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ALCALASE IMMOBILIZATION FOR TILAPIA SKIN GELATIN HYDROLYSIS

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ABSTRACT

The objective of this study was to investigate the alcalase immobilization on three different supports and their effect on the hydrolysis of tilapia gelatin. The study of alcalase immobilization contributes to biocatalysts reuse, to prevent the active sites from the self-degradation process and to facilitate the viability of industrial application of these biocatalysts reaction. In this work, glyoxil-agarose-alcalase showed to be the more stable derivate promoting hydrolysates with equal antioxidant activity even in the third reaction cycle.

1. INTRODUCTION

Proteases are the most important category of industrial enzymes, and they are becoming increasingly popular catalysts for peptide bond formation or hydrolysis. Unfortunately, their industrial application is often hampered by the lack of operational stability (Corici et al., 2011). The immobilization process is considered as more efficient and they have benefits in relation to the reuse and the stability of the enzymes, besides preserving the active sites of proteases against the process of self-degradation (Faria et al., 2016).

Some studies have investigated several supports for protease immobilization (Corici et al., 2011; Faria et al., 2016; Yust et al., 2010) and studied their performance in reactions. Corici et al. (2011),

for example, utilized sol-gel matrices to immobilize alcalase by entrapment. High degree of immobilization, reproducibility and efficiency in the synthesis of Z-Ala-Phe-NH2 was observed to alcalase entrapped in dimethyldimethoxysilane (DMDMOS) or tetramethoxysilane (TMOS). Also, alcalase-glyoxyl derivative investigated by Yust et al. (2010) showed to be a good biocatalyst to chickpea protein hydrolysis producing hydrolysates with better functional properties then protein isolate. In the present study, alcalase immobilization was evaluated on three different supports and their effect on the hydrolysis of tilapia gelatin.

2. MATERIAL AND METHODS

2.1. Material

Tilapia skin gelatin was donated by Embrapa Tropical Agroindustry and Alcalase 2.4L® by Novozyme. Immobead 350 was acquired from ChiralVision and agarose (Sepharose CL-6B) was purchased from GE Healthcare. Glycidol, DEAE-sepharose, N-Boc-L-alanine p-nitrophenyl ester (BANE) and 2,2- diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, EUA). All other chemicals were of analytical grade.

2.2. Immobilization procedure for three different supports

For immobilization in DEAE-sepharose, alcalase was immobilized by adsorption as defined by Faria et al. (2016). The immobilization on Immobead 350 was carried out by the formation of covalent bonds, for 24 hours, using the epoxy groups present on its surface. The immobilization on Glyoxil-agarose was performed by covalent interactions, after 96 hours, with the aldehyde groups inserted onto the surface. Proteolytic activity of free and immobilized alcalase was determinate by BANE hydrolysis and immobilization parameters were calculated (Faria et al., 2016).

2.3. Gelatin hydrolysis

Tilapia skin gelatin was hydrolyzed under controlled temperature (55°C) and pH (6) for 2 hours using an immobilized alcalase mass equivalent to 50U for system (Faria et al., 2016). At end of each reaction, derivate was filtrated, washed and dryed for a new reaction cycle. Sample of hydrolyzed gelatin was collected and antioxidant activity (DPPH) and peptidic profile analyzed. DPPH radical scavenging activity of hydrolyzed gelatin was tested according to the previously described method (Faria et al., 2016). RP-HPLC was used to analyze the peptidic profiles generated from tilapia gelatin hydrolysis. An analytical HPLC unit from Jasco (Jasco Corporation, Japan) with a Hypersil BDS C18 column (Thermo, USA) was used. The wave length used for detection was of 216 nm (Jasco UV 2077 Plus). Parameters of analysis were carried out as reported by Mellinger-Silva et al. (2015).

3. RESULTS AND DISCUSSION

Three different supports/methods were used to alcalase immobilization and parameters were reported in table 1. Since the immobead 350 is a support of high porosity, the protein load was higher for it than for glyoxil-agarose or DEAE-sepharose. However, greater activity was observed for DEAE. Immobilization yields obtained were greater than 90% for immobead and glyoxil-agarose and could be compared to Corici et al. (2011) that obtained values of protein immobilization in the range of 68 - 98% for alcalase immobilized by physical entrapment in glass sol-gel matrices. And, after application of immobilized alcalase in gelatin hydrolysis reaction, glyoxil-agarose-alcalase showed to be the more stable derivate promoting hydrolysates with equal antioxidant activity even in the third reaction cycle (Figure 1).

