ABSTRACT

This thesis deals with the evaluation of new chromogenic reagents for use with horseradish peroxidase and the subsequent use of one of these reagents in a flow injection method, involving the use of immobilized glutamate oxidase and horseradish peroxidase bioreactors, for the quantitation of monosodium glutamate in foods.

Nine different chromogenic reagent systems for use with peroxidase were characterized with respect to their optimum wavelength for detection of the chromogenic product, ratio and concentration of the reactants, pH and buffer type. Standard curves were generated for each system using the optimized reaction conditions. The viability of each system was assessed. 3-(*N,N*-dimethylaminobenzoic acid) (6 mM) and 4-aminoantipyrene (0.2 mM) in phosphate/ citrate buffer (0.25M in each component, pH 6.0), appeared to have the best sensitivity ($\varepsilon = 23\ 124\ M^{-1}\ cm^{-1}$), detection limit (4 μM) and stability.

The 3-(*N,N*-dimethylaminobenzoic acid)/ 4-aminoantipyrene chromogenic system was thus used in the carrier stream in an flow injection method for quantifying hydrogen peroxide using peroxidase immobilized onto a Fractogel support in a packed bed reactor. The optimized, compromised conditions for this system were found to be as follows: flow rate - 0.9 ml min^{-1}, working temperature - 25°C, buffer conditions - citrate/ phosphate buffer (0.25 M in each component, pH 6.0), bioreactor dimensions - 1.4 cm x 0.5 cm i.d. and sample injection volume – 165 μl. Kinetic studies carried out on the immobilized system

in a packed bed reactor indicate that at low temperatures (< 36°C) the system is operating under diffusion control conditions and at higher temperatures (40 - 60°C) the breakdown of the enzyme-substrate complex is rate-limiting. The maximum reaction capacity of the immobilized peroxidase system was 3.98 μmol min^{-1} with an apparent Michaelis constant, K'_m, of 3.23 x 10^{-5} M.

Glutamate oxidase was then immobilized onto a Fractogel support and the glutamate oxidase bioreactor arranged in tandem with the peroxidase bioreactor in a flow injection arrangement and used to quantify the monosodium salt of L-glutamate in various types of soups. The optimized conditions for operating the manifold were found to be: flow rate - 0.9 ml min^{-1}, temperature - 25°C, buffer conditions – citrate/ phosphate buffer (0.025 M in each component, pH6.5), bioreactor dimensions; peroxidase bioreactor - 1.0 cm x 0.5cm i.d., glutamate oxidase bioreactor - 2.0 x 0.5 cm i.d., and sample injection volume – 380 μl. The optimized system can analyse manually up to 45 samples an hour, 8 hours a day, 5 days a week for 12 weeks without any appreciable loss of activity. The within day C.V. ([L-glutamate]= 100 μM) was found to be 1.9 % and the day to day C.V. was 2.2 %. Recovery yields for glutamate spiked in soup matrices ranged from 96 to 100%. This new automated chromogenic assay was used to determine the monosodium glutamate content of various commercially available soups and was found to be in good agreement with the glutamate concentrations obtained from analyses of these same samples by the standard reference method of the Association of Official Analytical Chemists.