**ORIGINAL PAPER**

# Development of ground pork patty substitutes from mycelium of *Pleurotus pulmonarius*

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**Abstract**. As environmental sustainability continues to be emphasized globally, alternative food sources like meat substitutes have surged as an effective environmental remedy. While meat production accounts for more than 15% of total greenhouse gas emissions, the production of meat substitutes can be adopted to enhance efficacious environmental sustainability. *Pleurotus pulmonarius*, commonly known as oyster mushroom, is well recognized for its nutritional value and protein content. This study aims to develop meat substitutes derived from *P. pulmonarius*. Initially, *P. pulmonarius* was cultivated in brown rice solution by extracting brown rice in boiled distilled water. The mycelium of *P. pulmonarius* was dried in the oven at 60°C for 5 hours. The development of meat substitutes formulation consists of 34.4% dried *P. pulmonarius* mycelium, 34 % water, 31.2% wheat flour, 0.32% carrageenan, and 0.08% salt. The physical properties which were compression, pH, and color of meat substitute formulation were tested. The results revealed that on day 0 and day 9, compared to ground pork patties the color, pH and the strength of meat substitute was not significantly different statistically ($p$>0.05). Biological tests of meat substitutes were conducted to investigate protein content using Lowry's method, the result showed that meat substitutes contain significantly higher protein content than ground pork patties ($p \leq 0.05$). In addition, meat substitutes were analyzed for *Escherichia coli* presence in our meat substitutes on day 0 and day 9 using EMB agar, the result showed that there was no presence of *E. coli* in both days of investigation. Furthermore, according to sensory evaluation among 10 untrained consumers using a 9-point hedonic scale, the evaluation revealed no significant differences between our meat substitutes and ground pork patties ($p$>0.05).

**Keywords**: Alternative protein, Ground pork patties, Meat substitutes, *Pleurotus pulmonarius*,

## 1. Introduction

Nowadays, meat consumption has become a significant prevalence throughout numerous countries, as meat is an essential source of nutrients like protein. As environmental sustainability continues to be emphasized globally, alternative food sources like meat substitutes have surged as an effective environmental remedy (Poore & Nemecek, 2018). While various factors contribute to multifaceted environmental degradation, particularly meat production, accounts for more than 15% of total greenhouse gas emissions. Notably, cattle breeding also results in cutting wood and destroying the forest, resulting in increased carbon dioxide emissions in the atmosphere (Houghton, 2005). However, production of meat substitutes can be adopted to enhance efficacious environmental sustainability. It reduces the emission of carbon dioxide, which has been greatly reduced in comparison with mere food production processes. Fungi, an interesting alternative source of protein, are ubiquitously seen in numerous meat substitutes. Mycoprotein is low energy and protein-rich whole food source derived from the fermentation of filamentous fungi. According to Saeed et al. (2023) found a fungus, *Fusarium venenatum*, the most recognized fungi within the food industry given its use for production on a large industrial scale and mycoprotein containing high protein and essential amino acids, compared with animal-sourced protein such as favorable fatty acid and high fiber content. The edible mushroom was alternative used to prepare a fibrous meat analogue and providing nutrients, and promoting the development of chief organoleptic properties, such as the appearance, texture and flavor of the product (Yuan et al., 2022).

*Pleurotus pulmonarius*, commonly known as oyster mushroom is one of the most edible fungi and a saprophytic fungus. In addition, studies have shown that bioactive compounds, antioxidant activity, and antimicrobials against foodborne pathogens of *P. pulmonarius* (Castillo-Zamudio, 2024). Moreover, *P. pulmonarius* is essential for the human diet due to their nutritional components, including proteins, fats, minerals and vitamins (Rangel-Vargas, 2021). *Pleurotus pulmonarius* has grown due to the potential beneficial effects that its consumption can contribute to health. The edible fungi are well recognized for its nutritional value and protein content, meat substitutes derived from this might have the ability to be a pleasant food alternative (Doroški, 2022). The work has been reported that *P. pulmonarius* are considered an important source of an important source of proteins and amino acids, carbohydrates, minerals and vitamins (Torres-Martínez, 2021). The mycelium of *P. pulmonarius* is an alternative protein source due to its rapid growth on sustainable substrates. Fungal mycelium has nutritional and organoleptic properties (Torres-Martínez, 2021). Mycelium fungi can efficiently convert carbohydrates and overall nutrients from the growth media into protein and their efficiency can vary depending on the carbon source and its concentration. Pure culture of *P. pulmonarius* is very important in the early production. *In vitro*, mycelium of *P. pulmonarius* can be glowed in potato dextrose broth or can be used as boiled rice water.

