Research Article

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Olive pomace valorization by *Aspergillus* species: lipase production using solid-state fermentation

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Abstract

BACKGROUND: Pollution by olive mill wastes is an important problem in the Mediterranean area and novel solutions for their proper management and valorization are needed. The aim of this work was to optimize a solid-state fermentation (SSF) process to produce lipase using olive pomace (OP) as the main source of nutrients by several *Aspergillus* spp. Optimized variables in two different designs were: ratio between olive pomace and wheat bran (OP:WB), $NaNO_3$, Czapek nutrients, fermentation time, moisture content (MC) and temperature.

RESULTS: Results showed that the mixture OP:WB and MC were the most significant factors affecting lipase production for all fungi strains tested. With MC and temperature optimization, a 4.4-fold increase in *A. ibericus* lipase was achieved (90.5 $\pm$ 1.5 U g^{-1}), using a mixture of OP and WB at 1:1 ratio, 0.02 g $NaNO_3$ g^{-1} dry substrate, absence of Czapek nutrients, 60% of MC and incubation at 30 °C for 7 days. For *A. niger* and *A. tubingensis*, highest lipase activity obtained was 56.6 $\pm$ 5.4 and 7.6 $\pm$ 0.6 U g^{-1}, respectively.

CONCLUSION: *Aspergillus ibericus* was found to be the most promising microorganism for lipase production using mixtures of OP and WB.

Keywords: olive pomace (OP); solid-state fermentation (SSF); *A. ibericus*; *A. niger*; *A. tubingensis*; lipase production

INTRODUCTION

Lipases (triacylglycerol acylhydrolases EC 3.1.1.3) are enzymes which catalyze the hydrolysis of fats and oils, releasing free fatty acids, diglycerides, monoglycerides and glycerol, over an oil–water interface;[1,2] but they also mediate esterification, transesterification, acidolysis and alcoholysis.[3] The global market for industrial enzymes was estimated to be US $2 billion in 2004.[1] Lipases comprise a wide variety of applications, such as in laundry and household detergents, food, textile, cosmetic, paper, agrochemicals, pharmaceuticals and even in biodiesel production.[4,5] Lipases obtained from microorganisms are the most used for biotechnological applications. They may be obtained from bacteria, yeasts and filamentous fungi, through solid-state and submerged fermentation processes that use wild or recombinant strains.[5]

Solid-state fermentation (SSF) is defined as a fermentation process that occurs in the absence or near absence of free water, but with sufficient moisture to support microorganism growth.[6] In the last decade, an increasing interest in the development of SSF has been registered, mainly in applications for the bioremediation and biodegradation of toxic compounds and the detoxification of agricultural wastes, for example. Nonetheless, SSF is also being successfully applied in the production of enzymes, antibiotics, surfactants and other value-added products from agro-industrial wastes.[7] In particular, SSF represents an interesting alternative for producing industrial enzymes at low cost.[8] For example, the use of agro-industrial residues as substrate could result in a reduction in the cost of enzyme production, considering that the culture medium usually represents 25–50% of the total production costs.[9] Castilho *et al.*[10] found a total capital investment 78% higher for submerged fermentation (SmF) compared to SSF.

Olive mill wastes generated from olive oil extraction are a major environmental issue, particularly in Mediterranean area. Olive pomace (OP) is a sludgy waste generated by the olive oil two-phase extraction system. The two-phase extraction system is largely implemented in new olive mills, with OP being the most important residue produced. For example, in Portugal and Spain, this technology processes 86% and 90% of the olives for oil extraction, producing 250 000 and 5 million tons of OP in 2014, respectively.[11,12] Therefore, OP is a widely available and valueless residue that requires appropriate treatment and valorization. Biotechnological valorization approaches reported in the literature include composting,[13] production of hydrogen, methane[14,15] and ethanol as a biofuel;[16,17] also polysaccharides, polymers[18] and

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enzymes such as lignocellulosic enzymes[19] and lipase.[20] OP is an acidic and very humid solid by-product, which is rich in organic matter, potassium, nitrogen, carbohydrates and phenols and also contains residual fats.[21] Its properties make it an interesting substrate to induce lipase production by filamentous fungi under SSF. Recently, a growing interest in lipase production in low-cost agro-industrial wastes has emerged, and the use of substrates such as wheat bran (WB) has been proposed.[22,23] The access to starch fraction and high protein concentration add extra value to WB compared to other lignocellulosic feedstocks.[24]

