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Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain

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Abstract

Unpretreated spent beer grains were successfully used as a basic substrate material for the cultivation of *Pleurotus ostreatus*. The effects of spent grain types, additives, substrate moisture content, and substrate packing density on the yield and nutrition of fruit bodies were investigated. The cultivation results showed that few fruit bodies were formed on spent grain alone; however, a significantly high biological efficiency (19.1%) was obtained with the addition of wheat bran to (45%). The chemical analysis of fruit bodies indicated that *P. ostreatus* cultivated on spent grain substrate had a higher nutritional value than those grown on other reported types of substrates. The total amino acid content in the fruit bodies was 347.5 mg/g dry matter, and the crude protein content was as high as 53.3% on a dry weight basis. It was also found that the cultivation of *P. ostreatus* increased the crude protein content, while it decreased the ratio of lignin to cellulose, of the spent grain substrate.

Keywords: *Pleurotus ostreatus*; Spent beer grain; Cultivation; Biological efficiency; Nutrition

1. Introduction

Pleurotus ostreatus is a prospective source of valuable food protein, and an organism with the ability to effectively bioconvert various lignocellulosic materials (Zadrazil and Dube, 1992). In general, sawdust supplemented with wheat bran or rice bran is used in the commercial cultivation of *P. ostreatus* in Japan. However, the low availability of sawdust used for mushroom cultivation has in recent years become a serious problem. This shortage of sawdust has led to a continuing search for an alternative raw material which could serve as the substrate for mushroom cultivation (Yamashita et al., 1983; Shiratori et al., 1980; Terashita and Kono, 1984).

With this view, we turned our attention to spent grain by-product of beer brewing. In Japan, about one million tons of spent beer grain are discharged every year, of which about 95% is used for cattle feed, while disposal of the remaining 5% results in environmental problems, especially for local beer breweries. As is known, the spent grain is rich in lignocelluloses and protein, as well as having a particularly high moisture content. In addition, the physical properties of spent grain, such as particle size, volume weight, specific density, porosity, and water-holding capacity, are conducive to its use as a basic substrate material for mushroom cultivation (Levanon, 1988). Another advantage is that the spent grain is available at low or no cost throughout the year not only from large factories but also from a large number of local breweries.

The present investigation was undertaken with the aim of determining the feasibility of utilizing the lignocellulosic by-product of spent beer grain as a substrate for cultivation of the oyster mushroom, *P. ostreatus*, and evaluating the nutrition of the fruit bodies of *P. ostreatus* grown on spent grain substrate. Our interest lay not only in the search for a raw material to serve as substrate for mushroom cultivation, but also in the bioconversion of the substrate and the biodegradation of lignocelluloses in the substrate, and the reuse of the spent substrate. Thus, the present study is the first part of our series of work focusing on the optimization of the utilization of spent beer grain by using mushrooms.

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2. Methods

2.1. Materials and cultivation

2.1.1. Materials

The sawdust spawn of *P. ostreatus* Mori 39 (Mori & Company) was used in this study. Spent beer grain was obtained from Shinano Brewery, Vanryu. Three types of spent grains named A, B, and C were used. Their chemical compositions are listed in Table 1. Wheat bran, rice bran, corn bran, and Okara (a by-product of bean curd production) used as additives were obtained from Mori & Company.

2.1.2. Substrate preparation

Four groups of substrates were prepared as follows. (1) Effect of the ratio of supplement: wheat bran was added to spent grain by ratios from 0% to 50% on a dry weight basis. Spent grain A and wheat bran were employed as examples of spent grain and additive, respectively. (2) Effect of additive types: wheat bran, rice bran, corn bran, and Okara were added, respectively, to spent grain by a ratio of 50:50 on a dry weight basis. Spent grain B was used as an example of spent grain. (3) Effect of substrate moisture: the moisture content of the substrate was adjusted to 43–75%. Spent grain C with added wheat bran (50:50 on a dry weight basis) was used as the substrate. (4) Effect of substrate packing density: the effect of packing density was determined by filling the prepared substrate into bottles at packing densities of 42–96 g per 100 ml. The substrate of spent grain C supplemented with wheat bran and sawdust in a ratio of 65:10:25 (on a dry weight basis) was used. The sawdust supplemented with wheat bran by a ratio of 50:50 (on a dry weight basis) was used as a control substrate in each group of cultivations.

It should be pointed out that it was a random selection of the spent grain types A, B, or C in the above experiments, since the same type of fresh spent grain could not be obtained repeatedly in a shorter duration due to the producing schedule of the brewery. In other words, the fresh spent grains A, B, and C were accidentally available when we prepared the cultivations (1), (2), (3) and (4), respectively. In addition, since the main objective of the present study was not to find out the optimum conditions for a certain spent grain but to investigate the feasibility of utilizing spent beer grain in general as a mushroom substrate, it was considered that more general information could be obtained from such an arrangement as above.

