JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

REVIEWS

The Application of Biosensors to Fresh Produce and the Wider Food Industry

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The inherent specificity, selectivity, and adaptability of biosensors make them ideal candidates for use throughout the food industry. Potential applications within the supply chain range from testing of foodstuffs for maximum pesticide residue verification through to the routine analysis of analyte(s) concentrations, such as, glucose, sucrose, alcohol, etc., which may be indicators of food quality/acceptability. Biosensor formats include simple "one-shot" disposable devices that can be used either in the field or integrated into more sophisticated laboratory instruments. Until now, the main impact of these devices has been in the medical diagnostics field. However, with ongoing technical development, the food industry will be one of the prime beneficiaries of biosensor technology in the future. This report assesses the current and future trends in the application of biosensors to fresh produce and the wider food industry, focusing on both potential and current target analytes that are fundamental to fresh produce quality, traceability, and safety.

Keywords: Biosensors; food quality; food safety; fresh produce

INTRODUCTION

Quality is the key issue common to all horticultural products. In virtually all cases, fresh produce quality is set at harvest and then inevitably declines during postharvest senescence. To evaluate quality, one must be able to measure quality-related attributes (*1*). Instrumental measurements are preferred to sensory evaluations in research and commercial situations as they reduce variations in judgment among different individuals (*1*). This approach can provide a means of transferring objective information on quality throughout the supply chain and is, thus, fundamental to ensuring greater vertical integration and maintaining trust between supply chain actors.

Fresh produce quality assessment can be either destructive or nondestructive. Currently, most nondestructive techniques are not yet appropriate for large-scale commercial use. Various methods using either chlorophyll fluorescence, delayed light emission, electronic nose technology, nuclear magnetic resonance imaging, optical tomography, ultrasound, and X-ray are either still in their infancy or currently too expensive and/or unreliable to be adopted into most routine quality control (QC) operations. Despite this, some imaging technology (e.g., infrared spectroscopy/reflectance) is close to industrial adaptation (*2*, *3*). However, in the short to medium term, QC improvements for fresh produce should also be based on established technology that is proven and inexpensive. Biosensors may offer one opportunity to fulfill this niche by enhancing the relevance and extent of QC tests being carried out through measuring specific target analytes that are directly related to produce quality.

PRINCIPLES OF BIOSENSOR TECHNOLOGY

The commercial application of biosensors has had a significant impact in a number of areas, particularly in the field of medical diagnostics. Disposable blood glucose biosensors, frequently used by diabetes sufferers to monitor their blood sugar levels, make up nearly 87% of the current total biosensors market (*4*). Undoubtedly, this trend will continue with opportunities to exploit biosensor technology in areas other than medical diagnostics. One such industry where biosensor technology will be exploited is in the food industry. Currently, however, food testing represents a very small percentage of the total marketplace, but with advances in sensor longevity and stability and with new applications on the horizon, biosensors for food diagnostics are set to expand. Traditionally, the food industry has taken a very conservative approach to the introduction of biosensors but would benefit from improvements in QC, safety, and traceability that these relatively inexpensive devices can offer (*5*).

A biosensor can be defined as an integrated receptor−transducer device, which is capable of providing selective quantitative or semiquantitative analytical information using a biological recognition element (6). Accordingly, the basic principle of biosensor technology (**Figure 1**) is to convert a biologically induced recognition event (e.g., enzyme, antibody)

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10.1021/jf040319t CCC: $30.25
Published on Web 02/03/2005

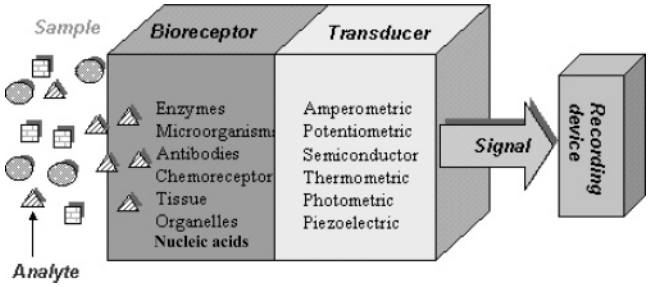

Figure 1. Key components of a biosensor showing examples of biological receptors, transducers, and the signal display (adapted from ref *4*).

Table 1. Main Transduction Systems Used for Biosensor Fabrication

transducer type	examples
electrochemical	clark electrode; mediated electrodes; ion selective electrodes (ISEs); field effect transistor (FET) based devices; light addressable potentiometric sensors (LAPS)
optical	absorbance, luminescence, fluorescence, photodiodes; waveguide systems; integrated optical devices
piezoelectric	quartz crystals; surface acoustic wave (SAW) devices
calorimetric	thermometric

into a detectable signal, via a transducer (**Table 1**) and processor. The end result is a display depicting both the presence and the concentration of the target analyte.

