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Optimization of L-asparaginase production by *Aspergillus niger* F.00721

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Introduction

The thermal process, or Maillard reaction, where reducing sugars and amino acids react at high temperatures to form other products, not just in starchy food, but in meat as well, is considered often desirable in the culinary arts for the flavor it imparts on food.

Naturally, cooking temperatures above 120°C cause a reaction between L-asparagine and reducing sugars resulting in the creation of acrylamide (Ciesarová, 2016).

The research supports a claim that acrylamide causes cancer in mice by interacting with the DNA, making it a probable cause of cancer in humans, as well.

The way to reduce its intake is by adapting the cooking and storing of food, as well as applying an enzyme treatment during production such as L-asparaginase. Decreasing the amount of L-asparagine, impulsively the acrylamide aggregate is reduced (Figure 2).

L-asparaginase has attracted significant attention in several investigations and in recent years fungi have occupied advanced rank among enzymes produced through microorganisms (Figure 1).

The fermentation with *A. niger* shows potential because it yields an exoenzyme, an extracellular enzyme, making it easier to extract and potentially cheaper for production (Aguirre-Pranzoni et al., 2011).

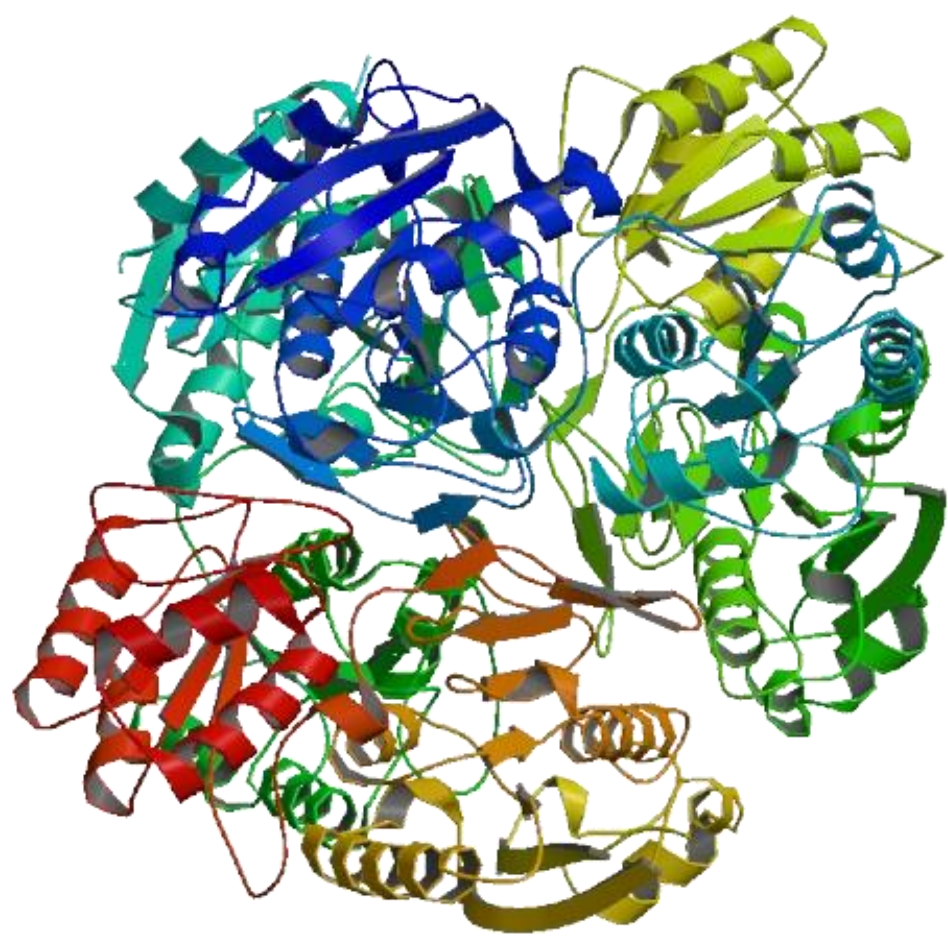

Figure 1. Structure of L-asparaginase (Pieters et al., 2010)

Materials and Methods

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

Culture inoculum was prepared in Potato Dextrose Broth, incubated in an orbital shaker at 28°C, 200 rpm for 48 hours.

Submerged enzyme fermentation was prepared with a modified Czapek-Dox medium in 250 ml Erlenmeyer flasks, and incubated at 28°C, 200 rpm for a period of time predetermined by the optimization step, between 24 and 120 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.