Table 1
Proximate compositions of spent beer grains (%, dry weight basis)

Spent grain	Crude protein	Fat	Ash	Cellulose	Lignin
A	23.9	6.8	3.3	14.0	9.1
B	24.2	6.1	3.9	15.8	10.8
C	27.5	–	3.8	16.9	16.3

2.1.3. Cultivation

The moisture content of the substrate was adjusted to 65% (unless otherwise stated) and the prepared substrate was filled in 140 ml glass bottles (with a wide opening of 4.2 cm in diameter) at a packing density of 60 g of substrate per 100 ml volume (unless otherwise stated). The bottles were autoclaved at 121°C for 20 min. The sterile substrates were inoculated by spreading the spawn on the surface of substrate with a weight percentage of about 8%, and then covered with aluminum foil. Each substrate condition was carried out in four replications. Spawn run was undertaken at 20°C and 70% R.H. for about three weeks. Fructification took another 2–3 weeks at 14 ± 2°C and 90 ± 5% R.H. After harvest, the fruit bodies and the spent substrates were dried for weight determination and chemical analysis.

2.2. Analytical methods

2.2.1. Fruit body analysis

Proximate composition. The proximate analysis was carried out according to the methods described in AOAC (1990). Total nitrogen was determined by Kjeldahl's method. The factor 6.25 was used to calculate the crude protein content (Crisan and Sands, 1978). Crude fat was determined using the acid hydrolysis method. Carbohydrate content was calculated by difference as total carbohydrates. The energy value was estimated based on the content of crude protein, fat, and carbohydrate in the fruit body using the factors 2.62, 8.37, and 4.2 kcal/g of each component, respectively (Crisan and Sands, 1978).

Amino acids. Amino acids were determined based on the acid hydrolysate of the dried fruit bodies using an amino acid auto-analyzer (Danell and Eaker, 1992; Cheung, 1997). Cysteine was measured as cysteic acid and methionine was measured as methionine sulfone after performing acid oxidation and 20% HCl hydrolysis at 150°C for 20 h. The tryptophan analysis was done on a $Ba(OH)_2$ hydrolysate (Fujihara et al., 1995).

Minerals. Calcium, magnesium, manganese, zinc in ashed samples were determined by atomic absorption spectrophotometry after mineralization by hydrochloric acid (M.F.A., 1982). Iron in ashed samples was determined using a 1,10-phenanthroline spectrophotometric method (M.F.A., 1982). Sodium, potassium and copper were extracted from dried samples by acids before being determined with an atomic absorption spectrophotometer (M.F.A., 1982). Phosphorus was measured spectrophotometrically after treating the ashed sample solution with ammonium molybdate, metavanadate, and nitric acid (Gujral et al., 1987; M.F.A., 1982).

Vitamins. Thiamin and riboflavin were determined by means of HPLC employing a fluorimetry detector after acid extraction and hydrolysis by takadiastase. Ascorbic acid was determined by means of HPLC with an ultraviolet detector after acid treatment. Niacin was measured by the microbial method (H.T.M., 1980).

2.2.2. Substrate analysis

The total nitrogen, cellulose, and lignin contents in spent beer grain, raw substrate, and spent substrate were estimated. Total nitrogen was determined by using a Perkin–Elmer (Norwalk, Conn, USA) CHN Analyzer, model 2400-II. The contents of cellulose and lignin were assayed according to a method described by Goering and Van Soest (1970). Namely, the contents of acid-detergent fiber (ADF) and acid-detergent lignin (ADL) were analyzed. The cellulose content was calculated by the difference between ADF and ADL. The lignin content was taken to be the ADL content. The substrate pH was determined from a homogeneous suspension of 5 g of substrate in 100 ml distilled water shaken at 25°C overnight.

3. Results and discussion

3.1. Biological efficiency of fruit bodies

P. ostreatus was cultivated on various spent grain substrates. The first flush was harvested in about 35 days, while the second flush was harvested in about 45 days. No third flush was obtained. More than 80% of the total fruit body was harvested in the first flush, while the fruit body obtained in the second flush was of lower quality as well as in a smaller amount.

