Applications of Biosensors to Analysis and Quality Control of Foods: An Overview

Youssef, M.M. & Nessma, N. El-Haddad

Food Science & Technology Dept., Fac. of Agric., El-Shatby, 21545

Alexandria Univ., Alexandria, Egypt

ABSTRACT

Biosensors have been described as the offspring of the marriage of biology and electronics. A biosensor is an analytical device consisting of a biocatalyst (enzyme, cell or tissue) and a chemical or physical transducer (electrochemical, mass, optical and thermal) which can convert a biological or biochemical signal or response into a quantifiable electrical signal. Modern biosensors evolved from the combination of the aforementioned two disciplines, with electronics/ information technology exemplified by micro circuits and optical fibers, and biology exemplified by molecular biology in the form of enzymes and antibodies.

Biosensors technology provides a new, substrate specific method enabling repeated measurements and possibility of *on-line* analysis for the process control in the food industry. It is obvious that *on-line* measurement is very important for the quality control and quality assurance (QC/QA) and hazard analysis at critical control point (HACCP). It is worth to mention that biosensors are very important analytical tools to detect any intended pollution of drinking water via bioterrorism. Biosensors are characterized by easy operation, rapid response and long stability. Such advantages suggested that biosensors can be used as economical analytical tools in the area of food analysis and quality control.

The present article deals mainly with the applicability of biosensors in the area of food analysis and quality control. For instance, many components in foods can be determined by means of biosensors. Meanwhile, the most important applications of biosensors in the area of food quality control include: microorganisms detection, sensory analysis, electronic tongue and nose, quality control of modified atmosphere packages, fish freshness analysis, meat and milk quality.

Keywords: ***biosensors-immunobiosensors, glucose, sulfite, pesticide residues, food pathogens, ELISA, microorganisms detection, sensory analysis, modified atmosphere packages, fish freshness, meat quality, milk quality.***

INTRODUCTION

The control of food quality and freshness is of growing interest for both consumer and food manufacturer. The quality of a food product is evaluated through periodic chemical and microbiological analysis. Such procedures conventionally use techniques as, chromatography, spectrophotometry, electrophoresis, titrations, and others. These methods do not allow an easily continuous monitoring, because they are expensive, slow, need well trained operators and in some cases, require steps of extraction or sample pretreatment, elongating the time of analysis (Mello & Kubota, 2002).

Biosensor technology provides a new, substrate specific method enabling repeated measurements and possibility of *on-line* analysis for the process control in the food industry. The main advantage of the biosensor analysis is its fast response time allowing an *on-line* measurement. It is obvious that *on-line* measurement is very important for the quality control and quality assurance (QC/QA) and hazard analysis at critical control points "HACCP" (Gürtas, 1997, Mello & Kubota, 2002). It provides less employment, fast, precise and cheap procedure beside the available methods which need experimental instruction, considerable technical skill and are tedious and time consuming. Accordingly, biosensors, combining a biological recognition element and a suitable transducer, represent very promising tools in this context (Castillo *et al.*, 2004).

Biosensors are expected to play an increasingly important role in the improvement of life quality (Castillo *et al.*, 2004). Meanwhile, in his article entitled "*Identifying Food Science & Technology Research Needs*", Heldman (2004) focused on the a pivotal role to be suspected for biosensors in the near future to act as effective

tool to protect human beings against the bioterrorism event.

A comprehensive analysis of papers published on immunoassay and biosensors used in food and environmental research since 1980 demonstrates a rapid increase of publications on "ELISA" and immunoassay since 1991 (more than 500 papers were published each year since 1996). Meanwhile, more than 200 papers on "biosensors" have been published each year since 2001 (Franek & Hruska, 2005).

What is a biosensor?

In the early days (the 1960s and 1970s) a sensor seemed to always be a probe of some sort, perhaps due to a vision inextricably linked to pH, ion selective or oxygen electrode. If you follow the old literature, you will find biosensors that were called bioelectrodes or enzyme electrodes or biocatalytic membrane electrode (Arnold & Meyerhoff, 1984).

Biosensors can be defined in two ways. The first implies that they are used to monitor living systems. The other defines them as devices that incorporate biological materials as a part of the sensing element. Most analysis currently use the term in its modern context as a sensor incorporating a biological element such as an enzyme, antibody, nucleic acid, microorganism or cell (Giese, 2002).

A biosensor (Figures 1, 2 & 3) is an analytical device consisting of a biocatalyst (enzyme, cell or tissue) and a chemical or physical transducer (electrochemical, mass, optical and thermal) which can convert a biological or biochemical signal or response into a quantifiable electrical signal. The biocatalyst component of most biosensors is immobilized on to a membrane or within a gel (Wilson & Walker, 1995, Mello & Kubota, 2002).

Biosensors may be categorized as first-, second- or third generation instruments according to the degree of intimacy between the biocatalyst and transducer. In first generation instruments, the two components (biocatalyst and transducer) may be easily separated and both may remain functional in the absence of the other. In second- generation instruments, the two components interact in a more intimate fashion and removal of one of the two components affects the usual functioning of the other. In third- generation instruments, the biochemistry and electrochemistry are even more closely linked and where the electrochemistry occurs at a semiconductor, the term biochip may be applied to describe such instruments (Wilson & Walker, 1995).

As a matter of fact, biosensors are of major commercial importance, and their significance is likely to increase as the technology develops. This is because they can be made to respond specifically and with high sensitivity to a wide range of molecules including those of industrial, clinical and environmental importance (Wilson & Walker, 1995).

