OPTIMIZATION OF SOLID-STATE CULTURE MEDIA FOR PRODUCTION OF ENZYME EXTRACT SUITABLE FOR CHLOROGENIC ACID EXTRACTION

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Introduction. The chlorogenic acid (ClA) presents anti-oxidant, anti-viral, anti-bacterial and anti-fungical activities[1]. This compound is hydrolyzed by chlorogenate esterase enzyme activity (CEA)[2]. The aim of this work was to determine optimal culture conditions for production of enzymatic extract that presents pectinase (PecA) and xylanase (XylA) activities required for ClA extraction from coffee pulp (CP) and avoiding the production of CEA.

Material and methods. Assays were realized in fermentation columns with a mixture of 1/3 of CP, 1/3 of olive cake and 1/3 sugarcane bagasse (mesh 16). The culture medium was (100g *SS*): maltose, 3.5 g; oligoelements solution 0.55 ml. The support/substrate (*SS*) was inoculated with 1×10^8 spores/g *SS,* 60% humidity at 2 VKgM at 35ºC. A central composite design was designed with the software STATISTICA 14th version. The assays factors were the concentrations of sucrose, $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$. The response variables were PecA, XylA and CEA.

Results. Table 1 shows the PecA, XylA and CEA measured in the central composite design. The PecA and XylA increased as concentration of sucrose increased from 2.9 to 5g/100g *SS*. The CEA decreased when the concentration of sucrose increased.

Table 1. Response variables measured in the CCD

A	B	C	PecA	XylA	CE
(g/100g)				(U/g DM)	
2.9	2.5	2.5	40.9 ± 0.7	22.9 ± 0.3	0.22 ± 0.01
3.5	1.5	1.5	40.9 ± 0.2	26.4 ± 1.3	0.26 ± 0.01
3.5	1.5	3.5	49.0 ± 0.8	27.2 ± 0.4	0.21 ± 0.00
3.5	3.5	1.5	47.2 ± 0.8	26.3 ± 0.7	0.20 ± 0.01
3.5	3.5	3.5	38.0 ± 1.1	23.3 ± 0.4	0.16 ± 0.01
5.0	1.1	2.5	53.8 ± 1.5	29.6 ± 0.6	0.16 ± 0.02
5.0	2.5	1.1	43.3 ± 0.0	25.4 ± 0.1	0.16 ± 0.01
5.0	2.5	2.5	64.8 ± 3.0	34.6 ± 0.6	0.16 ± 0.00
5.0	2.5	2.5	64.6 ± 1.3	34.2 ± 0.7	0.16 ± 0.01
5.0	2.5	3.9	64.2 ± 0.4	33.0 ± 1.3	0.15 ± 0.01
5.0	3.9	2.5	63.9 ± 0.5	33.4 ± 0.6	0.16 ± 0.00
6.5	1.5	1.5	51.1 ± 0.5	28.6 ± 0.2	0.14 ± 0.01
6.5	1.5	3.5	48.9 ± 0.3	28.0 ± 0.3	0.15 ± 0.02
6.5	3.5	1.5	57.9 ± 0.6	30.3 ± 1.7	0.13 ± 0.01
6.5	3.5	3.5	51.5 ± 0.7	27.7 ± 1.7	0.13 ± 0.01
7.1	2.5	2.5	56.2 ± 0.2	30.6 ± 0.1	0.12 ± 0.02

A: Sucrose; B: $(NH_4)_2SO_4$; C: $(NH_4)_2HPO_4$. Assays for duplicate

Figure 1 showed the response surface of central composite design for PecA and AXyl.

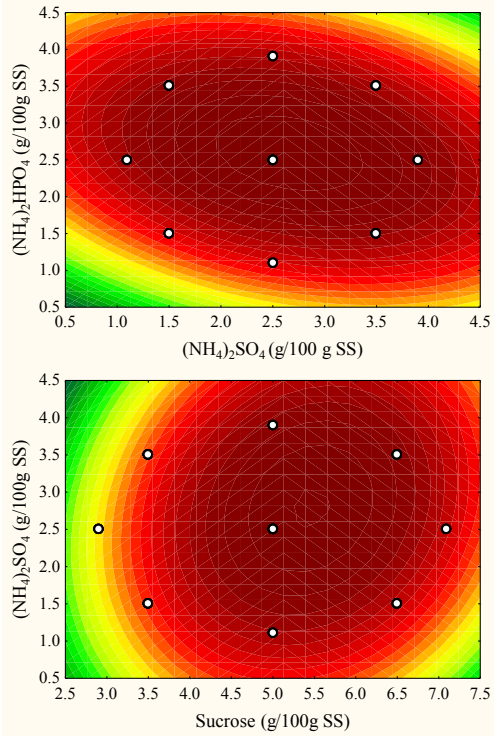

Fig.1 Contours levels plot for PecA and XylA

The intervals which presented the higher zone of production of PecA and XylA were (g/100g *SS*): 5 ≤ Sucrose ≤ 6; 2 ≤ $(NH_4)_2SO_4$ ≤ 3.3; 2.1 ≤ $(NH_4)_2HPO_4$ ≤ 2.9. The intervals which presented the lower zone of production of CEA were (g/100g *SS*): 5.4 ≤ Sucrose ≤ 7.5; 2.5 ≤ $(NH_4)_2SO_4$ ≤ 4; 2.5 ≤ $(NH_4)_2HPO_4$ ≤ 4.

The optimal culture conditions for production of enzyme extract was as followed: (g/100g *SS*): sucrose, 5.5; $(NH_4)_2SO_4$, 2.8 and $(NH_4)_2HPO_4$, 2.6. The enzyme extract allows the extraction of 68% of ClA esterified from CP.

Conclusions. The interval in which the higher zone of PecA and XylA was presented is within in the interval in which is presented the lower zone of CEA. The enzyme extract produced under the optimal culture conditions by SSF allow the extraction of 68% of ClA covalent linked to cell wall.

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References.

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