Characterization of a commercial fungal L-asparaginase for acrylamide mitigation in thermally processed starchy food

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Introduction

Acrylamide formation in thermally processed food remains a critical food safety challenge, with its classification as a probable human carcinogen necessitating effective mitigation strategies. The compound arises via the Maillard reaction, where reducing sugars react with free L-asparagine at temperatures exceeding 120°C, particularly in potato-based and cereal products [1]. L-asparaginase offers a targeted solution by hydrolyzing L-asparagine before thermal processing, thereby preventing acrylamide from forming .

Commercial L-asparaginase preparations, such as those from *Aspergillus oryzae*, are favored for their GRAS status and compatibility with food matrices [2]. This study characterizes a Novozymes enzyme Acrylaway to define its pH and temperature optima, catalytic efficiency, and operational stability. By aligning enzyme performance with industrial processing parameters, the work aims to bridge laboratory findings with scalable food safety applications.

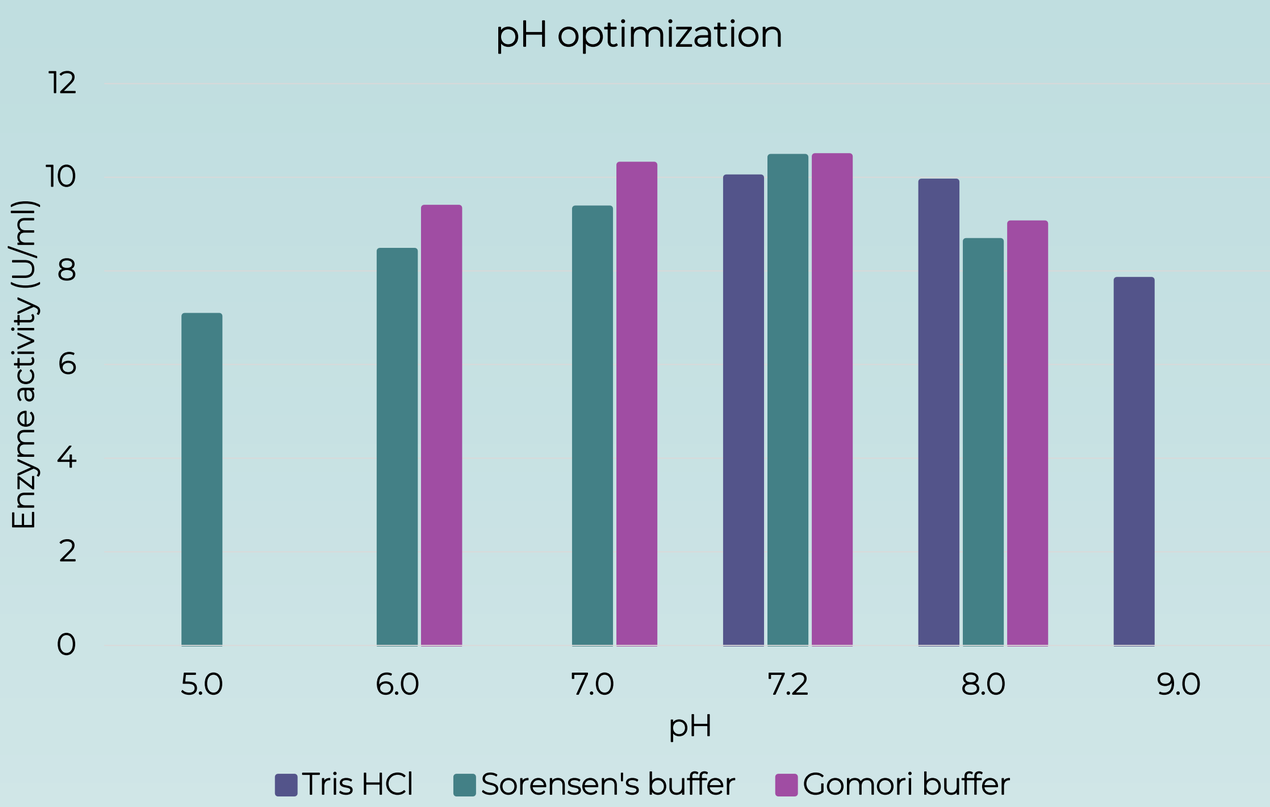

Figure 1. pH optimization of L-asparaginase

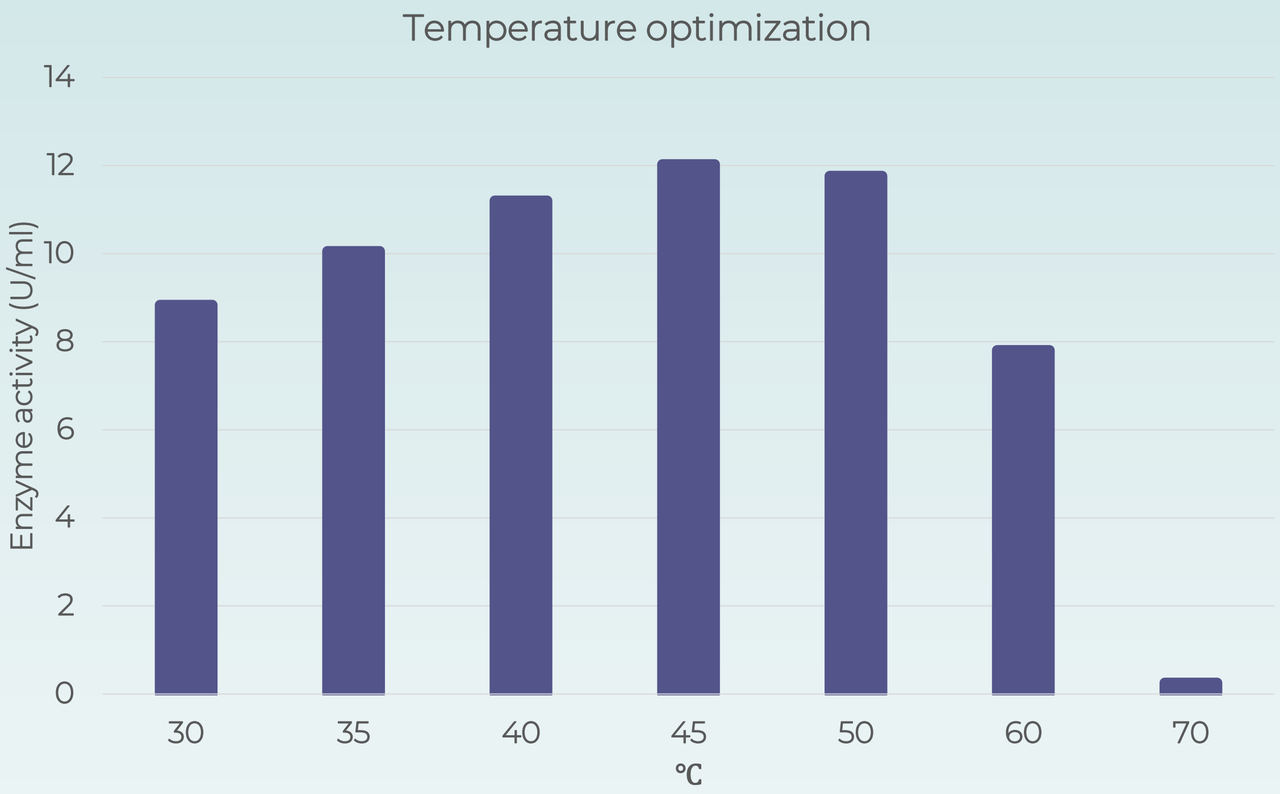

Figure 2. Temperature optimization of L-asparaginase

Methodology

The *Aspergillus oryzae*-derived L-asparaginase (Acrylaway - Novozymes) was prepared as a 10 mg/mL stock solution by dissolving 0.1 g enzyme powder in 10 mL distilled water, followed by 30-fold dilution for assays.

Enzyme activity was determined via Nesslerization: asparagine hydrolysis releases ammonia, which reacts with potassium mercuric iodide under alkaline conditions. pH optimization (5–9) used Tris-HCl (5–7.2), Sorensen (7.2–8.6), and Gomori buffers (8.6–9), while temperature profiling (30–70°C) employed water bath-controlled reactions at 5-10°C intervals, under pH 7.2.