Chang et al. (1981) has defined the term "biological efficiency" to express the yield of fresh fruit bodies per 100 g dry substrate, and some researchers have adopted this definition to evaluate the quality of organic waste as a substrate for mushroom cultivation (Madan et al., 1987; Patrabansh and Madan, 1997). In the present work, we prefer to define biological efficiency as the percentage of conversion of dry substrate to dry matter in fruit bodies as follows (Bisaria et al., 1987; Jwanny et al., 1995):

$$\text{Biological efficiency } \% = \frac{\text{Weight of dry fruit bodies harvested}}{\text{Initial weight of dry substrate}} \times 100\%.$$

The biological efficiencies of the fruit bodies of *P. ostreatus* harvested from various substrates based on one flush are shown in Tables 2 and 3 and Figs. 1–3. Each value of the graph point was the average from four replicates.

Table 2
Effect of spent grain type on biological efficiency (one flush)

Substrates (50:50)	Biological efficiency (%)
Spent grain A + wheat bran	16.9
Spent grain B + wheat bran	12.3
Spent grain C + wheat bran	11.2
Sawdust[a] + wheat bran	5.8

[a] Control substrate.

Table 3
Effect of additives on biological efficiency (one flush)

Substrates (50:50)	Biological efficiency (%)
Spent grain B + wheat bran	12.3
Spent grain B + rice bran	13.2
Spent grain B + corn bran	12.9
Spent grain B + Okara	2.3

3.1.1. Effect of spent grain type

According to the results listed in Table 2, the difference of spent grain type caused a difference in biological efficiency. Spent grain A produced a higher biological efficiency value than did spent grain B or C. The differences between spent grains resulted mainly from the type of malt, baking degree of malt, additive type, and addition ratio. However, there was no evident correlation found between the biological efficiency and proximate compositions of spent grains given in Table 1.

Comparing to the control substrate (sawdust supplemented with wheat bran), which is generally used in the commercial cultivation of *P. ostreatus* in Japan, about 2–3 times biological efficiency was obtained by substituting spent grain for sawdust. This might have

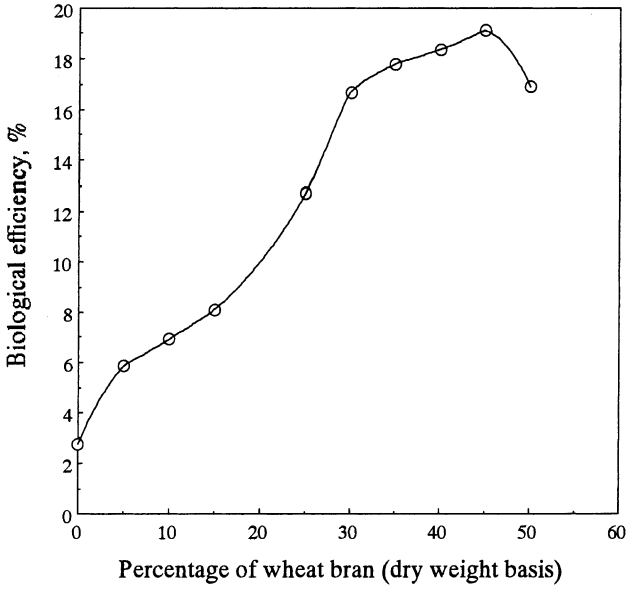

Fig. 1. Variation of biological efficiency of *P. ostreatus* with differnt percentages of wheat bran to spent grain A.

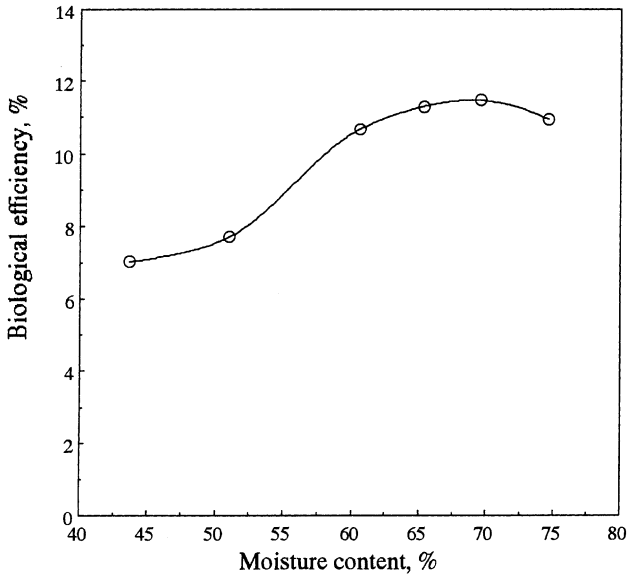

Fig. 2. Variation of biological efficiency of *P. ostreatus* with moisture content of substrate. Substrate: spent grain C + wheat bran (50:50 on a dry weight basis).

