SOLID-STATE FERMENTATION OF MUSHROOM-BASED MEDIA USING *Leuconostoc pseudomesenteroides.*

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ABSTRACT

Exopolysaccharides (EPS) are polysaccharides that can be produced by lactic acid bacteria, having great structural variety and application in various industrial sectors. The present work aimed to evaluate the fermentation of the lactic acid bacteria *Leuconostoc pseudomesenteroides* in four different media based on mushrooms from the fungus *Pleurotus ostreatus* with a view to preparing an analogue to fish fillet. Fermentation was carried out in glass bottles covered with 100g of medium with the formulations: T1 (mushroom stipe and 10.7% sucrose); T2 (stipe, 10.7% sucrose and 6% oat flour); T3 (stipe and 21.4% sucrose) and T4 (stipe, 21.4% sucrose and 6% oat flour), which were incubated at 28 °C for 30h. At the end of fermentation, cell concentration by plating on MRS agar, texture profile analysis, color parameters (CIELAB) and pH were determined, both in the analogues and in the non-fermented control sample, containing only mushroom stipe. It was concluded that the microorganism *L. pseudomesenteroides* grew well in the mushroom-based medium, reaching a concentration of 10^9 CFU/g and the best results were found in the treatment with 10.7% sucrose (T1), with respect to EPS (6.9 g/L) and texture parameters, especially hardness (68.2 N) and chewiness (10.1 N/m).

Keywords: *Pleurotus ostreatus*. fish analogue. Lactic bacteria. Color parameters. Texture parameters.

1 INTRODUCTION

The diet adopted by vegans, vegetarians and flexitarians, motivated by the philosophy of life, compassion for animals, religious reasons and concern for health and the environment, has increased the consumption of alternative sources of protein from grains, cereals, mushrooms and oilseeds, in parallel with the consumption of frozen and easy-to-prepare products. One of the raw materials with potential for formulating products for vegetarians and vegans are mushrooms, due to the wide variety of edible species, nutritional value and diverse flavors[1].

There are more than 12,000 species of mushrooms, and among them, there are about 300 species of edible mushrooms, but only 30 have been domesticated and ten are cultivated commercially. In Brazil, one of the most produced and commercialized species is *Pleurotus ostreatus*[2]. Mushrooms are organisms belonging to the kingdom of fungi, and they develop a reproductive structure, which is composed of a bulb, stipe (stalk), ring and pileus (cap). The appearance of these structures can vary significantly according to the mushroom species in question, presenting a wide diversity of shapes[3].

Exopolysaccharides (EPS) of microbial origin, especially those produced by lactic acid bacteria, have been a target of interest due to their remarkable structural variety and application in various industrial sectors. A good EPS-producing genus of lactic acid bacteria is *Leuconostoc sp.*[4].

The objective of this work was to evaluate the fermentation of the EPS-producing lactic acid bacteria *Leuconostoc pseudomesenteroides* in four different media based on mushrooms from the fungus *Pleurotus ostreatus* with a view to preparing an analogue to fish fillet.

2 MATERIAL & METHODS

2.1 Microorganisms and mushrooms

The microorganism *Leuconostoc pseudomesenteroides* was obtained from the Agricultural Microbiology Culture Collection - CCMA of the Federal University of Lavras. The *Pleurotus ostreatus* fungus mushrooms were purchased from the company Cogumelos do Japinha - Bom Sucesso/MG. They were kept under refrigeration (4 °C) until the experiments were carried out, with a maximum handling interval of 24 h after harvest.

2.2 Fermentation in mushroom-based media from the fungus *Pleurotus ostreatus*

Pre-activation of the microorganism was carried out by adding 1 mL of the inoculum to 9 mL of sterile MRS medium, in test tubes:

After inoculation, the tube was placed in an incubator oven (Solab model SL-223) without shaking, at 30º C for 24 hours. For fermentation, a mass of mushroom stipes from the *Pleurotus ostreatus* fungus was crushed using a 400W Turbo Vertical Mixer (Britânia/Brazil) in two different ways, with half being crushed to a puree texture and the other half crushed into chips. After crushing, the mushrooms were homogenized and used in the preparation of 5 treatments: control (mushroom stipe); T1 (stipe plus 10.7% sucrose); T2 (stipe, 10.7% sucrose and 6% oat flour); T3 (stipe plus 21.4% sucrose) and T4 (stipe, 21.4% sucrose and 6% oat flour), with the control being done in duplicate and each treatment in quadruplicate. The pH of the samples was adjusted with 0.1 M NaOH solution to pH 8.2. The treatments were divided into 16 airtight glass jars with a transparent lid, where each jar contained 100g of the treatment in question. Before adding sucrose and/or oat flour, the stipes were blanched in a thermostated bath (85ºC for 10 min), and cooled in an ice bath. Then, each pot was inoculated with *Leuconostoc pseudomesenteroides* at an initial concentration of 10^8 CFU/g, with the exception of the control, and subsequently incubated in an oven at 28 ºC for 30 h.