Filamentous fungi are interesting sources of lipase because they produce extracellular enzymes.[25] Moreover, the hyphal mode of fungal growth and their tolerance to low water activity make fungi extremely efficient in the bioconversion of the solid substrate.[7] The black aspergilli have a relevant importance in biotechnology since the most recognized species, *Aspergillus niger*, has GRAS status ('generally recognized as safe') from the Food and Drug Administration. *Aspergillus ibericus* is a new species from the black aspergilli group, which has been isolated from wine grapes.[26] In previous studies, it has been demonstrated that *A. ibericus* is able to produce lipase under SmF. In a 2 L bioreactor, *A. ibericus* produced up to 8.3 U mL^{-1} of lipase using olive mill wastewater.[27] Under solid-state fermentation using OP and winery wastes, Salgado *et al.*[20] reported that *A. ibericus* produced 18.7 U of lipase per gram of dry substrate.

In the work reported herein, OP was mixed with WB. The aim of the work was to optimize the production of lipase by *A. ibericus* MUM 03.49, *A. niger* MUM 03.58 and *A. tubingensis* MUM 06.152, under SSF. The process was optimized by evaluating parameters such as substrate composition (mixtures of OP and WB), amount of inorganic nitrogen supplementation, Czapek nutrients, fermentation time, moisture content (MC) and temperature.

MATERIALS AND METHODS

Substrates

OP samples were collected from a local two-phase olive mill plant in Vila Real, Portugal, in the 2011/2012 season, and stored at −20 °C for use throughout the study. OP characteristics were determined by Salgado *et al.*,[19] presenting an MC of 75.3 ± 0.1% (w/w, wet basis) and a lipid concentration of 102.5 ± 0.04 mg g^{-1} dry substrate. WB was purchased in a local supermarket and characteristics were determined as in the literature.[24]

Biological material

Aspergillus ibericus MUM 03.49, *A. niger* MUM 03.58 and *A. tubingensis* MUM 06.152 (MUM culture collection, Braga, Portugal) were used. These strains demonstrated high lipolytic activity in previous work.[20] They were revived on malt extract agar (MEA) plates (20 g L^{-1} malt extract, 20 g L^{-1} glucose, 1 g L^{-1} peptone and 20 g L^{-1} agar) at 25 °C from a frozen glycerol stock. Spore suspensions of the inoculum were prepared from 7-day-old culture plates with 1 g L^{-1} peptone solution (with 0.01 g L^{-1} Tween 80). The spore concentration of the suspension was adjusted to 10^6 spores mL^{-1} using a Neubauer counting chamber.

Optimization of SSF with Taguchi L9 orthogonal array

SSFs were performed in cotton-plugged 500 mL Erlenmeyer flasks containing 30 g dried substrate. Initial MC was adjusted to 75% (w/w, wet basis) with distilled water when necessary. Flasks were autoclaved at 121 °C for 15 min, cooled, inoculated with 1 mL of inoculum suspension and incubated at an initial temperature of 25 °C. Initially, a Taguchi L9 orthogonal array was designed using Qualitek-4 software (Nutek Inc., USA), in order to study the effect of several factors on lipase activity (Table 1). Four factors were evaluated at three levels, with nine runs performed for each fungus. The four factors were: (i) ratio of OP:WB (1:0; 2:1 and 1:1, w/w); (ii) supplementation with $NaNO_3$ (0.15; 0.3 and 0.6 g); (iii) supplementation with mineral nutrients (Czapek nutrients) (0 – no supplementation; 1× – 1 g L^{-1} K_2HPO_4, 0.5 g L^{-1} KCl, 0.5 g L^{-1} $MgSO_4.7H_2O$, 0.5 g L^{-1} $CaCl.2H_2O$, 0.01 g L^{-1} $FeSO_4.7H_2O$, 0.01 g L^{-1} $ZnSO_4.7H_2O$ and 0.005 g L^{-1} $CuSO_4.5H_2O$; 2× – same as 1× but with doubled concentration); and (iv) fermentation time (7, 14 and 21 days). Functions 'standard analysis' using 'average of results' and 'bigger is better' were used to evaluate the contribution of each factor on lipase activity and to determine the optimum conditions. Qualitek-4 was also used to perform analysis of variance (ANOVA) of the results obtained.

