#### **Original Research**



# The assessment of apple pomace as a basic substrate and a nutrient supplement for Oyster mushroom (*Pleurotus ostreatus*) cultivation in Bhutan

# Karma Thinley<sup>1, \*</sup>, Atsushi Kumata<sup>1, 2</sup>, Dorji<sup>1</sup>, Chencho Dukp<sup>1</sup>

<sup>1</sup>National Mushroom Centre, Department of Agriculture, Ministry of Agriculture and Livestock, Wangchutaba, Babesa, Thimphu, Bhutan, <sup>2</sup>Japan Overseas Cooperation Volunteer, Chhubachhu, Thimphu, Bhutan. <sup>\*</sup>Corresponding author: karmasonam22@gmail.com

#### Abstract

This study assessed the potential of using apple pomace waste as a substrate and nutrient for Pleurotus ostreatus cultivation. Paddy straw, a common substrate for P. ostreatus in Bhutan, was used as a control. In the case of using apple pomace as a substrate, the yield of 120 g/bottle in two strains was statistically more significant than the control ( $\alpha = 0.01$ ), achieving yieldincrease ratios of 50 % and 36 % compared to the control substrate yields of 80 g/bottle and 88 g/bottle in each strain. The yield from 135 tons of apple pomace wasted by a company in a year in Bhutan was estimated to be 39 tons, using a 29 % yield conversion rate per unit weight of apple pomace. The waste from the used substrate of apple pomace was calculated at 71 tons in wet weight, and the waste-reduction ratio of apple pomace was 47 % due to P. ostreatus cultivation. In the case of using apple pomace as a nutrient for paddy straw substrate, 124 g/bottle and 119 g/bottle of yield in the 4 % supplementation substrate were statistically more significant than 101 g/bottle and 104 g/bottle of the control without supplementation ( $\alpha = 0.05$ ), representing yield-increase ratios of 23 % and 14 % compared to the control substrate for each strain. If 135 tons/year of apple pomace were used as a 4 % nutrient for paddy straw substrate, it is estimated that an annual harvest of 1,001 tons could be achieved using a 33 % yield conversion rate and 3,034 tons of substrate quantity. It was not observed to reduce the commercial value of fruiting bodies in both methods of using the basic substrate and nutrient for paddy straw substrate. It was concluded that both methods of utilizing apple pomace waste were practical for *P. ostreatus* cultivation and efficient in reducing waste quantity.

**Keywords**: Efficient use cycle, Waste efficient use, Waste-reduction ratio, Yield ratio per unit substrate, Yield-increase ratio

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## 1. Introduction

The National Mushroom Centre (NMC) at Thimphu, Bhutan, has initiated studies on the efficient use of waste in mushroom cultivation to address issues related to water eutrophication caused by agricultural and food waste and to tackle the shortage of natural resources for mushroom substrate. Shiitake mushroom (*Lentinula edodes*) is cultivated with bed logs, while Oyster mushroom (*Pleurotus ostreatus*) is cultivated with a paddy straw substrate, representing Bhutan's major commercial cultivation. Agricultural and food industry waste, except for paddy straw, has not been used efficiently for mushroom cultivation. According to their records, the apple pomace wasted by Bhutan Agro Industry Limited (BAIL) in Thimphu is approximately 135 tons per year. NMC evaluated the potential of this apple pomace as a basic substrate and nutrient for *P. ostreatus* cultivation, comparing each use from economic and environmental perspectives.

Given the global mass production of apple juice, it is estimated that several million metric tons of apple pomace are generated each year worldwide (Lyu, Luiz, Azeredo, Cruz, Ajlouni, & Ranadheera, 2020). The carbohydrates in apple pomace mainly consist of insoluble sugars, including cellulose (127.9 g/kg dry weight (DW)), hemicellulose (7.2 to 43.6 g/kg DW), and lignin (15.3 to 23.5 g/kg DW). Simple sugars such as glucose (22.7 %), fructose (23.6 %), and galactose (6 % to 15 %) are also present (Dhillon, Kaur, & Brar, 2013). In addition to carbohydrates, some minerals were also identified in apple pomace, such as P (0.07 % to 0.076 %), Ca (0.06 % to 0.1 %), Mg (0.02 % to 0.36 %), and Fe (31.8 to 38.3 mg/kg, dry weight basis). Apple pomace is also characterized by a high proportion of polyphenols (31 % to 51 %), especially cinnamate esters, dihydrochalcones, and flavonols (Will, Olk, Hopf, & Dietrich, 2006). These data support the potential effectiveness of P. ostreatus cultivation substrates and highlight the risks of environmental pollution caused by waste at the same time. Previous work on the cultivation of P. ostreatus using apple pomace reported the best results for combinations of materials such as apple waste + 1.5 % barley (Petre & Petre, 2013), winery + apple waste (1:1) (Petre & Petre, 2012), and 0.5 kg apple pomace + 1.5 kg wheat (Pathania, Sharma, & Gupta, 2017).

