EVALUATION OF GLUE POWER AND COLOR IN THE CINETIC FORMATION OF *Pleurotus eryngii* MYCELIUM FOR THE CONNECTIVE TISSUE IN FISH FILLET ANALOGS

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ABSTRACT

Edible mushrooms are appreciated as alternative sources of nutrients and in the development of analogues to animal products, due to their functional characteristics, ease of cultivation and relatively neutral flavor. This research aimed to use mushrooms of the species Pleurotus eryngii to obtain mycelium for application in products similar to fish fillet. Mushroom stems were cut to simulate fish fillets and were incubated in BOD at two temperatures: 5°C and 25°C for 15 and 4 days, respectively. The formation of mycelium and the adhesion of the parts were monitored by means of macro and micro photographs, color and texture analysis of the samples. The formation of mycelium between the cuts and the maintenance of the junction were observed from the ninth day of incubation at 5°C, simulating the myotomes of fish. At 25°C the mycelium formed and there was junction from the 3rd day, but there were signs of decomposition. The potential application of *P. eryngii* mycelium in the development of intact muscle in fish analogues was demonstrated.

Keywords: Vegan connective tissue, plant-based, mycelium, King oyster mushroom.

1 INTRODUCTION

In recent years, there has been a clear increase in the supply of plant-based products to meet the demand of vegans and flexitarians, making it necessary to conduct research to develop meat analogues, such as biomimetics of whole fish muscle. Mushrooms have attracted human attention since ancient times due to their important role in the human diet. Mushrooms are sources of protein, non-starchy carbohydrates, dietary fiber, minerals, B vitamins, and do not contain cholesterol or significant amounts of fat. Mushroom consumption in Brazil has been growing with the adoption of oriental and vegetarian cuisine. Although they are not yet part of the regular diet of the Brazilian population, interest in mushrooms has been growing due to their recognized nutritional value and, recently, due to the potential that mycelium has for the development of plant-based and alternative proteins. Mycelium is the vegetative part of a fungus, essential for its development and survival. It consists of a set of branched or intertwined hyphae or filaments that make up the body of the fungus, mainly responsible for the absorption of nutrients and its reproduction. The mycelium consists of a network of branches formed by a set of intertwined hyphae. In addition to transporting nutrients, these hyphae also carry out symbiotic processes with some species. Furthermore, fungal mycelia have aroused interest in several areas, including agriculture, food production, medicine and industry, as they are an important source of bioactive compounds and biomaterials4. This project aims to verify the potential of the application of mycelium from *Pleurotus* eryngii (king oyster mushroom) in obtaining vegan connective tissue or microbial glue for the development of intact muscles similar to those of animal origin, giving them the desired appearance and texture. The ability of the mycelium formed to bond was observed by maintaining the adhesion of the parts, applying light manual and mechanical force (texture analysis). Changes in color at different temperatures and times were also accompanied by macro and microscopic photographs.

2 MATERIAL & METHODS

The *Pleurotus eryngii* mushrooms used in this study were produced in the Biology Department, Agricultural Microbiology Sector of the Federal University of Lavras. After harvesting, the mushrooms were sanitized using moistened cloths and exposed to ultraviolet light in a laminar flow chamber for 30 minutes. Afterwards, they were cut with a scalpel, separating the stems from the piles, and the stems were sliced into approximately 5 mm thick slices. These were placed side by side and packaged in PVC film previously pierced with a needle in order to maintain gas exchange and allow the metabolism of the mushrooms. Each set of sliced and packaged stems was considered a sample. They were placed in trays with lids in quadruplicate and incubated in BOD. Two temperatures were studied: 5 °C for each 3 days during 15 days and 25 °C for 4 days. A Konica Minolta CM-5 colorimeter was used to evaluate the color. The colorimeter was calibrated using a white plate as a standard; the samples were placed on a transparent plate and analyzed in triplicate. The following parameters were determined: L*, a* and b*. L* defines the luminosity (L* = 0 - black and L* = 100 - white) and a* and b* are responsible for the chromaticity (+a* red and -a* green; +b* yellow and -b* blue). The results were expressed according to the CIE LAB system (CIE, 1986) with reference to the illuminant D65 and a visual angle of 10°. In parallel, the formation of the mycelium was monitored by means of photographic images with a normal lens (macro) and under a microscope with a 100x objective. The strength/hardness (N) analysis was performed with a texturometer (TA.XT plus Texture Analyzer, Stable Micro Systems Ltd.), equipped with a 5 kg load cell and adjusted to perform the analysis at a height of 25 mm from the base, on day zero and at the end of the experiment. Both color and texture were compared with the standard: tilapia fish obtained by Silva (2023).

