MIXTURE DESIGN AND RESPONSE SURFACE METHODOLOGY FOR IMPROVING ENZYMATIC EXTRACTION OF CHLOROGENIC ACID

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Introduction. The chlorogenic acid (ClA) has various pharmacological applications such as anti-oxidant, anti-viral, anti-bacterial and anti-fungical[1]. Enzymatic extraction of ClA is selective and harmless to the environment compared with other extraction methods, obtaining a natural label product. The aim of this work was to determine the effect of depolymerizing enzyme activities as pectinase (PecA), xylanase (XylA) and cellulase (CellA) on the extraction of ClA from coffee pulp (CP), using an mixture and response surface methodology.

Material and methods. Assays were realized with 5 g of dried coffee pulp (mesh 100), 15 ml of water and 5 ml of phosphate buffer 100 mM at pH 5. The concentrations used (U/g dried CP) were 0 – 200 of each enzyme PecA, XylA and CellA. Enzymatic extraction was performed at 40°C and 100 rpm during 8 h. The mixtures and surface methodology was designed with the software STATISTICA 14th version.

Results. Table 1 shows the results of each enzyme extract assayed by triplicate.

Table 1. Enzymatic extraction of ClA

A	B	C	CIA (mg/kg)
1	0	0	1053.2 ± 17.5
0	1	0	1116.6 ± 15.4
0	0	1	243.5 ± 3.3
0.5	0.5	0	1638.0 ± 20.5
0.5	0	0.5	1107.5 ± 6.3
0	0.5	0.5	1077.3 ± 15.52
0.67	0.17	0.17	1170.0 ± 16.7
0.17	0.67	0.17	1137.2 ± 2.11
0.17	0.17	0.67	920.6 ± 1.35
0.33	0.33	0.33	1302.1 ± 0.3
0	0	0	187.7 ± 0.3

A: PecA; B: XylA; C:CellA; 0: 0 U/g dried CP; 1: 200 U/g dried CP

The enzymatic extract with 100/100 U per g of CP of pectinase/xylanase activities allow to extract 68.7% of ClA from CP.

The quadratic model with F = 29.8, α<0.001 and R^2 of 0.912 has the best fit with the experimental data[2].

$$Y= 1038.1A + 1089.5B + 284.9C + 1735.8AB + 1496.9AC + 1225.3BC \quad \text{(Eq. 1)}$$

Where: A: PecA; B: XylA; C: CellA

Figure 1 shows the contour levels plot from simplex centroid mixtures design for enzymatic extraction of ClA calculated with Eq. 1.
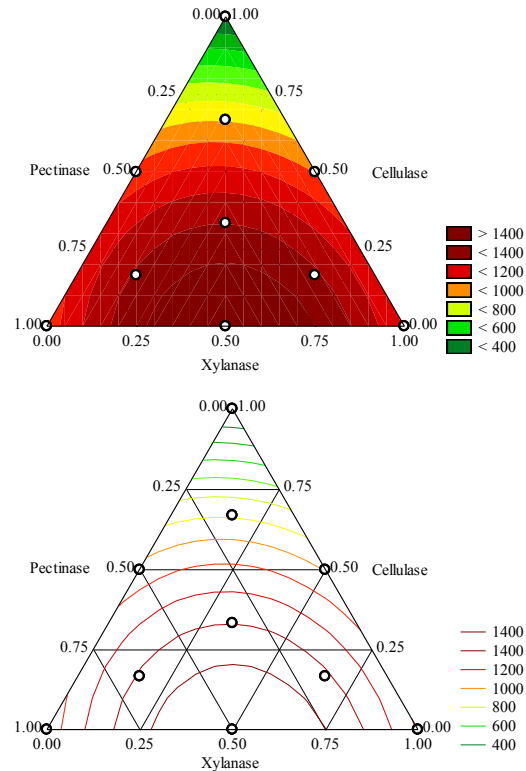

Fig.1 Contour levels plot for enzymatic extraction of ClA.

The enzymatic extraction of ClA increased as activities of Pec and Xyl increase and as activity of Cel decrease. The intervals that present the higher zone of extraction of ClA were: 0.3 ≤ Xyl ≤ 0.75, 0.25 ≤ Pec ≤ 0.70, 0 ≤ Cell ≤ 0.20.

Conclusions. The centroid simplex mixture design showed that the presence of pectinase and xylanase activities had a positive effect on the enzymatic extraction of chlorogenic acid.

References.
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2. Montgomery D.C. (2006). Diseño y análisis de experimentos. En *Experimentos con mezclas*. LIMUSA, México.