This paper evaluated apple pomace as the primary substrate without adding nutrients in test 1 since the above references reported evaluating apple pomace with added nutrients or in combination with another substrate. Similarly, it assessed apple pomace as a nutrient for the paddy straw substrate in test 2, as the referenced studies did not evaluate it as a nutrient. In Bhutan, all growers cultivate *P. ostreatus* using paddy straw as a basic medium without adding any nutritional material to avoid infections. Therefore, this study used paddy straw without added nutrients as the control substrate for two tests evaluating apple pomace as the basic substrate and nutrient.

On the other hand, the efficient use of waste through mushroom cultivation is expected to enhance the recycling of natural resources (Barshteyn & Krupodorova, 2016). This approach may also mitigate the risk of nitrogen pollution in water, soil, and air associated with agricultural and food industry waste (Ashrafi, Mian, Rahman, & Jahiruddin, 2014). Additionally, the waste generated from the used medium can serve as an important natural resource for compost (Fidanza, Sanford, Beyer, & Aurentz, 2010), fertilizer (Kwiatkowski & Harasim, 2021), and energy materials, such as biogas (Pérez-Chávez, Mayer, & Albertó, 2019). Thus, the quantity of waste produced from the apple pomace substrate after cultivation and the weight-reduced ratio of apple pomace waste from cultivation were estimated.



#### 2. Materials and methods 2.1 Materials

Fresh apple pomace collected from Bhutan Agro Industry Limited (BAIL), Thimphu in September 2023 was dried in a greenhouse at around 27 °C for about one month until no more moisture was left and kept in plastic bags until use (Fig. 1). Paddy straw, collected from a farm in October 2023, was kept under the roof until use. The chemical components of apple pomace and paddy straw used in this paper are shown in Table 1.

The control substrate for treated plots of apple pomace in each test was paddy straw without added nutrients, which is the most common substrate in Bhutan. A cultivation container for the substrate was a bottle of 800 ml with a 65 mm caliber made of polypropylene (PP).



Fig. 1 - Apple pomace after drying

Table 1 The chemical component of apple pomace and paddy straw used in this experiment												
Sample	Moisture	Total solid	total ash content	Crude Protein	Crude Fat	Total Carb	Energy	Ν	Р	К	Ca	Fe
	%	%	%	%	%	%	Kcal/100g	%	ppm	ppm	ppm	ppm
Apple pomace	10.9	89.1	2.1	9.6	2.2	75.2	359.0	1.53	0.11	10.5	15	1.62
Paddy straw	Paddy straw         7.9         92.1         11.4         4.1         0.6         76.0         325.6         0.66         0.02         7.5         10         0.7								0.71			
Notes: This data was provided by Royal University of Bhutan College of Natural Resources.												
The analysis methods of each component were as follows.												
Moisture Content and Total Ash; Gravimetric, Crude protein / N; Kjeldahl Method, Crude Fat; Soxhlet method,												

Phosphorus; UV Visible Spectrophotometer, Potassium and Calcium; Flame Photometer,

Iron; O-Phenanthroline (Spectrophotometric)

# 2.2 Preparation of substrate for each test

## The cultivation test for apple pomace as a basic substrate: test 1

The apple pomace and paddy straw were soaked in water as part of the preparation process. The paddy straw was soaked for 20 minutes and then drained overnight, while the apple pomace was soaked for two hours and drained for four hours. The moisture content of each substrate after substrate preparation was measured using a moisture analyzer, resulting in 65.9 % for the apple pomace substrate and 66.1 % for the control substrate. The filling weight of each substrate in the PP bottle was 420 g for apple pomace and 300 g for paddy straw. The replication number of the bottles was 6 or 7 for each plot.



