Biosensors applications in food analysis

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The food industry needs suitable analytical methods for quality control and contamination monitoring along the whole chain process, from the raw materials to the consumers; such methods must be rapid, reliable, specific and cost effective. Apart from a few important analytes, such as sugars, alcohols, aminoacids, flavours and sweeteners, food applications mainly focus on the determination of contaminants.
This article describes the development of biosensors as analytical tools in the food industry and discusses the technical and economic problems of applying this technology to the monitoring of foodstuffs quality and contamination. It is mainly focused on those biosensor systems which reached final phase of development, namely the industrialization and marketing.

Biosensors are devices employing biochemical molecular recognition properties as the basis for a selective analysis [1]. The major processes involved in any biosensor system are recognition, signal transduction and readout (Figure 1). A biosensor constitutes a foremost example of an interfacial technique, and as such, the rate of approach (flux) of analyte to the analytical surface, degree of exposure of the biolayer and immobilization effects, can have a significant bearing upon both dynamic and steady biosensor response.
Combining biology, physics and electronics, the biosensor is now picking up steam as more applications come into view. One of these is in the realm of food processing and quality control, as current wet chemistries and analytical practices are time-consuming and may require highly skilled labour and expensive equipment.
The food industry needs rapid and affordable techniques, both for compounds that have not previously been monitored, and to replace existing but complex and/or expensive procedures [2]. To establish a niche in this sector, biosensors must be inexpensive and reliable, and be robust enough to operate under conditions that may be hostile. Other requirements include increased speed in sample detection (instantaneous or just in time determinations), short sample preparation time, decreased requirement for skilled labour, measurements *in situ*, and a departure from the use of dangerous or 'environmentally unfriendly' organic reagents.
Besides of short description of biosensor concepts, the present survey is limited to those biosensor systems applied in food analysis which reached a final stage of technological and commercial development, i.e. industrialization and marketing. The reader interested in broader and more detailed reviews on biosensors research and development is addressed to various books or reviews [3-12]. It should be also underlined that

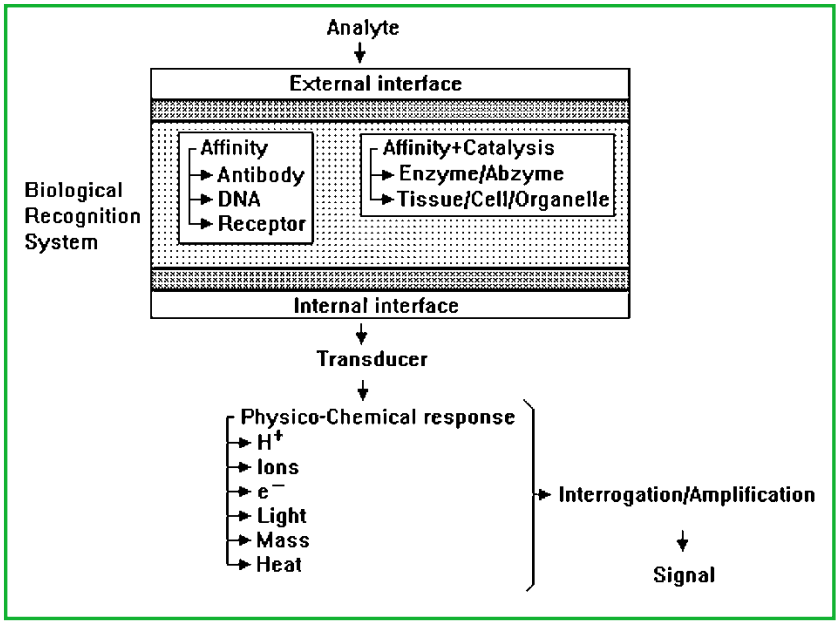

Figure 1 - Schematic diagram of possible biosensor analyte recognition cascade

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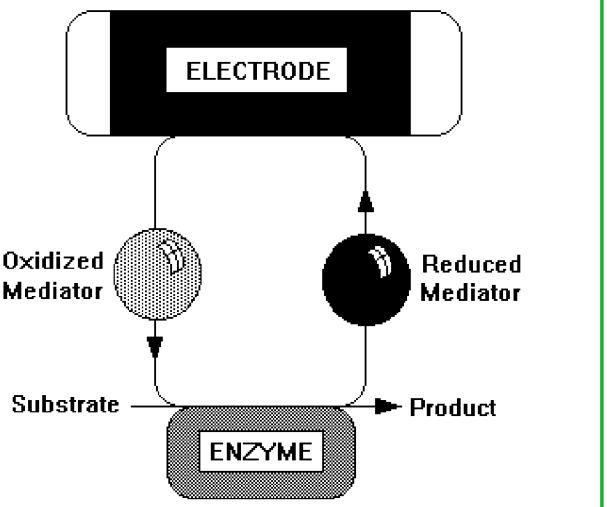

Figure 2 - Schematic diagram showing accumulation of mediator at surface modified enzyme electrode

the research on biosensors in Italy has been promoted in a variety of applicative fields thanks to internationally recognized activity of several scientific groups [13-20].