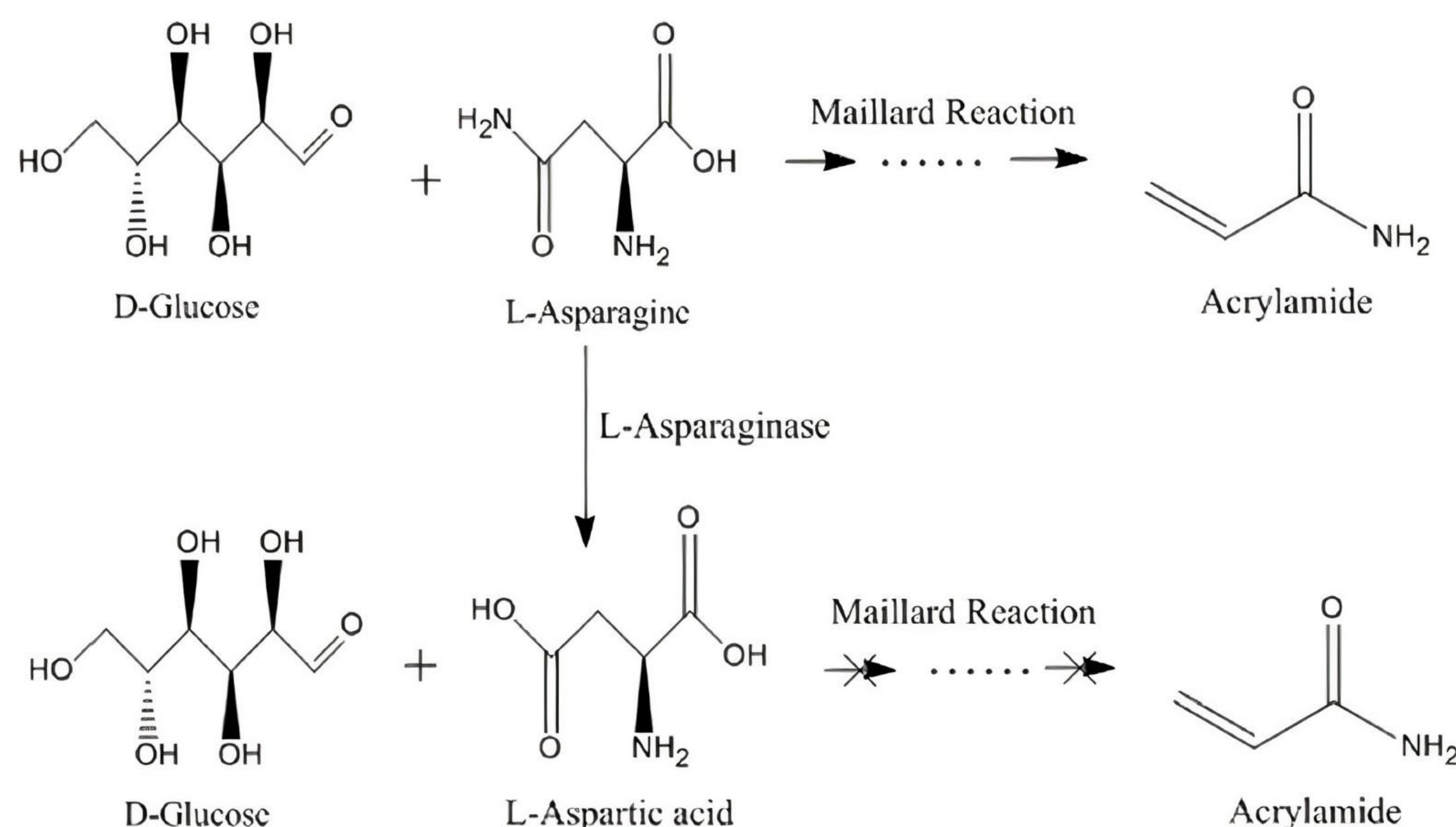

Figure 2. Effect of L-asparagine on acrylamide formation during Maillard reaction (Zuo et al., 2015)

Results

Four parameters were optimized during the experiments: substrate, carbon and nitrogen source, and the pH (Figure 3).

In the case of substrate optimization, L-asparagine was used (1-4%). The highest enzyme activity was with 1% of L-asparagine on the first day of fermentation. The activity was 0.207 U/ml.

Glucose was the optimized parameter for the carbon source (0-0.4%). The optimal conditions were in the sample with no glucose, on the first day with the value of 0.165 U/ml.

For nitrogen sources, yeast extract and peptone were tested in combination with different glucose concentrations. The highest values were in the case of 1% yeast extract and no glucose (0.212 U/ml) on the third day, and 1% peptone and 0.2% glucose (0.577 U/ml) on the fourth day.

pH was optimized, using Sörensen buffer (pH 5-8), with the fermentation medium being modified with 1% L-asparagine, 1% peptone, and 0.2% glucose, and the highest result was expressed on the fourth day in the case of pH 8 (2.0 U/ml).