## The cultivation test for apple pomace as a nutrient: test 2

The paddy straw was soaked in water for 20 minutes and drained overnight. The apple pomace was ground into powder using a kitchen grinder. This powdered apple pomace was added at concentrations of 2 %, 4 %, 6 %, and 8 % of the dried weight in a control substrate of paddy straw. The moisture content of each plot was measured: 67.2 % for the control, 69.1 % for the 2 % plot, 65.4 % for the 4 % plot, 65.4 % for the 6 % plot, and 68.4 % for the 8 % plot. The filling weight of each substrate plot in the PP bottle was 380 g. The replication number of the bottles was 6 or 7 for each plot.

# 2.3 Method of sterilization and inoculation

The substrate was sterilized for 60 minutes at 121  $^{\circ}$ C and cooled overnight. About 20 g of spawn of grain wheat substrate was inoculated in each substrate. Strains A and B, practical strains of *P. ostreatus* commonly cultivated in Bhutan, were used to evaluate the possibility of efficient use of apple pomace under close onsite conditions.

## 2.4 Conditions of incubation and formation of fruiting bodies of each test

For the cultivation test for apple pomace as a basic substrate (test 1), inoculated substrates were incubated for 48 days under non-heating conditions (5 to 20 °C in temperature, 50 to 80 % in humidity) in a room at NMC. Following incubation, the media were transferred to a natural room temperature (4 to 15 °C) at NMC, maintained at over 95 % humidity.

For the cultivation test for apple pomace as a nutrient (test 2), inoculated substrates were incubated for 36 days under non-heating conditions (7 to 22  $^{\circ}$ C in temperature, 50 to 75  $^{\circ}$  in humidity) in a room at NMC. Following incubation, the media were transferred to a natural room temperature (7 to 18  $^{\circ}$ C) at NMC, maintained at over 95  $^{\circ}$  humidity.

Scratching spawn on a substrate and irrigation, which was treated by filling a bottle with water, keeping it overnight, and draining, were carried out after each test's incubation was completed.

## **2.5 Factors for evaluation**

The parameters, including yields and the number of fruiting bodies per bottle, were measured until the second flush. The duration from the fruiting treatment to the first flush for each bottle was recorded and defined as the number of days to harvest from the fruiting treatment. For the first flush, the vertical length, horizontal length, and thickness dimensions of the pileus were measured for five pilei from each bottle. Similarly, the length and central diameter of five stipes were also measured. All data obtained from the test plots and control plots were statistically compared for each strain using a t-test (two-sample test assuming equal variances) using Excel (Microsoft).

For the experiment in evaluation as the basic substrate, the wet weight and moisture content of the substrate in each bottle were measured after the second flush. From this data, the annual quantity of waste, apple pomace substrate, after *P. ostreatus* cultivation was estimated.

## 3. Results

# **3.1** The evaluation of apple pomace as a basic substrate: test 1 Characteristics of cultivation

Fig. 2 presents the result of the comparative cultivation test 1 in paddy straw substrate as the control plot and apple pomace substrate. For strain A, the yield was 60 g/bottle for the control plot and 120 g/bottle for the apple pomace plot. For strain B, the yield was 88 g/bottle for the control plot and 120 g/bottle for the apple pomace plot (Fig. 2a). It was shown in strain A that



a significant difference between the control plot and the apple pomace plot ( $\alpha = 0.01$ ). The increase in yield on the apple pomace substrate, compared to the control plot, was 50 % for strain A and 36 % for strain B. This up ratio was calculated by dividing the difference in yield between the apple pomace and control plots by the yield of the control plot. There was no significant difference between the apple pomace and control plots present in the number of fruiting bodies (Fig. 2b), weight per fruiting body (Fig. 2c), or the number of days to harvest from the fruiting treatment (Fig. 2d) for both strains.



**Fig. 2** - The comparison of harvest characteristics of straw and apple pomace substrate with two *P. ostreatus* strains. Notes: Bars mean standard deviation.  $\star \star$  means significant difference ( $\alpha = 0.01$ ) with a t-test.  $\star$  means significant difference ( $\alpha = 0.05$ ) with a t-test.

#### The yield ratio per unit substrate

Fig. 3 shows the yield ratio per unit of wet substrate weight for straw and apple pomace substrate with two *P. ostreatus* strains. The yield ratios for all plots ranged from 48 % to 54 %, with no significant differences observed.

