice bran, and 1% $CaCO_3$. The symbols and C/N ratios of substrate formulas are depicted in Tables 1 and 2, respectively.

The water content of the substrate was adjusted to approximately 65% (w/w). Then, each polyethylene bag (height 38 cm, diameter 10 cm) was filled with 1 kg of substrate and sterilized at 121 °C for 80 min. Thirty-six replicate bags were used and divided into three replicates for each substrate. After the substrates were cooled down to room temperature, they were inoculated with 5 g/bag of sawdust spawn.

For substrate analysis, samples were dried at 60 °C to a constant weight and then ground into a coarse powder (8 openings/cm) using a mill. The carbon content was determined according to the report by Nelson and Sommers (1982), and the nitrogen content was determined using the Kjeldahl

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Table 1 Ten-culture substrate formula used for *Auricularia polytricha* cultivation (% by dry weight).

Material	Substrate[a]									
	Control	30PRS	45PRS	60PRS	30PPS	45PPS	60PPS	30ZMS	45ZMS	60ZMS[b]
Sawdust	90	60	45	30	60	45	30	60	45	30
Panicum repens stalk		30	45	60						
Pennisetum purpureum stalk					30	45	60			
Zea mays stalk								30	45	60

[a] All substrates also contained 9% rice bran and 1% $CaCO_3$.
[b] *Panicum repens* stalk, PRS; *Pennisetum purpureum* stalk, PPS; *Zea mays* stalk, ZMS.

Table 2 Substrate formulas and analysis (Carbon%, Nitrogen %, and C/N Ratio).

Substrate	Carbon (%)	Nitrogen (%)	C/N ratio
Control	52.12 ± 1.21	0.96 ± 0.05	54.29 ± 1.07
30PRS	51.22 ± 1.26	1.03 ± 0.06	49.73 ± 1.08
45PRS	50.16 ± 1.12	1.04 ± 0.05	48.23 ± 1.53
60PRS	49.98 ± 1.25	1.09 ± 0.05	45.85 ± 1.46
30PPS	51.25 ± 2.32	0.73 ± 0.03	70.21 ± 2.24
45PPS	49.02 ± 1.37	0.64 ± 0.02	76.59 ± 1.18
60PPS	46.89 ± 1.54	0.59 ± 0.04	79.47 ± 1.36
30ZMS	55.71 ± 1.90	0.88 ± 0.06	63.31 ± 1.35
45ZMS	54.15 ± 2.25	0.99 ± 0.04	54.70 ± 2.18
60ZMS	54.25 ± 2.64	1.16 ± 0.13	46.76 ± 2.55

method (Philippoussis et al., 2007). The carbon/nitrogen ratio of each substrate was then calculated.

2.3. Incubation and Harvest

The inoculated substrates were kept in a spawn running room at 25 °C and 70% relative humidity under dark condition. After the mycelium fully colonized the substrate, the height between the top substrate and bottom substrate (i.e., the height of mycelia) was calculated. The mycelial growth rate was defined as the height of the mycelia in the colonized culture bag divided by the incubation time (days). After the substrate surfaces were entirely covered with the mycelium, the temperature was controlled at 22 °C to stimulate primordia formation, and the bags were opened to allow the development of fruiting bodies under relative humidity of 90% or greater. The fruiting bodies in each polyethylene bag were harvested when they were grown to show the waveform margin. The harvested fruiting bodies were then counted and weighed. At the end of the harvest period, the accumulated data were used to calculate the biological efficiency. The biological efficiency is the ratio of the weight of the fresh fruiting body (g) per dry weight of substrate (g), expressed as a percentage.

2.4. Chemical analysis

The fruiting bodies of *A. polytricha* were collected after the first flush of every substrate and were dried at 60 °C to a constant weight. Mushroom samples were analyzed for chemical composition, including moisture, ash, crude protein and crude fat, using the AOAC procedures (1995). The nitrogen factor used for crude protein calculation was 4.38 (Chang and Miles, 1989). The carbohydrate content (g) was calculated by subtracting the contents of ash, crude protein and crude fat from 100 g of dry matter. The total energy was calculated according to the following equations (Manzi et al., 2004):

$$\text{Energy (kcal)} = 4 \times (\text{g protein} + \text{g carbohydrates}) + 9 \times (\text{g lipid})$$

2.5. Statistical analysis

Each data value was presented as the mean ± standard deviation. Differences between the means of individual groups were assessed by a one-way ANOVA with Duncan's multiple range tests at the 95% confidence level.

