Effect of different inoculation media on L-asparaginase produced by *Aspergillus niger* F.00721

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INTRODUCTION

Being a natural compound, acrylamide, a probable human carcinogen, is formed when the temperature exceeds 120 °C, through the Maillard reaction (L-asparagine reacts with reducing sugars) [1].

L-asparaginase hydrolyzes the amide group of the side chain of asparagine, resulting in aspartic acid and ammonia. This enzyme displays potential in the food industry because of the reduction of consumed acrylamide in starchy food such as potatoes, toast, root vegetables, etc. [2]

Fungal L-asparaginase is positively enthralling to the food industry, on account of filamentous fungi transpiring as enzyme-producing powerhouses. Optimization of the process is the first step toward accomplishing the goal of mass application. This research focused on the effect of different inoculation media on the activity of L-asparaginase produced by the *Aspergillus niger* F.00721 strain.

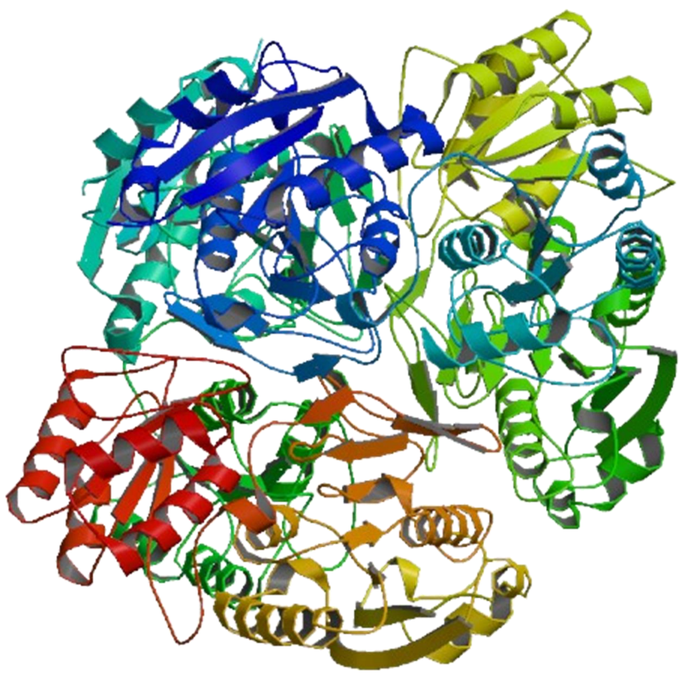

Figure 1. Structure of L-asparaginase [3]

MATERIALS AND METHODS

A. niger F.00721 strain was obtained from the National Collection of Agricultural and Industrial Microorganisms (NCAIM).

Four different fermentation media (modified Czapek-Dox media) were used to compare the effects of opposing inoculation media, as depicted in Table 1.

Inoculated samples were incubated in an orbital shaker at 28℃, 200 rpm for 48 hours. Submerged enzyme fermentation was prepared in 500 ml Erlenmeyer flasks, and incubated at 28℃, 200 rpm for 72 hours.

Enzyme activity assay was done with the Nesslerization method from the cell-free filtrate of the crude enzyme solution. Enzyme activity was measured in a spectrophotometer by determining the absorption at 450 nm.

Table 1. Inoculum and fermentation media used for the enzyme assay

Inoculation media	Fermentation media
Potato Dextrose Broth (PDB) - potato infusion and dextrose	MCDAG (1% asparagine, 0.2% glucose)
	MCDA (1% asparagine, 0% glucose)
Czapek-Dox Broth (CDB) - sucrose, $NaNO_3$, KH_2PO_4, $MgSO_4$, KCl, and $FeSO_4$	MCDPG (1% peptone, 0.2% glucose)
	MCDP (1% peptone, 0% glucose)

RESULTS AND DISCUSSION

The *Aspergillus niger* F.00721 strain synthetized L-asparaginase in all investigated media, and the results are presented in Figure 1. In the case of supplementation with 1% L-asparagine and 0.2% glucose, 0.207 U/ml and 0.011 U/ml L-asparaginase activities were assayed in the case of potato dextrose broth (PDB) and Czapek-Dox broth (CDB), respectively. Low enzyme activities were detected in the cultures without added glucose; 0.070 U/ml (CDB) and 0.165 U/ml (PDB).