Electrochemical Biosensors. Electrochemical biosensors are based on monitoring electroactive species that are either produced or consumed by the action of the biological components (e.g., enzymes and cells). Transduction can be performed using one of several methods under two broad headings: potentiometry and amperometry.

Potentiometric biosensors are based on monitoring the potential of a system at a working electrode, with respect to an accurate reference electrode, under conditions of essentially zero current flow. In operation, potentiometric measurements are related to the analyte activity (of a target species).

The use of ion selective and gas sensitive membranes coupled to enzyme systems, linked to the potentiometric sensor, allows the fabrication of a biosensor device specific to the enzyme substrate or product. By measuring either the ions or the gases that are generated or consumed as a result of the enzyme activity, an effective method for measuring the concentration of the target analyte can be realized.

Potentiometric biosensors can operate over a wide range (usually several orders of magnitude) of concentrations. The use of potentiometric biosensors for food analysis has not been as widely reported as for amperometric sensors. However, examples of where this approach has been used, for food analysis, include estimating monophenolase activity in apple juice (*7*), determining the concentration of sucrose in soft drinks (*8*), measuring isocitrate concentrations in fruit juices (*9*), and determining urea levels in milk (*10*).

Generally, the use of amperometry as the method of transduction has proved to be the most widely reported using an electrochemical approach. Both "one-shot" (disposable) sensors and on-line (multimeasurement) devices have been described, monitoring a wide range of target analytes. In contrast to potentiometric devices, the principle operation of amperometric biosensors is defined by a constant potential applied between a working and a reference electrode. The imposed potential encourages redox reactions to take place, causing a net current to flow. The magnitude of this current is proportional to the concentration of electroactive species present in solution. Both cathodic (reducing) and anodic (oxidizing) reactions can be monitored amperometrically.

Many amperometric biosensors described to date have been based on the use of enzymes. Typically, oxidase enzymes have been the most frequently exploited catalysts used for these biosensor formats. In operation, amperometric biosensors tend to monitor either the oxygen consumed or the hydrogen peroxide generated. Both are electrochemically active; oxygen can be electrochemically reduced, and hydrogen peroxide can be oxidized. The current generated is proportional to the concentration of the enzyme substrate (i.e., the target analyte) present. Biosensor technologists have also adopted other approaches, including the use of mediators. These compounds are able to replace oxygen as an electron acceptor and to operate at a much lower operating potential, reducing the effects of other electrochemically active species found in many food matrices (*11*).

Commercially available instruments, based on amperometric enzyme biosensors, are available. An example of this includes the range of analyzers manufactured and sold by YSI Inc. (Yellow Springs, OH). These instruments are designed for use in clinical diagnostics, environmental monitoring, and the food processing industries. The YSI 2700 SELECT Biochemistry Analyzer is designed to measure common food components such as glucose (dextrose), sucrose, lactose, lactate, galactose, glutamate, choline, glutamine ethanol, hydrogen peroxide, and starch. This is a fully automated instrument, at the heart of which is an electrochemical amperometric biosensor.

Calorimetric Biosensors. Sensors based on calorimetric transduction are designed to detect heat generated or consumed during a biological reaction. Many biochemical reactions are accompanied by either heat absorption or production; by using sensitive heat detection devices, biosensors for specific target analytes have been constructed. In the field of food analysis, several reports have described the use of such biosensors to detect metabolites. Thavarungkul et al. (*12*) described a thermometric biosensor system to determine sucrose in sugar cane. To measure sucrose, the enzyme invertase was immobilized on a thermistor system and the heat generated during the enzyme reaction was used to calculate the sucrose content of cane sugar.

Optical Biosensors. In addition to electrochemical transduction methods, optical-based biosensor systems have proved to be the most widely reported. These sensors are based on measuring responses to illumination or to light emission. Optical biosensors can employ a number of techniques to detect the presence of a target analyte and are based on well-founded methods including chemiluminescence, light absorbance, fluorescence, phosphoresence, photothermal techniques, light polarization and rotation, surface plasmon resonance (SPR), and total internal reflectance.

