

# ISOLATION AND PURIFICATION OF ASCORBATE OXIDASE FROM THE APPLE.

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Effective waste management and reuse are key challenges in achieving sustainable development and implementing zero waste policies. Approximately 44% of all waste consists of food and other biomass waste, a significant portion of which is generated by the fruit and vegetable processing industry. Juice production alone creates about 4–5 million tons of apple pomace annually, and its effective utilization is still underdeveloped [1]. In line with circular economy principles, this pomace should be reused, particularly for extracting biologically active compounds that could be applied in the food industry or biotechnology fields.

The aim of this study was to purify ascorbate oxidase from apple pomace and evaluate its potential for use. This enzyme is significant for the food industry and biotechnology, particularly in the development of biosensors [2]. To confirm the presence of ascorbate oxidase in apples, its activity was investigated in both fresh juices and pomace. "Golden Delicious" variety apple pomace and juice were processed with two different buffer solutions: 25 mM phosphate buffer (pH 7) and 25 mM acetate buffer (pH 4). Ascorbate oxidase activity was measured using two substrates—L-ascorbic acid and catechol—in different pH buffers solutions. The enzyme was precipitated with ammonium sulfate at 60% saturation, and further purification was performed using chromatographic methods. The purity of the sample was analyzed by protein electrophoresis method.

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[1] Golebiewska E., Kalinowska M., Yildiz G. Sustainable use of apple pomace (AP) in different industrial sectors. *Materials*, 2022, 15, 1788.  
[2] Wang X., Dong S., Wei H. Recent Advances on Nanozyme-based Electrochemical Biosensors. *Electroanalysis*, 2023, 35, e202100684.