The aim of the study was to develop meat substitutes from the mycelium of *P. pulmonarius*. Therefore, the potential of *P. pulmonarius* mycelium as alternative protein and a rich nutritional profile for health-conscious people and avoid eat meat. In addition, it can be further developed into a variety of products in the food business.

## 2. Materials and Methods

*2.1 Strain and growth conditions Main section*

Pure mycelia culture of oyster mushroom (*P. pulmonarius*) was obtained from Plant science Faculty of Science and Agricultural Technology of the Rajamangala University of Technology Lanna, Thailand. The oyster mushroom strain was grown on potato dextrose agar (PDA) at 28°C for 14 days. *Pleurotus pulmonarius* was confirmed the morphological characteristics such as colony characteristics.

*2.2 Making meat substitute products from mushroom mycelium*

Cultivate mycelium of *P. pulmonarius* in 20% w/v sterilized broken brown rice water and incubated at 28°C for 30 days. Mycelium of oyster mushroom were filtered and dried until constant weight at 65°C for 2 days. The mycelium obtained from the cultivation were made into meat substitute products. It is a patty with a diameter of 50 millimeters and a thickness of 10 millimeters, consisting of 34.4% mushroom fibers, 34% water, 31.2% wheat flour, and 0.08% salt by modifying meat in patty formulations (Rangel-Vargas et al., 2021). Carrageenan is used as a binder and textural characteristics of meat produced with only 0.32%. Then fry them in an oil-free fryer at 150 °C for 15 min. While the control treatment consisted of ground pork loin instead of mushroom fibers. Meat substitute products and ground pork patties were placed in a glass box and then stored at 4ºC for testing.

*2.3 Physical characterization of meat substitute*

*2.3.1 Uniaxial compression*

The compression of meat substitute products was tested compression using universal testing machine (UTM). Five-centimeter diameter and one centimeter thickness of meat substitute and ground pork patty samples were measured the compression force on the samples every 0.5 seconds compared to the changing thickness of the meat pieces. Then analyzed the relationship graph using Young's modulus relationship. When measuring the compression force, graph was showed the relationship between compression force and the change in thickness of the meat pieces in Figure 1.

Given that E = Young's modulus

F = exerted force (Y-axis)
A = surface area of sample

L0 = initial thickness of sample
ΔL = change in thickness of sample (X-axis)

*2.3.1 pH*

The samples were then let to stand in the refrigerated room (4°C) and the measurements on pH were conducted in day 0 and 9. The pH was determined on a blended homogenate of samples using mortar. Each sample was added 10 ml of peptone water and mixed the solution for 1 min by vortex mixer. The sample solution was centrifuged for 10 min at 4,000 rpm. Aliquots of supernatants meat substitutes were determined using a pH meter with a glass electrode in day 0 and 9.

*2.3.1 Color*

The samples were stanned in the refrigerated room (4°C) and the measurements on color were conducted in day 0 and 9. Samples of meat substitutes and ground pork patty were used in replicates and evaluation in day 0 and day 9 and conducted the Colorimeter App on the phone to assess each sample's color using the CIELAB system. The CIELAB color space, commonly used in food characterizations, defines color based on three parameters: L* (lightness, ranging from 0 to 100), a* (greenness to redness, ranging from negative to positive) and b* (blueness to yellowness, ranging from negative to positive) (Goñi et al., 2008).

*2.4 Biological characterization of meat substitute*

*2.4.1 Protein determination*

The protein content of samples was determined by modified Lowry protein measurement and bovine serum albumin (BSA) was used as the standard, using a standard calibration curve built by varying bovine serum albumin concentration at concentrations of 0, 20, 40, 60, 80, and 100 mg/ml. Each sample weight of 2.5 g of grounded meat substitute and pork meat (control) were added 10 ml of phosphate buffer and mixed. The sample solutions were incubated at 60°C by water bath and centrifuged at 4,000 rpm for 45 min. Fifty microliters of each sample supernatant were added 150 µl of Lowry solution (20% $Na_2CO_3$, 0.4% NaOH, 0.16% potassium sodium tartrate, 1% sodium dodecyl sulfate and 4% $CuSO_4$) and mixed. After that each sample solution was incubated at 25°C for 15 min. Then 50 µl of the Folin Ciocalteu phenol reagent was added the samples were mixed and incubated at 25°C for 45 min. To determine the protein concentration, the reaction in each sample was read at 660 nm using a Thermo Scientific Orion (AquaMate 8000) spectrophotometer.