Optical-based biosensors offer a number of advantages including speed and reproducibility of the measurement. Commercially, one of the most successful optical-based biosensor systems introduced has been the range of instruments supplied by BIAcore (Uppsala, Sweden). This instrument can be employed to study a wide range of biological interactions, both automatically and in real-time. The instrument is based on SPR, whereby biomolecular binding events cause changes at a metal/liquid interface, usually involving a complex that includes a specific antibody against a target analyte. On binding, these changes (in the refractive index) are recognized by a shift in the SPR signal, indicating a presence of the target analyte in a sample solution. One particular advantage for sensors based on SPR is that the system does not require the presence of a labeled

Table 2. Examples Showing the Use of Biosensors to Detect the Presence of Contaminating Microorganisms on Food

target organism	food matrix	detection method	detection limit	ref
Escherichia coli	dairy products	acoustic	3×10^5 to 6.2×10^7/mL	*16*
staphylococcal enterotoxin A	hot dogs, potato salad, milk, and mushrooms	optical	10–100 ng/g	*17*
Salmonella typhimurium	chicken carcass wash fluid	optical	1×10^5 to 1×10^7/mL	*18*
staphylococcal enterotoxin B	milk	optical	0.5 ng/mL	*19*
Salmonella groups B, D, and E	range of foods	optical	1×10^7 CFU/mL	*20*
E. coli	range of foods	QCM (acoustic)	1.7×10^5 to 8.7×10^7 CFU/mL	*21*
E. coli 0157:H7	range of foods	optical	1×10^3 CFU/mL	*22*

ligand (e.g., enzyme conjugated antibody) to function. SPR sensor systems have been used extensively to investigate the presence of harmful contaminating microorganisms in food and to determine food quality. For example, an optically based biosensor was recently used to screen poultry liver and eggs for the presence of the drug nicarbazin, a feed additive used to prevent outbreaks of coccidiosis in boiler chickens (*13*). The limits of detection for the sensor system were 17 and 19 ng g^{-1} for liver and eggs, respectively. Mohammed et al. (*14*) have also demonstrated the use of this technique to detect the presence of allergens, in particular peanuts, during food production.

Acoustic Biosensors. Piezoelectric quartz crystals can be affected by a change of mass at the crystal surface; this phenomenon has been successfully exploited and used to develop biosensors. For practical applications, the surface of the crystal can be modified with recognition elements (e.g., antibodies) that can bind specifically to a target analyte. If the crystal is placed in an alternating electric field, the crystals are subjected to mechanical deformations. At a particular frequency, a mechanical or acoustic resonance is induced. The frequency of this response will be dependent on the size and mass of the crystal. Hence, any change in mass (e.g., binding of the target analyte to the recognition element) is detected by the change in oscillation frequency of the crystal. As with the optical methods of detection, biosensors based on acoustic transduction have tended to be used mainly for the detection of contaminating microorganisms (see **Table 2**). Nonetheless, such sensor systems have been used to monitor other aspects of food production. Mannelli et al. (*15*) described the use of an acoustic sensor to detect genetically modified organisms. Such devices could pave the way to providing efficient screening tools in food analysis.

Immunosensors. Immunosensors are based on exploiting the specific interaction of antibodies with antigens. Typically, immunoassays (such as the widely used enzyme-linked immunosorbent assay technique) employ a label (e.g., enzyme, fluorescent marker) to detect the immunological reaction. The use of biosensor platforms, linked to an immunoassay format, offers a route to rapid quantitative measurements of target analytes. Both electrochemical and optical transduction systems have been exploited. For example, immunochromatographic methods can be coupled with electrochemical or optical detectors to yield simple dipstick style devices, combining the speed and convenience of sensors with the specificity and sensitivity of immunoassays.

To use labelled antibodies, a number of detection strategies are available including competitive competition and displacement assays (*23*). An example of where this approach has been exploited is illustrated by the detection of bovine progesterone during milking (*24*, *25*). With reproductive management a major financial concern of the dairy industry, these biosensor systems were designed to provide a rapid means for determining the onset of oestrus in dairy cattle. The sensor systems were designed to be operated in the dairy parlor during milking. Both approaches adopted a liquid handling system linked to a suitable immunoassay sensor. Similarly, flow injection analysis coupled to an electrochemical-based immunosensor system has been developed for the detection of gentamicin in milk (*26*). Again, the aim was to develop a field-based system that could be operated at-site. The system was able to distinguish between 0 and 100 μg/kg gentamicin in milk in less than 10 min with no sample pretreatment required.

