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# Food Biosensors

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 analysts 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.

Biosensors have been developed through a combination of electronics/information technology and biology. Information technology contributed microcircuits and data processing capabilities, while biology offers enzymes and antibodies as sensing elements. The biological or biologically derived sensing element is either integrated within or associated with a physicochemical transducer. The transducer is used to produce either discrete or continuous digital electronic signals, which are proportional to a single analyte or a related group of analytes.

Food biosensors have been developed for several analytical tasks, including determination of food composition, food sensory analysis, and food pathogen detection.

## Food Composition

Various biosensors for composition analysis have been developed for carbohydrate analysis, organic acid measurement, and determination of the content 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 high-performance liquid chromatography and gas chromatography, which may require high maintenance, expert operators, and long analysis times, making them less practical for food process monitoring.

The YSI 2700 from YSI, Inc. Yellow Springs, Ohio, may be used for food compositional analysis to measure common food ingredients, such as glucose (dextrose), 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.

The membranes contain three layers. The first layer, porous polycarbonate, limits the diffusion of the substrate into the second layer (enzyme), preventing the reaction from becoming enzyme-limited. The third layer, cellulose acetate, permits only small molecules, such as hydrogen peroxide, to reach the electrode, eliminating many electrochemically active compounds that could interfere with the measurement. For example, for glucose measurement, D-glucose is oxidized in the presence of glucose oxidase, producing hydrogen peroxide and glucono-lactone. For starch measurement, an indirect reading is obtained by measuring the dextrose liberated from the external hydrolysis of starch. Starch is hydrolyzed externally by the enzyme amylogluco-sidase to produce dextrose, which is then is oxidized in the presence of glucose oxidase, producing hydrogen peroxide.

Biacore Inc., Piscataway, N.J., offers biosensors based on surface plasmon resonance (SPR) to monitor interactions by measuring the mass concentration of biomolecules close to a surface. SPR occurs when light is reflected from a conducting film at the interface between two media of different refractive index. In the Biacore systems, the media are the sample and the glass of the sensor chip, and the conducting film is a thin layer of gold on the chip surface.

According to the company, SPR causes a reduction in the intensity of reflected light at a specific angle of reflection. This angle varies with the refractive index close to the surface on the side opposite from the reflected light (the sample side in Biacore). When molecules in the sample bind to the sensor surface, the concentration, and therefore the refractive index at the surface, changes and an SPR response is detected. Plotting the response against time during the course of an interaction provides a quantitative measure of the progress of the interaction. This plot is called a sensorgram.

What the Biacore systems actually measure is the angle of minimum reflected light intensity. The light is not absorbed by the sample. Instead, the light energy is dissipated through SPR in the gold film. Thus, the light used to detect interaction processes never enters the sample. SPR response values are expressed in resonance units (RUs). For most proteins, this is roughly equivalent to a change in concentration of about 1 pg/mm$^2$ on the sensor surface. The exact conversion factor between RU and surface concentration depends on properties of the sensor surface and the nature of the molecule responsible for

the concentration change.

The company offers several kits for specific analytes. Folic acid levels may be measured with quantitative, ready-to-use kits. AOAC method validation is pending for this kit. Other kits are available for biotin levels and vitamin B-12.

Future developments for the system may include protein quality measurement and detection of allergens, genetically modified organisms, and pathogens.

## Sensory Analysis

Two main categories of biosensors for sensory analysis are electronic noses and electronic tongues.

• **Electronic Noses.** Humans perceive odor as single chemicals or as combinations of many different chemicals. These odor 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.

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 odors. Essentially, each odor leaves a characteristic pattern or fingerprint of certain compounds. Known odors can be used to build a database to train a pattern recognition system.

The systems may be set up to either have a sensor for every chemical, although this would be costly, or use artificial neural networks. These networks are able to detect more chemicals than the number of sensors they are utilizing. They also allow for less-selective and therefore less-expensive chemical sensors. The artificial neural networks are trained to distinguish certain odors from certain chemical combinations. Pattern recognition is gained by giving the network known odors 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.

