

# Characterization Studies of Alcalase™ Enzyme Preparation for Production of Bioactive Peptides †

Emilse López <sup>1,\*</sup>, Enrique Mammarella <sup>1</sup>, Guillermo Sihufe <sup>1</sup>, Ricardo Manzo <sup>1,\*</sup>

<sup>1</sup> Instituto de Desarrollo Tecnológico para la Industria Química (INTEC-UNL-CONICET)

\* Correspondence: [eclopez@intec.unl.edu.ar](mailto:eclopez@intec.unl.edu.ar) (E.L.); [rmanzo@santafe-conicet.gov.ar](mailto:rmanzo@santafe-conicet.gov.ar) (R.M.);

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**Abstract:** The obtention of bioactive peptides from by-products using enzymatic approaches is intensively studied. This report aimed to make contributions to the characterization of Alcalase™, a commercial enzymatic preparation of dietary interest. For assaying enzymatic activity, different conditions of temperature, pH, time, and casein concentration were studied. The optimum temperature and pH conditions were 65°C and pH 9, respectively. Regarding stability, Alcalase™ maintained considerable activity values in a temperature and pH range of 50-60°C and 6,5-9,5. Moreover, we observed that the temperature of maximum thermal stability did not coincide with the optimum activity temperature. When evaluating productivity, a major hydrolysis percentage as temperature and reaction time increased (except for 50°C at 2 h) was observed. Finally, maximum enzyme activity was reached at optimum pH. These results would encourage the use of Alcalase™ at optimum pH and temperatures lower than 50°C for extensive hydrolysis reactions. To avoid a possible enzyme thermal deactivation, adequate conditions for enzymatic pre-incubation were 30-40°C and pH 6-9. In general, the increase in enzyme or casein concentration led to an increase in enzymatic activity.

**Keywords:** whey protein; hydrolysis; Alcalase™; enzyme characterization; reaction conditions; bioactive peptides.

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## Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.