

## Supplementary materials

### **Valorization of Macuba (*Acronia aculeata*) for Integrated Production of Lipase by *Yarrowia lipolytica* and Biodiesel Esters**

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**Table S1.**  $2^{6-2}$  Fractional factorial design matrix (real values in parenthesis) of all SSF tests with macauba fruit cake and respective lipase activity results after 24 h.

Run	Moisture (%)	Glucose (% m/m)	Yeast extract (% m/m)	Urea (% m/m)	Peptone (% m/m)	Inoculum (mg/g)	Lipase activity (U/g)
1	-1 (30)	-1 (1)	-1 (0)	-1 (0)	-1 (0)	-1 (0.67)	4.9
2	1 (50)	-1 (1)	-1 (0)	-1 (0)	1 (2)	-1 (0.67)	33.5
3	-1 (30)	1 (3)	-1 (0)	-1 (0)	1 (2)	1 (6.70)	10.8
4	1 (50)	1 (3)	-1 (0)	-1 (0)	-1 (0)	1 (6.70)	5.9
5	-1 (30)	-1 (1)	1 (2)	-1 (0)	1 (2)	1 (6.70)	6.4
6	1 (50)	-1 (1)	1 (2)	-1 (0)	-1 (0)	1 (6.70)	16.2
7	-1 (30)	1 (3)	1 (2)	-1 (0)	-1 (0)	-1 (0.67)	4.9
8	1 (50)	1 (3)	1 (2)	-1 (0)	1 (2)	-1 (0.67)	39.9
9	-1 (30)	-1 (1)	-1 (0)	1 (2)	-1 (0)	1 (6.70)	6.4
10	1 (50)	-1 (1)	-1 (0)	1 (2)	1 (2)	1 (6.70)	41.6
11	-1 (30)	1 (3)	-1 (0)	1 (2)	1 (2)	-1 (0.67)	12.8
12	1 (50)	1 (3)	-1 (0)	1 (2)	-1 (0)	-1 (0.67)	150.8
13	-1 (30)	-1 (1)	1 (2)	1 (2)	1 (2)	-1 (0.67)	8.4
14	1 (50)	-1 (1)	1 (2)	1 (2)	-1 (0)	-1 (0.67)	82.9
15	-1 (30)	1(3)	1 (2)	1 (2)	-1 (0)	1 (6.70)	10.8
16	1 (50)	1(3)	1(2)	1 (2)	1 (2)	1 (6.70)	57.2
17 (CP)	0 (40)	0 (2)	0 (1)	0 (1)	0 (1)	0 (3.70)	198.5
18 (CP)	0 (40)	0 (2)	0 (1)	0 (1)	0 (1)	0 (3.70)	220.7
19 (CP)	0 (40)	0 (2)	0 (1)	0 (1)	0 (1)	0 (3.70)	199.8

**Table S2.** Variance analysis for lipase activity obtained from macauba fruit cake.  $R^2 = 0.93$ ;  $F(0.10; 5; 4) = 4.05$ .

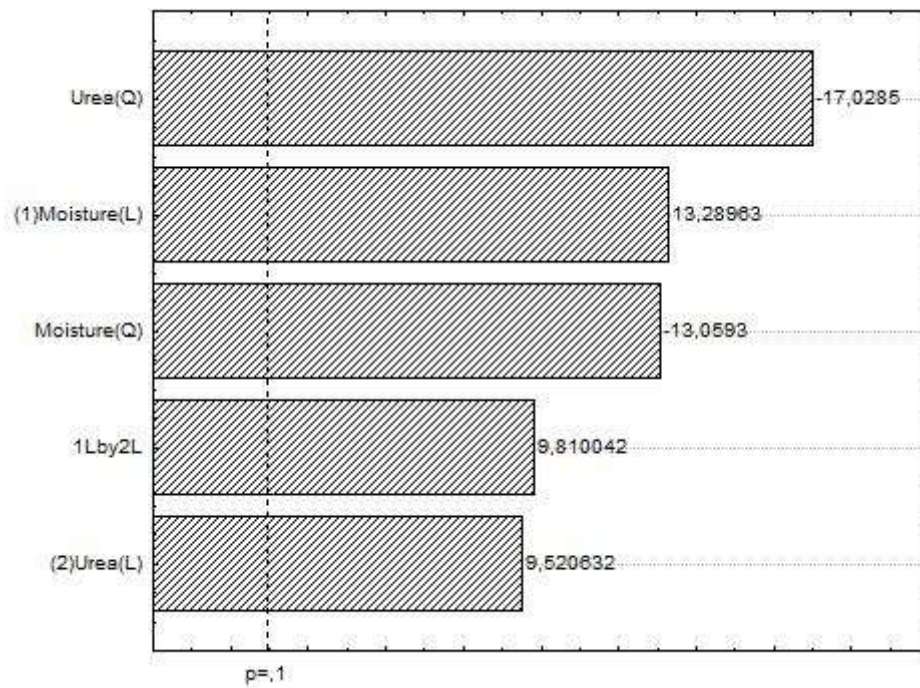
Variation source	Degree of freedom	Sum of squares	Mean square	$F_{\text{calc}}$
Model	5	162773.2	32554.6	20.9
Error	2	444.7	222.4	
Total fixed	10	169011.3	-	<b><math>F_{\text{tab}} = 4.05</math></b>

