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# Electrochemical Biosensors for Food Quality Control

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## 1. Introduction

The electrochemical biosensors are analytical devices designed by coupling biological recognition elements and electrochemical transducers. The transducer converts the analytical signal produced as a result of the biochemical and electrochemical interactions into measurable electrical one (Thévenot et al., 1999).
The electrochemical biosensors are self-contained, simple to handle, and able to provide specific, sensitive, accurate and cost-effective *in situ* and *on line* measurements in real time, without or with a minimum sample preparation. Because of these advantages over the conventional analytical methods, they are well suited for the detection of a large spectrum of compounds, entering food and subjects of analytical control.
The present work is intended to demonstrate the applicability of the electrochemical biosensors for arsenic determination in beverages.

## 2. Arsenic content in food and beverages

Arsenic is a chemically active, toxic, and carcinogenic element (Moore & Ramamoorthy, 1984). It is among the 129 priority pollutants of the environment and among the 25 hazardous substances representing a significant potential threat to human health (EPA: Toxic and priority pollutants). It occurs naturally in soil and groundwater, but additionally enters the environment in a large quantity because of the human industrial and agricultural activities. The most affected by arsenic pollution are fishes and other aquatic organisms, since they accumulate it. High arsenic concentrations in plants are registered when using for irrigation arsenic-rich groundwater or contaminated water because of the industrial discharges and the treatment of soils with fertilizers and pesticides. Lead arsenate insecticides were extensively used in some countries until 1981 (Peryea, 1998). Arsenic content in food from plant and animal origin, with the exceptions of seafood and animal and poultry offal, does not habitually exceed 0.25 mg $kg^{-1}$, according to WHO data (Arsenic.

WHO Food Additives Series 18). The average daily arsenic intakes for various countries are summarized in Fig. 1. Arsenic concentration in food and beverages, as evaluated by the US Food and Drug Administration (FAD, 2010) in its annual Total Diet Study, is shown in Table 1.

| Product | As, mg $kg^{-1}$ |
|---|---|
| Cheese, American, processed | 0.002 |
| Beef roast, chuck, oven-roasted | 0.001 |
| Turkey breast, oven-roasted | 0.004 |
| Liver (beef/calf), pan-cooked w/oil | 0.001 |
| Fish sticks or patty, frozen, oven cooked | 0.527 |
| Peanut butter, creamy | 0.013 |
| Peanuts, dry roasted, salted | 0.014 |
| Rice, white, enriched, cooked | 0.065 |
| Oatmeal, plain, cooked | 0.002 |
| Cream of wheat (farina), enriched, cooked | 0.001 |
| Corn, fresh/frozen, boiled | 0.001 |
| Bread, white, enriched | 0.001 |
| Bread, whole wheat | 0.002 |
| Muffin, fruit or plain | 0.007 |
| Corn/tortilla chips | 0.001 |
| Fruit-flavoured cereal, presweetened | 0.013 |
| Raisin bran cereal | 0.006 |
| Crisped rice cereal | 0.135 |
| Granola w/raisins | 0.021 |
| Oat ring cereal | 0.028 |
| Pear, raw (w/peel) | 0.001 |
| Strawberries, raw/frozen | 0.001 |
| Fruit cocktail, canned in light syrup | 0.002 |
| Grapes (red/green), raw | 0.003 |
| Cantaloupe, raw/frozen | 0.008 |
| Raisins | 0.014 |
| Avocado, raw | 0.001 |
| Apple juice, bottled | 0.005 |
| Prune juice, bottled | 0.004 |
| Spinach, fresh/frozen, boiled | 0.001 |
| Collards, fresh/frozen, boiled | 0.003 |
| Tomato, raw | 0.001 |
| Tomato sauce, plain, bottled | 0.001 |
| Cucumber, peeled, raw | 0.011 |
| Brownie | 0.006 |
| Syrup, chocolate | 0.001 |
| Jelly, any flavour | 0.002 |
| BF, cereal, rice, dry, prepared w/water | 0.041 |
| Beef steak, loin/sirloin, broiled | 0.001 |
| Chicken thigh, oven-roasted (skin removed) | 0.009 |
| Catfish, pan-cooked w/oil | 0.012 |
| Fruit juice blend (100% juice), canned/bottled | 0.005 |