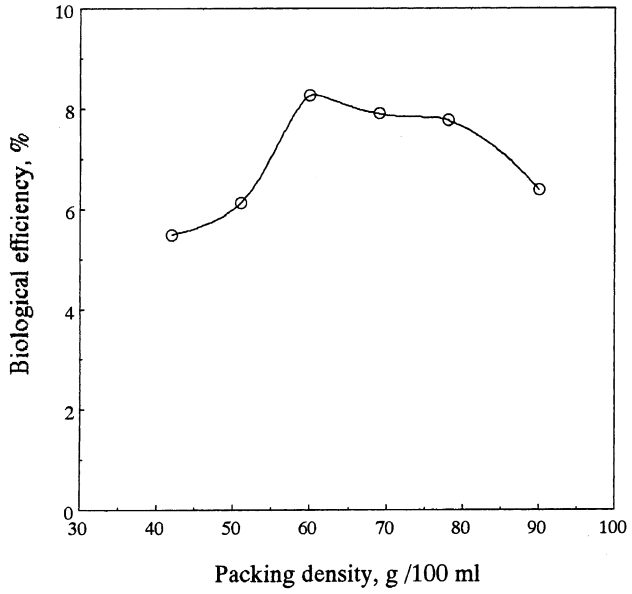

Fig. 3. Variation of biological efficiency of *P. ostreatus* with packing density of substrate. Substrate: spent grain C + wheat bran + sawdust (65:25:10 on a dry weight basis).

been due to the presence of the higher contents of crude protein, fat, low molecular sugars, and considerably lower lignocellulosic content in spent grains.

3.1.2. Effect of additive type

Table 3 shows the effect of additives on the biological efficiency. As depicted, wheat bran, rice bran, and corn bran proved similarly excellent as additives to the spent grain for the cultivation of *P. ostreatus*, while Okara showed less ability to improve the properties of spent grain for this purpose.

3.1.3. Effect of supplementary ratio of wheat bran

The effect of the percentage of wheat bran in spent grain substrate on biological efficiency is shown in Fig. 1. The biological efficiency increased rapidly with an increase in the ratio of wheat bran, and peaked at the additive percentage of around 45% with a maximum value of 19.1%. However, fewer fruit bodies were formed when the spent grain alone was used as substrate. This might have been due to lack of low molecular carbohydrates and/or vitamins in the spent grain.

3.1.4. Effect of substrate moisture content and packing density

It has been known that in addition to chemical components of the substrate, the substrate moisture content and packing density are the other two important factors affecting mycelia growth and fruit body formation (Zadrazil and Brunnert, 1981, 1982; Reid, 1985; Kumaran et al., 1996; Garcha et al., 1985).

Fig. 2 shows the variation of biological efficiency in relation to moisture content in the substrate. The biological efficiency value increased with the increase of moisture content and peaked at about 70%. The rate of mycelia extension was also seen to be fastest when the substrate moisture content was at 70%. These results suggest that the optimum moisture content of the spent grain substrate for the cultivation of *P. ostreatus* is around 70%. This is different from 65%, which is a value generally used in the cultivation of *P. ostreatus*. It could be expected that in this moisture condition with spent grain A the biological efficiency could be increased to over 19.1%.

Fig. 3 shows the effect of substrate packing density on the biological efficiency. The optimum value for biological efficiency was obtained at a packing density of 60 g substrate per 100 ml volume. This is in agreement with the value commonly reported.

In the present study, the effect of spent grain pretreatments such as drying and water washing was also investigated (the data are not shown in this paper). The results showed that biological efficiency decreased when dried spent grain was used, while it increased to some degree with the use of water-rinsed spent grain. The latter might be because the pH value of water-rinsed spent grain was nearer to the optimum value for *P. ostreatus* growth, and because the water removed some components in the spent grain, which inhibited mycelial growth. However, considering the use of a large amount of water and the problems brought by wasting water, the direct use of unpretreated spent grain is suggested. All the results presented in this paper were obtained by using unpretreated spent grain.

The above results indicate that spent beer grain is one of the best substrate materials for cultivation of *P. ostreatus*. The spent grain type, additive type, supplementary ratio of additive, and the moisture content and packing density of substrate affected the biological efficiency significantly. The obtained biological efficiency of 19.1% was the maximum value of *P. ostreatus* grown on various substrates reported so far. Based on the results of the present study, the optimum conditions of spent grain-based substrate (when wheat bran is used as an additive) might be around: moisture content 70%; packing density 0.6 g/ml; additive ratio 45%.

3.2. Composition of fruit bodies

To evaluate the nutritional value of *P. ostreatus* cultivated on the spent grain substrate, the proximate composition, amino acids, vitamins, and mineral constituents of the fruit bodies were analyzed.