Biosensors in food analysis

Table (1) shows the main uses of biosensors in food analysis. Various biosensors for composition analysis have been developed for carbohydrate analysis, organic acid measurement and the determination of vitamins and other compounds. Immobilization techniques for stabilization of biomolecules and their applications in food biosensors have been used for the analysis of sugars, ascorbic acid and lactic acid. Biosensors offer some advantages over traditional methods such as HPLC and GLC, which may require high maintenance, expert operators and long analysis times, making them less practical for food process monitoring (Giese, 2002).

The Y51 2700 from YS1, Inc. Yellow Springs, Ohio, may be used for food composition analysis to measure common food ingredients, such as glucose, sucrose, lactose, galactose, L-glutamate, choline and starch. The unit is an immobilized-enzyme biosensor. An enzyme specific for the substrate of interest is immobilized between two membrane layers, polycarbonate and cellulose acetate. The substrate is oxidized as it enters the enzyme layer, producing hydrogen peroxide which passes through cellulose acetate to a platinum electrode, where the hydrogen peroxide is oxidized. The resulting current is proportional to the concentration of the substrate (Giese, 2002).

It is worth to mention that a large variety of biosensors were described in the literature for monitoring heavy metals, using various biological recognition elements: enzymes, apoenzymes, metal binding proteins, antibodies, or whole cells coupled to different types of transducers, amperometric, potentiomtric, conductometric ... etc. (Castilla *et al.*, 2004).

A newly developed method for the quantification of folic acid in fortified food was

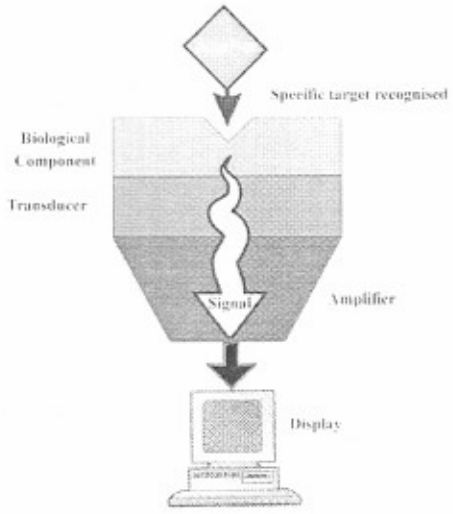

Fig. 1: Components of a generic biosensor

Source: Anonymous (2001)

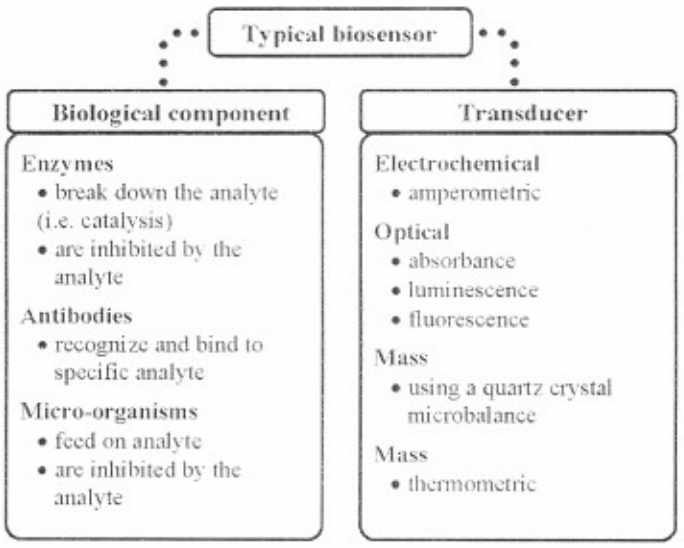

Fig. 2: Typical biosensor components and associated measurements

Source: Anonymous (2001)

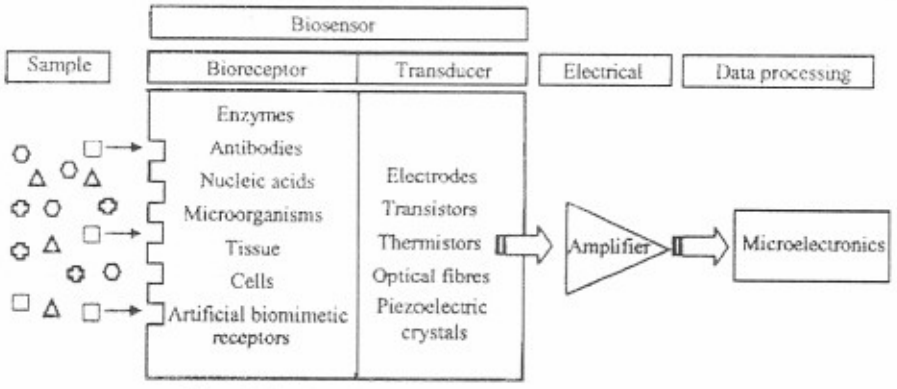

Fig. 3: Principles of operation of biosensor

Source: Velasco-Garcia & Mottram (2003)