Optimization of SSF with full factorial design

A full factorial design (3^2) was performed using Statistica 12 software (StatSoft, Tulsa, OK, USA) in order to study the influence of MC and temperature on the production of lipase. Optimal conditions determined previously by Taguchi L9 orthogonal array were used as the starting point for this second experiment. As shown in Table 4, three levels were assigned to each factor: MC (70%, 75% and 80%) and temperature (25, 30 and 35 °C), performing nine runs in total. Polynomial equations were fitted to experimental values of lipase activity using Statistica 12 software (StatSoft) and best levels of MC and temperature that produced the maximum lipase activity were determined using the Solver application in Microsoft Excel 2010. The relationship between the dependent (lipase activity) and independent (MC and temperature) variables was established by the polynomial eq (1), as follows:

$$y = a_0 + a_1x_1 + a_2x_2 + a_{11}x_1^2 + a_{22}x_2^2 + a_{12}x_1x_2 \quad (1)$$

where y is the predicted response, x_1 and x_2 are independent variables, a_0 is the intercept, a_1 and a_2 are linear coefficients, a_{11} and a_{22} are quadratic coefficients and a_{12} is the interaction coefficient.

Finally, for each fungus at optimum temperature found, a final optimization of MC was conducted at levels between 35% and 70%, performing eight runs in total. Data obtained were statistically analyzed using SPSS (IBM SPSS Statistics, Version 22.0; IBM Corp., Armonk, NY, USA) to study the effect of variables on lipase production. Data were tested for homogeneity, and submitted to one-way ANOVA and a pair-wise multiple comparison procedure (Tukey's test) at a confidence level of 95%.

Lipase extraction and determination

At the end of the incubation period, fermented substrates were homogenized with 150 mL of 10 g L^{-1} NaCl and 5 g L^{-1} Triton X-100 (solid:liquid ratio of 1:5) at 170 rpm and 20 °C for 2 h using a shaker. Homogenates were then centrifuged (12 000 × *g* and 10 min at 4 °C) and filtered using Whatman No. 1 filter paper. The resulting enzymatic extracts were immediately used for lipase determination.

Extracellular lipase activity was determined by colorimetric assay, using *p*-nitrophenyl butyrate as described by Gomes *et al.*[28] One unit of lipase activity (U) was expressed as the amount of enzyme that produced 1 µmol *p*-nitrophenol min^{-1} under the

Table 1. Factors and assigned levels in Taguchi L9 orthogonal array and experimental values of lipase activity obtained for the different filamentous fungi. Values are the mean ± standard deviation (SD) of triplicate analysis

					Experimental lipase activity ± SD (U g^{-1})		
Run	OP:WB	$NaNO_3$ (g)	Czapek nutrients	Time (d)	*A. ibericus*	*A. niger*	*A. tubingensis*
1	1:0	0.15	0	7	0 ± 0	0.2 ± 0	0.1 ± 0
2	1:0	0.3	1X	14	0 ± 0	0.5 ± 0	0.1 ± 0
3	1:0	0.6	2X	21	1.8 ± 0.5	0.2 ± 0	0.2 ± 0.2
4[a]	2:1	0.15	1X	21	1.0 ± 0.3	10.6 ± 0.4	**5.1 ± 0.6**
5	2:1	0.3	2X	7	1.4 ± 0.3	2.3 ± 0.6	1.4 ± 0.3
6[b]	2:1	0.6	0	14	4.2 ± 0.6	**10.9 ± 1.9**	1.9 ± 0.4
7	1:1	0.15	2X	14	11.9 ± 1.0	9.8 ± 1.5	1.8 ± 0.6
8	1:1	0.3	0	21	13.2 ± 1.1	10.9 ± 1.5	3.7 ± 0.3
9[c]	1:1	0.6	1X	7	**18.7 ± 0.3**	4.3 ± 1.2	1.3 ± 0.4

OP, olive pomace; WB, wheat bran; maximum lipase production for:
[a] *A. tubingensis*,
[b] *A. niger* and
[c] *A. ibericus*.