Protein concentration was measured using the Bradford assay, where Coomassie Brilliant Blue dye binding shifts absorbance to 595 nm. Specific activity (U/mg) was calculated by normalizing enzyme units to total protein.

All steps were performed in triplicates.

Results

The *Aspergillus oryzae*-derived L-asparaginase exhibited pH-dependent activity (Figure 1), with maximal hydrolysis rates observed at pH 7.2 (10.43 U/mL) in Gomori buffer. Activity remained above 80% between pH 6.8–7.6, demonstrating broad neutral-range compatibility. Below pH 6.0 and above pH 8.5, activity dropped.

Temperature profiling (Figure 2) revealed peak catalytic efficiency at 45°C (12.29 U/mL), aligning with common baking and frying conditions. Activity retention exceeded 90% between 40–50°C but declined precipitously above 60°C, reaching <5% residual activity at 70°C. This thermal inactivation profile confirms suitability for usage prior to thermal processing.

The Bradford assay quantified total soluble protein at 0.141 mg/mL, yielding a specific activity of 86.94 U/mg. This metric underscores the enzyme's high catalytic efficiency per unit protein, a critical factor for cost-effective industrial scaling [3].

Conclusion

This study demonstrates that commercial L-asparaginase from *Aspergillus oryzae* exhibits high catalytic efficiency under conditions relevant to food processing, with optimal activity at neutral pH and moderate temperatures. The enzyme's robust performance and high specific activity support its practical application for acrylamide mitigation in thermally processed foods. These findings reinforce the potential of enzymatic treatment as an effective strategy to reduce acrylamide formation, contributing to improved food safety and public health. Further studies could explore formulation, optimization and application across diverse food matrices to maximize industrial impact.

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