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Determination of Volatile Alcohols in Fruit and Vegetable Juices by an Amperometric Enzyme Electrode Measuring in the Headspace above the Liquid

C. Schlangen, M. Hämmerle, K. Hilgert, R. Moos
Functional Materials, University of Bayreuth, Universitätsstraße 30, 95440 Bayreuth, Germany
martin.haemmerle@uni-bayreuth.de

Abstract:
The determination of alcohol is relevant to the beverage industry, apparently for liquors, wines, or beers. However, alcohol can also be found in juices e.g. due to starting fermentation during storage of the fruits. We present an amperometric enzyme electrode that determines volatile alcohols (ethanol, methanol) in fruit and vegetable juices (apple, grape, currant, tomato). The biosensor analyses the headspace above the liquid sample. This mode of operation has the advantage that possible interferences are limited to volatile substances and that the biosensor is not contaminated by the sample. For example, no interference by ascorbic acid (vitamin C) can occur. The analysis results of the biosensor are compared to two reference methods, a commercial photometric enzyme test-kit and a HPLC analysis.

Key words: enzyme electrode, amperometry, alcohol oxidase, volatile alcohols, juices, and head space analysis

Sensor Principle

The sensor principle is based on the enzymatic oxidation of ethanol catalyzed by the enzyme alcohol oxidase (*P. pastoris*). The reaction product H_2O_2 is determined electrochemically at a platinum electrode (potential: +600 mV vs. Ag/AgCl).

$$CH_3CH_2OH + O_2 \xrightarrow{AOx} CH_3CHO + H_2O_2$$

$$H_2O_2 \xrightarrow{electrode} O_2 + 2\,H^+ + 2\,e^-$$

A porous Teflon membrane separates the headspace above the liquid sample and the aqueous inner electrolyte of the biosensors. The side of the Teflon membrane facing the sensor electrolyte is sputtered with a porous platinum layer that serves as the working electrode. Thus, the volatile alcohols enter the sensor through this porous Teflon membrane / working electrode. The enzymatic reaction and the electrochemical determination take place within the aqueous inner electrolyte of the biosensor. Details of the sensor set-up can be found in [1-3].

Sensor Characteristics

Characteristics of the biosensor are summarized in Tab. 1. The biosensor shows good stability, response time and detection limit. The working range is limited at high concentrations by enzyme saturation. The selectivity of the biosensor is related to the selectivity of the used enzyme alcohol oxidase. This enzyme catalyzes the oxidation of primary alcohols and shows a higher reaction rate for alcohols with a shorter alkyl group. Thus methanol exhibits a higher reaction rate than ethanol.

Tab. 1: Biosensor characteristics (data for three individual sensors)

detection limit (3<S/N)	8.9 ± 2.6 µM (0.4 mg/l)
sensitivity	0.74 ± 0.01 µA / mM
response time ($t_{90\%}$)	195 ± 25 s @ 1 mM
working range	0.1 - 20 mM
stability	95%, 24 h @ 5mM

Analysis of Fruit and Vegetable Juices

Various fruit and vegetable juices were analyzed with the biosensor and the results compared to two reference methods, a commercial photometric enzyme test-kit that is specific for ethanol, whereas it does not detect methanol, and a HPLC analysis. The results are summarized in Fig. 1. The EtOH content determined by the two reference methods agrees very well. In contrast, the apparent EtOH determined by the biosensor on the basis of its calibration with ethanol standards is much higher, especially for tomato juice. This is attributed to methanol present in the samples, which is confirmed by the HPLC analysis. It is quite usual to find methanol in juices. It is released during the hydrolysis of methyl esters present in pectins. From the biosensor signal a MeOH content can be calculated on the basis of the EtOH content determined by the two reference methods and taking into account the relative sensitivity of the sensor to methanol and ethanol, respectively. The calculated methanol content and the one determined by HPLC agree well.

Although it is not possible to derive quantitative values for the respective ethanol or methanol content of the analyzed juice samples based on the biosensor signal alone, the biosensor signal indicates the combined ethanol and methanol content of the sample. Thus it can be used as a screening method before expensive, detailed analysis is performed.

As the biosensor has no direct contact with the liquid sample, it is not contaminated by it, and turbid, unfiltered juices can be analyzed as well as filtered ones.

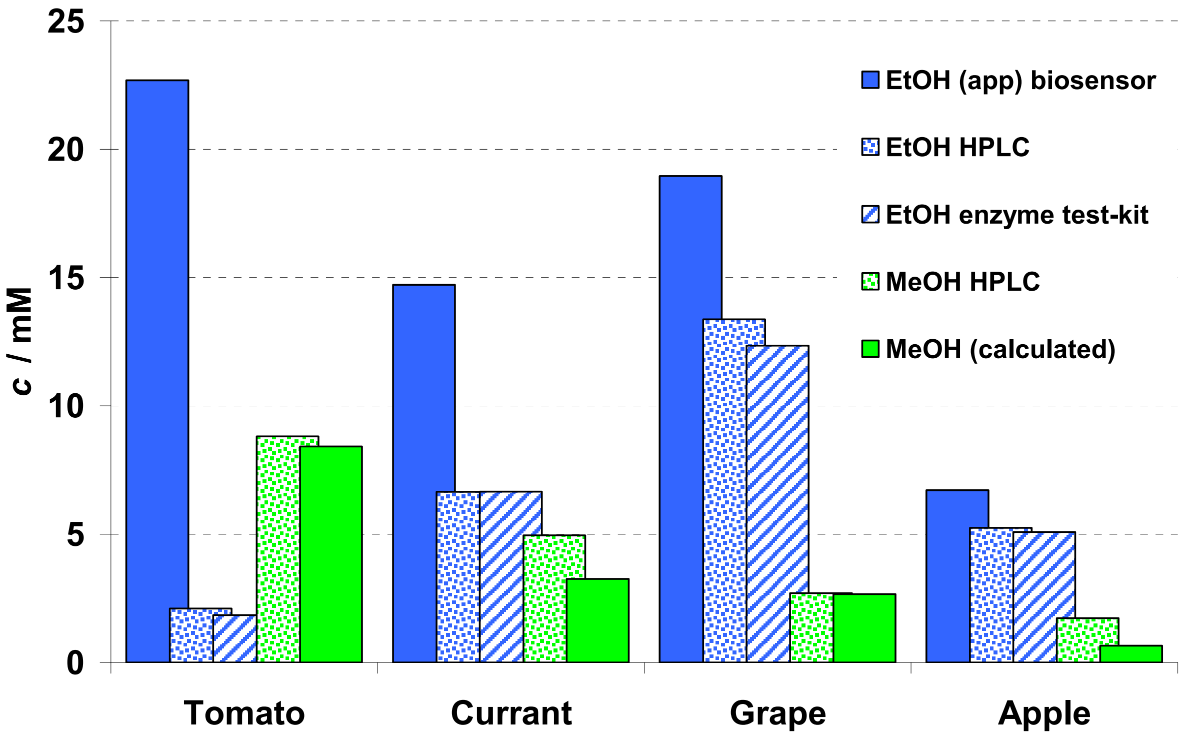

Fig. 2. Comparison of the analysis results of various fruit and vegetable juices. MeOH (calculated): calculated from the amperometric signal of the biosensor by taking into account the EtOH content of the sample given by the result of the enzyme test-kit and the relative sensitivity of the biosensor to MeOH and EtOH, respectively.

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