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Application of Fungi as Meat Alternatives in Industry: Mini Review

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ABSTRACT

Human consumption has outpaced meat production and manufacturing due to the rising human population and limited land for livestock agriculture. Meat consumption can have negative effects on human health, but meat production can negatively affect the environment by causing global warming and water pollution. Hence, this study produces the idea of using fungus as an alternative to replacing meat. Fungus is an ideal choice as a meat replacement because it has high nutritional content and a fast growth rate. The main objective of this review was to assess the nutritional potential of nine fungal species namely *Fusarium venenatum, Neurospora intermedia, Tuber sp., Xerocomus badius, Ganoderma lucidum, Pleurotuseryngii, Agaricus bisporus, Pleurotus sajor-caju* and *Lentinula edodes* and to determine which species is the best candidate for meat replacement. The nutritional values, toxicity, and growth rate of each fungus were assessed. Comparative data analysis suggests that *F. venenatum, N. intermedia, P. eryngii, A. bisporus, P. sajor-caju*, and *L. edodes* are found suitable for producing fungi-based meat.

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1 Introduction

Worldwide meat consumption was reported to be 360 million tonnes in 2018 alone (Curtain and Grafenauer 2019) and is steadily increasing annually. The primary reason for increasing meat demand is humans' preference for the superior protein source found in meat. The projected rising human population is believed to outpace the rising food resource (Ritson 2020). This is because livestock agriculture required huge land for the production of animal feed and raising of animals but the land available for livestock agriculture is limited (Nadathur et al. 2017). It was also pointed out by various researchers that the consumption of meat might have some negative impact on humans' health and the environment (González et al. 2020). Although laboratory cultured meat has been reported as an environmentally friendly meat alternative, but its safety for human consumption remains a public concern (Roy et al. 2021). Hence, an alternative solution is needed to overcome the shortcomings.

The fungus can be an excellent meat alternative (Hashempour-Baltork et al. 2020), because of its similarity to animal meat in terms of nutritional value, texture, and taste (Ismail et al. 2020; Michel et al. 2021). Fungus is also high in protein content (Finnigan et al. 2019) and possesses high growth and reproduction rate which shortened the period of conventional meat production (Battilani et al. 2008). The fungus also produces mycoprotein that can be processed into meat alternatives (Hüttner et al. 2020; Souza Filho et al. 2018). Currently, two manufacturing methods i.e. aerial mycelium and treating fungus at high temperature are available for fungi-based meat production (Wiebe 2004; Souza Filho et al. 2019). Although both methods contributed to successful mass production, the quality of the fungi meat varies, and it depends on the type of fungi species used for the meat production. Therefore, this review aims to assess the fungal species that have the potential for alternative meat based on the nutritional values, toxicity safety, and growth rate.

2 Criteria of Potential Fungi as Meat Alternatives

Fungi with high nutritional and similar protein content (%) to animal meat are the best potential species used as meat alternatives. However, species containing mycotoxins will not be safe for human consumption because these may cause disease; therefore, these are not a good candidate. Fungi with a high growth rate are also favored because they can be easily upscale by industry to meet the market demands. Nine fungal species namely *Fusarium venenatum, Neurospora intermedia, Tuber sp.* (edible truffles), *Xerocomus badius, Ganoderma lucidum, Pleurotus eryngii, Agaricus bisporus*, *Pleurotus sajor-caju* and *Lentinula edodes* were filtered from the available literature. These fungi were selected to be analyzed in this review because they are commonly found in the market, and demonstrate a higher level of acceptance amongst consumers.

2.1 Nutritional Value Evaluation

2.1.1 Protein Content

The protein content is one of the most important criteria when evaluating the nutritional value of potential fungus as meat alternatives. This is because the minimum protein dietary intake for humans is 1.0g per 1kg of body weight per day (Wu 2016). Animal meat has an average of 22% protein value (Ahmad et al. 2018). Data presented in table 1 revealed that among the tested nine species, *F. venenatum* has the highest amount of protein (65% to 76%), followed by. *N. intermedia* (56%), *P. sajor-caju* (29.3 %), and *A. bisporus* (27.1%), all surpassing the protein value found in meat (Kalač 2016). Further, *L. edodes* has a protein content of 20%, which is similar to the average protein content in meat while protein content for *P. eryngii*(16.39%), *X. badius* (1.62%), *Tuber sp* (0.72%), and *G. lucidum* (7% to 8%) was reported lower than the average protein value of meat. Overall, consuming *F. venenatum, N. intermedia, P. sajor-caju, A. bisporus,* and *L. edodes* can provide higher protein value to replace the meat diet. Although *P. eryngii* has slightly lower protein content than animal meat, but a higher intake of *P. eryngii* can easily resolve this minor issue. *G. lucidum, X. badius,* and *Tuber sp.* have too low protein value, relatively compared to meat, and so it is not a choice as a meat alternative.