Table 2. ANOVA for the Taguchi L9 orthogonal array

Fungus	Factor	Sum of squares	Variance	*F*-ratio	Percent P (%)
A. ibericus	OP:WB	1058.5	529.2	1568.4	90.6
	$NaNO_3$	81.5	40.7	120.7	6.9
	Czapek nutrients	10.8	5.4	16.1	0.9
	Time	11.3	5.7	16.8	0.9
	Error	6.1	0.3	-	0.8
A. niger	OP:WB	244.7	122.3	111.3	60.4
	$NaNO_3$	17.5	8.7	8.0	3.8
	Czapek nutrients	33.6	16.8	15.3	7.8
	Time	96.1	48.0	43.7	23.4
	Error	9.9	1.1	-	4.7
A. tubingensis	OP:WB	34.8	17.4	124.7	49.3
	$NaNO_3$	6.4	3.2	22.9	8.7
	Czapek nutrients	5.0	2.5	18.0	6.8
	Time	21.4	10.7	76.6	30.1
	Error	2.5	0.1	-	5.2

OP, olive pomace; WB, wheat bran.

assay conditions. All the analyses were performed in triplicate. The lipase activity obtained was expressed as units per gram of dry substrate (U g^{-1}).

RESULTS AND DISCUSSION

Optimization with Taguchi L9 orthogonal array

In general, all *Aspergillus* species were able to grow in all runs performed. However, in experiments conducted without WB, lipase activity was almost non-existent, probably due to the negative effect on microbial activity of OP organic acids and phenolic compounds,[29] or to the low protein content of OP. *Aspergillus ibericus* was found to be the best producer of lipase under the tested conditions, achieving lipase activities of 18.7 ± 0.3 U g^{-1}, as presented in Table 1. Also, Salgado *et al.*[20] found a similar *A. ibericus* lipase production using OP and winery wastes at 75% MC.

The factor OP:WB ratio presented a greater effect on lipase activity. Analysis of variance from Table 2 showed the highest *F*-ratio values, and an influence (percent P) of 90.6% for *A. ibericus*, 60.4% for *A. niger* and 49.3% for *A. tubingensis*. The combination of different substrates may favor fungal development and the production of enzymes, as presented in this study with the supplementation of OP with WB. It may act differently as a support matrix, as a nutrient source and as an inducer for the production of enzymes.[30] For example, Kumar *et al.*[31] found an optimum ratio of 1:1 of grease and WB, for lipase production using *Penicillium chrysogenum*, and concluded that fungi first uses WB for mycelial growth and then the grease waste.

According to *F*-ratio and percent of influence in Table 2, the second factor with the most pronounced effect on lipase activity was $NaNO_3$ for *A. ibericus* and time for *A. niger* and *A. tubingensis*. It is well known that different nitrogen sources may enhance differently the production of lipases by microorganisms. For example, Sun and Xu[32] reported that ammonium hydrogen phosphate had a positive effect on lipase production by *Rhizopus chinensis*, but that other nitrogen sources did not. In the present work, $NaNO_3$ had a positive effect on lipase production for *A. ibericus*

but the same was not observed for *A. niger* and *A. tubingensis*. On the contrary, fermentation time had a higher effect only for *A. niger* and *A. tubingensis*. Salgado *et al.*[20] observed a maximum *A. ibericus* lipase production on the 5th day of a 20-day SSF of OP with exhausted grape marc. Using *A. niger* in different substrates, Edwinoliver *et al.*[30] and Mahadik *et al.*[33] found an optimum fermentation time of 4 and 5 days, respectively. The only factor that did not present a clear influence on lipase production was the Czapek nutrients. Kumar *et al.*[31] also reported that Czapek Dox medium, used as salts and moisture facilitator for fungal growth, contributed to decrease lipase activity rather than to increase it. This work also showed that the optimum fermentation time for lipase production is extremely dependent on the fungus species used.

Table 3 presents the optimum fermentation conditions necessary to maximize the production of lipase for the fungi tested and the predicted lipase activity under those conditions, as determined by Qualitek-4 software. A production of 18.7 U g^{-1} for *A. ibericus*, 13.2 U g^{-1} for *A. niger* and 5.0 U g^{-1} for *A. tubingensis* was predicted.

Table 3. Optimum level of factors obtained with Taguchi L9 orthogonal array and predicted lipase activity at optimum conditions

Fungus	OP:WB	$NaNO_3$ (g)	Czapek nutrients	Time (d)	Predicted lipase (U g^{-1})
A. ibericus	1:1	0.6	1×	7	18.7
A. niger	1:1	0.15	0	21	13.2
A. tubingensis	2:1	0.15	1×	21	5.1

OP, olive pomace; WB, wheat bran.

Optimization with full factorial design

For a final optimization design, because some factors had a very small effect on lipase activity, SSF conditions were slightly changed: no Czapek nutrients were added for all strains (low *F*-ratio and percent P – Table 2); and for *A. niger*, an OP:WB ratio of 2:1 and 14 days of fermentation were used (since effects were similar – Fig. 1.b) and because it was more convenient to use more OP and to reduce the fermentation time. In the case of *A. ibericus* and *A. tubingensis*, it was not necessary to change the levels of the factors OP:WB ratio and time, as shown in Fig. 1.

