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Simultaneous Sensing of Five Compounds in Fruit by Amperometric Flow Injection System with Immobilized-Enzyme Reactors

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Five compounds in fruit samples, i.e., glucose, fructose, sucrose, L-malate and L-ascorbate, were determined simultaneously in a flow injection system using a parallel configuration of immobilized-enzyme reactors. Enzymes used were glucose oxidase (GOD) for glucose, GOD, mutarotase and invertase for sucrose, and malate dehydrogenase and NADH oxidase for L-malate. Hydrogen peroxide produced in the above enzyme reactions was monitored amperometrically on platinum electrodes. For fructose determination, p-benzoquinone (oxidized form) was coupled with fructose in the fructose dehydrogenase reaction, and the oxidation current of the hydroquinone produced (reduced form) was monitored on a platinum electrode. L-Ascorbate was determined by the direct oxidation of L-ascorbate on a platinum electrode. In this case, the signal difference between the ascorbate-oxidase-immobilized column and the blank column was measured as a net concentration of L-ascorbate. Linear relationships between sensor responses and concentration for all compounds were obtained in the ranges of 0.1 – 5.0 mM (for glucose, fructose and sucrose), and 2 – 100 μM (for L-ascorbate). For L-malate, the response was linearly related to the square root of the concentration in the range of 0.02 – 1.0 mM. The relative standard deviations for ten successive injections were less than 2% for each compound. This system was applied to the determination of the five compounds in several kinds of fruit, and the results showed good agreement with those obtained using conventional methods (F-kit methods).