| | |
|---|---|
| Lettuce, leaf, raw | 0.002 |
| Beef w/vegetables in sauce, from Chinese carry-out | 0.004 |
| Potato, baked (w/peel) | 0.002 |
| Chili con carne w/beans, canned | 0.003 |
| Quarter-pound hamburger on bun, fast-food | 0.001 |
| Meatloaf, beef, homemade | 0.001 |
| Chicken potpie, frozen, heated | 0.001 |
| Soup, tomato, canned, cond., prepared w/water | 0.003 |
| Cake, chocolate w/ icing | 0.013 |
| Sweet roll/Danish pastry | 0.001 |
| Gelatine dessert, any flavour | 0.001 |
| Wine, dry table, red/white | 0.010 |
| BF, beef and broth/gravy | 0.001 |
| BF, macaroni, tomato and beef | 0.002 |
| BF, peaches | 0.001 |
| BF, juice, apple | 0.022 |
| BF, vanilla custard/pudding | 0.002 |
| BF, fruit dessert/pudding | 0.003 |
| Chicken breast, oven-roasted (skin removed) | 0.004 |
| Shrimp, boiled | 0.265 |
| Bread, cracked wheat | 0.003 |
| Bagel, plain, toasted | 0.001 |
| English muffin, plain, toasted | 0.001 |
| Crackers, graham | 0.004 |
| Grape juice, frozen conc., reconstituted | 0.007 |
| Mushrooms, raw | 0.073 |
| Eggplant, fresh, peeled, boiled | 0.001 |
| Okra, fresh/frozen, boiled | 0.001 |
| Beef stroganoff w/noodles, homemade | 0.012 |
| Tuna noodle casserole, homemade | 0.164 |
| Fish sandwich on bun, fast-food | 0.380 |
| Egg, cheese, and ham on English muffin, fast-food | 0.002 |
| Clam chowder, New England, canned, cond., prepared w/ whole milk | 0.128 |
| Coffee, from ground | 0.0002 |
| BF, teething biscuits | 0.004 |
| Salmon, steaks/fillets, baked | 0.288 |
| BF, cereal, rice w/apples, dry, prepared w/water | 0.033 |
| Chicken breast, fried, fast-food (w/skin) | 0.013 |
| Chicken leg, fried, fast-food (w/skin) | 0.013 |
| Tuna, canned in water, drained | 1.00 |
| Cranberry juice cocktail, canned/bottled | 0.004 |
| Sweet potatoes, canned | 0.001 |

Table 1. Arsenic occurence in food (US Food & Drug Administration - Total Diet Study - Market Baskets 2006-1 through 2008-4).

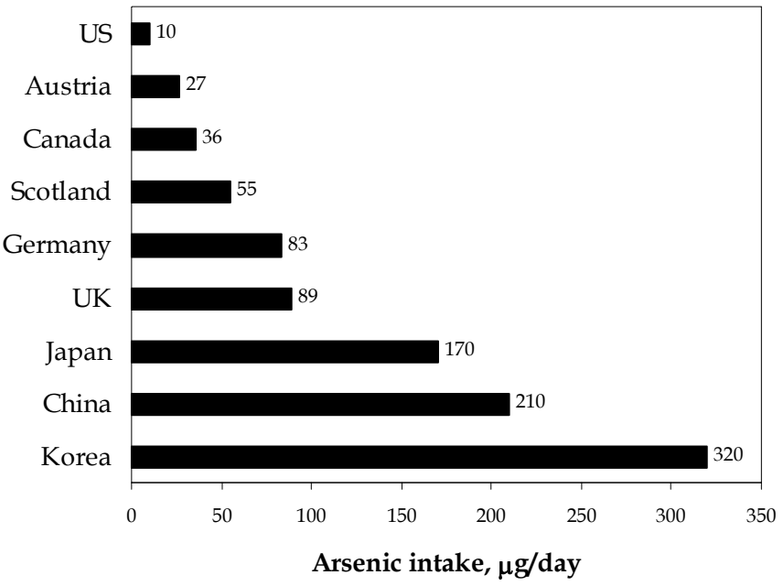


Fig. 1. Average daily arsenic intakes for various countries (Arsenic. WHO Food Additives Series 18)

## 3. Sample collection, preparation and treatment for arsenic determination in food and beverages

Sample collection, preparation and treatment for arsenic determination in food and beverages are performed according to the established procedures (WHO, 2011). These include: collection of samples, representative of the food consumed in a population; sample conservation in acid washed plastic containers; freezing of samples if necessary, to -80$^{o}$C; food preparation or cooking in a manner similar to those that would be used at home, if appropriate; sample homogenization and digestion, applying various techniques guided by the subsequent analysis technique.