Biological materials and immobilization technology for biosensors

Enzymes, whole cells (bacterial, fungal, animal or plant), organelles, tissues, receptors, antibodies and nucleic acids can all be used in biosensors as biorecognition elements [1].
Specificity is the main reason for the widespread use of enzymes; of these, oxidases are by far the most commonly used in biosensors, and several have been identified and/or isolated. Enzymes with a high specific activity may be immobilized on a water insoluble membrane and placed on the sensing area of the transducer. This method offers the advantage of simple preparation and linearity over a greater range of analyte concentrations [21]. Several proprietary hydrophilic microporous membranes have been developed and a few of these are on the market, such as Immunodyne TM, (Pall Biosupport, New York, Usa) and Immobilon TM (Millipore, Massachusetts, Usa). Enzymes can also be covalently immobilized onto water-soluble particles and held between two selectively permeable membranes. A number of such proprietary combination membranes have been developed and commercialized. The Yellow Spring Instruments (YSI, Yellow Spring, Ohio, Usa) glucose and lactate membranes are prepared in this way using a combination of cellulose acetate and polycarbonate, with the enzymes being covalently immobilized on glutaraldehyde activated resin particles. For enzymes with low activity, the resulting particles can be packed to form an enzyme column, and placed in close proximity to the transducer. The commercial Owens, Illinois (Toledo, Ohio, Usa) glucose analyzer was based on this concept, using glucose oxidase immobilized to a porous allumina support.

Transducers used in biosensors construction

Several types of transducer, including amperometric transducers, ion-selective electrodes (ISEs), ion-sensitive field effect transistors (ISFETs), conductometric electrodes, calorimetric devices, optical transducers, and piezoelectric crystals, have been used in biosensors (Table 1) [22,23]. In general, amperometric electrodes based on oxygen or hydrogen peroxide detection together with an oxidase system or an aerobic microorganism, dominate food and drink applications. The current technology for some of these transducers is discussed below, and the possible applications reviewed.
Amperometric biosensor systems receiving by far the greatest attention to date have been enzyme electrodes. Of these, oxidase enzyme mounted sensors have proved to be the most popular for study:

$$\text{Substrate} + O_2 \xrightarrow{\text{Oxidase}} \text{product} + H_2O_2$$

Table 1 - A comparison of transducers used in biosensor construction

Transducer	*Advantages*	*Disavantages*
Amperometric	Simple, high selectivity, wide linear range, high sensitivity	Interference resulting for redox active compounds
Conductometric	Microfabrication	Influence of pH
Optical systems	Remote sensing, low cost, can be miniaturized, free from electrical interference	Interference form ambient light, requires high energy sources, only applicable to a narrow concentration range
Piezoelectric	Fast response, simple, stable output signal, low cost for readout device, no special sample handling	Low sensitivity in liquid applications, interference resulting for non-specific binding
Calorimetric	Versatility, free from optical interferences such as colour or turbidity	Expensive, cumbersome, requires a large amount of enzyme
ISEs	Simple, reliable	Sluggish response, require a stable reference electrode, susceptible to electronic noise
ISFETs	Low cost, mass production, stable output, requires very small amount of biological material, can monitor several analytes simultaneously	Temperature-sensitive, fabrication of diferent layers on the gate is not perfected

allowing, for example, reagentless monitoring of O_2 consuption at a oxygen electrode, or of H_2O_2 production at a positively polarized (+0.7 V) electrode. The latter requires the application of a potential at which species, such as ascorbic acid, are also electroactive. The effect of such interferences renders this approach to the analysis of food samples difficult without tedious pre-treatment. Similarly, in applications using oxidases, for which dioxygen is the physiological electron acceptor, the problem of its fluctuation, overcome in commercial analyzers by pre-dilution of samples into oxygenated buffers, may be circumvented by choosing an alternative electron transfer acceptor. Usually the mediator is a low molecular weight species which shuttles electrons (Figure 2) between the redox centre of the enzyme and the working electrode (gold, platinum, carbon). Ideally, a mediator for use in an electrochemical device should react rapidly with the enzyme, exhibits reversible heterogeneous kinetics and possesses a low overpotential for regeneration. Furthermore it should be stable with respect to pH, temperature, redox state and oxygen.