## The estimated yield and substrate waste

Table 2 shows the annual yield of *P. ostreatus* and the estimated annual waste substrate quantity. The yearly estimated yield was calculated at 39 tons, derived from 135 tons of apple pomace yearly waste multiplied by 29 % yield ratio, yield (g/bottle)/dry weight of substrate  $\times$  100. The annual estimated quantity of waste substrate was calculated as 71 tons for wet weight and 32 tons for dry weight, considering a 53 % substrate weight loss ratio, calculated by



substrate weight in a bottle before and after cultivation. It showed that the waste-reduction ratio of apple pomace, 100 % minus 53 % of the substrate weight-loss ratio, was 47 % in wet weight by *P. ostreatus* cultivation.



**Fig. 3** - The yield ratio per unit substrate dry weight of straw and apple pomace substrate with two *P. ostreatus* strains. Notes: The yield ratio = yield (g/bottle)/dry weight of substraite×100, For bars, refer to Fig. 2.

Apple pomace	Waste su	Waste substrate quantity Waste substrate			
tons (wet)/y	%	tons (wet)/y	weight-loss ratio %	tons (wet)/y	tons (dry)/y
135	29	39	53	71	32

Notes: The yield ratio; refer to Figure 4, yield = Apple pomace (tons(wet)/y) × The yield ratio (%) Substrate weight loss ratio was calculated by substrate weight in a bottle before and after cultivation, Waste substrate (tons (wet/y) = Apple pomace (tons (wet)/y) × Substrate weight loss rate (%) Waste substrate (tons (dry/y) = Waste substrate (tons (wet/y)×(1- moisture content (0.55 %))

## **Characteristics of fruiting bodies**

Fig. 4 displays photos of fruiting bodies from each plot. Morphological differences, such as color in the fruiting bodies, were not observed with the naked eye between the plots, as depicted in the photos. Fig. 5 presents the sizes of the fruiting bodies. In strain B, only the horizontal length in the apple pomace plot was smaller than in the control plot, confirming a significant difference ( $\alpha = 0.01$ ). No significant differences were observed in thickness and vertical length for either strain. Fig. 6 illustrates the length and diameter of the stipe. For the stem length, the apple pomace plot showed larger values than the control plot in both strains, with a significant difference confirmed ( $\alpha = 0.01$ ); in contrast, no significant difference was observed for stem diameter.

# **3.2** The Evaluation of apple pomace as a nutrient: test 2 Characteristics of cultivation

Fig. 7 presents the result of cultivation test 2, adding 2, 4, 6, and 8 % ratio of apple pomace as a nutrient for paddy straw substrate. The control was paddy straw substrate without adding nutrients. For trains A and B, 4 % apple pomace plot showed a significantly larger yield than the control plot ( $\alpha = 0.05$ ). The yield for the control was 101 g for strain A and 104 g for strain B, while the 4 % plots yielded 124 g for strain A and 119 g for strain B (Fig. 7a). Regarding



the number of fruiting bodies, no significant differences were observed among the nutrient plots for strain A (Fig. 7b). However, all nutrient supplement plots for strain B showed a significant difference compared to the control. Regarding weight per fruiting body, no significant differences were found between the apple pomace plots and the control for both strains A and B (Fig. 7c). For the number of days to harvest from the fruiting treatment, the 6 % and 8 % apple pomace plots for strain A showed a significant delay compared to the control (Fig. 7d). The 6 % plot had a delay of 4 days, and the 8 % plot had a delay of 2 days.



**Fig. 4** - The comparison of fruiting bodies formation of each substrate with two *P. ostreatus* strains



**Fig. 5** - The comparison of pileus size of straw and apple pomace substrate with two *P*. *ostreatus* strains. Notes: For bars,  $\star \star$ , and  $\star$ , refer to Fig. 2.





**Fig. 6** - The comparison of the stipe size of straw and apple pomace substrate with two *P*. *ostreatus* strains. Notes: For bars,  $\star \star$ , and  $\star$ , refer to Fig. 2.



**Fig. 7** - The comparison of harvest characteristics on two *P. ostreatus* strains of straw substrate added several ratios of apple pomace. Notes: For bars,  $\star \star$ , and  $\star$ , refer to Fig. 2.



Table 3 shows the yield-increase ratio by the 4 % addition of apple pomace to the control of paddy straw substrate. The yield-increase rate was 23 % for strain A and 14 % for strain B.

