

Optimization Of An Enzymatic Methodology For Pesticide Determination In Vegetable Matrices

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Enzymatic indicators have been demonstrated as an alternative for the broad range analysis of substances in substitution of traditional analytical methods (chromatographic methods coupled with spectrometric procedures). The aim is to lower costs, reagent use and personnel qualification without losing result credibility. Bastos *et al* (1991) and Moura (1998) developed procedures in order to analyze anti-cholinergic pesticides in water and vegetable samples, respectively, using free AChE in solution, thus enabling the elimination of the analytical disadvantages of enzymatic biosensors. This study aimed to optimize the methodology of pesticide determination in vegetables through the use of the acetylcholinesterase (AChE) enzyme in solution. Samples of several types of vegetables were fortified with a commercial solution Methomyl (pesticide) and submitted to acetone extraction. The determinations of pesticide levels were conducted by incubation with a semi-purified AChE extract, obtained from Winstar mice brains, according to Lima (1996). An aliquot of the raw extract was incubated with a suspension of AChE in phosphate buffer (pH=7.4). After this procedure, the kinetic behavior of the enzyme activity was determined in a microplate reader. The extraction results of Methomyl showed a 75 to 95% recovery in the vegetable samples, indicating that this procedure is adequate for analysis of this pesticide. The specificity of the enzymatic response allowed the elimination of a clean-up step, which resulted in a quicker analytical routine, with less chance of analyte loss. The kinetic determination in microplates demonstrated that this is a quicker procedure in the detection of anti-cholinergic compounds, allowing for adequate sensibility.

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