## 4. Methods for inorganic arsenic determination in food and beverages

Inorganic arsenic determination could be performed applying a number of methods (WHO, 2011). Some of them, such as the spectrophotometric analysis with silver diethyldidhiocarbamate and certain modifications of the atomic absorption spectrometry (AAS) and the inductively coupled plasma (ICP) are standardised (DIN 38405-D12; APHA/AWWA/WPCF 3500-As C; AOAC 33.125-33.132 in combination with 25.041 and 25.042; EPA 7061; DIN 38405-D1; APHA/AWWA/WPCF 3500-As B: 3114 B; APHA/AWWA/WPCF 3500-As E: 3120 B; DIN 38406-E22) and are among the mostly applied for arsenic determination in food and beverages (Bingöl et al., 2010; Conklin, 2010; Husáková et al., 2007; Karadjova et al., 2005; Niu Jianjun & Wang Bingwu, 1992; Roberge et al., 2009; Stafilov et al., 2004; Syr-Song Chen et al., 2003; Tašev et al., 2005). Nevertheless, arsenic is one of the few elements for which AAS is not enough sensitive. Using special supplies such as arsine generators and electrothermal analysers allows lowering the detection limit, but causes difficulties in the routine analysis. The other advanced instrumental methods such as ICP, neutron activation analysis (NAA), and X-ray

fluorescence permit the determination of arsenic at trace levels, but they require expensive and sophisticated equipment. The spectrophotometric methods, although simple and cost effective, do not provide the required sensitivity.

The electrochemical methods for inorganic arsenic determination (Cavicchioli et al., 2004), including mainly anodic stripping voltammetry and differential pulse polarography, in spite of their limited application in food quality control, could be considered as an alternative to the above mentioned analytical techniques. For instance, their sensitivity is similar to this of mass spectrometry and NAA, but they are much more simple, require low costing equipment, and allow distinguishing the electro-active As(III) and the electro-inactive As(V), in contrast to the enumerated techniques. As(III) and As(V) have different toxicity, biological activity, and physiological action. The toxicity of As(III) is known to be greater of that of As(V). Thus, the distinction between the two forms is of primary importance.

The further development of the electrochemical methods is associated with the appearance, during the 1960s, of the so-called electrochemical biosensors. They combine the high sensitivity, accuracy and reproducibility of the electrochemical analysis with the substrate specificity and catalytic activity of the biological molecules. A number of them found an application in food industry, namely in food safety and quality control, and in the control of the fermentation processes (Mutlu, 2010; Scott, 1998; Prodromidis & Karayannis, 2002; Wagner & Guilbault, 1994).

## 5. Acylcholinesterase based sensor for arsenic determination in wine

Arsenic determination in wine, using the suggested in this work acetylcholinesterase electrochemical sensor, is based on the following reactions:

$$\text{acetylthiocholine} + H_2O \xrightarrow{ACh} \text{thiocholine} + CH_3COOH$$

$$\text{thiocholine} \rightarrow \text{dithio-bis-choline} + 2H^+ + 2e^-$$

The acetylcholinesterase Ach (EC 3.1.1.7) catalysed hydrolysis of acetylcholine generates the electroactive product thiocholine. The current of its oxidation is recorded amperometrically at a potential of +0.80 V/SCE. In the presence of As(III), because of the enzyme inhibition that it provokes, the quantity of the produced thiocholine decreases. Thus, the current of its oxidation also decreases as a function of As(III) concentration under similar conditions.

The acetylcholinesterase based electrochemical sensor was prepared as described in our previous works (Stoytcheva et al., 1998a, 1998b), i. e.: acetylcholinesterase was covalently immobilized onto the surface of a rotating disc electrode elaborated from spectrally pure graphite (Ringsdorf Werke, Germany). The analysis was carried out in an electrolysis cell of conventional type, at a temperature of 25$^o$C, with a rotation speed of the working electrode of 1000 rpm. The auxiliary electrode was a glassy carbon electrode. A saturated calomel electrode was used as a reference.

The response of the biosensor was measured for various acetylthiocholine iodide concentrations in the presence of different amounts of As(III) in the form of $AsO_3^{3-}$ in a Britton-Robinson buffer solution with pH 7. The obtained results are presented in Fig. 2, where $\Delta I$ is the difference between the registered steady-state currents of thiocholine oxidation in the absence and in the presence of inhibitor (to note that iodide oxidation to iodine occurs, too).