**Table S3.** Variance analysis for lipase production from macauba pulp and peel cake.  $R^2 = 0.86$ ;  $F(0.1; 5; 4) = 4.05$ .

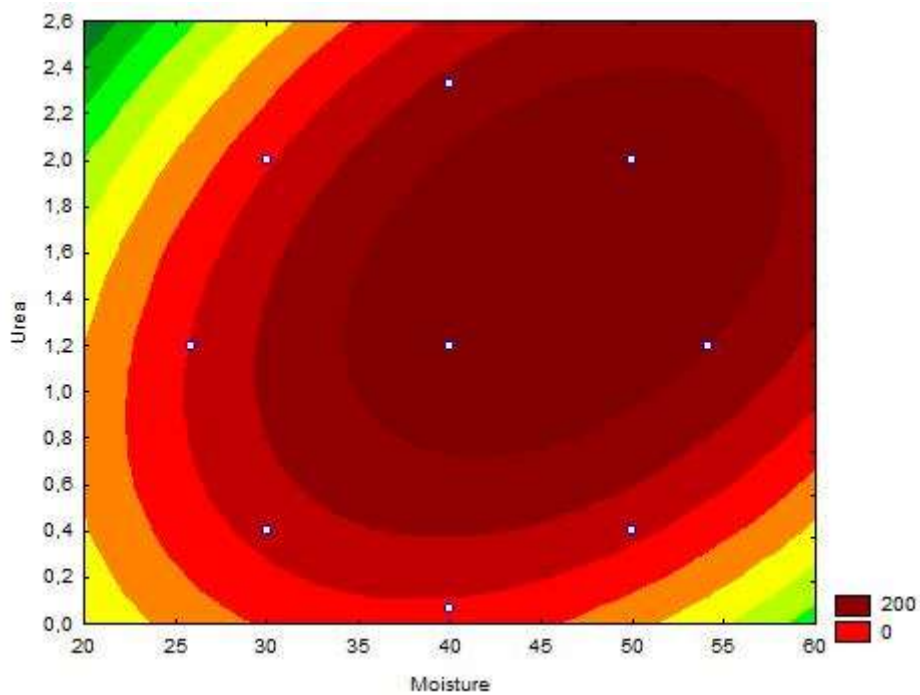
Variation source	Degree of freedom	Sum of squares	Mean square	$F_{\text{calc}}$
Model	5	43769.5	8753.9	4.8
Error	2	818	409	
Total fixed	9	51134.7	-	<b><math>F_{\text{tab}} = 4.05</math></b>

**Table S4.** Matrix of the CCRD (real values in parenthesis) for the synthesis of ethyl esters using FFAs from macauba acidic oil. \*Results after 48h reaction.