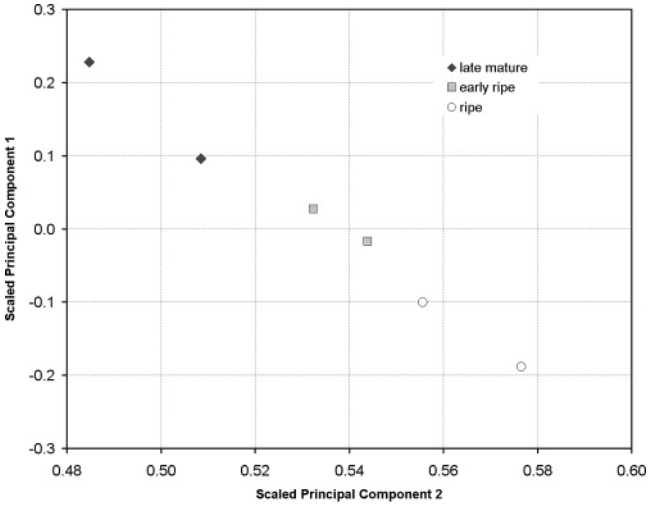

Figure 2. Chemometric (principle component analysis) output from biosensor array to score pineapple cv. Queen Victoria fruit ripeness. PCA scores plot for the scaled data matrix. PC1 and PC2 represent the first and second principal components, respectively (adapted from refs *28* and *29*).

In addition to the use of antibody-based immunosensors, receptor-based devices have also been described. Setford et al. (*27*) employed an amperometric affinity sensor for the rapid quantification of β-lactam in milk. Biorecognition was achieved using an immobilized β-lactam antibiotics specific receptor binding protein to measure penicillin G levels in milk. This device was designed to be a one-shot disposable sensor, based on screen-printed sensors, making it an ideal field-based screening tool.

Sensor Arrays. The ability to measure several analytes at the same time, using a single biosensor element, is becoming a reality (*28*−*30*). A one-shot disposable biosensor, comprised of individual sensor elements printed on one support matrix, has recently been described (*28*, *29*). The sensor array simultaneously measured glucose, sucrose, and ascorbic acid concentrations in tropical fruits such as pineapples (**Figure 2**). Such measurements can be used to determine the status of fruit maturation and ripening, hence, allowing growers, food transport operatives, and retailers the opportunity to rapidly determine the quality of the produce.

Whole Cell Biosensors. The use of immobilized whole cells (usually bacteria) as the recognition element for biosensor

Table 3. Examples of Using Microorganisms Incorporated into a Biosensor Format to Detect Various Analytes in Food

analyte	microorganism	ref
short chain fatty acids	*Arthrobacter nicotianae*	*31*
vitamin B-6	*Saccharomyces uvarum*	*32*
phenylalanine	*Proteus vulgarius*	*33*
sulfite	*Thibacillus thiooxidans*	*34*
glucose	*Aspergillus niger*	*35*

applications has been widely described (**Table 3**). Typically, electrochemical transduction methods have been used, particularly, the Clark oxygen electrode. These sensor systems rely on the interaction of a particular microorganism in the presence of a target analyte. By monitoring the respiratory activity of the microorganism, it has proved possible to detect and quantify the target analyte in a range of food matrices. However, traditional whole cell biosensors are inherently nonspecific in their action and, thus, may not be appropriate for some analytes that are associated with fresh produce flavor or taste. Improved specificity of whole cell biosensors has been achieved using recombinant microorganisms (*36*), but this has mainly been for detection of pollutants and/or toxins.

INADEQUANCIES OF FRESH PRODUCE QC

The fresh produce industry illustrates many of the problems encountered across the whole food industry in terms of still generally employing archaic QC methodologies (*37*). For example, fresh produce quality in the intact or minimally processed/fresh-cut form is initially assessed by sight: other important quality attributes include taste, smell, and texture. Each of these four quality attributes can be assessed either subjectively or objectively. Typically, fruit processors reject ca. 10% of fruit intakes. Better selection through improved quantitative QC at low-cost will inevitably result in improved overall quality for intact and minimally processed fruit products. Improved QC will probably, in the short-term, lead to increased rejections and reduce the number of "concessions" for fruit processors. A concession is where fruit material can be used but is expected to incur a cost penalty due to greater QC costs. Improved QC may result in a ca. 25% saving on concessions for fruit processors (Cockerill, M. Orchard House Foods, United Kingdom. Personal communication, 2004).

