USE OF SEMISOLID FERMENTATION WITH A SUBSTRATE BASED ON *Pleorotus ostreatus* MUSHROOMS BY *Weissella cibaria* IN THE DEVELOPMENT OF FISH FILLET ANALOGS

Joana M. Silva[1], Emerson T. Martos[2], Olga L. Mondragón-Bernal[3] & José Guilherme L.F. Alves[3]*

[1] *Food Engineering/Department of Food Science, Federal University of Lavras, Lavras, Brazil.*
[2] *Food Engineering/Department of Food Science, Postgraduate Program in Food Engineering, Federal University of Lavras, Lavras, Brazil.*
[3] *Department of Food Science, University of Lavras, Lavras, Brazil.*
** Corresponding author's email address: *joana.silva2@estudante.ufla.br, jlembi@ufla.br, olga@ufla.br/Departamento de Ciência dos Alimentos /DCA – Universidade Federal de Lavras (UFLA)*

ABSTRACT

Edible mushrooms have been considered as raw materials with potential for application in the development of plant-based foods due to their nutritional characteristics, variety, rapid production, and pleasant texture and flavor. Some lactic acid bacteria produce exopolysaccharides (EPS) that could be useful in improving the texture and adhesion of ingredients in products analogous to those of animal origin. The aim of this study was to evaluate the effect of fermentation variables of the *Pleurotus ostreatus* (PO) mushroom-based medium by EPS-producing *W. cibaria* in the preparation of fish fillet analogs. A Plackett & Burman (PB12) experimental design was performed with 8 variables and 4 central points. The production of EPS, color, pH, and cohesion were evaluated. Oatmeal and white bean flour had a significant positive effect on cohesion. Added proteins such as soybean and pea isolates had a positive effect on color +a* or increased red color, an undesirable effect. No variable showed an effect on EPS production and cell growth. *W. cibaria* demonstrated the ability to ferment mushroom-based medium enriched with alternative proteins, producing EPS, indicating potential for the development of a fish analogue.

Keywords: Hiratake. Exopolissacarídeos. Fungos comestíveis. Cola microbiana. *Weissella Cibaria*

1 INTRODUCTION

The search for foods similar to those of animal origin is on the rise, especially among vegan, vegetarian and flexitarian consumers, who are looking for sustainable products that meet not only their nutritional and sensory requirements, but also their animal empathy[1]. Therefore, meat analogues are expected to increasingly resemble conventional muscle tissue in aspects such as flavor, appearance and texture[2]. Mushrooms are a promising option for developing plant-based alternatives, due to their nutritional value, wide variety, ease of cultivation, fast production cycle and sensory characteristics[3]. However, replicating the structure of muscle and connective tissues is a challenge, especially without the use of ingredients of animal origin[4]. Exopolysaccharides (EPS), produced by fungi and bacteria, are being investigated for their functional properties, including improvements in texture (such as cohesion or binding), stability of food products, health benefits and tissue simulation [5,6]. EPS production can occur through fermentation processes in bioreactors, with selected microorganisms and optimized culture conditions7. Semi-solid fermentation, using raw materials from the agroindustry as substrate, is a viable approach to produce these compounds[8]. Therefore, the objective of this study was to evaluate the effect of process variables and inputs on EPS production, cell growth, cohesion and color of prototypes of fish fillet analogues applying semi-solid fermentation by the lactic acid bacteria *W. cibaria* of substrates based on *P. ostreatus* mushrooms, enriched with plant proteins and other inputs.