How odors are sampled is a major concern for electronic noses. The instruments work well if they are kept under constant conditions, but if they are being used outside, for example, the temperature, pressure, and humidity may have an impact on the output. The sensors drift and give varying results from day to day.

Much research is being performed to try to solve this problem. Researchers at North Carolina State University are currently working on an electronic nose to detect dangerous odors emitted from

hog farms. They are running into many problems with varying conditions, but are making progress toward solutions.

Sampling techniques are used to bring the sample into an area where the conditions can be controlled. There are two basic types of sampling techniques: flow injection analysis (FIA) and static headspace analysis (SHA). FIA involves a known gas constantly being pumped across a sensor. Then a known concentration of the gas in question is injected into the air stream before the sensor. SHA is the more common practice, since it is easier to perform. It basically involves allowing the headspace above a certain sample to become saturated with the odor, which is then pumped across the sensor. In each method, the temperature, humidity, and pressure can be controlled.

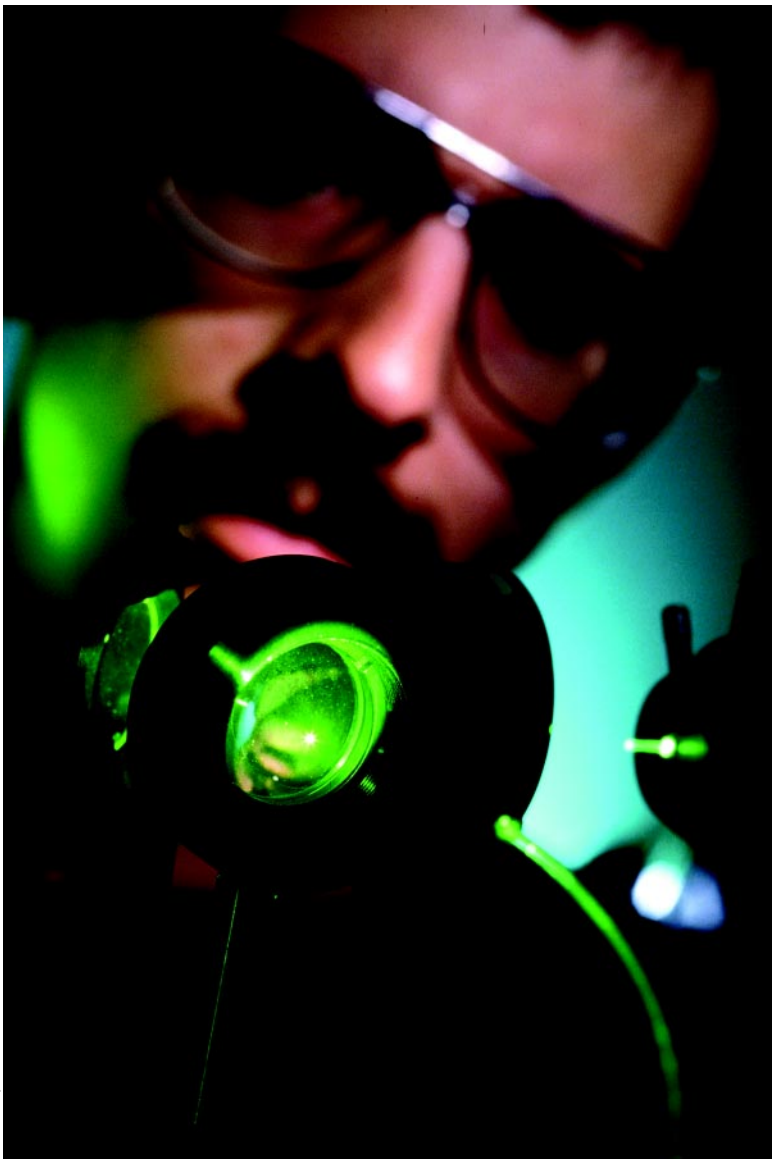

Photo courtesy of USDA/ARS

**Optical biosensor developed by the U.S. Dept. of Agriculture allows the measurement of aflatoxin or fumonisin in corn.**

• **Electronic Tongues.** In comparison to electronic nose systems, which measure the volatile chemical components that constitute a sample's odor, 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 aroma/odor and taste.

