

## **Obtaining of Bioactive Hydrolysates from Protein of Californian Red Worm (*Eisenia fetida*) Through Enzymatic Hydrolysis and Crossflow Filtration.**

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The objective of this study was to optimize the enzymatic hydrolysis of California red earthworm meat to obtain peptides with antioxidant capacity and it's scaled up from laboratory scale to bench scale, and then to separate it in 3 and 1kDa fractions in a membrane system of cross-flow filtration. The worms were manually separated, washed with water, purged for 4 hours with 4% sodium bicarbonate, then sacrificed using saline solution. The optimization of the hydrolysis used a spherical composite central response surface design with five points at the center using 4 factors, pH (7-9), temperature (40-60°C), substrate (100-200g) and alkalase enzyme (500-1500uL) and as response variables, soluble protein percentage (PP), degree of hydrolysis (DH), antioxidant capacity (AC) ABTS and FRAP, implementing a 7.5 L reactor. The procedure to achieve the dimensional analysis consists of three steps: Listing important variables, verifying dimensional homogeneity by transferring it to a dimensionless form and determining dimensionless Pi numbers from a transformation matrix, Pi theorem. Fractionation was performed using 7-channel membranes with molecular weight cut-off of 3 and 1 kDa. The optimal hydrolysis conditions are pH 8.5, temperature 45°C, with 125.01g substrate and 1243uL of enzyme, obtaining DH of 16.52%, PP of 3.38% and AC of 2055 and 170  $\mu\text{mol-eq trolox/g}$  protein for ABTS and FRAP, respectively. Additionally, the optimal hydrolysate has an ORAC of 823 $\mu\text{mol-eq trolox/g}$  protein and iron chelation with IC50 at 150ppm. The dimensional analysis of the hydrolysis process from 0.5L to 7.5L showed that the dimensionless number for the scale-up is the Reynolds, the scaling was performed with geometric similarity modifying the impeller speed which went from 240 rpm in 0.5L to 122.45rpm in 7.5L. The purification of the peptides by means of the membrane system concentrated the proteins of the retained with respect to the initial fluid, while the concentration of the permeate is significantly lower compared to the original fluid, both in the 3 and 1kDa membrane. It is concluded that the enzymatic hydrolysate of Californian red worm has a high AC and a low IC50 in iron chelation, making it a substrate of interest for application in different industries.