2 MATERIAL & METHODS

P. ostreatus mushrooms were purchased from local producers (*Cogumelos do Japinha* - Bom Sucesso/Brazil). The mushrooms were cleaned and kept refrigerated (4°C) for up to 24 hours after harvest. A Plackett-Burman 12 (PB12) experimental design was performed to study the effects of variables in the preparation of fish fillet analogues. There were 8 independent variables: Soy protein isolate - SPI (%m/m)– x1; Pea protein – PE (%m/m)– x2; Oat flour - OF (%m/m)– x3; White bean flour - WBF (%m/m)– x4, Vegan microalgae oil - Ô3 (%m/m)– x5; Sucrose – S (%m/m)– x6; Heat treatment time - HTT (min)– x6; Ultrasound time - UT (min)–– x7, being 12 treatments and 4 central points, totaling 16 tests. The heat treatment was carried out at 80°C to inactivate enzymes, after the pre-activated *W. cibaria* was inoculated and incubated at 37°C for 24 hours. The following responses were analyzed: color parameters L* – Y1; a*– Y2; b* – Y3; pH variation (final fermentation pH - start fermentation pH) – Y4; EPS production (g/L) – Y5, Log growth (CFU/g) (final - initial) – Y6 and Cohesion (mm) - Y7. The color, pH, EPS, count and cohesion analyses were performed after fermentation (fresh sample). Color measurement was performed with a Konica Minolta CM-5 colorimeter, following the CIE LAB system[10], with samples analyzed in triplicate. The parameters L*, a* and b* were determined. 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 cohesion (mm) was determined with a texturometer (TA.XT plus Texture Analyzer, Stable Micro Systems Ltd.), equipped with a 5 kg load cell. The pH was measured before and after fermentation using a potentiometer. Quantification of exopolysaccharides (EPS) was performed using the anthrona method according to adapted methodology[5]. Cell growth was assessed using the pour plate method[6]. The results were analyzed using the STATISTICA 10.0 software (StatSoft, 2010)[9].

3 RESULTS & DISCUSSION

The results obtained using PB12 are presented in Table 1 below.

Table 1 PB12 Responses

	Real levels								Responses								
Trat.	**X_1**	**X_2**	**X_3**	**X_4**	**X_5**	**X_6**	**X_7**	**X_8**	**L***	**a***	**b***	**pHi**	**pHf**	**ΔpH**	**EPS (g/L)**	**Log (CFU/g)**	**Cohes. (mm)**
1	8	0	6	0	0	0	20	20	66	5.1	23	6.4	4.9	1.5	0.4	3.3	0.34
2	8	8	0	4	0	0	10	20	63	5.3	22	6.7	5.3	1.4	0.2	4.7	0.31
3	0	8	6	0	1.4	0	10	0	64	4.6	21	6.5	5.8	0.7	0.2	3.9	0.29
4	8	0	6	4	0	10.7	10	0	63	5.7	24	5.8	4.9	0.9	0.1	2.4	0.39
5	8	8	0	4	1.4	0	20	0	64	5.5	23	6.7	5.6	1.1	0.2	2.6	0.26
6	8	8	6	0	1,4	10.7	10	20	59	6.0	23	6.5	5.2	1.3	0.2	4.1	0.36
7	0	8	6	4	0	10.7	20	0	59	5.2	22	6.9	5.1	1.8	0.0	4.3	0.44
8	0	0	6	4	1.4	0	20	20	65	4.0	21	6.4	4.8	1.6	0.1	**5.6**	0.44
9	0	0	0	4	1,4	10.7	10	20	64	4.1	25	6.4	4.7	1.7	0.3	4.4	0.34
10	8	0	0	0	1.4	10.7	20	0	66	5.4	24	6.5	4.8	1.7	0.2	2.7	0.28
11	0	8	0	0	0	10.7	20	20	65	4.4	20	6.8	4.7	**2.1**	0.2	2.2	0.28
12	0	0	0	0	0	0	10	0	69	3.5	24	6.9	5.5	1.4	0.4	2.3	0.29
13	4	4	3	2	0.7	5.4	15	10	66	4.5	21	6.6	4.8	1.8	**0.5**	1.5	0.34
14	4	4	3	2	0.7	5.4	15	10	67	4.6	22	6.7	4.8	1.9	0.3	2.5	0.30
15	4	4	3	2	0.7	5.4	15	10	65	4.6	21	6.7	4.8	1.9	0.4	2.5	0.29
16	4	4	3	2	0.7	5.4	15	10	66	4.8	21	6.8	4.8	2.0	0.3	2.0	0.31
					Fresh tilapia [10]				**54**	**3.5**	**12**	**6.2**	**6.2**		**-**	**-**	0.41

Treat.=Treatment; X1= Soy protein isolate (%m/m); X2=Pea protein (%m/m); X3=Oat flour (%m/m); X4=White bean flour (%m/m); X5=Omega 3 (%m/m); X6=Sucrose (%m/m); X7=Heat treatment time (min); X8=Ultrasound time (min); pHi=pH at the beginning of fermentation, pHf=pH at the end of fermentation, EPS=Exopolysaccharide produced (g/L); Log CFU=logarithm of the Colony Forming Units of *W. cibaria*; Cohes.=Cohesion.