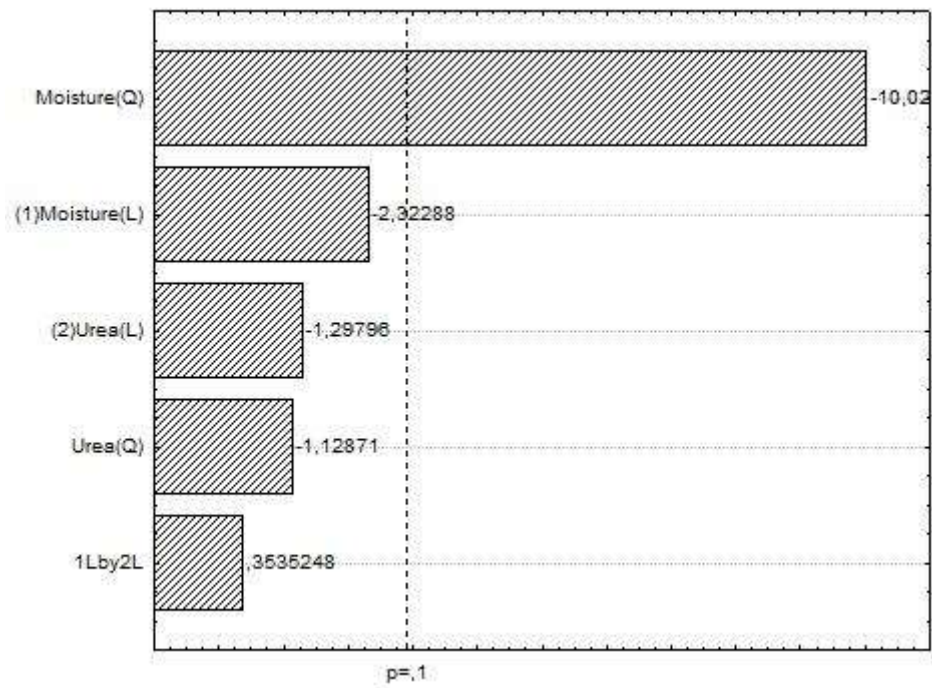
Run	Levels and variables			Conversion (%)*	Ester yield (%)*
	Lipase activity (U/g FFA)	Molar ratio (alcohol:acid)	Temperature (°C)		
1	-1 (10)	-1 (2)	-1 (32)	32	28
2	+1 (20)	-1 (2)	-1 (32)	34	31
3	-1 (10)	+1 (4)	-1 (32)	29	17
4	+1 (20)	+1 (4)	-1 (32)	33	24
5	-1 (10)	-1 (2)	+1 (42)	37	36
6	+1 (20)	-1 (2)	+1 (42)	20	27
7	-1 (10)	+1 (4)	+1 (42)	40	37
8	+1 (20)	+1 (4)	+1 (42)	28	14
9	0 (15)	0 (3)	-1.41 (29)	48	49
10	0 (15)	0 (3)	+1.41 (46)	23	22
11	0 (15)	-1.41 (1.3)	0 (37)	35	36
12	0 (15)	+1.41 (4.7)	0 (37)	27	24
13	-1.41 (6.6)	0 (3)	0 (37)	20	15
14	+1.41 (23.4)	0 (3)	0 (37)	30	31
15	0 (15)	0 (3)	0 (37)	35	32
16	0 (15)	0 (3)	0 (37)	38	35
17	0 (15)	0 (3)	0 (37)	36	35



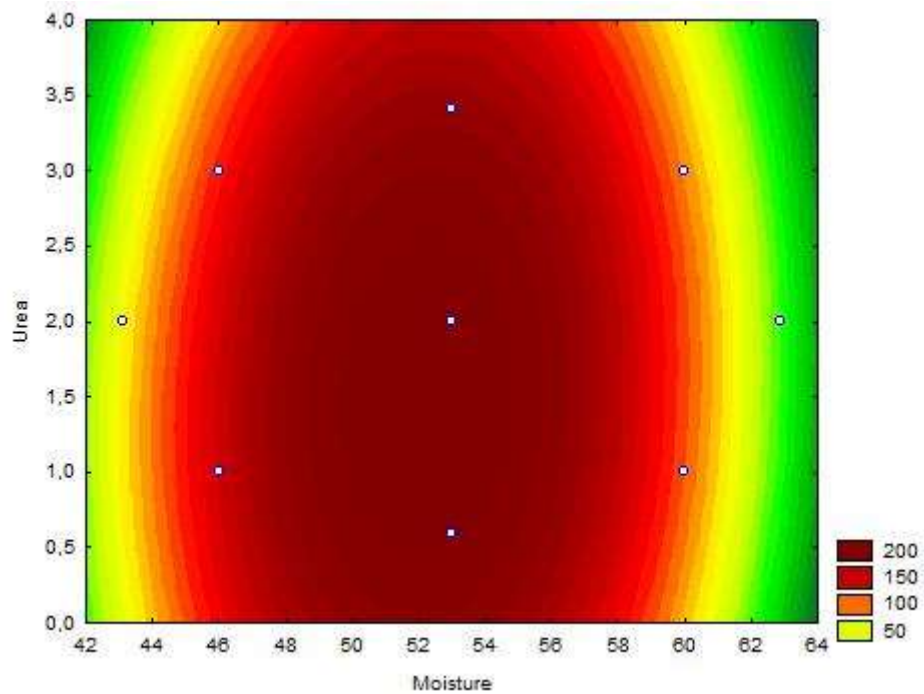
**Figure S1.** Pareto diagram of the effects of independent variables on lipase production in SSF using macauba fruit cake (24 h).



