## Immobilization of enological pectinase in polyamide microparticles for wine clarification

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## Abstract

Nowadays enzymes are widely used as catalysts in food industry, particularly in winemaking.[1] Pectinases have a considerable influence on the sensorial properties of wines. They catalyze the hydrolysis of pectin, which is responsible for wine turbidity, thus contributing to the improvement of the clarification and filtration processes.[2] Pectinases act under very mild conditions without destroying any valuable wine components.[3] However, native enzymes are very sensitive to extreme conditions e.g., pH and cannot be re-used. To overcome these limitations, enzymes can be immobilized on polymer supports.[4] Polyamides could be good supports because they are biocompatible and possess appropriate physical and chemical properties that allow for both covalent and not-covalent enzyme immobilization.[5]

The main objective of this work is the synthesis of new particulate polyamide/pectinase complexes and their application for wine clarification. New polyamide 6 (PA6) microparticles (MP) with controlled shape, size and porosity were synthesized by activated anionic ring-opening polymerization of caprolactam in solution. These PA6 MP were used as supports for non-covalent immobilization of pectinase varying the enzyme concentration. The immobilization was performed at room temperature for 24 hours, at pH 4.5. The influence of the immobilization conditions on the amount of the immobilized enzyme and on its activity was studied. The resulting complexes were used for clarification of industrially produced rosé wine must samples. As compared to the free pectinase, the pectinase/PA6 MP complexes displayed higher activity, better clarification for shorter treatment periods, as well as possibility for reutilization in various consecutive cycles.

## References

[1] Jegannathanand, K. R. & Nielsen, P. H. Environmental assessment of enzyme use in industrial production – a literature review. *J Clean Prod* **42**, 228–240 (2013).

[2] Busto, M. D., García-Tramontín, K. E., Ortega, N. & Perez-Mateos, M. Preparation and properties of an immobilized pectinlyase for the treatment of fruit juices. *Bioresour Technol* **97**, 1477–1483 (2006).

[3] Moreno-Arribas, M. V. & Polo, M. C. Wine Chemistry and Biochemistry. Wine Chemistry and Biochemistry vol. 1. Springer New York (2009).

[4] Nguyen, H. H. & Kim, M. An Overview of Techniques in Enzyme Immobilization. *Applied Science and Convergence Technology* **26**, 157–163 (2017).

[5] Goldstein, L., Freeman, A. & Sokolovsky, M. Derivatized Nylon: A New Support for the Immobilization of Enzymes. in *Enzyme Engineering Volume 2* 97–104, *Springer US* (1974).