In reality, current standard product-orientated QC operations are inadequate and consider only appearance (e.g., color, size, and shape), presence/absence of disease, and the concentration of total soluble solids (TSS). TSS is commonly expressed as °Brix and is typically still measured using a hand-held refractometer. There is often poor correlation between TSS and total sugar concentration. Fruit sugars are one of the main soluble components in fresh produce that are important for flavor. In addition to sugar composition, fruit acid concentration can affect flavor directly and can regulate cellular pH, influencing the appearance of fruit pigments within the tissue during processing. The total titratable acidity (TTA) of fruit is not routinely measured as part of the standard QC procedures that are implemented by growers, suppliers, fresh produce distribution centers, and fruit processors (*37*). Titratable acidity is a measure of the buffering capacity of the fruit and is generally expressed as a percent of the predominant organic acid. Current standard QC operations do not use TTA due to the cumbersome and time-consuming nature of titrations. Fruit sugar/acid ratios can be used as an important index of consumer acceptability and act as one determinant of overall fruit quality. However, sugar/acid ratios are not frequently assessed for all fruit types due to primitive QC instrumentation and the requirement for skilled analytical scientists.

An initial step to improving routine QC assessment would constitute producing a simple and low-cost alternative to refractometry and titrations so that specific sugar and organic acid ratios can be standardized for fresh produce types. Biosensors may offer the opportunity to fulfill this niche and allow industry to adjudge fruit quality on the basis of taste (sugar:acid ratios) rather than just appearance alone. Introducing biosensor technology within the fresh produce industry may provide the ideal solution to providing improved QC, safety, and traceability methodologies (*11*, *29*). It follows that biosensor applications could be extended across the whole food industry, e.g., meat, dairy foods, and beverages.

ADVANTAGES OF BIOSENSORS FOR FOOD ANALYSIS

Unquestionably, biosensors have made their greatest impact in the field of medical diagnostics. The use of screen-printed electrochemical biosensors by diabetics to regularly monitor their blood glucose levels has provided sufferers with a powerful method for controlling this pernicious condition. Using modern printing methods, these devices are manufactured on a scale of millions per month and sold globally. It is the accuracy, comparative low cost, and ease of use that has led to their widespread application. Adapting this technology for use not only for fresh produce (**Table 4**) but also in the wider food industry could lead to immense improvements in QC, food safety, and traceability. For instance, Abayomi et al. (*11*) demonstrated that pungency in bulb cvs. Renate and SupaSweet (SS1) onions (as measured by pyruvate concentration in macerated tissue) could be determined 20-fold more rapidly using a mediated biosensor format in comparison to the standard colorimetric assay used by industry (*38*) (**Figure 3**) with no loss in resolution. Moreover, biosensors have been developed for the determination of concentration of metabolites such as glucose, sucrose, lactate, alcohol, glutamate, and ascorbic acid, typically found in many food items (*39*; **Table 5**), and for rapidly detecting the presence of contaminating agents such as microorganisms, pesticide residues (*40*), and antibiotics (*41*, *42*).

Biosensor systems can be operated either as simple one-shot measurement tools or, when incorporated into a suitable fluid handling system, as multimeasurements devices. Both approaches can also be adapted to measure several different analytes, using the same sample solution. It is this versatility, coupled to a high degree of sensitivity and selectivity, that has prompted worldwide interest in both the fundamental research and the commercial exploitation of biosensor technology. Biosensor systems can be designed such that they can be operated at-site on a real-time basis, removing the reliance on expensive centralized laboratory-based testing. Moreover, the process of miniaturization can be adapted to biosensors. Hence, an array of sensors can be integrated into a small portable device for multiple parameter determination for use by nonspecialized persons with a minimum of manual manipulation. This is one of the major advantages of using biosensors, as measurements can be made either during raw material preparation, food processing (e.g., as QC devices), or for checking the reliability of storage conditions. Hence, these devices can act as cost effective tools for QC, for process control, and for the determination of food safety.

The majority of biosensor research for food industry have been enzyme-based amperometric electrochemical biosensors (**Tables 4** and **5**). However, to obtain functional biosensor

Table 4. Examples of the Range of Analytes Monitored in Fresh Produce Matrices by Amperometric Biosensors

analyte	food matrix	enzyme	detection limit	ref
fructose	citrus fruits	fructose dehydrogenase	10 μM	*43*
amines	apricots and cherries	diamine oxidase and polyamine oxidase	2×10^{-6} mol/L	*44*
L-ascorbic acid	fruit juices	ascorbate oxidase	5.0×10^{-5} M	*45*
sucrose	fruit juices	sucrose phosphorylase; phosphoglutaminase; glucose-6-phosphate 1-dehydrogenase	9.25 g/L in pineapple juice	*46*
malic acid	apples, potatoes, and tomatoes	malate dehydrogenase	0.028 mM	*47*
polyphenols	vegetables	horseradish peroxidase	1 μmol/L	*48*
β-D-glucose; total D-glucose; sucrose; L-ascorbic acid	tropical fruits (mango, pineapple, and papaw)	glucose oxidase; glucose oxidase, mutarotase; invertase, mutarotase, and glucose oxidase; ascorbate oxidase	7 mM	*28*, *29*
cysteine sulfoxides	alliums (e.g., onion and garlic)	allinase	5.9×10^{-6} M	*49*
pyruvic acid	onion	pyruvate oxidase	2 μmol/g	*11*