The electronic tongue is unaffected by carbon dioxide. For orange juice and apple juice, it will more typically measure the nonvolatile components, including chemical molecules responsible for sweetness, bitterness, saltiness, and sourness.

A group of researchers in Malaysia recently developed a taste-sensing system for herbal sample analysis. Zuraini Dahari and other researchers at the School of Electrical and Electronic Engineering, Universiti Sains Malaysia, developed the system to assist in herbal standardization techniques. Research focused on two main problems: qualitative (to distinguish certain herbs from another) and quantitative (to ensure that the amount of original herbs used are as claimed by the manufacturers). Analysis was done in three successive steps: identify the herbs, determine the technology used, and classify the sample according to the discrete concentration. Results indicate that the sensor was able to distinguish selected herbs from others, even within the same sample.

## Pathogen Detection

Many of the biosensors for microbial detection have been developed for clinical applications. The devices use a variety of electrochemical, optical, and mechanical mechanisms as transducing elements. As in clinical settings, there is a need for rapid methods to detect pathogenic bacteria in food products as an alternative to current laborious, expensive, and time-consuming culture procedures. Some of the more recent developments in this area are described below:

• Biosensors being developed at the University of Arkansas may provide fast, reliable methods for detecting *Escherichia coli*, *Salmonella typhmurium*, and other illness-causing bacteria during poultry processing. Most of the sensors the researchers have developed use antibodies to trap specific bacteria. An immuno-optical capillary column–based biosensor, for example, pumps the sample solution through capillary columns lined with antibodies that can capture bacteria. The sensor then uses secondary antibodies labeled with an enzyme, such as alkaline phosphatase, to produce a signal that can be measured optically or electrochemically. The researchers are working with a Fayetteville, Ark., firm to create a company through which this technology will be transferred to the poultry industry.

• A team at Pennsylvania State University has developed a technique for detection of *Salmonella enteritidis* and *E. coli* in milk and related food products using SPR-based biosensors. The research team investigated two SPR-based biosensors to detect *S. enteritidis* and *E. coli* in spiked milk samples at cell concentrations from $10^1$–$10^7$ cfu/mL,

using antibodies for specific binding of the pathogens. A fully automated angle-dependent Biacore 2000 SPR-based biosensing system and a portable, low-cost integrated Spreeta biosensor from Texas Instruments were evaluated against traditional standard plate counts for rapid, sensitive, and specific detection of the pathogens.

Direct assays were employed for both detection techniques. This involved binding of whole bacteria cells with antibodies bound to the sensor surface. The limit of detection for *S. enteritidis* and *E. coli* was determined to be $10^1$ cfu/mL for both pathogen detection methods. The Spreeta biosensor had a shorter detection time per cycle (8 min), compared to the Biacore 2000 (15 min). Background noise level was higher for the Spreeta unit than for the more widely used Biacore system. In both cases, cross-binding antibodies and bacteria did not generate a notable response.

### Recent Developments

Some promising recent developments in the field are the use of optical biosensors. Optical biosensors have been developed for rapid detection of contaminants in foods, including pathogens, and several have evolved into commercial prototype systems. The propagation of light through a fiber or waveguide can be very sensitive to the surroundings, making optical fibers excellent detectors. The development of these optical biosensors was fully described in the March 2002 issue of *Food Technology*. A copy can be found online at www.ift.org/publications/docshop/ft_shop/03-02/fttoc-56-03-Mar02.shtml.

## PRODUCTS & LITERATURE

**Pathogen Tests**, called Pathigen7, are intended for the detection and presumptive identification of *E. coli* O157, *Salmonella*, *Listeria*, and *Campylobacter* in food and environmental samples. The tests utilize a sandwich immunoassay format. One antibody specific for the pathogen to be measured is immobilized on paramagnetic particles. The other antibody is labeled with a proprietary label. The sample is mixed with these reagents. When the specific pathogen to be measured is present in the sample, both antibodies bind to it, effectively linking together the magnetic particles, the organism, and the special label. The reaction mixture is then transported with an assay buffer to a flow cell with an electrode, which stimulates the bound label to emit light.