3. Results

3.1. Chemical composition and colonization of different substrates

The chemical composition and C/N ratio after sterilization varied considerably among substrates (Table 2). The carbon and nitrogen contents determined in substrates containing PPS were all lower than those of the control. However, their C/N ratios were higher than those of substrates containing PRS, ZMS and the control, whereas the C/N ratio of substrates containing PRS was lower than that of the control.

The mycelial growth on substrate 30PPS was shown to be significantly faster ($P < 0.05$) than that of the control; however, the mycelial growth of 45PRS and 60PPS was significantly slower ($P < 0.05$) than that of the control. The results indicated that 30PPS was the best substrate for mycelial growth of *A. polytricha*. The corresponding results of the total colonization time are shown in Table 3. The mycelium of *A. polytricha* totally colonized the substrates within a period of 38.7 days of spawn run. The shortest period of time in which this mushroom colonized substrate 30PPS was 32.0 days, followed by the control substrate at 35.5 days.

The primordia began appearing for all substrates before the spawn fully colonized the substrate (Table 3). The shortest period of primordia formation was 25.2 days for 30PRS, followed by 45PRS at 26.5 days.

Among the different substrates for the cultivation of *A. polytricha*, 30PRS for *P. repens* stalk, 30PPS for *P. purpureum* stalk and all of the substrates for *Z. mays* stalk were found to be the best substrate combinations for mycelial growth in the three different grass plants, with the mycelium fully colonizing the substrates at 37.8, 32.0 and 35.6 days,

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Table 3 Comparison of mycelial growth of *Auricularia polytricha* on different substrates.

Substrate	Mycelial growth rate (mm/day)	Total colonization time (days)	Time to primordia formation (days)
Control	4.84 ± 0.35b[a]	35.5 ± 0.3b	26.7 ± 0.2b
30PRS	4.52 ± 0.31bc	37.8 ± 0.5ab	25.2 ± 0.1c
45PRS	4.41 ± 0.28c	38.7 ± 0.6a	26.5 ± 0.1bc
60PRS	4.49 ± 0.32bc	38.1 ± 0.8ab	26.7 ± 0.1b
30PPS	5.35 ± 0.40a	32.0 ± 0.7c	27.0 ± 0.1ab
45PPS	4.80 ± 0.34b	35.6 ± 0.6b	27.3 ± 0.2ab
60PPS	4.42 ± 0.36c	38.7 ± 0.7a	27.0 ± 0.1ab
30ZMS	4.77 ± 0.29b	35.8 ± 0.5b	26.8 ± 0.2b
45ZMS	4.80 ± 0.21b	35.6 ± 0.6b	27.2 ± 0.1ab
60ZMS	4.80 ± 0.25b	35.6 ± 0.6b	27.8 ± 0.1a

[a] Data were analyzed by Duncan's Multiple Range Test. Each value is expressed as the mean ± S.E. ($n = 3$). Means within a column followed by the same letter are not statistically significantly different ($P < 0.05$).

respectively. However, 30PRS and 60PRS for *P. repens* stalk were not significantly different from one another. The length of time to fully colonize the substrate was directly reflected by the mycelial growth rate. Shorter lengths of times for spawn to fully colonize the substrates were obtained from those substrates that had a quicker mycelial growth rate.

3.2. Yield and biological efficiency

Flushes of mushroom yield varied among different substrates (Table 4). There were four flushes for all of the substrates during the 3-month cultivation period. In the first flush, the highest yield of a substrate was seen in 45ZMS, with 54.05% biological efficiency, which showed no significant difference with the yield of the 60PRS (53.36%), 45PPS (52.53%), 30ZMS (52.31%) and 30PRS (52.26%) substrates. In the second flush, the highest yield of a substrate was seen in 60PRS, with 52.70% biological efficiency. This showed no significant difference with 30PRS (52.02%), but was significantly different from the other substrates. In the third and fourth flush, the highest yield of a substrate was seen in 30ZMS and 45ZMS, with the corresponding biological efficiencies of 41.20% and 37.83%, respectively. These showed a significant difference with the other substrates. Generally speaking, the biological efficiency of the first flush was clearly higher ($P < 0.05$) than that of the second flush for all of the substrates, whereas the biological efficiency of the third flush was clearly lower ($P < 0.05$) than that of the other flushes for all of the substrates, with the exception of the control, 60PPS, 30ZMS and 60ZMS.