Table 4 presents experimental values of lipase activity from the full factorial design for the filamentous fungi tested, as a function of MC and temperature. *Aspergillus ibericus* and *A. niger* reached the highest lipase activity (28.0 ± 1.0 U g^{-1} and 16.6 ± 0.5 U g^{-1}, respectively) at 70% MC and 30 °C. For *A. tubingensis* a maximum of 6.7 ± 0.6 U g^{-1} was obtained at 70% MC and 25 °C. Compared to the previous results from optimization conditions using the

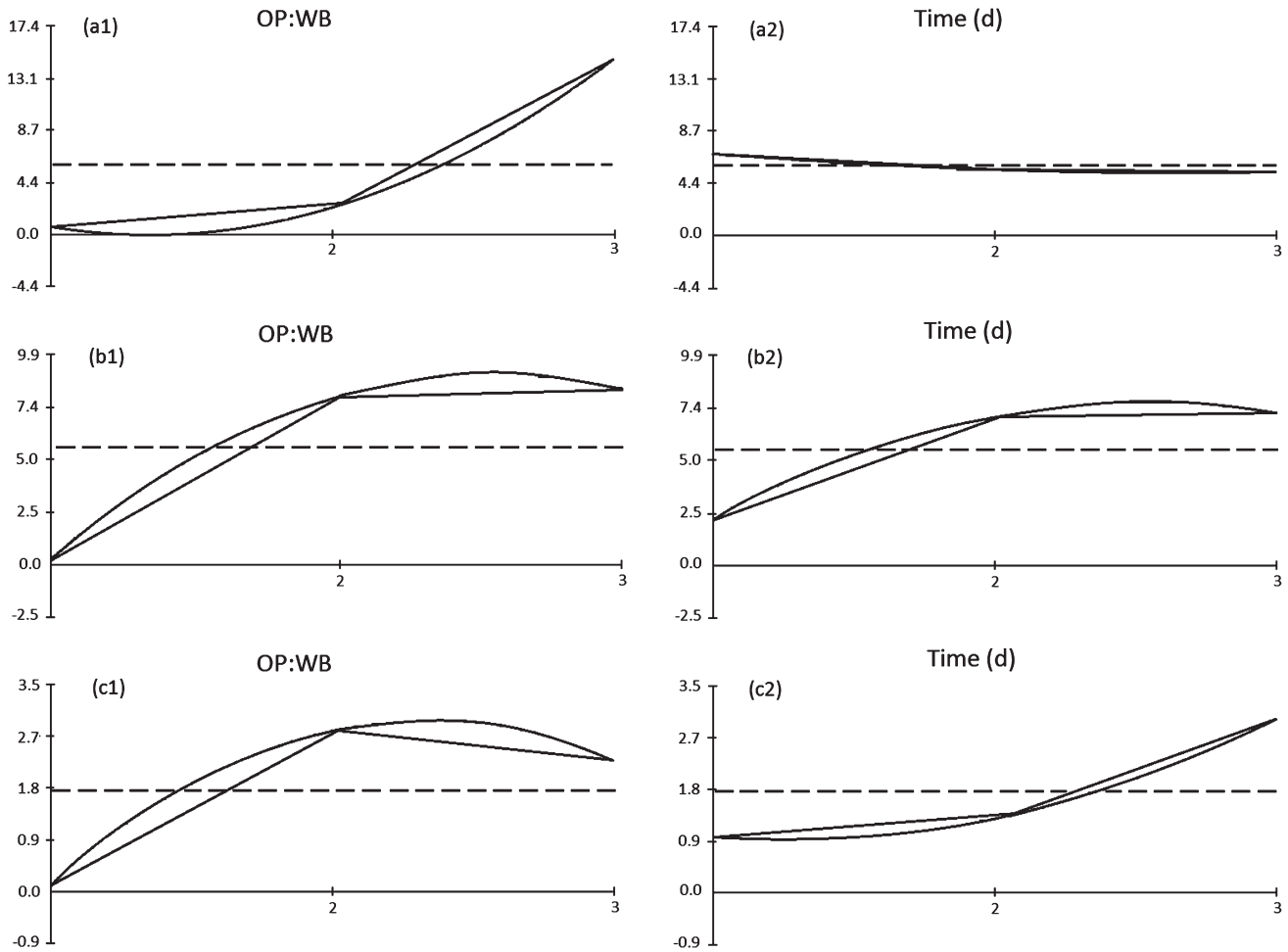
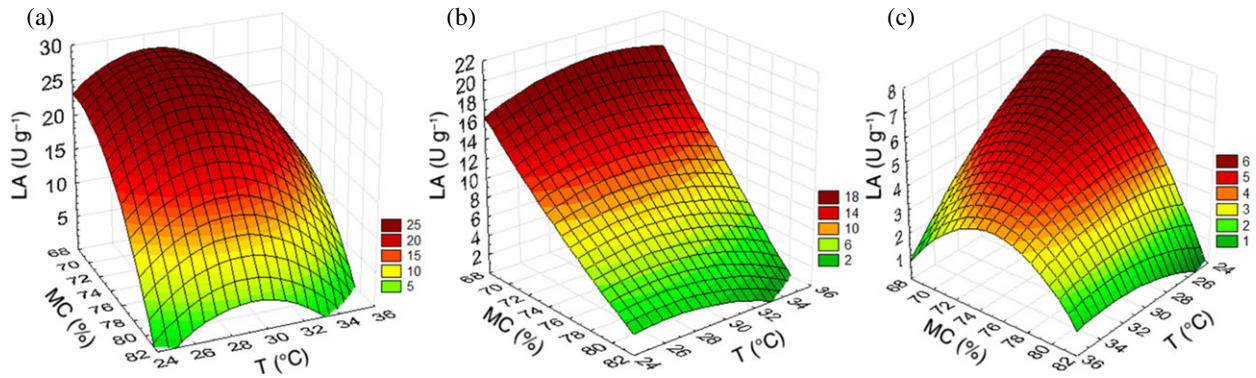