The bead-based format has been shown to provide robust performance in many different applications, even when challenged with the most demanding and complex food matrices. For more information, contact Igen International Inc., 16020 Industrial Dr., Gaithersburg, MD 20877 (phone 301-869-9800 or 800-336-4436; fax 301-947-6990; www.igen.com).

**Sample Preparation**, NIR spectroscopy, and rapid determination of fat content are topics covered in a series of informational bulletins from Buchi Analytical, Inc. Rapid determination of fat content and individual fatty acids by the Caviezel method is covered in Bulletin 01-2000. The method is based on the fat definition given by the Food and Drug Administration and involves extracting fat from the sample matrix using n-butanol as a solvent and simultaneously saponifying the fat in the presence of potassium hydroxide. The limiting step in the analytical pathway, particularly in food analysis, is sample preparation.

Bulletin 05-2001 discusses the relevance of the mixing, grinding, and homogenization processes and their influence on the accuracy of the sample analysis. Bulletin 10-2001 discusses the importance of the right sample preparation of food samples for NIR spectroscopy. It evaluates the ideal sample preparation tool to find the optimal measurement conditions for NIR analysis of meat samples.

For a copy of any of the bulletins, contact Buchi Analytical, Inc., 19 Lukens Dr., New Castle, DE 19720 (phone 302-652-3000; fax 302-652-87770).

**Rapid Sugar Determination** in turbid samples is possible with the OXI 50 UV analyzer. The unit can oxidize different kinds of glucose into aldehydes, which can be detected through UV spectrophotometric reading. The company claims that its innovative technique can be used with turbid samples and is not sensitive to chemical interferences. The photo-oxidation module and UV analyzer are used as a unit for titration control of sugar in soft drinks, wine, fresh juices, and other beverages. For more information, contact Secomam, 1/3, rue des Charbonniers, 95335 Domont Cedex, France (phone 011-330-139-35-4200; e-mail info@secomam.fr).

**Mini Digital Refractometer**, Model RF 80, may be used to measure both %Brix concentration and refractive index. Measurements range from 0 to 45% Brix with 0.1% resolution and accuracy. Features include a large, easy-to-read LCD display in Brix (%), refractive index (nD), energy kcal/100 g of solution (cal), or temperature (°C), automatic temperature compensation, zero adjustment function, and auto power off. For more information, contact Extech Instruments Corp., 285 Bear Hill Rd., Waltham, MA 02451-1064 (phone 781-890-7440; fax 781-890-7864; www.extech.com).

**Sterile-Pack Swabs** for industrial surface sampling are available are combined with a tube filled with rinse solution. Designed for hard-to-reach places, the swabs can fit easily into equipment recesses, nooks, and crevices. They are double wrapped, gamma-irradiated, and validated after exposure to the vaporized hydrogen peroxide atmosphere used during isolator facility decontamination cycles. The swabs are said to be ATP-free and have a shelf life of up to one year. For more information, contact BD Diagnostic Systems, 7 Loveton Cl., Sparks, MD 21152 (phone 410-316-4467).

**New Testing Method** for the identification of *E. coli* and total coliforms is available with the use of MI Agar. The agar is said to produce results in a fraction of the time of other testing methods. It utilizes a select antibiotic that suppresses non-coliform organisms, making positive identification more accurate than other conventional media. The presence of total coliforms is indicated by the development of fluorescence under UV light. *E. coli* present in a sample are similarly identified by the development of fluorescent blue colonies. The chromogenic and fluorescent results make the test easier to interpret, contributing to a claimed 95% accuracy rate. For more information, contact S&S Biopath, 950 N. Congress Ave., Riviera Beach, FL 33404 (phone 800-645-2302). ●