With the exception of 30PPS, the total biological efficiency of all of the substrates containing *P. repens* stalk, *P. purpureum* stalk and *Z. mays* stalk was higher ($P < 0.05$) than that of the control. The most suitable substrate for the high yield of *A. polytricha* was 60PRS, followed by 30ZMS, 45ZMS and 30PRS. More than 50% of the yield was obtained in the first two flushes in all of the substrates, in particular, over 60% of the yield was acquired in the same period for the substrates containing *Panicum repens* stalk.

3.3. Chemical contents of A. polytricha

Table 5 indicates the chemical composition of *A. polytricha* fruiting bodies grown on different substrates. The moisture and ash contents of *A. polytricha* ranged from 6.33 to 7.01 and 3.49 to 4.81, respectively. The ash contents of *A. polytricha* cultivated on substrate containing *Z. mays* stalk were higher than those of the control, but were lower ($P < 0.05$) than those of the control when cultivated on a substrate containing *P. purpureum* stalk. The protein contents of fruiting bodies cultivated on 30PRS and 60PRS were significantly greater ($P < 0.05$) than those of the others substrates. The protein contents of *A. polytricha* cultivated on the substrate containing *P. repens* stalk (except substrate 45PRS) were higher ($P < 0.05$) than that of the control. The highest fat content of *A. polytricha* was seen in 45ZMS, followed by substrates 30ZMS and 60ZMS. In general, the fat contents of *A. polytricha* cultivated on the substrate containing *Z. mays* stalk were higher ($P < 0.05$) than that of the control. The carbohydrate contents of fruiting bodies cultivated on 30PPS and 45PPS were significantly greater ($P < 0.05$) than that of the other substrates. The carbohydrate content of *A. polytricha* cultivated on the substrate containing *P. purpureum* stalk (except substrate 60PPS) was higher ($P < 0.05$) than that of the control, but was lower ($P < 0.05$) than that of the control

Table 4 Comparison of biological efficiency of *Auricularia polytricha* on different substrates.

Substrate	Biological efficiency (%)				
	1st flush	2nd flush	3rd flush	4th flush	Total
Control	44.63 ± 1.51c[a]	15.23 ± 0.52e	25.74 ± 0.45bc	13.89 ± 0.05e	99.49 ± 0.64d
30PRS	52.26 ± 2.01a	52.02 ± 1.22a	5.12 ± 0.06e	27.28 ± 0.57b	136.68 ± 0.97b
45PRS	50.59 ± 1.36b	38.88 ± 0.95b	7.76 ± 0.07e	27.47 ± 0.50b	124.70 ± 0.72c
60PRS	53.36 ± 1.75a	52.70 ± 1.26a	18.52 ± 0.10c	23.54 ± 0.35bc	148.12 ± 0.85a
30PPS	41.13 ± 1.02d	26.18 ± 0.85d	9.35 ± 0.05de	18.65 ± 0.12d	95.31 ± 0.52e
45PPS	52.53 ± 1.38a	33.68 ± 0.84c	12.43 ± 0.06d	30.90 ± 0.21ab	129.54 ± 0.63bc
60PPS	45.30 ± 1.04c	30.51 ± 0.67c	29.07 ± 0.08b	21.87 ± 0.25c	126.75 ± 0.51c
30ZMS	52.31 ± 1.59a	32.95 ± 0.65c	41.20 ± 0.84a	18.59 ± 0.35d	145.05 ± 0.85ab
45ZMS	54.05 ± 1.57a	35.57 ± 0.34bc	16.70 ± 0.06c	37.83 ± 0.85a	144.15 ± 0.71ab
60ZMS	41.03 ± 1.12d	27.73 ± 0.38d	30.14 ± 0.55b	29.97 ± 0.34ab	128.87 ± 0.60bc

[a] Data were analyzed by Duncan's Multiple Range Test. Each value is expressed as the mean ± S.E. ($n = 3$). Means within a column followed by the same letter are not statistically significantly different ($P < 0.05$).

