

**Document category** : ISI Web of Science

**Title** : A Simple Visual Ethanol Biosensor Based on Alcohol Oxidase Immobilized onto Polyaniline Film for Halal Verification of Fermented Beverage Samples

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**Abstract** : A simple visual ethanol biosensor based on alcohol oxidase (AOX) immobilised onto polyaniline (PANI) film for halal verification of fermented beverage samples is described. This biosensor responds to ethanol via a colour change from green to blue, due to the enzymatic reaction of ethanol that produces acetaldehyde and hydrogen peroxide, when the latter oxidizes the PANI film. The procedure to obtain this biosensor consists of the immobilization of AOX onto PANI film by adsorption. For the immobilisation, an AOX solution is deposited on the PANI film and left at room temperature until dried (30 min). The biosensor was constructed as a dip stick for visual and simple use. The colour changes of the films have been scanned and analysed using image analysis software (i.e., ImageJ) to study the characteristics of the biosensor's response toward ethanol. The biosensor has a linear response in an ethanol concentration range of 0.01%-0.8%, with a correlation coefficient ( $r$ ) of 0.996. The limit detection of the biosensor was 0.001%, with reproducibility (RSD) of 1.6% and a life time up to seven weeks when stored at 4 degrees C. The biosensor provides accurate results for ethanol determination in fermented drinks and was in good agreement with the standard method (gas chromatography) results. Thus, the biosensor could be used as a simple visual method for ethanol determination in fermented beverage samples that can be useful for Muslim community for halal verification.

**Subject** : Halal; Polyaniline; Alcohol Oxidase Ethanol; Biosensor; Fermented Beverage

**Type** : Article

**Journal** : Sensors

**ISSN** : 1424-8220

**e-ISSN** :

**Publisher** : Mdpi Ag

**Year issue** : 2014

**Language** : English