Table 1: Applications of the biosensors in food analysis

Analyte	Application	Biocomponent	Transducer
Glucose	Soft drink Fruit drink Juice Honey Milk Yoghurt	Glucose oxidase (GDO) Glucose oxidase (GDO) β-galactosidase and mutarotase	AMP
Fructose	Honey, Milk Juice, Cola	D-Fructose dehydrogenase (FDH)	AMP.
Lactose	Milk	β-Galactosidase, Lactozym and *Saccharomyces cerevisia*	Potent.
Lactulose	Milk	D-Fructose dehydrogenase (FDH) and β-Galactosidase (β-gal)	AMP.
Starch	Wheat flour	α-Amylase, Amyloglucosidase (AMG) and glucose oxidase (GOD)	AMP.
Ethanol	Alcoholic beverage	Alcohol dehydrogenase (ADH)	AMP.
Acetaldehyde	Alcoholic beverage	Aldehyde dehydroganase	AMP.
Glycerol	Monitoring fermentation	Glycero kinase and glycerol-3 phosphate oxidase	AMP.
Polyphenols	Olive oil	Tyrosinase	AMP.
Catechol	Beer	Polyphenol oxidase	AMP.
Ascorbic acid	Juices	Ascorbate oxidase	AMP.
Pyruvic acid	Fruits	Pyruvate oxidase (POD)	AMP.
Citric acid	Juice and Sport drinks	Citratelyase (CL)	AMP.
Folic acid	Fortified foods	Anti-folic acid antibody	SPR.
Biotin	Infant formula and milk	Anti-biotin antibody	SPR.
Folate	Infant formula and milk	Anti folic acid antibody	SPR
L-amino acids	Milk and fruit juices	D-Amino acid oxidase (D-AAO)	AMP
L-glutamate	Seasonings	L-Glutamate oxidase (Glu OD) and NADH oxidase (NOD)	AMP.
L-Lysine	Milk and Pasta	L-lysine α-oxidase or lyase oxidase	AMP
Amines	Fish, anchovy, fruits and vegetables	Diamine oxidase (DAO)	AMP
	Meat	Xanthine oxidase (XOD)	O_2 electrode
Amines	Fish freshness	Hypoxanthine oxidase and xanthine oxidase (XOD)	AMP.
Biogenic amines	Fish	Diamine oxidase (DAO)	AMP.
Histamine	Sea foods	Histamine oxidase	AMP.
Hypoxanthine	Fish and its freshness	Xanthine oxidase (XOD)	AMP.
Oxalate	Spinach, sesame, seeds, tea leaves and strawberries	Oxalate oxidase (OXO)	AMP.
Phosphate	Drinking water	Poly phenol oxidase and alkaline phosphatas	AMP.
Antibiotics	Milk, food	Antibodies	SPR.
Bacteria	Chicken	Anti-*Salmonella* antibody	Fiber optic
Bacteria	Beef	Anti-*Escherichia coli* 0157:H7	AMP.
Pesticide	Milk	Chlinesterase (ChE)	AMP.
Pesticide	Fruits and vegetables	Choline oxidase, Acetyl cholinesterase (AChE)	AMP.
Toxin	Foods	Anti-*Staphylococcal*, Anti-*Staphylococcal* enterotoxin β antibody	SPR
Aspartame	Foods	Alcohol oxidase, α-chymotrypsin and catalase	AMP.

AMP : Amperometric Potent : Potentiometric SPR. : Surface plasmon resonance

Cited with modification from Mello & Kubota (2002)

presented by Caselunghe & Lindeberg (2000). An immunoaffinity-based optical biosensor was used to determine folic acid concentration levels in milk powder, infant formula and cereal samples. Accuracy of the method (88-101%) was demonstrated with the analysis of five reference samples. A collaborative precision study, where ten participants at four different laboratories analysed a set of ten samples, resulted in repeatability relative standard deviations of 2-8% and reproducibility relative standard deviations of 4-10%.

Here we will give some detailed examples to elucidate how biosensors act as analytical tools:

1- Determination of glucose

Since the pioneering work of Updike & Hicks (1967) for the determination of glucose and the enzymatic electrodes to this analyze in particular, the amperometric have dominated the literature about biosensors. The principal reason is that glucose is an analyte of great importance in biotechnology (Mello & Kubota, 2002).

A good example of a commercially available second- generation biosensor is provided by the ExacTech blood glucose meter. In this device, the rate of oxidation of glucose is measured not by the rate of disappearance of substrate or appearance of product, but by the rate of electron flow from glucose to an electrode surface. The reactions that occur in this device may be summarized as follows:

$$\underset{(red)}{\text{Glucose}} + \underset{(ox)}{\text{GO/FAD}} \longrightarrow \underset{(ox)}{\text{Gluconic acid}} + \underset{(red)}{\text{GO/FADH}_2}$$

$$\underset{(red)}{\text{GO/FADH}_2} + 2M^+ \longrightarrow \underset{(ox)}{\text{GO/FAD}} + \underset{(red)}{2M} + 2H^+$$

At electrode:

$$\underset{(red)}{2M} \longrightarrow \underset{(ox)}{2M^+} + 2e^-$$

Where

GO/FAD: represents the FAD redox center of glucose oxidase in its oxidized form.

$GO/FADH_2$: represents the FAD redox center of glucose oxidase in its reduced from.

M: is a mediator, which in the ExacTech blood glucose meter is ferrocene.

The electrons donated to the electrode surface then go to form a current that is proportional to the rate of oxidation of glucose and hence proportional to the glucose concentration in the blood. Devices of this type are far more suitable for miniaturisation. Whilst the ExacTech meter is itself only the size of a pen, devices using similar technology are now being produced that are so small they can be implanted under the skin to produce a blood glucose measuring system *in situ*. Work is ongoing to link such sensors to appropriate logic circuits and an insulin reservoir to provide diabetic patients with exactly the insulin they need throughout the day (Wilson & Walker, 1995).

2- Determination of sulfite

Determination of sulfite is important particularly from biological and industrial point of view. Sulfite is widely used as food additive to prevent oxidation and bacterial growth and to control enzymatic reactions during production and storage. Nowadays, due to the reported harmful effects towards hypersensitive people, the sulfite content in food and beverages have been strictly limited in many countries (Wedzicha, 1984).