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Table 5 Comparison of the chemical contents and energetic contribution of *Auricularia polytricha* cultivated on different substrates (100 g of dry matter).

Parameter	Substrate[a]								
	Control	30PRS	45PRS	60PRS	30PPS	45PPS	60PPS	30ZMS	45ZMS	60ZMS[b]
Moisture (g)	6.33 ± 0.13c [a]	6.70 ± 0.11a	6.78 ± 0.26a	7.01 ± 0.18a	6.55 ± 0.22b	6.54 ± 0.11b	6.53 ± 0.15b	6.73 ± 0.11a	6.73 ± 0.14a	6.97 ± 0.13a
Ash(g)	4.15 ± 0.07c	4.00 ± 0.12d	3.49 ± 0.11e	4.47 ± 0.13b	3.54 ± 0.07e	3.94 ± 0.09d	4.05 ± 0.09d	4.53 ± 0.08b	4.35 ± 0.05b	4.81 ± 0.13a
Protein (g)	10.22 ± 0.15c	11.46 ± 0.12a	10.37 ± 0.17c	11.41 ± 0.20a	9.40 ± 0.18d	9.13 ± 0.12d	11.05 ± 0.23b	11.06 ± 0.22b	10.12 ± 0.19c	11.10 ± 0.17b
Fat (g)	0.85 ± 0.09c	0.90 ± 0.07c	0.87 ± 0.12c	0.98 ± 0.03b	0.93 ± 0.24c	0.74 ± 0.07d	0.84 ± 0.06c	0.98 ± 0.07b	1.06 ± 0.06a	0.98 ± 0.05b
Carbohydrates (g)	78.45 ± 0.44b	76.94 ± 0.42c	78.49 ± 0.66b	76.13 ± 0.54c	79.58 ± 0.52a	79.65 ± 0.39a	77.53 ± 0.53b	76.70 ± 0.48c	77.74 ± 0.44b	76.14 ± 0.48c
Energy (kcal)	362.33 ± 1.12b	361.70 ± 1.08b	363.27 ± 1.87a	358.98 ± 1.10c	364.29 ± 1.22a	361.78 ± 0.98b	361.88 ± 1.15b	359.86 ± 0.96b	360.98 ± 1.02b	357.78 ± 1.25c

[a] Data were analyzed by Duncan's Multiple Range Test. Each value is expressed as the mean ± S.E. ($n = 3$). Means within the same row followed by the same letter are not statistically significantly different ($P < 0.05$).

when cultivated on the substrate containing *Z. mays* stalk (except substrate 45ZMS). Substrates 30PPS and 45PRS showed the highest energy values, which were 364.29 and 363.27 kcal/100 g of dry matter, respectively. Meanwhile, substrates 60ZMS and 60PRS showed the lowest energy values and lower ($P < 0.05$) than that of the control, which were 357.78 and 358.98 kcal/100 g of dry matter, respectively.

4. Discussion

In this study, all of the *P. repens*, *P. purpureum* and *Z. mays* stalks were found to support the growth of *A. polytricha*. This result is consistent with our previous reports regarding *P. citrinopileatus* (Liang et al., 2009) and *P. pulmonarius* (Liang et al., 2011) cultured on these three grass plants. It is also in accordance with the reports of other researchers (Murugesan et al., 1995; Velázquez-Cedeño et al., 2002; Obodai et al., 2003; Mukherjee and Nandi, 2004; Pant et al., 2006; Sainos et al., 2006; Das and Mukherjee, 2007; Li et al., 2016) indicating that *Pleurotus* spp. have a high saprophytic ability to grow on a variety of agro-industrial wastes, weed plants, aquatic plants or perilla.