*2.4.1 Escherichia coli determination*

A 1 g of grinded sample was aseptically added to 10 ml of buffered peptone water and mixed using a vortex mixer for 1 min. Each sample solution was streaked on Eosin-methylene blue (EMB) agar and incubated at 37°C for 9 days. On EMB agar, strong acid producers like *E. coli* usually form colonies with green metallic sheen.

*2.5 Sensory evaluation*

All meat samples (meat substitutes and grounded pork patties) were coded with randomized codes and rotated to prevent bias. Meat samples were cut into smaller cubes of 1 x 1 x 1 cm to the fried in an oil-free fryer at 150°C for 15 min. Samples were given to 10 untrained testers to evaluate characteristics of meat samples. The participants were then presented with each blinded sample one at a time and asked to evaluate their liking of overall preference, taste, texture, color, and odor on a 9-point hedonic scale from 1 (extreme dislike) to 9 (extreme like).

*2.6 Statistical Analysis*

The means and standard deviations of the results of physical characterization, biological characterization and sensory evaluation of meat substitutes were calculated. An independent t-test was used to compare delta values between groups. Statistical significance was defined as $p \leq 0.05$.

## 3. Results and Discussion

### *3.1 Physical characterization of meat substitute*

The compression, pH and color were important physical factor that influence meat substitute characterization. Table 1 showed that the compression characteristic of meat substitute and ground pork patty was on average compressive force, 403,200.00 N and 428,007.03 N respectively. There was no significant difference in the meat substitute and ground pork patty ($p$>0.05), indicated potential textural properties of meat substitute comparing ground pork patty. The compression of meat substitute indicates due to protein structural and quantity protein. The study of Kumar *et al*. (2011) reported that 22.5% mushroom replacing texturized soya protein increased the sensory attributes of analogue meat nuggets due to increase in flavor and overall acceptability. In addition, Table 2 showed CIELAB color of meat substitute and ground pork patty. No significant difference in any of CIELAB color values at Day 0 and Day 9 between the meat sample groups was found ($p$ >0.05). The result indicated that 34.4% oyster mushroom fibers was suitable color of meat substitute. Similar to previous reports, chicken patties containing different value of oyster mushroom was one of the major factors of the color of chicken patties (Wan et al., 2011). However, significant difference was found between pH values at day 0 and day 9 between the meat substitute and ground pork patty ($p$≤0.05). pH values of meat pork range 5.4-6.0 (Bidner et al., 2004) but meat substitute had pH values more than 6 because the protein of meat substitute could be breakdown basic compounds (Al-Dalain, 2018).

### *3.2 Biological characterization of meat substitute*

#### *3.2.1 Protein determination*

In Table 3 the results of the protein analysis and the modified Lowry protein analysis of meat substitute and grounded pork patty were showed the values at 0.084 and 0.049 % w/w, respectively. The protein of meat substitute was significantly higher than grounded pork patty ($p$≤0.05). It may be due to the fact that mycelium has a relatively low density compared to ground pork (Awad & Pena, 2023).

**Table 1.** Compression of meat substitute and ground pork patty

| Treatment | Compression (N) (Mean ± standard deviation) | Independent t-test *P* value |
|---|---|---|
| Meat substitute | 403,200.00±123,016.06 | 0.65 |
| Ground pork patty | 428,007.03±19,943.50 | |

Data were presented as mean ± standard deviation, * significantly independent t-test, $p$≤0.05

**Table 2.** pH and color of meat substitute and ground pork patty

| Physical characterization | Day | Samples | Mean ± standard deviation | Independent t-test *P* value |
|---|---|---|---|---|
| pH | 0 | Meat substitute | 6.63±0.01 | 0.00* |
| | | Ground pork patty | 6.49±0.02 | |
| | 9 | Meat substitute | 6.50±0.02 | 0.00* |
| | | Ground pork patty | 6.37±0.01 | |
| CIELAB color | 0 | Meat substitute | 41.33±18.18 | 0.16 |
| | | Ground pork patty | 42.21± 23.26 | |
| | 9 | Meat substitute | 42.00± 25.63 | 0.18 |
| | | Ground pork patty | 39.00± 34.12 | |