2.3 Physicochemical, microbiological and statistical analyzes

The pH values was measured before and after fermentation using a portable pH meter (Model HI 99163, Hanna). Texture profile 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. A Konica Minolta CM-5 colorimeter was used to perform the color analysis. After calibration using a standard (white plate), the samples were placed on a transparent plate and made in triplicate. The results obtained were expressed according to the CIELAB system, using as reference the illuminant D65 and a visual angle of 10°. For cell counting, 1 g of fermented medium sample was collected, diluted in 0.1% peptone water and plated on MRS agar containing 0.1% aniline blue. The plates were incubated in triplicate for 48 hours at 30 °C in an oven. For the quantification of EPS, an adapted methodology[5] was used, extracting 1 g of fermented sample with water, followed by centrifugation, precipitation with absolute ethanol, centrifugation and determination of EPS by the anthrone method. The results of this study were subjected to statistical analysis using Tukey tests using the STATISTICA 7.0[6] software, applying a 95% confidence level.

3 RESULTS & DISCUSSION

The texture parameters results of the control samples, 4 treatments carried out after fermentation with *L. pseudomesenteoides* and raw tilapia fillet are found in Table 1 and the respective results for color, pH and EPS in Table 2. Regarding the parameters of texture, the control sample (without addition of inoculum, sucrose and oat flour) presented higher values and statistically differed ($p<0.05$) from samples from all other treatments, which indicates that the additions of sucrose, oat and of inoculum significantly influenced the texture parameters, in order to reduce them, which also made the treatments more distant from the texture values obtained for the raw fish fillet. The only exception was for cohesiveness, in which all treatments did not differ significantly from the tilapia fillet value.

The color parameters of L* and b* obtained in the experiment were superior when compared to those of tilapia, with T3 being the closest in terms of these parameters. Regarding the final pH values in the treatments, the results of which are directly linked to fermentation, they showed a significant difference in relation to the control, proving that the added microorganism consumed the nutrients available in the inoculated medium and produced acids, reducing the pH value. It was observed that treatments 4 (T4), composed of 21.4% sucrose and 6% oats, and treatment 1 (T1), composed of 10.7% sucrose were the most efficient in the production of exopolysaccharides. For the final cell concentration values, it was found that they were around 10^9 CFU/g, with no significant differences between treatments.

Table 1 Texture parameters for control samples, treatments 1, 2, 3 and 4 and for raw tilapia fillet.

	Hardness	Elasticity	Cohesiveness	Gumminess	Chewiness	Resilience
Control	136 ± 17 a	0,62 ± 0,02 a	0,50 ± 0,04 a	69 ± 13 a	43 ± 9 a	0,14 ± 0,02 a
T1	68 ± 12 b	0,35 ± 0,04 b,c	0,40 ± 0,03 b,c	28 ± 6 b	10 ± 3 b	0,07 ± 0,01 b
T2	54 ± 7 c	0,32 ± 0,03 c	0,37 ± 0,04 c	21 ± 4 b,c	7 ± 2 b	0,06 ± 0,01 b
T3	54 ± 12 b,c	0,38 ± 0,05 b	0,43 ± 0,04 b	23 ± 7 b,c	9 ± 4 b	0,07 ± 0,01 b
T4	49 ± 10 c	0,33 ± 0,03 c	0,40 ± 0,04 b,c	20 ± 5 c	7 ± 2 b	0,06 ± 0,01 b
Raw tilapia fllet	2060 ± 176	0,49 ± 0,03	0,41 ± 0,14	799 ± 206	390 ± 123	0,15 ± 0,05

Table 2 Color and pH parameters for control samples, treatments 1, 2, 3 and 4 and for raw tilapia fillet.

	L*	a*	b*	Final pH	Final EPS (g/L)
Control	70,10 ± 0,90 a,c	2,53 ± 0,27 b	22,02 ± 0,44 b,c	5,32 ± 0,02 a	
T1	70,57 ± 0,61 a	1,84 ± 0,44 b,c	21,45 ± 0,44 c	3,94 ±0,05 b	6,8 ± 0,8 a,b
T2	70,38 ± 0,62 a	4,14 ± 0,25 a	22,31 ± 0,64 b	3,87 ±0,04 b	5,5 ± 0,8 b
T3	67,35 ± 0,86 b	1,63 ± 0,32 c	21,36 ± 0,39 c	4,02 ±0,04 b	6,4 ± 0,3 a,b
T4	68,45 ± 0,27 b	4,71 ± 0,17 a	23,47 ± 0,37 a	4,01 ± 0,04 b	7,2 ± 0,5 a
Raw tilapia fllet	53,69 ± 2,91	3,54 ± 2,52	11,55 ± 1,22	6,31 ± 0,12	

Caption: Control – mushroom stipe without inoculum; T1- mushroom stipe and 10.7%m/m sucrose; T2 - mushroom stipe, 10.7% sucrose and 6% oat flour; T3 - mushroom stipe and 21.4% sucrose; and T4 - mushroom stipe, 21.4% sucrose and 6% oat flour. L*- brightness (L = 0 black and L = 100 white); a* and b*- chromaticity (+a red and -a green; +b yellow and -b blue).
a, b, c...- Different lowercase letters in the columns indicate statistical difference using the Tukey test ($p < 0.05$)

4 CONCLUSION

The lactic acid bacterium *L. pseudomesenteroides* was able to grow in the mushroom-based medium, reaching a concentration of 10^9 CFU/g, decreasing its pH and texture parameters, when compared to the control without microorganism. Regarding exopolysaccharides, no major differences were observed and the medium with mushroom stipes added with 10.7% sucrose produces a significant amount (6.8 g/L). The parameters of texture, color and final pH of the analogues differed significantly from the parameters related to raw tilapia fillet, indicating that the process of preparing the fish fillet analogue must be improved.

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