The addition of *P. purpureum* stalk to the substrates decreased the mycelial growth rate and delayed the total colonization time, which corresponded with the C/N ratio gradually increasing from 70.21 to 79.47. The lowest mycelial growth rates of substrates were seen in 45PRS and 60PPS, with corresponding C/N ratios of 48.23 and 79.47, respectively. These did not correspond with the most suitable C/N ratios for *P. citrinopileatus* (Liang et al., 2009) and *P. pulmonarius* (Liang et al., 2011), which ranged from 46 to 51 and from 46 to 55, respectively. This finding indicates that the mycelial growth of *A. polytricha* probably has different requirements, such as the addition of lipids to the substrate (Onyango et al., 2011; Lau et al., 2014).

The mycelial growth rates of the experimental substrates did not seem to correspond with the biological efficiency. The substrate 30PPS showed the highest mycelial growth rate and shortest colonization time, whereas the biological efficiency of this substrate was lower than the others. In contrast, the lower mycelial growth rate of the substrate 60PRS yielded the highest biological efficiency. This result is similar to our previous studies on the cultivation of *P. citrinopileatus* (Liang et al., 2009) and *P. pulmonarius* (Liang et al., 2011) using grass plants to partially replace sawdust. All of our study results on the cultivation of mushroom with grass plants were consistent with other research findings that showed that the mycelial growth rate of rice husk (Obodai et al., 2003) or wheat/rice straw (Yang et al., 2013) as substrate for the cultivation of *P. ostreatus* did not accord with the yield.

In this study, the biological efficiencies of *A. polytricha* cultivated on the experimental substrates were between 124.70% and 148.12% (except for substrate 30PPS). This was 3.33–4.32 folds higher than the efficiency reported for rubber wood sawdust with rice bran and rubber wood sawdust with empty fruit bunch substrates by Lau et al. (2014), and was 1.77–2.16 folds higher than that reported for maize cobs with wheat bran substrate by Onyango et al. (2011) for the cultivation of *A. polytricha*. In the experimental substrates, higher C/N ratios showed slightly lower biological efficiencies, which indicates that a high nitrogen content in substrates, could improve the

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mushroom yield. This result is consistent with the report by Xu et al. (2016).

Application of different lignocellulosic materials for use as substrates in the cultivation of other mushrooms has also been reported; these studies indicated variable ranges of yield and biological efficiency. A biological efficiency of 139.0% was obtained from the combined substrates of rice straw and weed plant (*Leonotis* sp.) at a 1:1 proportion for *P. ostreatus* cultivation (Das and Mukherjee, 2007). Uhart et al. (2008) achieved a biological efficiency of 179% for *Agrocybe cylindracea* with wheat straw and soybean flour, which was the highest reported for this mushroom and higher than that obtained by plain willow sawdust and wheat straw. Increasing the amount of cotton seed hull to wheat straw for *P. ostreatus* cultivation could increase the yield by up to 125.6% of biological efficiency (Yang et al., 2013).

In this study, considerable differences were observed in moisture, ash, protein, fat and carbohydrate, depending on the supplementation with different proportions of grass plant stalk. These results were in agreement with the report by Xu et al. (2016). The protein and fat contents of this mushroom varied from 9.13 to 11.46 g and 0.74 to 1.06 g, respectively, which were lower than those of *P. ostreatus* grown on perilla stalks (Li et al., 2016) and *Oudemansiella canarii* cultivated on different lignocellulosic wastes (Xu et al., 2016). Our results are in agreement with earlier findings that showed the chemical contents of mushroom are affected by both the species (Calzada et al., 1987; Reis et al., 2012) and substrate (Bisaria et al., 1987; Das and Mukherjee, 2007; Gupta et al., 2013).

In conclusion, the biological efficiencies of the substrates containing PRS, PPS and ZMS for the cultivation of *A. polytricha* were higher than that of the control (except 30PPS), with corresponding percentages ranging from 25.3% to 48.9%, 27.4% to 30.2% and 29.5% to 45.8%, respectively. Based on the biological efficiencies of the substrates tested, the stalks of the three grass plants used in this study can be considered to be practical and economically feasible for the cultivation of *A. polytricha* due to their availability in large quantities throughout the year. The stalks of *P. repens* and *Z. mays* in particular yielded relatively high biological efficiencies and could be an alternative material for *A. polytricha* cultivation.

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