Data were presented as mean ± standard deviation, * significantly independent t-test, $p$≤0.05

Previous research conducted by Mazumder *et al*. (2023) has further validated the effectiveness of protein content of edible *Pleurotus* sajor-caju mushroom-based minced meat substitute was higher than that of minced beef (25.53% w/w) and minced pork (25.7% w/w). Therefore, when controlling the ratio of mycelium and ground pork in the same patty size, the mycelium has a higher quantity compared to the same weight, resulting in a higher protein content. However, the high protein content in the meat substitute demonstrates its potential and good qualities as a meat alternative.

*3.2.2 Escherichia coli determination*

The results of microbiological analysis of meat samples relative to the contamination levels of *E. coli* are represented. In the study, out of the total samples analyzed, which include meat substitute and ground pork patty were no contaminated with *E. coli*. *Escherichia coli* is a bacterium that is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products. Some strains of *E. coli can* cause severe foodborne disease such as diarrhea, fever and vomiting. According to the results, the meat substitute's *E. coli* levels were within the standard standards, which were less than 3 MPN/gram for ready-to-eat foods. *Escherichia coli* is a direct fecal contamination indicator since they are an index of *E. coli* contamination. Therefore, particularly *E. coli* analysis is important for routine food control (Dog˘an-Halkman et al., 2023). It is safe and good quality to use oyster mushroom fibers as a meat replacement in place of ground pork patties.

*3.3 Sensory evaluation*

The sensory evaluation that was summarized in Table 4. The hedonic of affective tests, assess the degree of liking of a product based on its sensory appeal. In the sensory evaluation, taste, texture, color and odor were not significantly different ($p>0.05$). This showed that the sensory characteristics of the meat substitute and ground pork patty can be replaced. However, because no other flavors or seasonings were introduced. As a result, sensory attributes all has score range from 5.5 to 6.5 indicator “neither like nor dislike”. The meat substitute from oyster mushroom had good taste because the compounds of mushrooms contained glutamic acid, aspartic acid, and 5’-nucleotides responsible for their umami taste (Dermiki et al., 2013). This result also agrees with a study by Tokarczyk et al. (2023) that found that he sensory evaluation of the tested burgers, according to the evaluators, was characterized by a good general appearance and did not differ significantly from burgers with a 20% addition of oyster mushrooms.

**Table 3.** Comparison of protein analysis of meat substitute and grounded pork patty

| **Treatment** | **Protein analysis (% w/w) (Mean ± Standard deviation)** | **Independent t-test** |
|---|---|---|
| | | ***P* value** |
| Meat substitute | 0.084±0.32 | 0.02* |
| Ground pork patty | 0.049±0.29 | |

Data were presented as mean ± standard deviation, * significantly independent t-test, $p<0.05$

**Table 4.** Treatment means of sensory characteristics for meat substitute and ground pork patty

| **Sensory evaluation** | Meat sample | | **Independent t-test** |
|---|---|---|---|
| | Meat substitute | Ground pork patty | ***P* value** |
| Taste | 5.5±1.43 | 5.5±1.96 | 1.00 |
| Texture | 6±1.70 | 5.6±1.96 | 0.63 |
| Color | 6.4±1.90 | 5.8±2.04 | 0.50 |
| Odor | 5.8±1.55 | 6.3±1.42 | 0.46 |
| Overall preference | 5.9±1.37 | 6.1±1.66 | 0.77 |

Data were presented as mean ± standard deviation, * significantly independent t-test, $p\leq0.05$

## 4. Conclusions

The development of meat substitute from mycelium of *P. pulmonarius* has the ability to be a meat substitute. The meat substitute from mycelium of *P. pulmonarius* contains a high protein content. Moreover, physical characteristics and sensory characteristics of meat substitute were similar to grounded pork patty. Therefore, the mycelium of the *P. pulmonarius*, which is used to produce meat substitutes, is a new and highly promising method. Protein may be produced sustainably and ecologically by growing mycelium in regulated conditions. With the use of this technology, meat substitutes that taste and feel like real meat may be produced, giving consumers a more ethical and sustainable option.

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