A biosensor was developed for the determination of sulfite in food. The *Malva vulgaris* tissue homogenate containing sulfite oxidase enzyme (EC.1.8.3.1) was used as the biological material. This homogenate was cross linked with gelatin using glutaraldehyde and fixed on a pretreated Teflon membrane. Sulfite was enzymatically converted to sulfate in the presence of the dissolved oxygen as follows :

$$SO_3^{2-} + O_2 + H_2O \xrightarrow{\text{Sulfite oxidase}} SO_4^{2-} + H_2O_2$$

The dissolved oxygen was monitored amperometrically. Sulfite determination was carried out by standard curves, which were obtained by the measurement of consumed oxygen level related to sulfite concentration. Several operational parameters had been investigated: the amounts of plant tissue homogenate and gelatin, percentage of glutaraldehyde, optimum pH and temperature. There was linearity in the range between 0.2 and 1.8mM of sulfite at 35°C and pH 7.5. The results of real sample analysis obtained with the biosensor agreed well with the enzymatic reference method using spectrophotometeric detection (Sezgintürk & Dinckaya, 2005).

3- Determination of pesticide residues

The analysis of pesticide residues is an important concern due to their high toxicity and the serious risk that they represent for the environment and human health. Analysis of pesticides is usually carried out by GLC or HPLC. How-

ever, these methods require laborious extraction and clean up steps that increase analysis time and the risk of errors. The development of biosensors is a growing area, in response to the demand for rapid, simple, selective and low cost techniques for pesticides analysis. The main principle of the biosensors developed is based on the correlation between toxicity of a pesticide and a decrease in the activity of a biomarker such as an enzyme. This activity can be registered by employing different transducers, e.g. amperometry, potentiometry, spectrometry, fluorimetry or thermometry for detection of different substrates or products of enzymatic reaction (Velasco-Garcia & Mottram, 2003).

Organophosphorus and carbamate insecticides selectively inhibit cholinesterases. The enzyme acetylcholinesterase (AChE) (EC.3.11.7) catalyses the hydrolysis of acetyl choline to acetic acid and choline:

$$(CH_3)_3\,N\,(OH)\,CH_2\,CH_2\,OCO\,CH_3 + H_2O \xrightarrow{\text{AChE}} CH_3\,COOH + (CH_3)_3\,N\,(OH)\,CH_2\,CH_2\,OH$$

Several authors have used a pH– sensitive transducer in the development of AChE-based biosensors (Andres & Narayanaswamy, 1997). On the other hand, Xavier *et al.* (2000) described an optical fiber biosensor for the determination of the pesticides propoxur and carboxyl, widespread insecticides in vegetable crops.

Biosensors in food quality

It is obvious that many batch operations in the food industry are being replaced by continuous processing and high degree of automation. Accordingly, there is an increasing demand for instruments suitable for automatic quality control through the process and at the end of the line so that the real time state of the process can be described. Biosensors obviously offer food industry monitoring of specific analyte at real-time and a feedback control as shown in Figure (4). This will not only increase the food safety but also provide less effective control, less employment, time and energy saving (Gürtas, 1997).

Biosensors can be used as analytical tools in some food industries, especially applied to the determination of the composition, degree of contamination of raw materials and processed foods, and for the *on-line* control of the fermentation process. Despite the enormous diversity of research involving biosensors for the food industry, its application in this area for any analyte is still restricted (Mello & Kubota, 2002).

Biosnesors or immunosensors reduce assay time and cost or increase the product safety. These methods have been adapted to detect or measure analytes in *on-line* system (Rasooly, 2001). Hazard analysis at critical control points (HACCP) system is, generally accepted as the most effective system to ensure food safety, can utilize biosensors to verify that the process is under control. The high sensitivity of enzymatic biosensors or immunosensors enabled detection of microorganisms like *E. coli, Salmonella, S. aureus*, pesticides, herbicides ... etc. in hours or minutes (Fitzpatrick *et al.*, 2000, Killard & Smyth, 2000). Some commercial biosensors for food industry are given in Table (2). It is worth to mention that a book on commercial biosensors has been published (Ramsay, 1998).

In the present overview we will shade a light on some of important applications of biosensors to the area of food-quality control:

1- Detection of microorganisms

Conventional methods to determine and specify microorganisms are time consuming and laborious. They are based on so-called colony counts on solid media and often include different enrichment and isolation steps on selective media. The confirmation of the

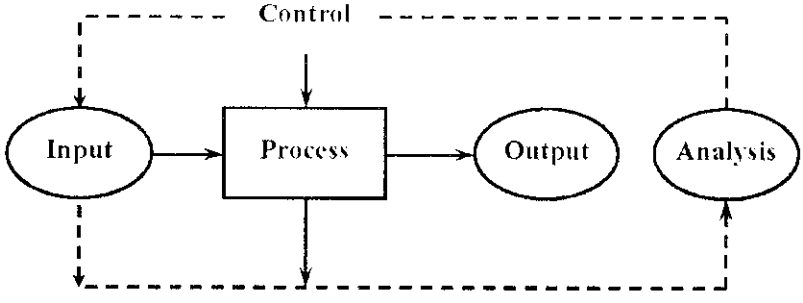

Fig. 4: Desired feedback control of a process

Source: Gürtas (1997)