Figure 1. Effect of the factors (1) OP:WB ratio and (2) fermentation time on lipase production of (a) *A. ibericus* MUM 03.49, (b) *A. niger* MUM 03.58 and (c) *A. tubingensis* MUM 06.152, using Taguchi L9 orthogonal array.

Table 4. Factors and assigned levels of full factorial design. Experimental and predicted values of lipase activity obtained for each fungus. Values are the mean ± standard deviation (SD) of triplicate analysis.

			Values of lipase activity ± SD (U g^{-1})					
			A. ibericus		*A. niger*		*A. tubingensis*	
Run	MC (%)	Temp. (°C)	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	70	25	23.2 ± 5.2	24.9	13.9 ± 1.2	14.4	6.7 ± 0.6	6.5
2	70	30	28.0 ± 0.6	27.2	16.6 ± 0.5	16.1	5.2 ± 0.6	5.0
3	70	35	16.7 ± 1.1	16.4	15.8 ± 0.8	15.3	2.6 ± 0.6	2.6
4	75	25	20.8 ± 1.0	19.3	10.1 ± 0.7	8.8	5.9 ± 1.1	5.6
5	75	30	22.9 ± 1.5	22.4	9.6 ± 0.9	9.5	4.8 ± 1.0	5.2
6	75	35	9.8 ± 0.6	12.4	6.9 ± 0.4	7.7	4.3 ± 1.1	3.8
7	80	25	6.8 ± 1.1	7.1	3.6 ± 0.8	3.9	1.9 ± 0.3	2.0
8	80	30	9.0 ± 0.5	10.9	3.7 ± 0.5	3.7	3.3 ± 0.2	2.7
9	80	35	3.2 ± 0.2	1.6	1.7 ± 0.4	0.9	2.2 ± 0.2	2.4



	Fungus	Equation
a)	*A. ibericus*	$LA = -753.07 + 17.52MC + 12.67T - 0.13MC^2 - 0.26T^2 + 0.03MCT$
b)	*A. niger*	$LA = 55.92 - 2.31MC + 5.91T + 0.015MC^2 - 0.05T^2 - 0.04MCT$
c)	*A. tubingensis*	$LA = -197.58 + 6.60MC - 2.22T - 0.05MC^2 - 0.02T^2 + 0.04MCT$

Figure 2. Response surfaces of lipase activity (LA) as a function of moisture content (MC) and temperature (*T*) according to the polynomial equations of (a) *A. ibericus* MUM 03.49, (b) *A. niger* MUM 03.58 and (c) *A. tubingensis* MUM 06.152.