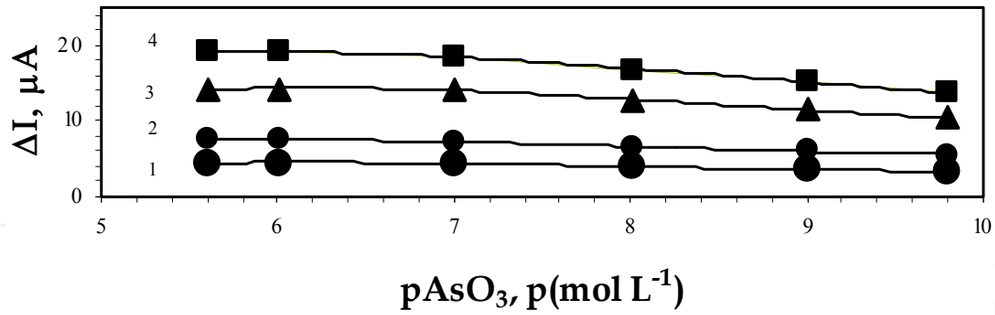


Fig. 2. Calibration curves for $AsO_3^{3-}$ determination using different substrate concentrations: 1) 0.2 mmol $L^{-1}$; 2) 0.4 mmol $L^{-1}$; 3) 0.6 mmol $L^{-1}$; 4) 1 mmol $L^{-1}$; 5) 1.2 mmol $L^{-1}$. $pAsO_3$ is the negative decimal logarithm of the $AsO_3^{3-}$ concentration.

As shown, the linear dynamic range of the calibration curves suitable for $AsO_3^{3-}$ determinations varies from 0.2 nmol $L^{-1}$ to 0.02 μmol $L^{-1}$. $AsO_3^{3-}$ concentrations superior to 10 μmol $L^{-1}$ caused an increase of the sensor response, due to the following concurrent reactions:

$$3I^- - 2e^- = I_3^-$$

$$H_3AsO_3 + I_3^- + H_2O = H_3AsO_4 + 3I + 2H^+$$

The sensitivity of the determinations, as shown in Table 2, increased with the increase of the acetylthiocholine iodide concentration until the enzyme saturation with 1.0 mmol $L^{-1}$ acetylthiocholine iodide.

| Substrate concentration, mmol $L^{-1}$ | Sensitivity, μA/p(mol $L^{-1}$) |
|---|---|
| 0.2 | 0.39 |
| 0.4 | 0.65 |
| 0.6 | 1.25 |
| 1.0 | 1.65 |

Table 2. Sensitivity of As(III) determination

The method allows As(III) and As(V) differentiation, due to the fact that $AsO_4^{3-}$ does not inhibit the acetylcholinesterase.

These preliminary results served for the development of a method for As(III) determination in wine. As known, arsenic content in some type of wines exceeds 0.1 mg $L^{-1}$ (Crecelius, 1997). Arsenic concentration in contaminated illicit whiskey (moonshine) was found to be more than 0.4 mg $L^{-1}$ (Gerhardt et al., 1980).

For the purposes of the analysis, commercially available wine was artificially contaminated with $AsO_3^{3-}$ 0.0133 mmol $L^{-1}$ (0.001 mg $L^{-1}$). The sample, without any pretreatment, was analysed according to the following protocol: (i) registration of the amperometric response of the electrochemical biosensor for a substrate concentration of 1.0 mmol $L^{-1}$, for which the sensitivity of the $AsO_3^{3-}$ determination is maximal (25°C, Britton-Robinson buffer 0.1 mol $L^{-1}$, pH 7, 1000 rpm, +0.80 V/SCE) in the presence of no contaminated wine; (ii) registration of the amperometric response of the electrochemical biosensor in similar conditions, but in the presence of the contaminated wine sample; (iii) ΔI calculation and $AsO_3^{3-}$ concentration

evaluation using a preliminary constructed calibration curve. The relative error of the analysis was found to be inferior to 3%.

## 6. Conclusion

The modern food analysis requires sensitive, accurate, and express methods for food safety, food quality, and food technology control. The growing field of the biosensors in food industry represents an answer to this demand. Thus, the method for As(III) determination in wine described in this work is an example demonstrating the viability of the electrochemical biosensors in food quality control.

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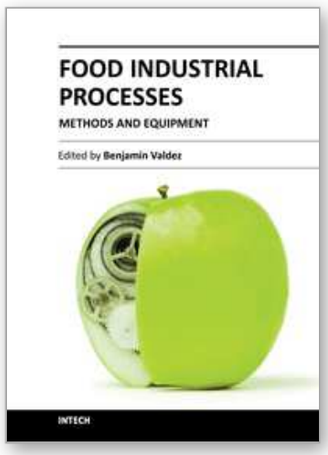


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The global food industry has the largest number of demanding and knowledgeable consumers: the world population of seven billion inhabitants, since every person eats! This population requires food products that fulfill the high quality standards established by the food industry organizations. Food shortages threaten human health and are aggravated by the disastrous, extreme climatic events such as floods, droughts, fires, storms connected to climate change, global warming and greenhouse gas emissions that modify the environment and, consequently, the production of foods in the agriculture and husbandry sectors. This collection of articles is a timely contribution to issues relating to the food industry. They were selected for use as a primer, an investigation guide and documentation based on modern, scientific and technical references. This volume is therefore appropriate for use by university researchers and practicing food developers and producers. The control of food processing and production is not only discussed in scientific terms; engineering, economic and financial aspects are also considered for the advantage of food industry managers.

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