Table 2: Commercial biosensors for food industry

Companies (country)	Biosensors	Target compounds
Danvers (USA)	Apec glucose analyzer	Glucose
Biometra Biomedizinische Analytik GmbH (Germany)	Biometra Biosensors for HPLC	Glucose, ethanol and methanol
Eppendorf (Germany)	ESAT 6660 Glucose Analyzer	Glucose
Solea – Tacussel (France)	Glucoprocesseur	Glucose and lactate
Universal Sensors (USA)	Amperometric Biosensor Detector	Glucose, galactose, L-amino acids, ascorbate and ethanol
Yellow Springs Instruments (USA)	ISI Analysers	Glucose, lactose, L-lactate, ethanol, methanol, glutamate and choline
Toyo Jozo Biosensors (Japan)	Models: PM-1000 and PM-1000 DC (*on line*), M-100, AS-200 and PM-1000 DC	Glucose, lactate, L-amino acids, cholesterol, tryglicerides, glycerin, ascorbic acid, alcohol
Oriental Electric (Japan)	Oriental Freshness Meter	Fish freshness
Swedish BIACORE AB (Sweden)	BIACORE	Bacteria
Malthus Instruments (UK)	Malthus 2000	Bacteria
Biosenson SpA (Italy)	Midas Pro	Bacteria
Biotrace (UK)	Unilite	Bacteria

Source: Mello & Kubota (2002).

identity of the isolated microorganism is achieved by microscope, biochemical and immunological characteristics. This leads to total detection times of several days which is the major disadvantage of conventional plating methods. However, improved analytical methods have been developed which predominantly use the advantages supplied by immunological or DNA-based methods for the past decade, biosensors have become more and more important for the determination of microorganisms. Very specific antibodies can be produced against surface antigens of various microorganisms. In this way, an immunosensor can discriminate between different organisms. In combination with different transducers (e.g. piezoelectric materials or optical fibers) antibodies have been successfully employed for the detection of microorganisms. Most applications focus on confirming the absence of pathogenic organisms like *Salmonella* species and *Escherichia coli* species. It is worth to mention that many strains of *Escherichia coli* are known to be dangerous human pathogens that can cause life-threatening conditions including blody diarrhea, hemorrhagic colitis, renal failure and meningitis (Kuhnert *et al.*, 2000).

In recent years, various types of biosensors have been developed which could help in overall quality control in food processing plants by detecting pathogens within minutes of sampling. If pathogens are found with *on-* or *near-line* biosensors, then food processors can make decisions more quickly about applying treatments, minimizing the chance of a contaminated final product (Velasco-Garcia & Mottram, 2003).

The general approach for the immunoaffinity steps to capture and concentrate bacteria on beads, a membrane or a fiber optic prob tip, followed by detection of bound bacteria by LASER excitation of bound fluorescent antibodies, a coustogravimetric wave transduction, surface plasmon resonance or electrochemical methods. The infectious dosage of pathogens such as *Salmonella* or *Escherichia coli* 0157:H7 is 10 cells and the existing coliform standard for *E. coli* in water is 4 cells 100 ml^{-1} (Velasco-Garcia & Mottram, 2003).

According to Rand *et al.* (2002) optical biosensors have been developed for rapid detection of contaminates in foods and several of these biosensors have evolved into commercial prototype systems. For instance, *E. coli* 0157: H7 in seeded ground beef samples was detected

at 3-30 cfu/ml, with results obtained within 20 min. of sampling (Demarco *et al.*, 1999).

The limit of detection of immunobiosensor (Silicon chip-based biosensor) is substantially better than the values obtained using enzyme-linked methods and comparable with a polymerase chain reaction (PCR)-chemiluminescent method. Experiments involving the detection of *Salmonella* from chicken carcass washing (showing a recovery of 90%) indicated that this technology could be placed into onsite facilities and used to evaluate the extent of *Salmonella* contamination in the poultry industry (Dill *et al.*, 1999).

Ertl *et al.* (2003) developed a rapid identification of viable *E. coli* species (*E. coli* B, *E. coli* Neotype, *E. coli* JM 105 and *E. coli* HB101) with an electrochemical screen-printed biosensor array. In this method, selective recognition is accomplished using lectins that recognize and bind to cell-surface lipopolysaccharides and colourimetric transduction exploits non-native external oxidants to monitor respiratory cycle activity in lectin-bound cells.

Alocilja & Radke (2003) reviewed pathogen detection markets and their prospects for the future. Potential markets include the medical, military, food, and environmental industries. Those industries combined have a market size of $563 million for pathogen detecting biosensors are expected to grow at a compounded annual growth rate of 4.5%. The food market is further segmented into different food product industries. The trend is pathogen testing emphasize the need to commercialize biosensors for the food safety industry as legislation creates new standards for microbial monitoring. With quicker detection time and reusable features, biosensors will be important to those interested in real time diagnostics of disease causing pathogens. As the world becomes more concerned with safe food and water supply, the demand for rapid detecting biosensors will only increase.

2- Sensory analysis

Two main categories of biosensors for sensory analysis are electronic noses and electronic tongues. It is known that humans perceive odour as single chemicals or as combinations of many different chemicals. Those odour molecules usually have three basic properties. They are small and light, with molecular masses below 300 Da/polar and hydrophobic. An electronic nose recognizes these molecules and certain combinations of them (Giese, 2002).

Electronic noses are generally made up of two main parts: a sensing system and a pattern recognition system. In the past, gas chromatography and mass spectrometry have been used as the sensing systems, although these are usually expensive and time consuming. Currently, these techniques have been replaced by chemical sensors to analyze odour. Essentially, each odour leaves a characteristic pattern or fingerprint of certain compounds. Known odours can be used to build a database to train a pattern recognition system (Giese, 2002).

The artificial neural networks are trained to distinguish certain odours from certain chemical combinations. Pattern-recognition is gained by giving the network known odours and classifying them with a signature. Then the nose is tested to see how well the network has learned. The sensors basically measure the change in voltage due to the presence of certain chemicals. The chemicals in the air change the oxygen content over the sensors, which are electronic circuits. By changing the oxygen content, the resistance across the sensor is changed. This change can be measured as a voltage drop from the normal or standardized conditions (Giese, 2002).

