

Title An electrochemical DNA sensor for the detection of chlorophyllase gene expression in fresh cut broccoli

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Abstract

A technique for the semi-quantitative determination of chlorophyllase gene expression in tissues of fresh cut broccoli based on electrochemical biosensor was developed. This method employed RT-PCR amplification of a specific domain corresponding to a target chlorophyllase gene and a common housekeeping 18S rRNA gene, and a phenomenon of DNA aggregation induced by Hoechst 33258 in conjunction with changes in anodic current peaks measured via a carbon screen printed electrode on linear sweep voltammetry. Anodic current peaks of the resulting cDNA products from broccoli tissues during storage were between 1.12-2.13 μ A. Semi-quantitative analysis for the level of chlorophyllase gene expression was measured using the comparative ratio between copy numbers of chlorophyllase and housekeeping 18S rRNA genes. Expression levels were up to 81% of the level of the housekeeping and corresponded with the results obtained using RT-PCR with gel visualisation. Maximum expression of the chlorophyllase gene was obtained after 3 days of storage at 27°C, while little expression was detected when fresh cut broccoli was kept cool (4°C) at same period of time. The technique had several merits based on its rapidity and simplicity in quantifying gene expression, and on its cost effectiveness since no sophisticated devices and expensive reagents were needed.