Taguchi design, these results correspond to an increase in lipase production of 35% for *A. ibericus*, 64% for *A. niger* and 14% for *A. tubingensis*, by MC and temperature optimization.

The effects of those factors on lipase production were determined using Statistica software and it was observed that an increase of MC from 70% to 80% had a negative effect ($P < 0.005$) on lipase production by *A. ibericus* and *A. niger*, but was insignificant ($P > 0.05$) for *A. tubingensis*. Concerning temperature, a significant effect on lipase production was only observed for *A. ibericus* ($P < 0.05$). In other works, MC was also considered one of the most important factors affecting SSF processes,[34] as well as temperature. Maximum saturation of the enzyme active site occurs at an optimum temperature, as enzymes become denatured at high temperature. Also at high temperature, protease production occurred, denaturing the enzymes.[31] However, at lower temperature, microorganism growth slows down and, consequently, enzyme production.

Next, polynomial equations as a function of MC and temperature (*T*) were fitted to the experimental values of lipase activity (LA) for each fungus (Fig. 2). ANOVA indicated a good fitting of the equations to the experimental values ($R^2 > 0.96$), with the exception of *A. tubingensis* ($R^2 < 0.80$). According to the model equations, the variation of LA with MC and temperature were represented as response surface plots, using Statistica software (Fig. 2). In this way the negative effect on LA of increasing MC levels and the existence of optimum values of MC and temperature, for which LA is maximized, using Solver application, can be clearly observed (Fig. 2 and Table 5). In general, results revealed that optimum values of temperature were around 30 °C for *A. ibericus* and *A. niger*, and 25 °C for *A. tubingensis*, but that the optimum MC was close to the lower level of the experimental design for all strains (around 70%). Thus further experiments with MC lower than 70% were conducted to find the optimum.

Figure 3 presents the results of LA as a function of MC for these experiments. Lipase production was significantly higher for all strains at MC values lower than 70% ($P < 0.0001$) and a maximum was obtained at 60%, 50% and 35% for *A. ibericus*, *A. niger* and *A. tubingensis*, respectively. Comparing with MC at 70%, a threefold increase in lipase production was observed for *A. ibericus*, sevenfold for *A. niger* and fourfold for *A. tubingensis*, yielding significantly higher lipase production. Again, *A. ibericus* produced more lipase (90.5 ± 1.5 U g^{-1}) than *A. niger*

Table 5. ANOVA of the polynomial equations and optimum predicted conditions and respective lipase activity for each fungus

	ANOVA						Optimum predicted		
Fungus	R^2	R^2 adjusted	SS equation	SS residual	*F*-value	*P*-value	MC (%)	T (°C)	LA (U g^{-1})
A. ibericus	0.9654	0.9308	580.16	20.80	27.89	0.0035	68.7	28.3	28.1
A. niger	0.9692	0.9383	236.58	7.53	31.44	0.0028	70.0	30.9	16.2
A. tubingensis	0.7603	0.5205	17.10	5.39	3.17	0.1449	70.9	25.0	6.6