Electronic tongues have been developed to measure taste. Recently, Alpha MOS, Toulouse, France, introduced an electronic tongue for the analysis of taste and nonvolatile chemicals typically found in liquids. The objective is to complement the electronic nose and, more important allow the food and beverage industry to cover a larger proportion of the sensory perception of consumers. The use of both instruments allows food manufacturers to test for both aromal odour and taste (Giese, 2002).

3- Quality control of modified – atmosphere packages

Improper package design or temperature abuse during handling may cause fruits and vegetables in modified – atomosphere packages to be exposed to low, injurious O_2 levels associated with the production of fermentation volatiles, quality loss and eventually product breakdown (Velasco-Garcia & Mottram, 2003). Excessively low package O_2 also may promote growth of dangerous pathogens (e.g. *Clostridium botulinum*).

The detection of ethanol would provide a sensitive technique for low-O_2 injury identifica-

tion. A commercial ethanol biosensor composed of a chromagen and immobilised enzymes: alcohol oxidase (EC.1.1.1.1) and peroxidase (EC.1.11.1.7) have been tested. Alcohol oxidase catalyses oxidation of ethanol into acetaldehyde and H_2O_2 in the presence of O_2 and peroxidase (an H_2O_2 decomposing enzyme) catalyses oxidation of the chromagen causing a colour change. The biosensor detects ethanol to levels below the human olfactory threshold (10µl/l) ethanol in gas phase at 50°C with a 15s exposure. The onset of low O_2 injury was detected in lightly processed lettuce, cauliflower, broccoli and cabbage modified- atmosphere packages as measured by accumulation of headspace ethanol (Smyth *et al.*, 1996). The response of the biosensor was very similar to the one measured by gas chromatography, which is expensive and requires technical expertise. The biosensor could also be useful to monitor ethanol during controlled – atmosphere storage of apples, rot development in stored potato tubers or any application where ethanol accumulation can be associated with a loss of quality.

4- Fish freshness analysis

Fish freshness has been evaluated chemically and expressed as *K*-value which is useful index of raw fish grade. However, the *K*- value approach requires the sample preparation and the complicated sensor system with several kinds of biochemical substances because the *K*-value is calculated from the concentrations of inosine 5'-monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx) in the fish-extract solution, with several kinds of biochemical process and reagents. Then, a newly approach is required at fish markets, restaurants and kitchens, i.e. non-destructive methods with simple biochemical reaction, such as smell evaluation of putrid fish-odour with higher sensitivity of human smell sense (Mitsubayashi *et al.*, 2004).

Trimethylamine (TMA) is typical and common fish-odour substance in seafood, and is produced by the decomposition of trimethylamine N-oxide (TMAO) in sea creatures. The fact is that fresh marine products contain little TMA. Mitsubayashi *et al.*, (2004) constructed a TMA biosensors by immobilizing flavine-containing mono oxygenase type 3 (FMO3), as one of drug metabolizing enzymes in human liver, onto a sensitive area of a dissolved oxygen electrode. This sensor with flow injection analysis (FIA) was calibrated against TMA solutions (Putrefactive substance of fish) from 1.0 to 50 mmol/l. It was obvious that the TMA sensor with FMO3 would be convenient device for evaluating fish freshness (Coefficient of variation: 4.39%, n=5). The behaviour of the biosensor was evaluated using standard TMA solutions as the typical putrefactive substance in fish, with a flow injection analysis system including a computer-controlled potentiostat at a fixed potential of –600 mV versus Ag/AgCl as counter/reference. The sensor output induced by FMO3 enzyme reaction was continuously monitored on a computer display and saved on the hard disk for later analysis (Figure 5).

The TMAO formation from TMA takes place according to the following reaction:

$$TMA + NADPH + H^+ + O_2 \xrightarrow{FMO3} TMAO + H_2O + NADP^+$$

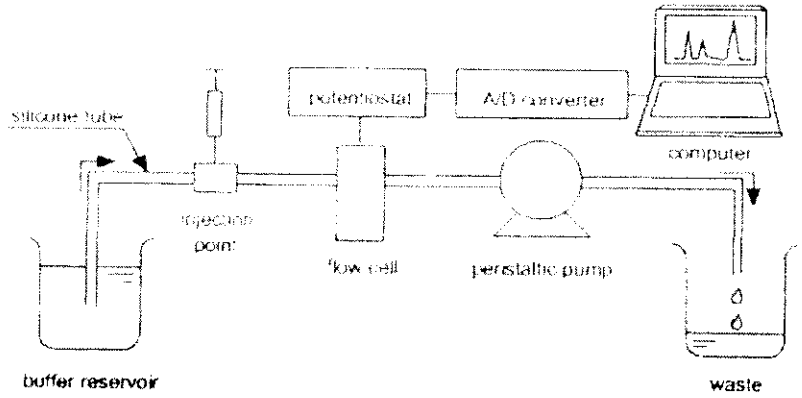

Fig. 5: Schematic diagram of experimental set-up for the flow injection analysis with the FMO immobilized biosensor

Source: Mitsubayashi *et al.* (2004)

5- Quality control of meat

Examples of successful commercialised sensing instruments are meatcheck and biocheck sensors. The meatcheck is a four – electrode array attached to a knife which can be inserted into meat to measure the glucose gradient immediately below the surface. The size of the gradient is related to microbial activity on the surface of the meat and is regarded as a sound indicator of meat quality. The device provides in seconds what laboratory-based microbiology takes days to test. The biocheck method transformed the glucose sensor into a device capable of detecting and quantifying microorganisms in aqueous solutions. The system transfers electrons from the respiratory pathways of microorganisms, and it is capable of detecting bacteria in under two minutes (Maines *et al.*, 1996).

