ORT_29 - Production of a recombinant L-asparaginase and an immobilized biocatalyst to decrease the carcinogenic potential of French fries

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Introduction: When producing foods such as French fries, crackers and roasted coffee, the heat treatment above 120 °C induces the Maillard reaction that can produce acrylamide. Acrylamide is classified as probably carcinogenic to human, a neurotoxic and genotoxic compound derived from the reaction between short-chain reducing sugars and L-asparagine (Asn), through the Maillard reaction. After various studies involving the human dietary exposure to acrylamide, the World Health Organization (WHO) recommends the development of methods to mitigate the presence of acrylamide in largely consumed foods, such as the application of L-asparaginase (L- ASNase). The enzyme L-ASNase can hydrolyze Asn in foods before the heat treatment, thus preventing the formation of acrylamide.

Objectives: This work aims to develop a fermentation procedure to obtain high L-ASNase yield in the fermented broth through recombinant expression of L-ASNases in *Escherichia coli* cells. The development of a biocatalyst through L-ASNase immobilization should enable the reuse of the enzymes in French fries' production.

Methodology: The genes *ansB* and *ansZ*, encoding *E. coli* (EcAII) and *Bacillus subtilis* L-ASNases II (BsAII) respectively, were cloned in constitutive expression vectors, containing a signal sequence for periplasmic transport. Sequences for His-tags were added upstream (HisN) and downstream (HisC) of the genes. The plasmids were cloned into *E. coli* cells and fermentations were performed in Falcon tubes and Erlenmeyer flasks. The immobilization procedure with EcAII was performed through covalent bonding in the mesoporous silica Santa Barbara Amorphous-15 (SBA-15). Operational stability experiments were performed to evaluate the biocatalyst's reuse capacity.

Results: Through Western Blotting, it was showed that both expressed HisC constructs, EcAII and BsAII, had no affinity for an anti-poly-histidine antibody, while HisN constructs were marked. Besides, BsAII HisC had no enzyme activity. The fermentation process was then conduced only with HisN constructs. The enzyme production on the fermented broth was successful, but it was higher in Falcon tubes, reaching 14800 $U \cdot L^{-1}$, than in Erlenmeyer flasks, reaching 275 $U \cdot L^{-1}$. Covalent immobilization of EcAII on SBA-15 was successful, reaching 97% global yield. After 101 cycles of operation at 37 °C, the biocatalyst retained more than 80% of its initial activity.

Conclusion: An efficient method for recombinant L-ASNase production was developed, obtaining the enzymes directly from the fermented broth. The developed biocatalyst showed great performance and feasibility to industrial application in French fries' production.

Keywords: Acrylamide; Enzyme Production; Enzyme Immobilization