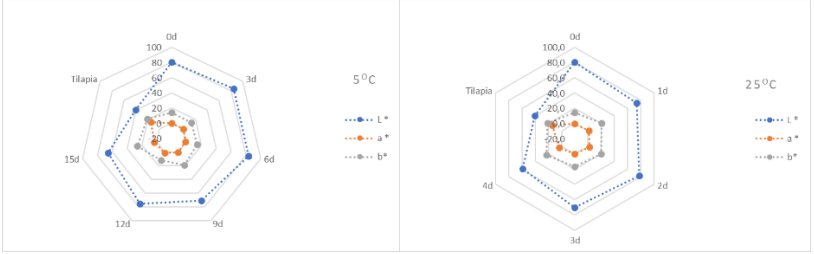
3 RESULTS & DISCUSSION

Mushrooms incubated at 25°C formed mycelium more quickly than those at 5°C on the third day of incubation, but yellowing and drying of the surface were observed, followed by a strong odor probably caused by the formation of biogenic amines[9] after 4 days of incubation. However, at 5°C, the formation of mycelium and glue of the parts occurred from the 9th day onwards, maintaining desirable characteristics of color and aroma during the formation of the mycelium until day 12th. Table 1 shows the averages for color and texture parameters, compared with the tilapia standard. Figure 1 shows the variation in the parameters L, a* and b* in the upper phase for both temperatures. Changes in color at different temperatures and times were also accompanied by macro and microscopic photographs (Figure 2). At both temperatures, a decrease in luminosity and hardness was observed over time, probably caused by biological activity, which is a positive aspect when approaching the characteristics of fish fillets. The luminosity of the previous phase of the cut king oyster stems changed from 80.5 to 64.4 in 15 days, slightly approaching that of tilapia (L=40,4)[8]. It is observed that in raw tilapia the yellow color is b*=21.6, according to Silva (2023)[8] and that as the incubation time increases up to the 15 days studied, the color of the mushroom became very close to the standard fish at 5°C (b*=21.6) on day 12th, while at 25°C on the 3rd day it moved away from the color of tilapia, reaching 22,6, a fact also observed in the photographs (Figure 2). The hardness of the *P.eryngii* stipe decreased after 15 days of mycelium formation at 5°C (from 3169.0 to 1249.0 N) approaching the texture of raw tilapia (1507 N), more than at 25°C (1192.7 N) (Table 1).

Table 1 Color and texture of analogues with *Pleurotus* sp. Mycelium

Time (days)		Color (5ºC) L *	Color (5ºC) a *	Color (5ºC) b*	Force (N)	Time (days)	Color (25ºC) L *	Color (25ºC) a *	Color (25ºC) b*	Force (N)
0	Sp	80,5 ± 1,5	-0,3 ± 0,1	13,9 ± 0,1	3169,0 ± 231,8	0	80,5 ± 1,5	-0,3 ± 0,1	13,9 ± 0,0	3169,0 ± 231,8
	Ip	75,9 ± 0,0	1,2 ± 0,0	18,1 ± 0,1			75,9 ± 0,0	1,2 ± 0,0	18,1 ± 0,0	
3	Sp	84,8 ± 0,0	0,0 ± 0,0	12,9 ± 0,1		1	73,3 ± 1,5	1,0 ± 0,1	20,7 ± 0,0	
	Ip	74,4 ± 0,0	1,5 ± 0,0	19,2 ± 0,1			68,9 ± 0,0	1,9 ± 0,0	22,0 ± 0,0	
6	Sp	83,9 ± 0,0	-1,0 ± 0,0	14,4 ± 0,1		2	77,3 ± 0,0	2,0 ± 0,0	19,8 ± 0,0	
	Ip	75,1 ± 0,0	1,4 ± 0,0	20,4 ± 0,1			61,8 ± 0,0	6,2 ± 0,0	25,1 ± 0,0	
9	Sp	70,8 ± 0,0	0,2 ± 0,0	18,7 ± 0,1	1265,1 ± 177,2	3	70,6 ± 0,0	0,2 ± 0,0	16,9 ± 0,0	
	Ip	74,4 ± 0,0	1,3 ± 0,0	22,8 ± 0,3			59,1 ± 0,0	5,4 ± 0,0	24,4 ± 0,0	
12	Sp	75,8 ± 0,1	1,1 ± 0,0	12,3 ± 0,1		4	59,4 ± 0,0	3,6 ± 0,0	22,6 ± 0,1	1192,7 ± 148,5
	Ip	75,2 ± 0,0	1,0 ± 0,0	21,6 ± 0,6			53,6 ± 0,0	5,8 ± 0,0	22,3 ± 0,0	
15	Sp	65,8 ± 0,0	3,8 ± 0,0	26 ± 0,2	1249,0 ± 231,3					
	Ip	64,4 ± 0,0	4,8 ± 0,0	26 ± 0,4						
Tilapia*		40,4	14,5	21,2	1507,0		40,4	14,5	21,2	1507,0

Values obtained by Silva (2023)[8]. Sp=Superior phase, Ip=Inferior phase. L= Luminosity; chromaticity: +a red and -a* green; +b* yellow and -b* blue.



(a) (b)

Figure 1 Color behavior in the upper phase of the stipe during mycelium formation at (a) 5ºC and (b) 25°C.

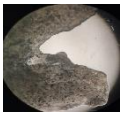
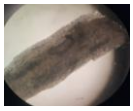
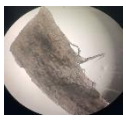
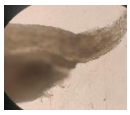
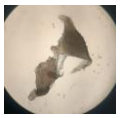
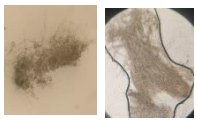
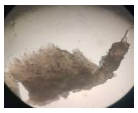
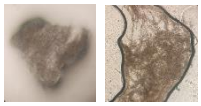
Day	Photo macro 5ºC	Photo 100X 5ºC	Day	Photo macro 25ºC	Photo 100X 25ºC
0			0		
3			1		
6			2		
9			3		
12			4		
15					

Figure 2 Imagens macro e micro de formação de micélio a 5 e 25ºC. Source: Authors.

4 CONCLUSION

The ability of the mycelium formed to stick together was observed by maintaining the adhesion of the parts of stipes when incubated at 5°C from the ninth day onwards, with a slight variation in the color and aroma of the fruiting body. At 25°C, it was observed that on the third day the parts joined via mycelial growth; however, after the fourth day, the stems showed yellowing and signs of decomposition were observed due to the appearance of off-flavor similar to biogenic amines. The mycelium of *P. erynguii* has the potential to be used as connective tissue or microbial glue in the development of plant-based fish analogues.

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