Notwithstanding, concentration of lactic acid is an important parameter for the meat industry as it characterises the state of fresh meat. Lactic acid is caused by an aerobic glycolysis from glycogen *post-mortem* in muscles. The lactic acid concentration leads to conclusions concerning the *pre-mortem* metabolic situation, physical stress and deficiency in the meat quality. Bergann *et al.* (1999) reported on enzymatic biosensor based on immobilised lactate oxidase as bioreceptor and an amperometric transducer. The biosensor estimate lactic acid without special sample preparation, very quickly and at low cost.

6- Quality control of milk

The increasing demand for *on-line* evaluation of milk quality directs the industry to look for practical solutions, and biosensors are a promising possibility. Eshkenazi *et al.* (2000) developed a multi-enzymatic amperometric biosensor for lactose in fresh raw milk. The characteristics of the biosensor (easy operation, rapid response, long stability) suggested that this method could be used as an economical, *on-line* lactose measurement technique in the milking parlour. Also, some biosensors were designed to determine fat in milk (Velasco- Garcia & Mottram, 2003).

Schmidt *et al.* (1999) reported a microbial biosensor based on thick film technology for free fatty acids. The biosensor measures the oxygen uptaken by respiratory activity of the immobilised microorganisms. Oxygen was determined by electrochemical reduction. The sensor could be applied to milk samples without previous pretreatment, having a short response high sensitivity and easy handling. However, biological research is needed to determine how sensor derived information can be used to improve the product quality other than by separating the milk into sources of high and low quality.

REFERENCES

Alocilja, E.C. & Radke, S.M. **2003**. Market analysis of biosensors for food safety. Biosensors and Bioelectronics, **18**: 841-846.

Anonymous. **2001**. Biosensors for industrial applications: A review of biosensor technology. Bio Wise UK Government Programme Funded by the Department of Trade and Industry. www.biowise.org.uk/core-files/biosensor-review.pdf.

Andres, R.T. & Narayanaswamy, R. **1997**. Fiber-optic pesticide biosensor based on covalently immobilized acetyl cholinesterase and thymol blue. Talanta, **44**: 1335-1352

Arnold, M.A. & Myerhoff, M.E. **1984**. Ion selective electrodes. Anal. Chem., **56**: 20R-48R.

Bergann, T., Gifley, K. & Abel, P. **1999**. Concentration of lactic acid in carcasses and fresh meat-estimation with an enzymatic-biosensors or measuring system. Fleischwirtschaff, **79**: 84-87.

Caselunghe, M.B. & Lindeberg, J. **2000**. Biosensor-based determination of folic acid in fortified food. Food Chemistry, **70**: 523-532.

Castillo, J., Gaspar, S., Leth, S., Niculescu, M., Mortari, A., Bontidean, I., Soukharev, V., Dorneanu, S.A., Ryabov, A.D., & Csoregi, E. **2004**. Biosensors for life quality design, development and applications. Sensors and Actuators, **102**: 179-194.

Demarco, D.R., Soaski, K.W., McCrae, D.A. & Lim, D.V. **1999**. Rapid detection of *Escherichia coli* 0157:H7 in ground beef using a fiber optic biosensor. J. Food. Protect., **62:** 711-716.

Dill, K., Stanker, L.H. & Young, C.R. **1999**. Detection of *Salmonella* in poultry using a silicon-chip based biosensor. Journal of Biochemical and Biophysical Methods, **41**: 61-67.

Ertl, P., Wanger, M., Corton, E. & Mikkelsen, S.R. **2003**. Rapid identification of viable *Escherichia coli* subspecies with an electrochemical screen-printed biosensor array. Biosensors and Bioelectronics, **18**: 907-916.

Eshkenazi, I., Maltz, E., Zion, B. & Rishpon, J. **2000**. A three-cascaded-enzymes biosensor to determine lactose concentration in raw milk. Journal of Dairy Science, **83**: 1939-1945.

Fitzpatrick, J., Fanning, L., Hearty, S., Leonard, P., Manning, B.M., Quinn, J.Q. & O'Kennedy, R. **2000**. Application and recent develop-

ments in the use of antibodies for analysis. Analytical Letters, **33**: 2563-2609.

Franek, M. & Hruska, K. **2005**. Antibody based methods for environmental and food analysis. A Review. Vet. Med. Czech., **50**: 1-10.

Giese, J. **2002**. Food biosensors. Food Technology, **56** (7): 72-75.

Gürtas, F. **1997**. Use of *on-line* biosensors in food industry. Journal of Qafqaz University, **1** (1): 138-150.

Heldman, D.R. **2004**. Identifying food science and technology research needs. Food Technology, **58** (12): 32-34.

Killard, A.J. & Smyth, M.R. **2000**. Separation-free electrochemical immunosensor strategies. Analytical Letters, **33**: 1451-1465.

Kuhnert, P., Boerlin, P. & Frey, J. **2000**. Target genes for virulence assessment of *Escherichia coli* isolates from water, food and the environment. FEMS Microbiol. Rev., **24**: 107-117.

Maines, A., Ashworth, D. & Vadgama, P. **1996**. Enzyme electrodes for food analysis. Food Technology and Biotechnology, **34** : 31-42.

Mello, L.D. & Kubota, L.T. **2002**. Review of the use of biosensors as analytical tools in food and drink industries. Food Chemistry, **77**: 237-256.

