Influence of biopolymers in the thermal stability of the L-Asparaginase

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ABSTRACT

L-asparaginase (L-ASNase, L-asparagine-amydohidrolase, EC 3.5.1.1) is an enzyme widely used in both food and pharmaceutical industries. The enzyme, however, exhibits low enzymatic activity and several characteristics that hinder its use, such as low thermal stability and difficulties in solid substrate adherence. The focus of this study was to evaluate the influence of two different biopolymers, namely Alginate and Methylcellulose (Methocel), in the L-ASNase thermal stability to develop new formulations for industrial purposes. The biopolymers were dispersed in pH 7.0 PBS solution at 0.25 mg/mL and 0.5 U mL^{-1} of L-ASNase, the solution was then incubated at 55 ºC for one hour and the enzymatic activity was analyzed by the Nessler colorimetric method. It was possible to conclude that Alginate does not significantly influence enzymatic activity at the tested condition, showing the same relative activity as the control (61%), while Methylcellulose was able to maintain enzymatic activity at 98% of the initial activity. Therefore, the biopolymer in question is capable of increasing the L-asparaginase thermal stability at 55 ºC and may be useful for improving the efficiency and cost of industrial enzymatic processes.

Keywords: L-asparaginase. Biopolymers. Thermal stability.

1 INTRODUCTION

Enzymatic technology has been extensively studied and improved with the primary objective of modifying and enhancing the efficiency of enzymes that have various applications. One such enzyme of note is L-asparaginase (L-ASNase, L-asparagine-amydohidrolase, EC 3.5.1.1), which holds significant potential in the biopharmaceutical industry, due to its efficacy as an oncological agent, and in the food industry, primarily for its ability to mitigate the formation of acrylamide, a carcinogenic compound found in many processed foods such as coffee and french fries. Its activity in both contexts is linked to the catalysis of the L-asparagine hydrolysis into L-aspartate and ammonia. Consequently, it has high global demand and significance in the enzyme market.[1, 2, 3]

However, both industries face some obstacles related to the properties of this enzyme, such as its stability, half-life, and thermostability. Temperature, for instance, directly affects its catalytic potential and stability.[4] Typically, its optimal activity temperature ranges between 25°C and 45°C depending on the species.[2]

Certain components can directly influence the enzymatic activity of L-ASNase, such as cellulose-derived biopolymers, for example, which can be dispersed and included in an enzymatic solution to modify its properties, thus potentially improving its thermal stability.[5] Therefore, the exploration of formulations combining enzymatic and biopolymeric solutions becomes of significant interest for research as it can directly impact the efficiency of enzymatic reactions, optimizing such processes and reducing production costs. Thus, this study aimed to evaluate the influence of Alginate and Methylcellulose (Methocel) in the thermal stability of commercial L-ASNase.

2 MATERIAL & METHODS

The L-ASNase solution (1 U mL^{-1}) was diluted in the same volume (1:1) of each biopolymer, alginate and methylcellulose, and then let at room temperature for 30 min. After that, these samples and the positive control (without the biopolymers) were submitted to a thermal treatment at 55°C for 1h in a heating dry bath (Kasvi®). A negative control was left at room temperature for the same time.

After the thermal treatment, those samples were submitted to a Nessler colorimetric quantification to check the enzyme activity, based on the ammonia release due to the asparagine hydrolysis. The first step consisted of the L-asparagine hydrolysis at 37°C for 10 min, which was realized by mixing 150 µL of Tris-HCl pH 8.6, 150 µL of the enzymatic sample and 15 µL of L-asparagine 189 mM solution. The reaction was stopped by the addition of 75 µL trichloroacetic acid (TCA) 1.5 M solution and then centrifuged. In a new microtube, it was added 270 µL of Tris-HCl pH 8.6, 270 µL of the hydrolysis samples and 135 µL of Nessler reagent. After 30 min the colorimetric quantification was realized at 465 nM in a microplate reader (Multimode Plate Reader-EnSpire, PerkinElmer) and the relative activity was measured.

The residual activity of the L-ASNase was calculated based on comparing the enzyme's activity before and after the thermal treatment by Eq 1.

$$Residual\ Activity\ (\%) = \frac{Activity\ after\ treatment}{Initial\ activity} \times 100 \qquad (1)$$

3 RESULTS & DISCUSSION

The results obtained are presented in Figure 1. The Enzyme + buffer control without heating represents 100% of the enzyme activity. From this data, we have the relative enzymatic activity for the other tests. Initially, the Enzyme + buffer control with heating (positive control) showed a relative activity of 61.35%, similar to the value obtained by the solution containing Alginate, which indicates this biopolymer has not shown influence in the L-Asparaginase thermal stability at 55 ºC at the used concentration (0.25 mg mL^{-1}). Regarding the results from the Methocel-added enzymatic solution, it is possible to notice an outstanding increase in thermal stability, maintaining 98% of the enzymatic activity relative to the negative control.

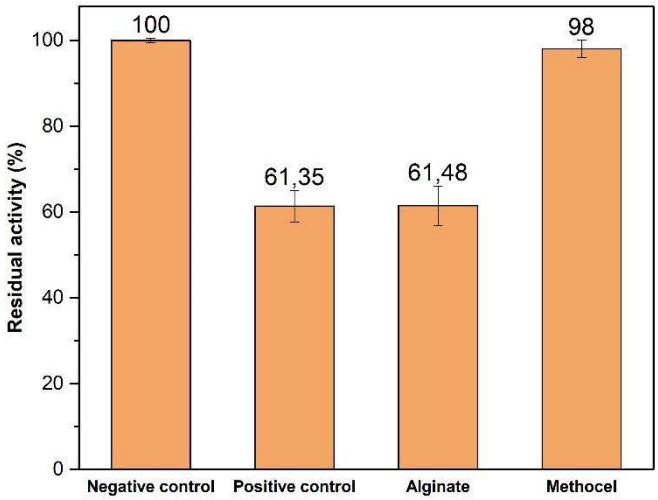

Figure 1 – Relative L-Asparaginase activity in the presence of biopolymers after 1 h treatment at 55 ºC. Negative control: room temperature. Positive control: Enzymatic solution without biopolymers. Error bars: standard deviation

From these results, it is possible to note that Methocel biopolymer at a concentration of 0.25 mg/mL was able to maintain enzymatic activity under heating causing some protection to the enzyme regarding thermal denaturation, while the Alginate solution, as also the enzymatic solution without biopolymers, lost almost 39% of L-ASNase activity when subjected to 55 ºC for 1 h. These results indicate the potential of using Methocel biopolymer in new enzymatic formulations able to contribute to the development of new bioprocesses in food and pharmaceutical industries.

4 CONCLUSION

Based on the obtained results, it can be concluded that exposure to a temperature of 55°C indeed negatively influences the enzymatic activity of L-asparaginase, which loses approximately 39% of its activity when exposed for 1 hour. However, upon adding a biopolymeric solution of Methocel at 0.25 mg mL^{-1} to the enzymatic solution it is observed that after the thermal incubation, its enzymatic activity is maintained almost equal to that of the control that was not exposed to heat degradation (98%). Therefore, the exploration and application of biopolymeric compounds in enzymatic formulations could be an excellent tool to improve enzymatic processes, making them more efficient and reducing, to some extent, the production and utilization costs of this enzyme widely used in both the biopharmaceutical and food industries.

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