Table 4 lists the proximate compositions of fruit bodies harvested from various types of spent grain substrates. For comparison, the corresponding data from the Standard Tables of Food Composition in Japan (STFC, 1982) are also included. Considerable differences were observed in the crude protein, carbohydrate, and ash content in fruit bodies cultivated on different substrates, while the fat contents of fruit bodies grown on all types of substrates were quite similar. This tendency is in agreement with the analyses of *P. ostreatus* cultivated on substrates composed of straw, cotton waste, and tea leaves reported by Chang et al. (1981). When compared with the data from the Standard Tables of Food Composition in Japan (STFC, 1982), generally, the fruit bodies of *P. ostreatus* grown on spent grain substrates had higher protein and fat contents but lower carbohydrate and ash contents.

According to Table 4, the supplementation with wheat bran had a greater potential to improve the accumulation of protein in *P. ostreatus* than did rice bran or corn bran. Also, the type of spent grain affected the protein content, even though initial protein contents were similar in spent grains A and B (Table 1). These results show that not only the amount but also the nature of the nitrogen source present in the substrate influences the protein content of fruit bodies (Rapior et al., 1988; Delmas and Poitou, 1965; Tshinyangu, 1996). Khanna and Garcha (1984), Gujral et al. (1987), and Chang et al. (1981) reported protein contents of *Pleurotus* species being 26.9–37.2%, 26.6–35.5%, and 26.6–35.6%, based on their fruit bodies cultivated on various kinds of substrates. However, the protein content of *P. ostreatus* grown on the spent-grain-based substrates was 41.5–53.3% with the highest value obtained from the substrate of spent grain B with wheat bran (50:50 on a dry weight basis) in the present experiment conditions. It is obvious that the use of spent grain increased the protein content of fruit bodies.

The variation of crude protein content in fruit bodies with the added wheat bran to spent grain A is shown in Fig. 4. The protein content was 43.1–52.1% when the amount of wheat bran was 20.0–50.0%, with the highest value of 52.1% observed at around 35% bran.

The amino acid profile of *P. ostreatus* cultivated on a spent grain substrate (spent grain B supplemented with wheat bran 50:50 on a dry weight basis) was determined (Table 5). The fruit bodies harvested from the spent grain substrate contained all the essential amino acids, which comprised 36.5% of the total amino acid content. The contents of total amino acids and total essential amino acids were 347.5 and 126.7 mg/g of dry matter, respectively. The first four major components were the non-essential amino acids, glutamic acid, aspartic acid, alanine, and arginine, which comprised 40.5% of the total amino acids. The next three components were essential amino acids, leucine, lysine, and valine, which comprised 20.0% of total amino acids, while the sulfur-containing amino acids cysteine and methinine were in least amount.

To evaluate the protein quality of *P. ostreatus* cultivated on the spent grain substrate, the percentage of total amino acids in crude protein (TAA/CP) was calculated and compared with those calculated from other researchers' results (Jwanny et al., 1995; Khanna and Garcha, 1984) (Table 6). The crude protein content and total amino acids content of *P. ostreatus* grown on spent grain substrate were 1.9 and 2.0–2.3 times, respectively,

Table 4
Proximate composition of *P. ostreatus* grown on different substrates (%, dry weight basis)[a]

Substrates (50:50)	Crude protein	Fat	Carbohydrate	Ash	Energy value (kcal/100 g)
This work					
SGA + wheat bran	46.3	4.4	42.0	7.3	330.3
SGB + wheat bran	53.3	4.3	35.7	6.7	325.6
SGB + rice bran	41.5	4.6	45.5	8.4	338.3
SGB + corn bran	44.1	4.7	44.5	6.7	341.8
Reference					
STFC[b]	34.4	3.1	54.2	8.3	343.7

[a] SGA and SGB present spent grains A and B, respectively.
[b] Data from STFC (1982), sawdust-based substrate.

as high as those cultivated on paddy straw substrate (Khanna and Garcha, 1984) and date waste substrate (Jwanny et al., 1995). *P. ostreatus* grown on spent grain substrate also showed a higher level of TAA/CP. Therefore, not only the amount but also the quality of protein indicated that *P. ostreatus* grown on spent grain substrate was a good protein resource. The high amino acid content in *P. ostreatus* grown on spent grain might be attributed to the higher amounts of nitrogen and amino acids in this substrate (Kissmeyer-Nielsen et al., 1966; Fujihara et al., 1995; Rapior et al., 1988; Delmas and Poitou, 1965; Tshinyangu, 1996).