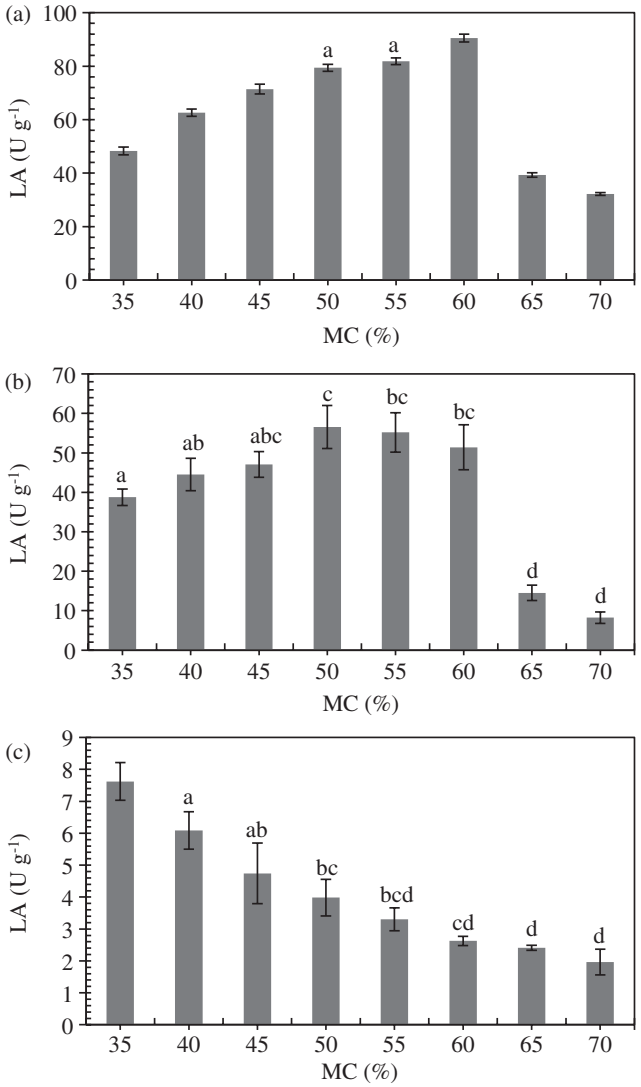


Figure 3. Influence of moisture content (MC) on lipase activity (LA) of (a) *A. ibericus* MUM 03.49, (b) *A. niger* MUM 03.58 and (c) *A. tubingensis* MUM 06.152. Depicted values are the mean of triplicate analysis ± standard deviation. Means with the same letter do not differ significantly at $P > 0.05$ (*t*-test).

(56.6 ± 5.4 U g^{-1}) and *A. tubingensis* (7.6 ± 0.6 U g^{-1}). Compared to *A. ibericus* lipase production from Taguchi design (at 75% MC and at 25 °C), 20.8 ± 1.0 U g^{-1}, a 4.4-fold increase was found.

The observed decrease of lipase activity with increasing MC has been observed by other researchers as well,[32,35–37] and has been attributed to the impact of moisture on the physical properties of the solid substrate.[38] High MC decreases substrate porosity, alters substrate particle structure, promotes development of stickiness and reduces gas volume and exchange, leading to oxygen diffusion limitation in the substrate layer,[30,32,34,39] and microbial growth decreases.[39] This study revealed the great importance of performing SSF at optimum MC to improve lipase production.

In general, after the whole optimization process, *A. ibericus* continued to be the best lipase producer, and a final increase of 4.4 fold in lipase production was achieved as a result of MC and temperature optimization. The obtained maximum lipase activities (90.5 ± 1.5 U g^{-1} for *A. ibericus* and 56.6 ± 5.4 U g^{-1} for *A. niger*) were higher than those obtained by other researchers, which also used SFF and filamentous fungi to produce lipase. For example, Falony *et al.*[23] reported a lipase activity of 9.1 U g^{-1} at 65% MC with *A. niger* using WB with olive oil as inductor; Gutarra *et al.*[25] obtained a lipase production of 19.6 U g^{-1} in 72 h at 30 °C growing *Penicillium simplicissimum* in babassu cake supplemented with sugar cane molasses at 70% MC; and Sun and Xu[32] reported the production of 24.5 U g^{-1} by *Rhizopus chinensis* in wheat flour with WB at 70% MC.

As mentioned previously, *A. ibericus* is also capable of producing lipase under SmF using another olive residue: olive mill wastewater. The maximum concentration of lipase activity obtained in that case was 8.3 U mL^{-1},[27] which was approximately 1.9-fold less than that obtained in the present work via SSF of OP:WB with *A. ibericus* (15.6 U mL^{-1} in the extracting solution). These results agree with other researchers, which also reported that SSF indeed leads to higher lipase concentration than SmF.[23,40] Additionally, for *A. ibericus*, the observed optimum MC (60%) turned out to be very convenient because it is exactly the MC obtained when OP and WB are mixed at a ratio of 1:1, thereby contributing to the reduction of costs and simplification of an eventual process at a large scale.

CONCLUSIONS

Substrate ratio of OP:WB and MC were found to be the most critical factors for lipase production by SSF with *Aspergillus* spp. Using Taguchi design, *A. ibericus* lipase production of 20.8 ± 1.0 U g^{-1} was achieved, but after the subsequent optimization of MC and temperature a lipolytic activity of 90.5 ± 1.5 U g^{-1} was attained, corresponding to 4.4-fold increase in lipase production. The optimum conditions were OP:WB in a 1:1 ratio, 0.02 g $NaNO_3$ g^{-1} dry substrate, absence of Czapek nutrients, 60% of MC and incubation at 30 °C for 7 days. *Aspergillus ibericus* was found to be the best lipase producer among the tested strains, being a promising microorganism for the production of this enzyme under SSF of OP with WB, and enabling an interesting approach for OP valorization.

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