Table 3 Yield-increase rate for two P. ostreatus strains by the 4 % addition of applepomace to paddy straw substrate									
	Strain A		Strain B						
Yield	(g/bottle)	Yield-	Yield (	Yield-					
Conrol	+ 4 % apple pomace	increase rate (%)	Conrol	+ 4 % apple pomace	increase rate (%)				
101	124	23	104	119	14				

## **Characteristics of fruiting bodies**

The sizes of the pileus are shown in Fig. 8. Only the 4 % apple nutrition plot of strain B showed a significant difference ( $\alpha = 0.01$ ) from the control plot for thickness and vertical and horizontal length. The length and diameter of the stipe are shown in Fig. 11. Regarding stipe length, strain A's 8 % apple nutrition plot ( $\alpha = 0.01$ ) and strain B's 4 % apple nutrition plot ( $\alpha = 0.05$ ) showed significant differences from their respective controls. The stipe length of strain A's 8 % apple nutrition plot was longer than its control, while the stipe length of strain B's 4 % apple nutrition plot was shorter than its control. For stem diameter, no significant differences were observed in any of the plots compared to the control.

Fig. 12 shows photos of the fruiting bodies of the control plot and the 4% added apple pomace plot. There were no noticeable differences between the 4% plot and the control, including visual characteristics such as color and malformation.



**Fig. 8** - The comparison of pileus size on two *P. ostreatus* strains of straw substrate added several ratios of apple pomace. Notes: For bars,  $\star \star$ , and  $\star$ , refer to Fig. 2.





**Fig. 9** - The comparison of stipe size on two *P. ostreatus* strains of paddy straw substrate added several ratios of apple pomace. Notes: For bars,  $\star \star$ , and  $\star$ , refer to Fig. 2.



**Fig. 10** - Photos of fruiting bodies on two *P. ostreatus* strains of the control substrate (paddy straw) and the 4% apple pomace added substrate.

## 4. Discussion

## 4.1 The evaluation of apple pomace as a basic substrate: test 1

## The effect of increased yield with apple pomace as a basic substrate

It has been reported that mixing 0.5 kg of apple pomace with 1.5 kg of wheat straw resulted in the highest yield of 110 g compared to 85 g obtained with 2 kg of wheat straw in *P. ostreatus* cultivation (Pathania et al., 2017). With a biological efficiency of 54.23% in the highest yield of 110 g, the paper mentioned that optimal N levels provided by apple pomace account in part for its effectiveness. In another study, the cultivation of *P. ostreatus* and *P. sajor-caju* on apple pomace and sawdust, both separately and in combination, showed that higher yields were achieved using pomace alone compared to sawdust (Worrall et al., 1992).



In our experiment, using 100 % apple pomace resulted in a yield of two strains with an increase of 39 % and 50 % compared to 100 % paddy straw. Chemical components of apple pomace exhibited higher concentrations than those of the paddy straw, except for total solids and total ash, as shown in Table 1. Generally, the proper C/N ratio of the substrate was 20 % for vegetative growth and 30 to 40 % for reproductive growth (Takabatake, 2021). For *P. ostreatus* cultivation, it was reported that a C/N ratio below or above 36:1 decreased the yield (Ashrafi, Mian, Rahman, & Jahiruddin, 2014). The C/N ratio was 49 % for apple pomace and 115 % for paddy straw, and apple pomace and close to proper C/N ratio were considered one of the reasons for this increased yield. However, despite these differences, the yield ratios across all plots consistently remained around 50 %, as shown in Fig. 5. It was suggested that the amount of substrate, 420 g/bottle for apple pomace substrate and 300 g/bottle for paddy straw substrate, was also considered another contributing factor to the increased yield in apple pomace substrate.

#### The efficient use cycle on apple pomace with *P. ostreatus* cultivation

The substrate waste can serve various purposes, such as compost (Fidanza et al., 2010) or biogas material (Pérez-Chávez, Mayer, & Albertó, 2019). Improper disposal of agricultural waste often leads to water and soil contamination. It has been reported that for oyster mushroom cultivation, there can be a significant reduction in the C/N ratio from an initial value of 115 to 60, along with a 64 % reduction in the lignin content of paddy straw (Udayasimha & Vijayalakshmi, 2012). This study suggests that mushroom cultivation can help mitigate air pollution associated with the burning of agricultural waste. Therefore, the efficient utilization of apple pomace through *P. ostreatus* cultivation is expected to enhance product value and reduce pollution risks to water, soil, and air (Petre & Petre, 2012).