2.1.2 Fat Content

The dietary reference intake for fats in adults is 25 - 30% of total daily energy intake (Ministry of Health Malaysia 2017). Data given in table 1 suggests that the *Tuber sp* has the highest fat content (4.4%) followed by *A. bisporus* (4.3%), *P. sajor-caju* (0.91%), *L. edodes* (0.8%), *X. badius* (0.71%), *G. lucidum* (3% - 5%), *N. intermedia* (3.5%), *F. venenatum* (2.0% - 3.5%) and *P. eryngii* (0.16%). Both *N. intermedia* (Gmoser et al. 2020) and *A. bisporus* (Kalač 2016) contained α-linolenic acid up to 77.7% in their fats, which can help to reduce cardiovascular diseases (Feeney et al. 2014). Overall, all the selected fungal species have relatively lower fats content compared to meat. The fat content of meat varies according to the type and parts of the animal, for example, pork consists of 31.7% fats (Ahmad et al. 2018).

Based on the protein and fat nutritional value, it can be concluded that *F. venenatum, N. intermedia, P. eryngii, A. bisporus, P. sajor-caju,* and *L. edodes* have the greatest potential to replace meat protein while *Tuber sp., X. badius* and *G. lucidum* have least non-potential of meat alternatives.

Table 1 Potential fungi species and the nutrient content

Species	Ingredients	Amount	References
Fusarium venenatum	Protein	65% - 76%	Reihani and Khosravi-Darani 2018; Wiebe 2002
	Fats	2.0% - 3.5%	Hoseyni and Khosravi-Darani 2010; Rodger 2001
Neurospora intermedia	Protein	56%	Nair et al. 2016
	Fats	3.5%	Karimi et al. 2019
Tuber sp.	Protein	0.72%	Saritha et al. 2016
	Fats	4.4%	
Xerocomus badius	Protein	$1.62 \pm 0.18\%$	Jaworska et al. 2015
	Fats	$0.71 \pm 0.06\%$	
Ganoderma lucidum	Protein	7% - 8%	Wang et al. 2017
	Fats	3% - 5%	
Pleurotus eryngii	Protein	16.39%	Nie et al. 2019
	Fats	$0.16 \pm 0.03\%$	Reis et al. 2012
Agaricus bisporus	Protein	27.1%	Kalač 2016
	Fats	4.3%	
Pleurotus sajor-caju	Protein	29.3%	Gogavekar et al. 2014
	Fats	0.91%	
Lentinula edodes	Protein	20%	Rahman and Choudhury 2012
	Fats	$0.8 \pm 0.01\%$	Cohen et al. 2014; Kalač 2016

2.2 Evaluation of Toxicity Safety

Toxicity is a crucial criterion that needs to be assessed while evaluating the possibility of fungus as a meat substitute. Results presented in table 2 suggested the presence of mycotoxin in some fungal species that can harm the human being when consumed.

F. venenatum was approved for sales by the Ministry of Agriculture, Fisheries, and Food in the United Kingdom for human consumption after 12 years of intensive testing (Wiebe 2002). The FDA has granted GRAS (Generally Recognized as Safe) status to a Quorn product produced from *F. venenatum* (FDA 2020). However, allergic reactions like pruritus, breathing difficulties, nausea, and diarrhoea due to allergen in the mycoprotein of *F. venenatum* were reported by Quorn's consumers (Jacobson and DePorter 2018). However, it was argued that based on the systematic evidence the prevalence of allergic responses towards mycoprotein remains extremely low and uncommon. Thus, *F. venenatum* can be accepted as safe for human consumption (Finnigan et al. 2019).

N. intermedia was also classified as GRAS with no detectable mycotoxins (Gmoser et al. 2018). This species is widely used in the production of antibiotics, enzymes, animal foods, and pharmaceutical products (Gmoser et al. 2020). So far, no study has implicated on the disease caused by *Neurospora* species in humans, animals, or plants (Perkins and Davis 2000).

Further, *X. badius* is an edible wild mushroom without mycotoxins (Proskura et al. 2017). Although no cases of allergic reaction were recorded after consuming *X. badius*, this specie can bioconcentrate heavy metals such as mercury from polluted soil (Falandysz et al. 2012). To avoid having heavy metals inside the fungus, it is imperative to choose an unpolluted environment to grow the mushroom.

P. eryngii is also commonly consumed by humans without any reported case of allergies or disease to human beings (Hu et al. 2019). Daily consumption of *P. eryngii* is recommended because it has anti-inflammatory properties that can help prevent acute lung injury caused by a bacterial infection (Kawai et al. 2014). Furthermore, consuming *P. eryngii* was reported to lower blood cholesterol in humans (Alam et al. 2011).