Table 5. Examples of the Range of Analytes Monitored in Food Matrices Other than Fresh Produce by Amperometric Biosensors

analyte	food matrix	enzyme	detection limit	ref
essential fatty acids	fats and oils	lipoxygenase, lipase, and esterase	0.04 mM in an FIA system	*50*
lysine	range of foods	lysine oxidase	1×10^{-5} mol/L	*51*
glucose and maltose	beer	glucose oxidase and amyloglucosidase	40 mM to glucose (only upper limit stated)	*52*
glucose and glutamate	beverages	glucose oxidase and glutamate oxidase	10 μM for glucose and 3 μM for glutamate	*53*
rancification indicators	olive oils	tyrosinase	0.2–2.0 μM in different oils	*54*
lactate	wine and yoghurt	lactate oxidase	1.4×10^{-6} mol/L	*55*
D- and L-amino acids		amino acid oxidase	0.1 or 0.2 mM for L- and D-amino acids	*56*
choline	dairy produce	choline oxidase	5 μmol/L	*57*
organophosphorus pesticide	range of foods	acetyl cholinesterase	0.2–1.8 ppm	*58*
insecticide residues	infant food	acetyl cholinesterase	5 μg/kg	*59*
laminarin	seaweed	1,3-glucanase and glucose oxidase	50 μg/mL	*60*
alcohol	beer and wine	alcohol oxidase and horseradish peroxidase	5.3×10^{-6} mol/L	*61*

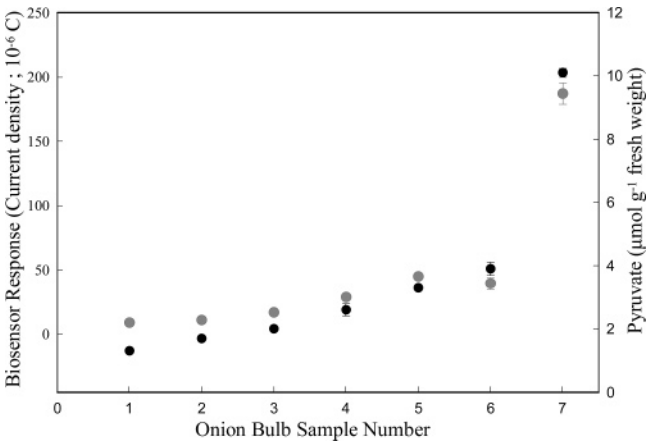

Figure 3. Mediated enzyme biosensor response (gray circle) operating at +200 mV to extracts from seven onion (cvs. SupaSweet and Renate) bulbs of increasing pyruvate concentration (black circle) against conventional colorimetric analysis assay (*38*). Standard error bars represent the means of three experiments (adapted from ref *11*).

devices, which can be manufactured within the necessary performance and cost constraints, other components and technologies must also be considered.

BIOSENSOR OPTIMIZATION FOR FOOD ANALYSIS

Membranes. Almost all biosensors rely on membranes for improved functionality. Membranes can play different roles in the sensor format and are typically used to retain the biological component, while allowing the analyte to pass; one of the key features of a biosensor is the close proximity of the biological recognition element to the transducer. Usually, this is achieved using an immobilization process. Another useful function of membranes is their ability to extend the linear range of a biosensor by acting as a mass transport barrier. A limiting factor for a linear response from an enzyme-based biosensor may be the K_m of the catalyst. By imposing an analyte diffusion barrier (i.e., a membrane) over the enzyme, a pseudo K_m may be produced, extending the linear range of the sensor. Membranes can provide a protective barrier for the sensor system, preventing fouling of the sensor by components in the sample matrix and, conversely, contamination of the sample solution by the sensor. Membranes can also act to provide stability for the sensor, for both the long-term storage and the operational capability of the sensor. By the use of a suitable membrane, a high degree of selectivity can be achieved, through either allowing only the target analyte to reach the sensor surface or by eliminating other interfering compounds that may affect the sensor signal. Numerous materials have been used as membrane materials for biosensors including cellulose acetate (CA), PVC, and nafion (a polyflurosulfonated hydrocarbon). Jawaheer et al. (*28*, *29*) demonstrated that interferences related to electrochemically active compounds present in tropical fruits could be significantly reduced by inclusion of a suitable CA membrane on a rhodinized carbon electrode. CA membranes improved the linear range of biosensors for β-D-glucose, total D-glucose, sucrose, and L-ascorbic acid by as much as 5-fold as compared to sensors without an additional diffusion barrier.