The results indicated variation in the luminosity parameters: L* between 59 and 69, being higher than those obtained by the *in natura* tilapia fillet (54), the chromaticity a* varied between 3.5 and 6.0 and b* between 20 and 25. The pH variation was between 0.9 and 2.1 in the treatments, with the final pH being very far from the fish muscle (pH6.2[10]). EPS production varied between 0.0 g/L and 0.5 g/L. The highest microbial growth (5.6 CFU/g) occurred in treatment 8, which contained the highest levels of WBF, Ô3 and S. The cohesion varied from 0.28mm to 0.44mm (treatments 7 and 8), being the closest to the tilapia fillet (0.41mm).

According to Table 2, it can be seen that the variables that negatively influenced the luminosity were PE and FA ($p<0.1$), thus, the higher the content of these ingredients, the lower the luminosity, favoring the approximation to the luminosity of the fish (54) as in treatments 6 and 7. SPI (X1), PP (X2), OF (X3) and S (X6) had a positive effect on the parameter a* indicating an increase in the undesirable red. PP (X2) had a significant negative effect on the parameter b* decreasing the yellow hue. Only OF and WBF had a significant positive effect on the cohesion within the limits of this study, either due to the properties of the starches and proteins present or contributing to the formation of EPS of *W. cibaria*. Although no effect of the variables was found on EPS and *W. cibaria* growth, the literature explains that EPS present different behaviors depending on their structure regardless of the content and their production is not necessarily associated with growth [11]. HTT (X7) positively influenced the pH variation during fermentation due to possible bioavailability of nutrients for fermentation.

None of the variables at the levels studied had a significant effect on EPS production and microbial growth. EPS derived from different strains of lactic acid bacteria (LAB) vary greatly in composition, charge, spatial arrangement, stiffness and ability to interact with proteins and other substances [11,12]. Factors such as the species (LAB) and culture conditions (medium composition, incubation period, pH, temperature and aeration) exert a broad influence on the functional characteristics of EPS[13].

Table 2 Effect of variables on PB12 responses (p-value <0.1)

		Mean	SPI (%m/m)	PP (%m/m)	OF (%m/m)	WBF (%m/m)	Ô3 (%m/m)	S (%m/m)	HTT (min)	UT (min)
L*	Eff.	**64.50**	-1.16	**-2.94**	**-2.78**	-1.89	-0.48	-2.22	0.43	-0.61
	Pe.	0.45	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
	p	0	0.310	0.026	0,033	0.115	0.66	0.072	0.692	0.576
a*	Eff.	**4.82**	**1.17**	**0.54**	**0.43**	0.14	0.05	**0.44**	0.06	-0.17
	Pe.	0.055	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
	p	0	0	0.004	0,012	0.323	0.694	0.011	0.643	0.23
b*	Eff.	**22.35**	0.84	**-1.55**	-0.74	0.18	0.18	0.55	-1.03	-0.57
	Pe.	0.34	078	0.78	0.78	0.78	0.78	0.78	0.78	0.78
	p	0	0.310	0.086	0.370	0.821	0.827	0.50	0.229	0.49
△pH	Eff.	**1.55**	-0.24	-0.03	-0.26	-0.07	-0.16	0.28	**0.37**	0.35
	Pe.	0,08	0,19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
	p	0	0.261	0.883	0,228	0.725	0.443	0.189	0.09	0.12
EPS (g/L)	Eff.	**0.25**	0.02	-0.10	-0.08	-0.08	-0.01	-0.08	-0.02	0.07
	Pe.	0.04	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
	p	0	0.860	0.311	0.405	0.413	0,960	0.421	0.856	0.471
Log (CFU/g)	Eff.	**3.19**	-0.47	0.18	0.79	0.90	0.67	-0.40	-0.19	0.99
	Pe.	0.296	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68
	p	0	0.514	0.80	0.288	0.23	0.362	0.58	0.793	0.192
Cohes. (mm)	Eff.	**0.33**	-0.03	-0.02	**0.08**	**0.06**	-0.02	0.03	0.01	0.02
	Pe.	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	p	0	0.198	0.262	0.003	0.017	0.448	0.183	0.568	0.35

SPI=Soy protein isolate (%m/m); PP=Pea protein (%m/m); OF=Oat flour (%m/m); WBF=White bean flour (%m/m); Ô3=Omega 3 (%m/m); S=Sucrose (%m/m); HTT=Heat treatment time (min); UT=Ultrasound time (min); Cohes.=Cohesion; Eff.=Effect; Pe.=Pure error; p=p-value; t. EPS: exopolysaccharides. CFU (Colony Forming Units). Values in **bold** showed significant effect (*p-value*<0.1).