Although *A. bisporus* is an edible mushroom (Usman et al. 2021) but it contains mycotoxin agaritine which is a potential carcinogen (Mohamed 2012). Ingestion of *A. bisporus* can cause anaphylaxis in patients with allergic reactions (Cunha et al. 2020; Gabriel et al.

Table 2 Mycotoxin and the reported effect on human in fungal species

Species	Mycotoxin content	Effect/Response	References
Fusarium venenatum	Absent	Allergic reactions	FDA 2020; Jacobson and DePorter 2018; Wiebe 2002).
Neurospora intermedia	Absent	-	Gmoser et al. 2018
Tuber sp.	Absent	Addiction	Pacioni et al. 2015; Yan et al. 2017
Xerocomus badius	Absent	Bioconcentration potential in fruiting body	Falandysz et al. 2012; Proskura et al. 2017
Ganoderma lucidum	Absent	Adverse effects (insomnia, digestive upsets, skin rashes and hepatotoxicity)	Boa 2004; Jin et al. 2012; Wachtel-Galor et al. 2011
Pleurotus eryngii	Absent	-	Hu et al. 2019
Agaricus bisporus	Present (agaritine)	Stomach bloating, anaphylaxis	Blumfield et al. 2020; Cunha et al. 2020; Gabriel et al. 2015; Usman et al. 2021
Pleurotus sajor-caju	Absent	-	Gogavekar et al. 2014; Mazidi et al. 2020
Lentinula edodes	Absent	Allergic reactions, shiitake dermatitis	Goikoetxea et al. 2009; Grotto et al. 2016; Mendonça et al. 2015

2015). However, the concentration of agaritine in *A. bisporus* is naturally low and the cooking method of *A. bisporus* such as boiling and frying greatly reduces the agaritine content (Mohamed 2012). Consumption of *A. bisporus* did not impair cognitive function in a human, but minor side effect such as stomach bloating has been documented (Blumfield et al. 2020) and attributed to allergy reaction (Cunha et al. 2020). Long-term consumption of *A. bisporus* was able to prevent the development and progression of type 2 diabetes (Calvo et al. 2016).

P. sajor-caju is a popular culinary and medicinal mushroom (Mazidi et al. 2020). Since it does not contain mycotoxin, no cytotoxic effect on cells has been observed and is safe to be consumed by humans (Elhusseiny et al. 2021). Although traces of heavy metals like nickel, lead and chromium was occasionally reported in *P. sajor-caju* (Gogavekar et al. 2014), but the concentrations were within the FAO/WHO safety guideline 2001 (Gogavekar et al. 2014).

L. edodes is one of the most consumed mushrooms worldwide (Grotto et al. 2016; Mao et al. 2021). There were cases of reported allergic reactions, including itching toxicoderma, asthma, rhinitis, and hypersensitivity pneumonitis (Goikoetxea et al. 2009). Raw or undercooked *L. edodes* can cause shiitake dermatitis if consumed (Mendonça et al. 2015).

Tuber sp. or truffles are a non-toxic mushroom delicacy (Yan et al. 2017). However, the species *T. melanosporum* naturally consists of 7.0 ± 5.8 pmol anandamide per 1mg protein (Pacioni *et al*. 2015). The anandamide is similar to tetrahydrocannabinol which is a marijuana's active compound that can cause addiction (Pacioni et al. 2015).

In the end, *G. lucidum* is widely used in various medicinal applications (Boa 2004; Wachtel-Galor et al. 2011). However, there are studies showing patients treated with *G. lucidum* experience adverse effects such as insomnia, digestive upsets, skin rashes, and hepatotoxicity (Jin et al. 2012). Long-term intake of *G. lucidum* was also reported to cause liver failure in humans.

In summary, *F. venenatum, N. intermedia, X. badius, P. eryngii, P. sajor-caju* and *L. edodes* are the least toxic and safest, so they are suitable as meat alternatives since they do not contain mycotoxins, and ingestion of them have no severe side effects. *A. bisporus* contains low concentration mycotoxin but is safe for consumption with the proper cooking method (heat) to reduce the toxin. Although *Tuber sp.* does not contain mycotoxin but it contains anandamide that can cause addiction making the fungus unsuitable to be consumed as a meat alternative. *G. lucidum* also does not produce mycotoxin, but long-term consumption causes liver failure in humans, and thus cannot serve as a meat alternative. However, the conclusion in this review has to be taken with caution, as data on the pattern of consumption, individual body weight, and eating habits might influence the toxicity outcome. Thus, the safety of fungi species discussed earlier can only estimate the real situation for the vast majority of consumers, but not for every individual consumer.