Immobilization. Immobilization of the recognition element on or close to the transducer is a major factor in biosensor design and fabrication. Adopting this approach allows an efficient transfer of signal from the biological element to the transducer and hence to the biosensor user. In addition, with an immobilized biological element, the opportunities for reuse of the biosensor are greatly enhanced. The main methods of immobilization, particularly for enzymes, include physical adsorption, entrapment in a matrix (using gels, polymers, or printing inks),

covalent binding, or electrochemical polymerization and photopolymerization. Physical adsorption is generally based on interactions such as van der Waals forces between the biological element and the transducer (e.g., a carbon electrode surface). Jawaheer et al. (*29*) demonstrated that pectin, a natural polysaccharide present in plant cell walls, could be used as a novel matrix to enhance enzyme entrapment on rhodinized carbon electrodes. Pectin also assisted in prolonging enzyme storage stability rather than improving operation performance and could be applied as a viscous paste of screen printable consistency.

Fabrication Techniques. It has long been realized that advanced fabrication techniques are key to the successful development of commercially viable biosensors. Fortunately, many technologies have been developed for other applications, such as the microelectronics industry, that can be adapted to biosensor fabrication.

Screen printing is a thick film process, which has been used for many years in artistic applications and, more recently, for the production of miniature, robust, and cheap electronic circuits. This technique has been successfully exploited by electrochemical biosensor manufacturers. The process has been one of the major reasons for the commercial success of many biosensors and is the process by which a number of medical diagnostic companies annually produce over 1 billion (electrochemical) biosensor strips for home blood glucose monitoring. It follows, therefore, that the inexpensive nature of biosensor fabrication lends itself to an increasingly price competitive industry, such as the fresh produce sector. Given this fact, it is perhaps surprising that biosensor fabrication techniques have not been transferred to the measurement of important target analytes in the food industry.

Sample Handling. The ability to handle small volumes of liquids with high precision will be one of the key areas of development for some of the next generation of biosensors. In particular, where high value reagents, such as particular enzymes or antibodies, are needed, screen printing may not (because of cost implications) be the most appropriate method of production. For example, fructose, which normally increases during physiological fruit ripening, is a potentially desirable target for fresh produce biosensor development. However, the commercial availability of the fructose enzyme, fructose dehydrogenase, is relatively expensive and, therefore, not economically viable at present unless significant research is forthcoming. In addition, the same economic barrier exists for malate, which is the principle organic acid found in pome fruits (e.g., apples and pears) and an important parameter characterizing wine quality (e.g., malolactic fermentation). Malic acid measurement can be cost prohibitive when using either the malic acid enzyme (L-malate: $NADP^+$ oxidoreductase) associated with $NADP^+$ and pyruvate oxidase (*62*) or malate dehydrogenase and diaphorase immobilized on gold electrodes using glutaraldehyde (*63*). Other printing methods can be used to overcome high price enzyme usage, including inkjet printing and Cavro deposition. The deposition of biological agents, such as enzymes, can be carried out accurately and reproducibly using these print methods and are suitable for depositing droplets of less than 1 nL in volume. Furthermore, noncontact technology, such as inkjet printing, allows fluid to be placed on almost any surface, irrespective of texture and shape.

Sampling. In addition to the variety of biosensor devices available, there are a number of methods that can be adapted to facilitate sampling. By their very nature, many food items are complex mixtures of many compounds; this can present a significant challenge to the efficient operation of a biosensor. Hence, whenever biosensor technologists design sensors for applications in the food industry, careful consideration must be given to sampling. For solid or semisolid foods, this usually involves an extraction process, possibly followed by a simple preparation step such as filtration.

Broadly, the main sampling methods that are used with biosensors can be categorized in several ways. At-site sampling involves taking a sample from the matrix and carrying out an extraction process followed by measurement by the biosensor. This approach to sampling can be detrimental in terms of efficiency. Manually removing and pretreating a sample prior to measurement may require some limited degree of technical skill. However, careful design of the sensor system should reduce this complexity to a level at which the procedure can be carried out rapidly and easily. At-site sampling is probably more suited to liquids (e.g., beer, wine, and fruit juices) where (generally) the target analyte of interest is more readily available. Similarly, sampling from fresh produce is usually not as challenging as for other more heterogeneous foodstuffs as many of the potential target analytes are in solution when tissue is disrupted/decompartmentalized and, thus, available for measurement in extracted fruit juice (*11*, *28*, *29*, *46*). This said, significant variation in the spatial and temporal distribution of target analytes does exist in all fresh produce; a fact that reconfirms that sampling procedures must be optimized.