Meanwhile, 0.069 U/ml for CDB was detected for the sample with 1% peptone and no glucose, whereas 0.174 U/ml was for PDB. This trend was not observed in the case of 1% peptone and 0.2% glucose supplementation, where 0.168 U/ml and 0.132 U/ml were detected for CDB and PDB, respectively.

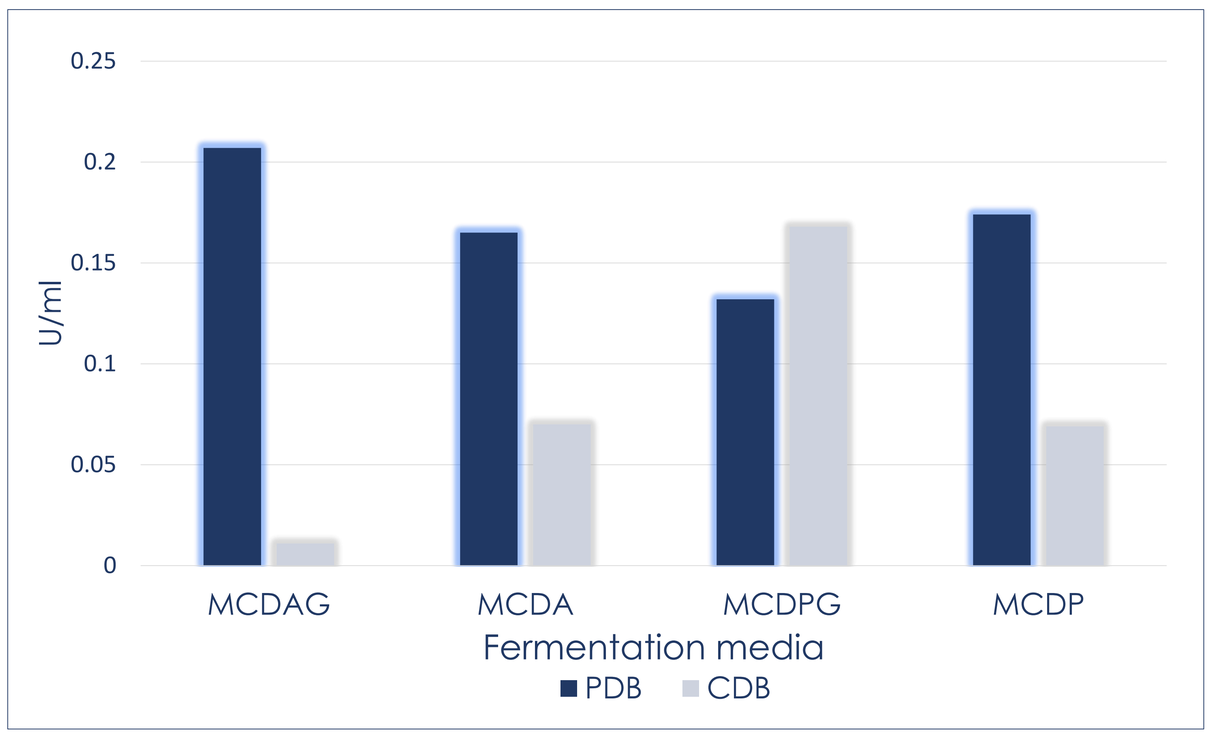

Figure 2. Results of the enzyme assay representing the relationship between two inoculation media on the enzyme activity measured from four fermentation media

CONCLUSIONS

The effort was made to determine the effects of different inculcation media on the fermentation process to obtain the L-asparaginase enzyme. In three out of four cases, potato dextrose broth displayed a higher enzyme activity. In the most extreme case, MCDAG, 18 times higher enzyme activity compared to Czapek-Dox Broth.

MCDPG was the single case where CDB had a 1.2 times higher enzyme activity compare to the PDB counterpart.

The results are preliminary, but they provide a good basis for the production of L-asparaginase to reduce acrylamide in foods.

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