2.3 Evaluation of Growth Potential

Meat producing animals like chickens, pigs, lambs, and cattle needs 48 days, 118 days, 140 days, and 400 days respectively to achieve the standard weight to be slaughtered and marketed (Penn State Extension 2020). In comparison to these, the growth rate of fungus is fast and these can be easily produced on artificial media or other organic compounds. Further, the growth of the fungus is crucial if it is to be used as a meat alternative because it will have a significant impact on cost. Fungus with a slow growth rate will need more nutrients to accelerate the growth, resulting in a higher production cost.

Table 3 Potential fungi species and the growth rate with optimal growth parameters

Species	Growth rate	Optimal condition		References
		Temperature	Substrate	
Fusarium venenatum	0.19g / hours	25°C	Modified Vogel's medium	Farnworth et al. 2003
Neurospora intermedia	7.632mm /day	30°C	PDA agar	Osadolor et al. 2017
Tuber sp.	0.1-0.35mg /90 days	23°C	MMN agar	Nakano et al. 2020
Xerocomus badius	14.3mm / day	25 °C	PDA agar	Liu et al. 2019
Ganoderma lucidum	14.8 ± 0.4 days for 86.1 ± 2.6g /kg	25°C	poplar sawdusts	Atila 2020
Pleurotus eryngii	3.35mm / day	25°C	PDA agar	Uysal andSoylu 2016
Agaricus bisporus	6.17mm / day	25°C	WHS2 medium	Rashid et al. 2018
Pleurotus sajor-caju	7.11mm / day	25°C	PSA medium	Go et al. 1984
Lentinula edodes	7.5mm / day	25°C	PDA agar	Ohga 1990

As indicated in table 3, most of the selected fungi species can grow relatively faster than the chicken (48 days), and in this manner, these fungi grow at the fastest rate among all the poultry animals. The only exception is the *Tuber sp.* (edible truffles), which took 90 to 120 days (Nakano et al. 2020). All the growths were recorded with growing temperature in the optimal range of 23°C to 30°C on potato dextrose agar (PDA).

F. venenatum shows the highest growth rate since the growth rate was calculated per hour. In terms of the day, *X. badius* has the highest growth rate (14.3mm /day), followed by *N. intermedia* (7.63mm /day), *L. edodes* (7.5mm /day), *P. sajor-caju* (7.11mm /day), *A. bisporus* (6.17mm /day) and lastly *P. eryngii* (3.35mm /day). *G. lucidum* takes about 14 days to produce a yield of 86.1 ± 2.6g /kg fungus. The slowest growth is for *Tuber sp* with only 0.1-0.35mg /90 days.

In summary, *F. venenatum*, *X. badius, N. intermedia*, *L. edodes, P. sajor-caju*, *A. bisporus*, *P. eryngii,* and *G. lucidum* have relatively high growth rates and require less than a month to produce the mycelium. The fungi can produce 300 kg of mycelium in hours, whereas pig, which weighs around 130 kg, takes about 4 months to mature (Wiebe 2002). However, in this review, one of the main limitations while assessing the growth parameters is that the units used to calculate the fungus growth rate are not fixed, making it difficult to compare growth rates between different fungal species.

2.4 Complications of using Fungi as Meat Alternatives

Some difficulties might be encountered when assessing which fungi species is the best to replace meat protein. There are too many fungal species with different growth characteristics or nutritional parameters under a single genus. Under a single genus, some species remain unidentified and assumed to share similar characterises as the other species within genus. This assumption will lead to safety issues since some of these unidentified fungi might produce harmful mycotoxins. Toxicity studies are complex since researchers might only be able to reveal that a particular fungus is toxic only after long-term exposure to humans. Therefore, the toxic fungus might be mistaken as safe to be developed as meat alternatives. Although this review narrows down some of the fungi identify, more information on toxicity is needed to better determine which fungi species have the best potential to be developed as meat alternatives to replace animal meat.

Conclusion

In this review, nine fungi species were examined in terms of nutritional values, toxicity, and growth rate to determine which fungus can be used as meat alternatives. This review concludes that *F. venenatum, N. intermedia, P. eryngii, A. bisporus, P. sajor-caju,* and *L. edodes* have the best potential fungi as meat alternatives due to their high nutritional content, high growth rate, and non-toxic. *Tuber sp., G. lucidum* and *X. badius* are not suitable due to relatively low protein content, slower growth rate, and higher toxicity risk.

Although this review allows the identification of fungi as meat alternatives, more empirical studies on the selected species need to be conducted and refine these findings. The primary concern of this review is to access the nutritional value, toxicity, and growth rate of the selected fungi. However, the flavor and texture of fungus are equally important, since differences in taste and texture between alternative meat made from fungus and animal meat may cause consumers to lose faith in purchasing fungi-based meat.

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Conflict of Interest Statement

There are no conflicts of interest.

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