Table 1. Immobilization parameters for alcalase onto different supports.

	Derivative Activity (U/g)	Immobilized Protein (mg/g)	Immobilization Yield(%)	Recovered Activity (%)
Immobead 350	57.13	12.48	99.2	7.0
Glyoxil-Agarose	25.36	2.00	93.1	20.2
DEAE-Sepharose	116.5	1.63	*	*

* It was not possible to calculate.

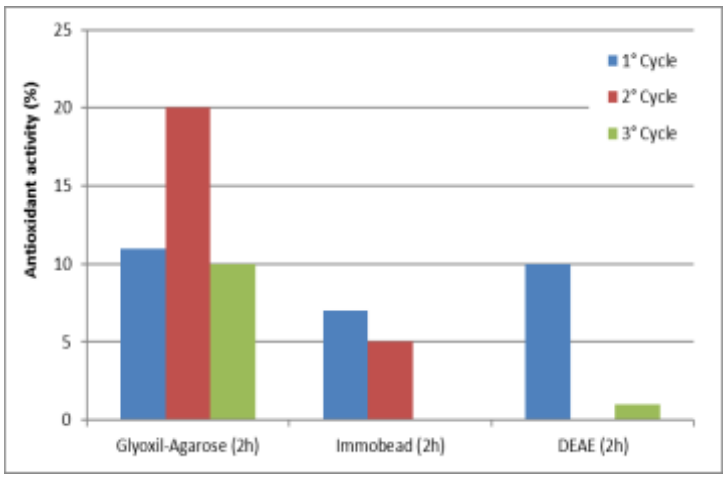

Figure 1. Antioxidant activity of hydrolyzed gelatin after 2 hour of hydrolysis reaction catalyzed by immobilized alcalase and recycles.

Analyzing the peptidic profile through RP HPLC (Fig. 2) it is possible to observe that the free enzyme (Fig. 2B) was able to broadly hydrolyze the main proteins from the gelatin (Fig. 2A), generating a

rich peptidic profile, formed by many peaks in lower intensity. Comparing the chromatograms of the free (Fig. 2B) and different supports of immobilization (Fig. 2 C-E), it is possible to observe that the peptidic profiles were quite different among themselves, as all the immobilizations showed the main peaks in different retention times, although DEAE and Glyoxil-Agarose immobilized alcalase had showed similar antioxidant activity (Fig. 1). The difference between free and immobilized alcalase may be related to a decreased affinity of enzyme for its substrate, probably caused by an increase in mass transfer limitation for substrate as observed by Corici et al. (2011).

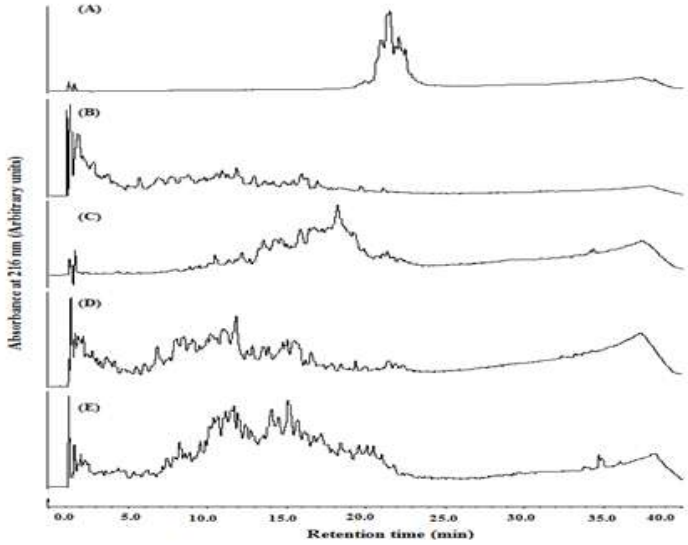

Figure 2. RP HPLC chromatograms of gelatin hydrolysates after 2h-reaction. (A) Gelatin solution, control sample; (B) Free alcalase; (C) Immobead-alcalase; (D) Glyoxil-Agarose-alcalase; (E) DEAE-alcalase.

4. REFERENCES

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