For this paper, it has been estimated that 135 tons of apple pomace waste from a BAIL company can be converted into 35 tons of mushroom product through *P. ostreatus* cultivation, resulting in a reduction of waste quantities to 71 tons for wet weight and 32 tons for dry weight. This result indicated that *P. ostreatus* cultivation could be an effective method for reducing the environmental pollution risks of apple pomace waste.

# 4.2 The evaluation of apple pomace as a nutrient: test 2

#### The effect of increased yield by adding apple pomace as a nutrient

The results of applying 2.5 %, 5 %, and 10 % apple pomace to sawdust and then cultivating *P. ostreatus*, indicated that the addition of 2.5 % apple pomace supplement enhanced the mycelial growth rates in both solid and liquid culture, however, adding more than 5 % apple pomace was observed to affect the mycelial growth negatively (Park, Park, Kim, Yoon, Son, Kwon, Han, & Lee, 2012). Moreover, the optimum conditions for *P. ostreatus* to produce laccase were a 2.5 % apple pomace addition and a cultivation time of 9 days (Park, Yoon, Kim, Kwon, Yoo, Kong, & Lee, 2014). The results of these reports align with our result; the yield of 4 % added plot was statistically more significant than the control plot. For strain A, the 4 % apple pomace plot did not show any significant differences from the control in terms of the number of days to harvest and the size of the fruiting bodies and stems. There were no noticeable differences between the 4 % plot and the control, including visual characteristics such as color and malformation. These results indicate that adding 4 % apple pomace (by dry weight) to the straw substrate is effective for commercial cultivation.



## 4.3 Comparison of basic substrate and nutrient uses in apple pomace

The efficiency of using apple pomace as a basic substrate versus as a nutrient for *P. ostreatus* cultivation is shown in Table 4. In the case of using apple pomace as a substrate, the yield from 135 tons of apple pomace wasted by a company in a year in Bhutan was estimated to be 39 tons, using a 29 % yield conversion rate per unit weight of apple pomace. If 135 tons/year of apple pomace were used as a 4 % nutrient for paddy straw substrate, it is estimated that an annual harvest of 1,001 tons could be achieved using a 33 % yield conversion rate and 3,034 tons of substrate quantity. Therefore, from the perspective of efficiently utilizing the limited apple pomace resource and distributing it among multiple farmers, adding 4 % apple pomace to the paddy straw substrate, which is relatively easy and cost-effective to procure in this country, is evaluated to be a more valid approach than using it as a basic substrate. To support this strategy, it is necessary to establish a system for drying, powdering, and providing this valuable waste to individual farmers.

Table 4 The comparison of basic substrate and nutrient on efficient use of apple pomacefor P. ostreatuscultivation								
Apple pomace	Use		Quantity (tons(wet)/y)		The yield ratio	Yield		
tons(wet)/y	Purpose	Rate	ate Paddy straw Substrate		%	tons(wet)/y		
135	substrate	100%	0	135	29 <sup>×1</sup>	39		
	nutrient	4%	2,903	2,903 3,034 3		1,001		
Notes: The moisture content of apple pomace and substrate were assumed 66 %.								
The yield ratio (%) =( yield per bottle / quantity of medium per bottle) $\times$ 100								
$^{\times 1}$ ; (120 g/bottle / 420 g/bottle) × 100 = 29 %								
$^{3}$ ; (124 g/bottle / 380 g/bottle) × 100 = 33 %								

## 5. Conclusion

The yield-increased ratios of the apple pomace showed 50 % and 36 % for the two strains compared with the paddy straw substrate, respectively, when evaluated as a basic substrate for *P. ostreatus* cultivation. In the evaluation as a nutrient, adding 4 % apple pomace to the paddy straw substrate resulted in yield-increased ratios of 23 % and 14 % for the two strains, respectively. It was not observed that inference reduced the commercial value of fruiting bodies in all apple pomace treatment plots. In the case of using apple pomace as a substrate, the waste from the used substrate of apple pomace was calculated at 71 tons in wet weight base, and the waste-reduced ratio of apple pomace was 47 % due to *P. ostreatus* cultivation. It was concluded that both methods of utilizing apple pomace waste were practical for *P. ostreatus* cultivation and efficient in reducing waste quantity.