4 CONCLUSION

This study found that OF and WBF had a positive effect on cohesion, however, they had a negative impact on color. Omega 3 and ultrasound had no effect on the levels and responses of this study, therefore, they could be used at the lowest level. The use of *P. ostreatus* mushrooms proved promising for the preparation of fish fillet analogues using the semi-solid fermentation technique by LAB. The LAB *W. cibaria*, chosen for fermentation, was able to produce EPS in the formulated substrates, which suggests the potential for application in improving cohesion for the development of fish analogues.

REFERENCES

[1] LUPETTI, C.; CASSELLI, R. 2024. Olhar 360° sobre o consumidor brasileiro e o mercado plant-based 2023/2024– São Paulo: Tikbooks;The Good Food Institute, 2024. E-Book: PDF, 74 p.; IL. Color.

[2] PALHARES, M. DE P. P. 2023. Produtos vegetais análogos de cárneos e pescado comercializados no Brasil: caracterização a partir de informações da rotulagem. (Dissertação de Mestrado em Ciência dos Alimentos). Universidade Federal de Minas Gerais, Belo Horizonte.

[3] SILVA, D. S. H. DA, et al. 2020. Secagem de cogumelos comestíveis como prática sustentável. Rev. Gestão Sustentab. Ambient., 9, 830.

[4] RAMACHANDRAIAH, K. 2021. Potential Development of Sustainable 3D-Printed Meat Analogues: A Review. Sustainability, 13(2), 938.

[5] MONDRAGÓN-BERNAL, O. L., et al. 2007. Lactobacillus rhamnosus exopolysaccharides (EPS) production and growth in soybeans water extract synbiotic beverage. J. Biotechnol. - J BIOTECHNOL, 2(131), S180-S181.

[6] YUHARA, T. T., et al. 2014. Produção de queijo tipo quark funcional contendo exopolissacarídeos. Rev. Inst. Laticínios Cândido Tostes, 69(6), 387-394.

[7] ORLANDELLI, R. C., et al. 2012. Enzimas de interesse industrial: produção por fungos e aplicações. SaBios-Rev. Saúde Biol., 7(3).

[8] SOUZA, R. L. A. DE, et al. 2010. Caracterização da poligalacturonase produzida por fermentação semi-sólida utilizando-se resíduo do maracujá como substrato. Rev. Bras. Eng. Agric. Ambient., 14(9), 987–992.

[9] STATISTICA ANALYSIS. 2010. Data Analysis Software System 10.0. Stat-Soft, Tulsa.

[10] MONTOYA-CAMACHO, N. et al. 2021. Evaluation of biochemical, chemical, physical and microbiological quality of tilapia (Oreochromis niloticus) muscle during 0 and 5 °C storage. Biotecnia, 23(2),127–133, 2021. DOI: 10.18633/biotecnia.v23i2.1418.

[11] MONDRAGÓN-BERNAL, O. L. et al. 2017. Stability and functionality of synbiotic soy food during shelf-life. J. Funct. Foods, 35, 134-145. https://doi.org/10.1016/j.jff.2017.05.021

[12] DUBOC, P., MOLLET, B. 2001. Applications of exopolysaccharides in the dairy industry. *Int. Dairy J.*, 11(9), 759-768.

[13] DABA, G. M., ELNAHAS, M. O.,ELKHATEEB, W. A. 2021. Contributions of exopolysaccharides from lactic acid bacteria as biotechnological tools in food, pharmaceutical, and medical applications. Int. J. Biol. Macromol., 173, 79-89.

ACKNOWLEDGEMENTS

The authors would like to thank The Good Food Institute (GFI-USA and Brasil), the Foundation for the Coordination of Improvement of Higher Education Personnel (CAPES), the Department of Food Science at the Federal University of Lavras (UFLA/DCA), PhD. Maria Isabel Rodrigues (PROTIMIZA) and Cogumelos do Japinha.