Mitsubayashi, K., Kubotera, Y., Yano, K., Hashimoto, Y., Kon, T., Nakakura, S., Nish, Y. & Endo, H. **2004**. Trimethylamine biosensor with flavin containing monoxygenase type 3 (FMO3) for fish-freshness analysis. Sensors and Actuators, **103**: 463-467.

Ramsay, G. **1998**. Commercial Biosensors. John Willey, New York.

Rand, A.G., Jianming, Y., Chris, W., Brown, W. & Letcher, S. **2002**. Optical biosensors for food pathogen detection. Food Technology, **56** (3): 32-39.

Rasooly, A. **2001**. Surface plasmon resonance analysis of staphylococcal enterotoxin B in food. Journal of Food Protection, **64**: 37-43.

Schmidt, A, Standfub-Gabisch, C. & Bilitewski, U. **1999**. Microbial biosensor for free fatty acids using an oxygen electrode based on thick film technology. Biosensors and Bioelectronics, **11**: 1139-1145.

Smyth, A.B., Talasila, P.C. & Cameron, A.C. **1996**. An ethanol biosensor can detect low-oxygen injury in modified atmosphere packages of fresh-cut produce. Post harvest Biology and Technology, **15**: 127-134.

Sezgintürk, M.K. & Dinckaya, E. **2005**. Direct determination of sulfite in food samples by a biosensor based on plant tissue homogenate. Talanta, **65**: 998-1002.

Updike, S.J. & Hicks, G.P. 1967. Enzyme electrode. Nature, **214**: 986-991.

Velasco-Garcia, M.N. & Mottram, T. **2003**. Biosensor technology addressing agricultural problems: A Review Biosystems Engineering, **84**: 1-12.

Wedzicha, B.L. **1984**. Chemistry of Sulpher Dioxide in Foods. Elsevier, Amsterdam.

Wilson, K. & Wallker, J. (Eds.) **1995**. Practical Biochemistry Principles and Techniques. Fourth edition. Cambridge University Press.

Xavier, M.P., Vallejo, B., Marazuda, M.D., Moreno Bondi, M.C., Baldini, E. & Falai, A. **2000**. Fiber optic monitoring of carbamate pesticides using porous glass with covalently bound chlorophenol red. Biosensors and Bioelectronics, **14**: 895-905.

تطبيقات المستشعرات الحيوية في تحليل ومراقبة جودة الأغذية: نظـرة شـاملة

محمد محمود يوسف، نسمة نبيل الحداد

قسم علوم وتكنولوجيا الأغذية – كلية الزراعة (الشاطبي) – جامعة الإسكندرية – الرقم البريدي ٢١٥٤٥ الإسكندرية – جمهورية مصر العربية

المستشعرات الحيوية هي نتاج تزاوج بين البيولوجي والإلكترونيات، فالمستشعر الحيوي ما هو إلا أداة تحليلية تتكون من عامل حيوي (إنزيم، خلية، نسيج) ومحول إما أن يكون كيماويا أو فيزيقيا (كهروكيماوي، كتلي، ضوئي، حراري) في مقدوره تحويل الإشارة البيولوجية أو الكيموحيوية أو الاستجابة إلى إشارة كهربية كمية تكون دالة للمكون موضع التقدير. وفي الحقبة الأخيرة تم تطوير مستشعرات حيوية حديثة تتكون من المكونين سابقي الذكر (المكون البيولوجي، المكون الإلكتروني) مع الاستفادة من تكنولوجيا المعلومات الممثلة من المنظور الإلكتروني في الدوائر الكهربية الميكرو والألياف الضوئية في حين تمثل الإنزيمات والأجسام المضادة مقومات المكون البيولوجي الذي يدخل في تركيب المستشعر الحيوي.

لاشك أن تقنية المستشعرات الحيوية توفر طريقة جديدة تعتمد على تخصصية مادة التفاعل مع إمكانية تكرارية القياس وكذا اجراء التحليل على خط الإنتاج نفسه، الأمر الذي يعني إمكانية مراقبة جودة العمليات التصنيعية في مجال إنتاج الغذاء. وتعد القياسات على خط الإنتاج من الأهمية بمكان بالنسبة لنظم مراقبة وتوكيد الجودة وكذا في نظام تحليل المخاطر عند نقاط التحكم الحرجة (HACCP)، وناهيك عن أهمية المستشعرات الحيوية كأدوات تحليلية فاعلة وسريعة في الكشف عن تلوث مياه الشرب حال حدوث أي إرهاب بيولوجي. وتتميز المستشعرات الحيوية بعدة مزايا أهمها سهولة التشغيل، سرعة الاستجابة، طول الثبات، مما حدا بالمعنيين بمجال تحليل ومراقبة جودة الأغذية إلى اقتراح استخدام المستشعرات الحيوية كأدوات تحليلية ذات مردود اقتصادي في هذا المجال.

وتتناول هذه المقالة على وجه الخصوص تطبيقات المستشعرات الحيوية الحالية والواعدة في مجالي تحليل ومراقبة جودة الأغذية. من ناحية التحليل نوه إلى أن عديداً من مكونات الأغذية يمكن تقديرها باستخدام المستشعرات الحيوية، أما بالنسبة لتطبيقات هذه المستشعرات في مجال مراقبة جودة الأغذية فهي متعددة. وسنتناول منها تفصيلا الأمثلة التالية: الكشف عن الكائنات الحية الدقيقة، التحليل المسمى باللسان والأنف الإلكترونيين، مراقبة جودة عبوات الأغذية في جو معدل، تقدير طزاجة الأسماك، تقويم جودة كل من اللحوم والألبان.