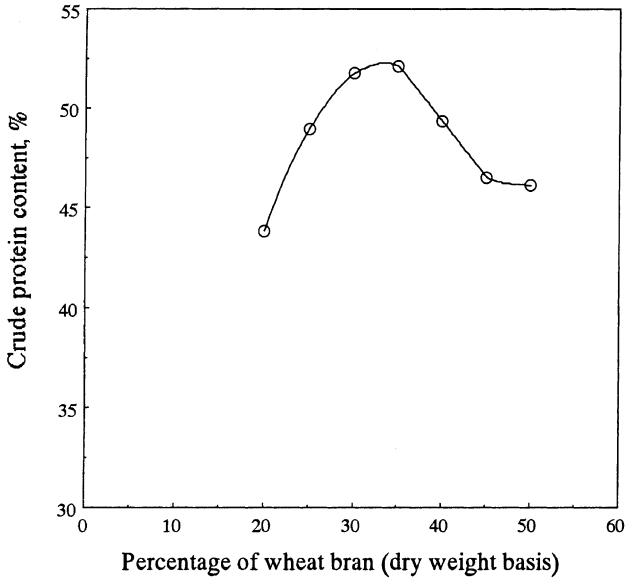

Fig. 4. Variation of crude protein content in *P. ostreatus* with increasing percentage of wheat bran to spent grain A.

Table 5
Amino acid composition of *P. ostreatus* (spent grain B + wheat bran, 50:50, dry weight basis)

Amino acids	mg/g dry weight
Aspartic acid	31.4
Threonine[a]	17.1
Serine	18.1
Glutamic acid	53.3
Glycine	17.1
Alanine	28.6
Valine[a]	21.0
Cysteine	3.8
Methionine[a]	3.8
Isoleucine[a]	16.2
Leucine[a]	25.7
Tyrosine	13.3
Phenylalanine[a]	15.2
Lysine[a]	22.9
Histadine	12.4
Arginine	27.6
Tryptophan[a]	4.8
Proline	15.2
Total essential amino acids	126.7
Total amino acids	347.5

[a] The essential amino acids.

The vitamin contents of *P. ostreatus* grown on spent grain are listed in Table 7. Thiamin and riboflavin were at low levels, and particularly, ascorbic acid was not detected. In contrast, the niacin content was comparatively high. When compared with the values quoted, all of the vitamin contents in *P. ostreatus* cultivated on spent grain were lower than those given in the Standard Tables of Food Composition in Japan (STFC, 1982); however, they were considerably higher than those reported by Chang et al. (1981).

The mineral components given in Table 8 showed that potassium and phosphorus were the main constituents of ash in *P. ostreatus* grown on spent grain. The order of content of the metals was quite similar to that given by the Standard Tables of Food Composition in Japan (STFC, 1982) (Table 8) and reported by Chang et al. (1981); however, it was different from that of Jwanny et al. (1995) who reported that phosphorus and

Table 6
Comparison of crude protein and total amino acids (%, dry weight basis)[a]

Data from	Crude protein	TAA	TAA/CP (%)
This work[b]	53.3	34.8	65.2
Khanna[c]	27.4	15.3	56.0
Jwanny[d]	27.4	17.5	64.4

[a] CP: crude protein; TAA: total amino acids.
[b] Spent grain B + wheat bran, 50:50 on a dry weight basis.
[c] Khanna and Garcha (1984).
[d] Jwanny et al. (1995).

Table 7
Vitamin content of *P. ostreatus* (mg/100 g dry matter)

Vitamins	This work[a]	STFC[b]
Thiamine	1.91	4.17
Riboflavin	3.62	4.17
Ascorbic acid	ND[c]	ND
Niacin	90.0	111.5

[a] Spent grain B + wheat bran, 50:50 on a dry weight basis.
[b] Data from STFC (1982).
[c] ND – not detected.

Table 8
Mineral content of *P. ostreatus* (mg/100 g dry matter)

Minerals	This work[a]	STFC[b]
Na	21.9	74
P	1647.6	1061
Fe	7.1	7.8
Ca	ND	3.9
K	2171.4	2720
Mg	181.9	156
Cu	2.5	1.6
Zn	13.7	10.8
Mn	1.6	1.5

[a] Spent grain B + wheat bran, 50:50 on a dry weight basis.
[b] Data from STFC (1982).