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#### References

- Ashrafi, R., Mian, M. H., Rahman, M. M., & Jahiruddin, M. (2014). Recycling of spent mushroom substrate for the production of oyster mushroom. *Research in Biotechnology*, 5(2), 131.
- Barshteyn, V., & Krupodorova, T. (2016). Utilization of agro-industrial waste by higher mushrooms: Modern view and trends. Journal of Microbiology, Biotechnology and Food Sciences, 5(6), 563–577.
- Dhillon, G. S., Kaur, S., & Brar, S. K. (2013). Perspective of apple processing wastes as lowcost substrates for bioproduction of high value products: A review. *Renewable and Sustainable Energy Reviews*, 27, 789–805. https://doi.org/10.1016/j.rser.2013.06.046
- Fidanza, M. A., Sanford, D. L., Beyer, D. M., & Aurentz, D. J. (2010). Analysis of fresh mushroom compost. *HortTechnology*, 20(2), 449–453. https://doi.org/10.21273/HORT TECH.20.2.449
- Kwiatkowski, C. A., & Harasim, E. (2021). The effect of fertilization with spent mushroom substrate and traditional methods of fertilization of common thyme (*Thymus vulgaris* L.) on yield quality and antioxidant properties of herbal material. *Agronomy*, 11(2), 329. https://doi.org/10.3390/agronomy11020329
- Lyu, F., Luiz, S. F., Azeredo, D. R. P., Cruz, A. G., Ajlouni, S., & Ranadheera, C. S. (2020). Apple pomace as a functional and healthy ingredient in food products: A review. *Processes*, 8(3), 319. https://doi.org/10.3390/pr8030319
- Park, Y.-J., Park, H.-R., Kim, S.-R., Yoon, D.-E., Son, E.-S., Kwon, O.-C., Han, W., & Lee, C.-S. (2012). Apple pomace increases mycelial growth of *Pleurotus ostreatus*. *African Journal of Microbiology Research*, 6(5), 1075–1078. https://doi.org/10.5897/AJMR 11.1546
- Park, Y.-J., Yoon, D.-E., Kim, H.-I., Kwon, O.-C., Yoo, Y.-B., Kong, W.-S., & Lee, C.-S. (2014). Overproduction of laccase by the white-rot fungus *Pleurotus ostreatus* using apple pomace as inducer. *Mycobiology*, 42(2), 193–197. https://doi.org/10.5941/ MYCO.2014.42.2.193
- Pathania, S., Sharma, N., & Gupta, D. (2017). A study on cultivation and yield performance of oyster mushroom (*Pleurotus ostreatus*) on wheat straw mixed with horticultural waste (apple pomace) in different ratio and their nutritional evaluation. *International Journal* of Current Microbiology and Applied Sciences, 6(8), 2940–2953. https://doi.org/ 10.20546/ijcmas.2017.608.353
- Pérez-Chávez, A. M., Mayer, L., & Albertó, E. (2019). Mushroom cultivation and biogas production: A sustainable reuse of organic resources. *Energy for Sustainable Development*, 50, 50–60. https://doi.org/10.1016/j.esd.2019.03.002
- Petre, M., & Petre, V. (2012). The semi-solid state cultivation of edible mushrooms on agricultural organic wastes. *Scientific Bulletin, Series F, Biotechnologies, 16*, 36–39.
- Petre, V., & Petre, M. (2013). Biotechnology for controlled cultivation of edible mushrooms through submerged fermentation of fruit wastes. *AgroLife Scientific Journal*, 2(1), 117–120.
- Takabatake, K. (2021). Development of edible mushroom production technology using waste biomass. *Mushroom Science and Biotechnology*, 29(1), 5–14. (*In Japanese*).
- Udayasimha, L., & Vijayalakshmi, Y. C. (2012). Sustainable waste management by growing mushroom (*Pleurotus florida*) on anaerobically digested waste and agro residues. *International Journal of Engineering Research & Technology (IJERT)*, 1(5), 1–8.
- Will, F., Olk, M., Hopf, I., & Dietrich, H. (2006). Characterization of polyphenol extracts from apple juice. *Deutsche Lebensmittel-Rundschau*, 102(7), 297–302.



Worrall, J. J., & Yang, C. S. (1992). Shiitake and oyster mushroom production on apple pomace and sawdust. *HortScience*, 27(10), 1131–1133. https://doi.org/10.21273/HORTSCI. 27.10.1131