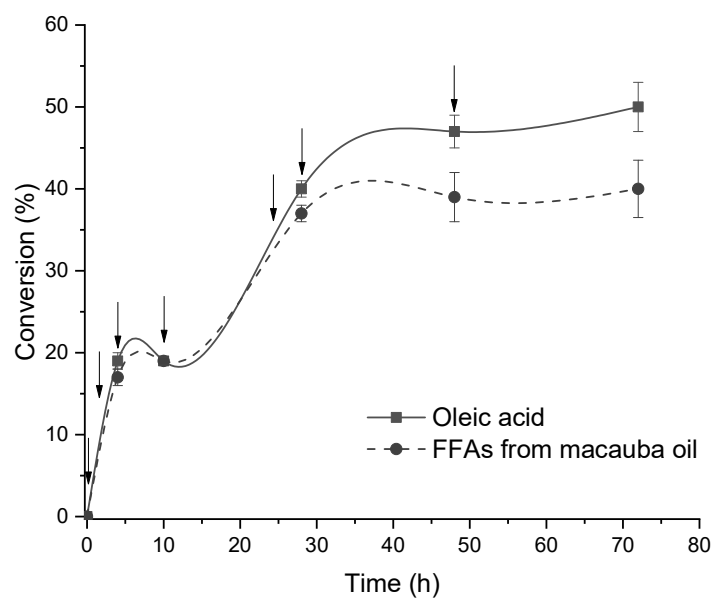
**Figure S2.** Response surface of lipase production as a function of moisture and urea contents using macauba fruit cake.



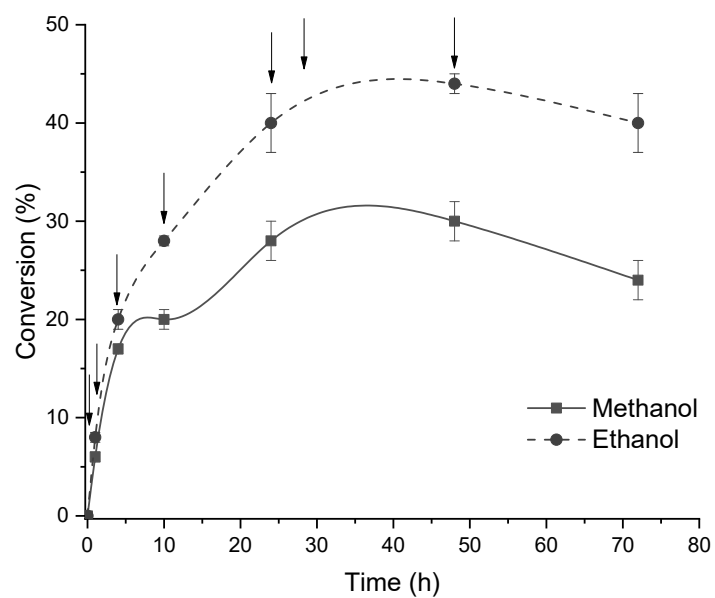
**Figure S3.** Pareto diagram of the effects on lipase activity in SSF using macauba pulp and peel cake (20 h).



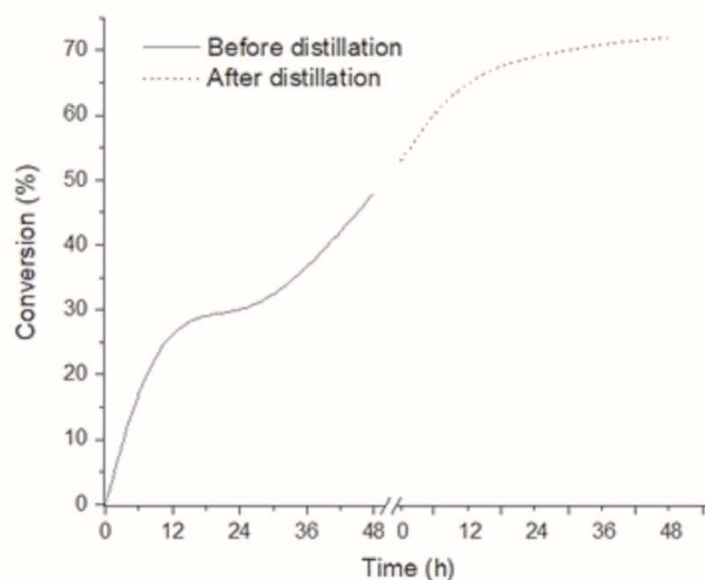
**Figure S4.** Response surface of lipase production as a function of moisture and urea contents using macauba pulp and peel cake.



**Figure S5.** Enzymatic esterification of FFAs over 72 h of reaction at 37 °C, 5 U/g enzyme load, 1:2 (alcohol:acid) molar ratio. Stepwise addition of ethanol at times 0, 1, 4, 10, 24, 28 and 48h.



**Figure S6.** Enzymatic esterification of oleic acid over 72 h of reaction at 37 °C, 15 U/g enzyme load, molar ratio 1:1. Stepwise addition of methanol and ethanol at times 0, 1, 4, 10, 24, 28 and 48h.



**Figure S7.** Esterification reactions of macuba acidic oil with ethanol as acyl-acceptor. After 48h reaction the water was withdrawal by distillation and esterification reaction continued for 48h. Reaction conditions: 15 U/g enzyme load, 1:3 molar ratio (acid:alcohol), at 29 °C.