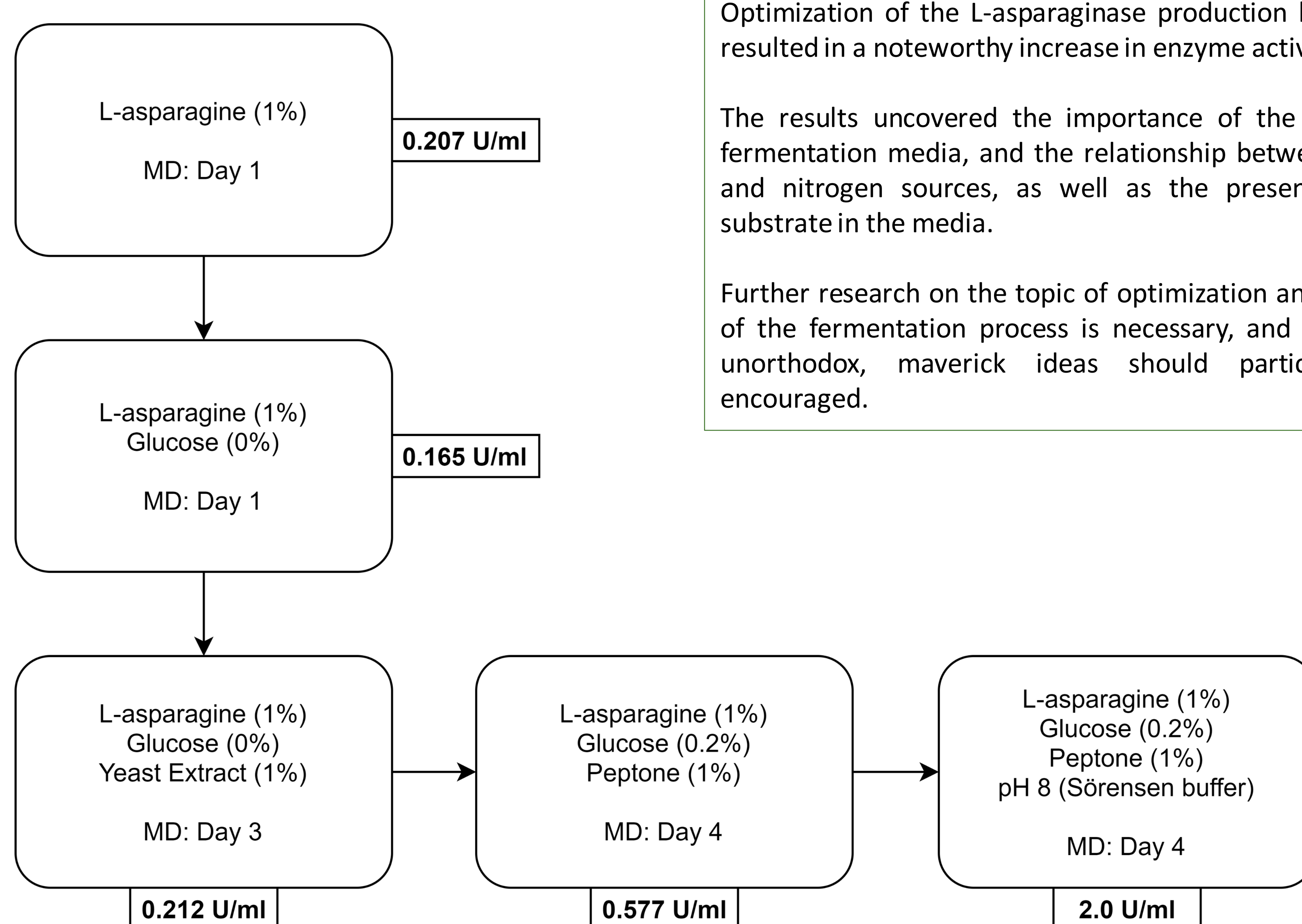

Figure 3. Flowchart of the optimization steps (MD: Fermentation day with maximum measured enzyme activity)

Discussion

Based on the results, the optimum concentration of the substrate in the fermentation medium (L-asparagine) is 1%.

Glucose was found to have an inhibiting effect on the enzyme activity, even in the presence of nitrogen sources, L-asparagine, and yeast extract. But in the case where 0.2% glucose was combined with 1% peptone, an inhibitory effect was not detected, and a carbon – nitrogen relationship was expressed with a 5-fold increase in the enzyme activity.

All the other tested parameters had a positive effect on the enzyme production, but the highest level of improval was found in the case of pH adjustment.

pH was optimized using the optimal values of the other variables. pH 8 displayed the highest enzyme activity with increased 10-fold compared to the initial values of the experiments.

Conclusions

Optimization of the L-asparaginase production by *A. niger* resulted in a noteworthy increase in enzyme activity.

The results uncovered the importance of the pH in the fermentation media, and the relationship between carbon and nitrogen sources, as well as the presence of the substrate in the media.

Further research on the topic of optimization and improval of the fermentation process is necessary, and the use of unorthodox, maverick ideas should particularly be encouraged.

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