In situ sensors are placed directly in the matrix containing the target analyte. The use of in situ sensors has long been established in the bioprocess industry, where "dip in" devices are used to monitor a number of parameters such as pH and dissolved gas concentrations. A number of advantages are gained by operating sensors in this fashion, including real-time monitoring and a continuous output signal from the sensors (any rapid change in the analyte concentration can be readily observed). In addition, labor requirements are significantly reduced. This approach would be most applicable in the food processing field, where the careful (automatic) sampling and measurement of processed food (e.g., processes that incorporate a fermentation or distillation step) would be very useful. However, given the complex nature of many foods, in situ sampling and monitoring can be very difficult. Components in the food matrix can adhere to and foul the sensor surface, leading to erroneous signals. Calibration of the sensor (in situ) may be difficult. In addition, the sensor operation may be affected by varying conditions during the process cycle, such as temperature, pH, and salinity.

Methods of on-line sampling involve the automatic removal and measurement of a sample, or sample stream, from the food matrix (e.g., flow injection analysis, FIA). FIA is a liquid handling technique that has proved flexible in adapting to most chemical and biochemical reaction procedures (*9*), representing an effective compromise between the desirability of in situ monitoring and the technical ease of off-line measurements. The use of liquid handling systems can be used to present a sample in an appropriate format to the sensor. Flow operations are comparatively easy to automate, miniaturize, and control as closed tubing avoids evaporation of fluids and provides an exactly repeatable environment for highly reproducible mixing of compounds. Moreover, sensors can be protected from fouling during contact time and from interfering compounds that may be present in the food sample. This is especially relevant when considering target analytes within fresh produce matrices. For example, phenolic compounds (e.g., catechin and epicatechin) and ascorbic acid, which are common place in many fresh produce products, are electrochemically active; thus, their influence must be greatly reduced or eliminated (*29*).

FUTURE DEVELOPMENTS IN BIOSENSOR TECHNOLOGY AND MARKETPLACE

Overall, the use of biosensors for food analysis can provide a route to a specific, sensitive, rapid, and an inexpensive method for monitoring a range of target analytes. This applies to monitoring carried out not only under laboratory conditions but also (e.g., with the use of screen-printed sensors) at on-site locations. These devices can be designed such that the non-specialist operator can use them effectively. However, as with most technologies, there is still room for improvement. Perhaps one of the main areas where biosensor technology can be improved is the actual recognition element itself. Developments in both enzyme (e.g., protein engineering), antibody (e.g., antibody fragments) technology, and complementary DNA probes continue apace (*4*). Advances in computational techniques now allow the modeling of both electron transfer reactions and receptor binding interactions with increasing accuracy. This not only enhances understanding of the receptor/transducer interface but also allows consideration of the design of new receptors based on biological molecules.

In contrast to the development of purely biological recognition elements, the use of synthetic material (to perform the same task) has increased. Undoubtedly, these new techniques (e.g., molecularly imprinted polymers) will impinge on the evolving biosensor field. Most importantly, biomimetics have the potential to overcome some of the shortfalls associated with biological components, primarily poor stability and higher cost of production (*4*). The successful introduction of such materials would enable biosensors to be used in many difficult environments. Along with these improvements in the biological recognition elements, other developments in areas such as further miniaturization and advanced fabrication procedures should lead to more robust and inexpensive sensors. Linked to advances in sampling and extraction, the use of biosensors for monitoring target analytes in a range of foods that are at present difficult to access will become a real possibility.

In conclusion, the global food analysis market currently stands at ∈1.1 billion with rapid methods accounting for ∈115 million (*64*). Therefore, although biosensors are likely to see growth in this area, it is probable that standard food analysis for microorganisms will remain a difficult market to penetrate. There are, however, several areas where biosensors are ideal candidates for improving food diagnostics. These potential opportunities include improved QC and assurance of food-derived raw materials (*11*), testing for absence/presence of genetically modified constituents where feasible (*15*), food authenticity/traceability, and incorporation into smart packaging. Fundamentally, however, the increasing trend from a yield-driven to quality-driven provision of agricultural products in response to consumer demands for improved food quality, safety, and traceability is set to increase in the developed world. The demand for reliable and inexpensive methods for assessment of quality is set to expand; biosensors offer the opportunity to fulfill this niche.

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Received for review July 9, 2004. Revised manuscript received December 9, 2004. Accepted December 10, 2004.

JF040319T