Table 9
Crude protein and lignocelluloses in substrates before inoculation (*R*) and after harvest (*S*) (%, dry weight basis)

Substrate (50:50, dry weight basis)	Crude protein		Cellulose		Lignin		Lignin/cellulose	
	R	*S*	*R*	*S*	*R*	*S*	*R*	*S*
Spent grain A + wheat bran	19.2	22.8	9.1	19.2	6.2	12.5	0.68	0.65
Spent grain B + wheat bran	19.7	22.3	10.2	20.4	7.1	11.0	0.70	0.54
Spent grain C + wheat bran	20.8	23.6	10.7	20.9	9.8	10.4	0.92	0.50

magnesium were the main constituents of ash in their *P. ostreatus*, followed by iron and sodium. The variation of mineral content in *P. ostreatus* grown on different substrates might reflect the variation in the mineral contents of the substrates. A higher content of a desirable mineral might be introduced into *P. ostreatus* by adding this mineral to the substrate.

3.3. Protein and lignocelluloses in substrate before inoculation and after harvest

The compositions of substrates of spent grains supplemented with wheat bran before and after cultivation of *P. ostreatus* were determined and are shown in Table 9. All analytical values are expressed without taking into account the dry weight loss of substrate because our purpose here was to show the final quality of the spent substrate. The results show that the crude protein in the substrates increased during the process of *P. ostreatus* cultivation. The accumulation of protein in spent substrate can be seen as result of the metabolic activity of growing mycelium and the substrate decomposing into CO_2 and H_2O (Zadrazil and Dube, 1992).

In regard to the acid–agent–cell components in the spent substrates produced from spent grains, generally, the lignin and cellulose contents increased, while the ratio of lignin to cellulose decreased when compared with the raw substrates. The cellulose content in spent substrate was around twice the level of that in raw substrate for all types of spent grains. However, the increased amount of lignin after the cultivation of *P. ostreatus* varied significantly with the spent grain type. The lignin contents in spent substrates were 2.02, 1.55, and 1.06 times as much as those in raw substrates for spent grains A, B, and C, respectively. Considering the weight loss of the substrate during the cultivation of *P. ostreatus* (about 50% dry matter), cellulose was almost non-utilized by *P. ostreatus*, so concentrated in the spent substrates, while lignin was degraded by *P. ostreatus* except for the spent grain A. The fact that the lignin in spent grain A was almost not utilized by *P. ostreatus* might have been due to there being enough C-source, easier to be absorbed by the mycelia of *P. ostreatus*, in spent grain A. This might be one of the main reasons for the higher biological efficiency obtained from spent grain A (Section 3.1). More detailed study is needed here.

4. Conclusions

In summary, unpretreated spent beer grain was successfully used as a basic substrate material for the cultivation of *P. ostreatus*. Wheat bran, rice bran, and corn bran proved excellent as additives to the spent grain, while Okara showed less ability to improve the properties of spent grain for the cultivation. The optimum substrate moisture content was around 70%, and the optimum substrate packing density was around 60 g per 100 ml. The fruit bodies of *P. ostreatus* grown on spent grain substrate had higher biological efficiency, higher amino acid content, and higher crude protein content than those cultivated on other reported substrates. In addition, the cultivation of *P. ostreatus* increased the protein content, while it decreased the ratio of lignin to cellulose, in the substrates.

The pH value, high moisture content, and physical parameters of the spent grain made it possible to be used directly as a mushroom substrate. The other advantage of using spent grain as a mushroom substrate is that spent grain is available at low or no cost throughout the year not only from large factories but also from a large number of local breweries.

References

AOAC, 1990. Official Methods of Analysis, 14th ed. Association of Official Analytical Chemists, Washington, DC.

Bisaria, R., Madan, M., Bisaria, V.S., 1987. Biological efficiency and nutritive value of *Pleurotus sajor-caju* cultivated on different agro-wastes. Biol. Wastes 19, 239–255.

Chang, S.T., Lau, O.W., Cho, K.Y., 1981. The cultivation and nutritional value of *Pleuortus sajor-caju*. European J. Appl. Microbiol. Biotechnol. 12, 58–62.

Cheung, P.C.-K., 1997. Chemical evaluation of some lesser known edible mushroom mycelia produced in submerged culture from soy milk waste. Food Chem. 60 (1), 61–65.

Crisan, E.V., Sands, A., 1978. Nutritive value. In: Chang, S.T., Hayes, W.A. (Eds.), The Biology and Cultivation of Edible Mushrooms. Academic Press, New York, pp. 137–168.

Danell, E., Eaker, D., 1992. Amino acid and total protein content of the edible mushroom *Cantharellus cibarius* (*Fries*). J. Sci. Food Agric. 60, 333–337.

Delmas, J., Poitou, N., 1965. Nitrogen compounds and more especially amino acids in cultivated mushrooms and in composts. Mushroom Sci. 6, 193–202.

Fujihara, S., Kasuga, A., Aoyagi, Y., Sugahara, T., 1995. Nitrogen to protein conversion factors for some common edible mushrooms. J. Food Sci. 60, 1045–1047.

Garcha, H.S., Dhanda, S., Khanna, P., 1985. Efficacy of container system for the production of *Pleurotus*. Mushroom Newsletter for the Tropics 5, 16–20.

Goering, H.K., Van Soest, P.J., 1970. Forage fiber analysis. Agric. Handb. 379, 1–19.

Gujral, S.S., Bisaria, R., Madan, M., Vasudevan, P., 1987. Solid state fermentation of *Saccharum munja* residues into food through *Pleurotus* cultivation. J. Ferment. Technol. 65, 101–105.

H.T.M., 1980. Hygiene Testing Method. Nihon Yakugakukai, Tokyo.

Jwanny, E.W., Rashad, M.M., Abdg, H.M., 1995. Solid-state fermentation of agricultural wastes into food through *Pleurotus* cultivation. Appl. Biochem. Biotechnol. 50, 71–78.

Khanna, P., Garcha, H.S., 1984. *Pleurotus* mushroom – a source of food protein. Mushroom Newsletter for the Tropics 4 (3), 9–15.

Kissmeyer-Nielsen, E., Mcclendon, J.H., Woodmansee, C.W., 1966. Change in amino acid and urea in the cultivated mushroom, agaricus bisporus, as influenced nutrient supplementation of the compost during the growth cycle. J. Agric. Food Chem. 14, 633–636.

Kumaran, S., Sasatry, C.A., Vikineswary, S., 1996. Reusing spent sago 'hampas' from solid substrate fermentation for mushroom cultivation. Malays. Appl. Biol. 25 (1), 119–122.

Levanon, D., 1988. Chemical and physical parameters in recycling organic wastes for mushroom production. Biol. Wastes 26, 341–348.

M.F.A., 1982. Methods of Food Analysis. Nippon Shokuhin Kogyo Gakkai, Tokyo.

Madan, M., Vasudevan, P., Sharma, S., 1987. Cultivation of *Pleurotus sajor-caju* on different wastes. Biol. Wastes 22, 241–250.

Patrabansh, S., Madan, M., 1997. Studies on cultivation, biological efficiency and chemical analysis of *Pleurotus sajor-caju* (FR.) SINGER on different bio-wastes. Acta Biotechnol. 17 (2), 107–122.

Rapior, S., Moussain, D., Plassard, C., Andary, C., Salsac, L., 1988. Influence of nitrogen source on growth of *Cortinarius orellanus* and on accumulation of nitrogen and phosphorus in mycelium. Trans. Br. Mycol. Soc. 90 (2), 181–185.

Reid, I.D., 1985. Biological delignification of Aspen wood by solid-state fermentation with the white-rot fungus *Merulius tremellosus*. Appl. Environ. Microbiol. 50 (1), 133–139.

Shiratori, T., Kakimoto, Y., Muraoka, S., Okimura, Z., 1980. Studies on sawdust–ricehull mixed media for Enokitake (*Flammulina ueltipes*) cultivation in bottle culture system. Bull. Nagano Veg. & Ornam. Crops Exp. Sta. Japan 1, 57–63.

STFC, 1982. Standard Tables of Food Composition in Japan, 4th revised ed. Resources Council, Science and Technology Agency, Japan.

Terashita, T., Kono, M., 1984. Utilization of industrial wastes for the cultivation of *Pleurotus ostreatus*. Mem. Fac. Agr. Kinki Univ. 17, 113–120.

Tshinyangu, K.K., 1996. Effect of grass hay substrate on nutritional value of *Pleurotus ostreatus var. columbinus*.. Nahrung 40 (2), S.79–83.

Yamashita, I., Mori, T., Iino, K., Yanai, S., 1983. Utilization of Job's-tears husk, peanut shell, lawn grass and porous stone for cultivation of oyster mushroom (*Pleurotus ostreatus* (Jacq. ex Fr). Quel.). Nippon Shokuhin Kogyo Gakkaishi 30 (12), 693–697.

Zadrazil, F., Brunnert, H., 1981. Investigation of physical parameters important for the solid state fermentation of straw by white rot fungi. European J. Appl. Microbiol. Biotechnol. 11, 183–188.

Zadrazil, F., Brunnert, H., 1982. Solid state fermentation of lignocellulose containing plant residues with *Sporotrichum pulverulentum* Nov. and *Dichomitus squalens* (Karst.). European J. Appl. Microbiol. Biotechnol. 16, 45–51.

Zadrazil, F., Dube, H.C., 1992. The oyster mushroom. Importance and prospects. Mushroom Res. 1 (1), 25–32.