Several biosensors [24] that use amperometric transducers have been developed for analytes that may be of interest to the food and beverage industries (Table 2).

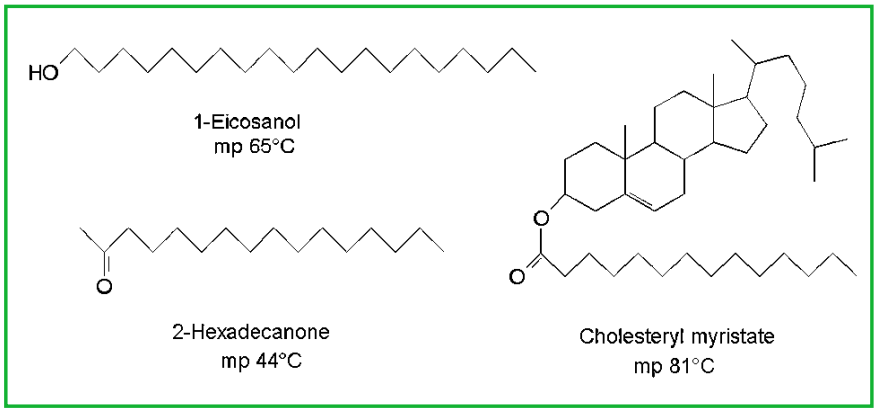

Figure 3 - SBMs molecular structures and melting points

Concept of solid binding matrix

Until recently, amperometric biosensor technology (and in biosensors field generally) suffered from several drawbacks, namely it showed short operational and storage stability, poor reproducibility and a very little versatility of the systems when they were used to test real samples and to undergo scaling-up in industrial production. To solve these problems, the concept of solid binding matrix (SBM) has been developed [25-28] and now, this innovative technology hold an important position among industrialized amperometric (mediated transducers) biosensor systems presently available and have already found a wide commercial market [29-33]. Solid binding matrices are compounds having a hydrophobic skeleton, mostly substituted by polar groups, which results in an amphiphilic character of their molecules (Figure 3). Substituted hydrocarbons, esters of fatty acids with glycerol or sterols were used for the construction of several kind of biosensors (Table 3).

SBMs are solid at room temperature, insoluble in water and in most organic solvents. They show a good compatibility with biocatalysts, co-factors, redox mediators, and they have a good mechanical properties, especially compactness, plasticity, polishability, with good resistance against progressive erosion of the electrode surface, a very common occurrence in the case of carbon paste biosensors. Several methods were described for preparation of SBMs-based bioelectrodes. Nevertheless, mixing of modified graphite (with biocatlyst and possibly also with co-factors or mediators) with the melted SBM at a temperature suitably higher than its melting point (50-90 °C), yielded biosensors with the best electrochemical and mechanical properties. Enzymes inside the composite matrices are protected against environmental deactivating factors, such as oxygen, humidity, and biological contaminants. Besides their structure proved to be more stable and protected against undesired conformational stretching which can lead to their deactivation. In fact, the original biocatalytic sensitivity was found practically unchanged when the electrode surface was renewed after several months of controlled storage. Moreover, it has been demonstrated the extreme versatility of the SBM composite transducer concept, that can be used in the construction of tip-type, niddle type, screen printed and spray coated renewable and/or disposable biosensors for different analyte detection employing a very small sample volume. Therefore, the simplicity, reproducibility, and inexpensiveness of the SBM biosensors conjugated with an extended storage stability allow commercialization of these composite bioelectrodes. The commercial SBM biosensing system dedicated to determination of glucose,

Table 2 - Amperometric biosensors that could be relevant to the food and drink industries

Target compound (analyte)	*Biosensing material*
Glucose	Glucose oxidase, glucose dehydrogenase, Pseudomonas fluorescences
Sulphite	Sulphite oxidase
Xanthine	Xanthine oxidase
Cholesterol	Cholesterol oxidase
Acetic acid	Trichosporon brassicae
Glutamate	Glutamate oxidase
Lysine	Trichodema viride
Galactose	Galactose oxidase
Alcohol	Alcohol oxidases Trichosporon brassicae
Hydrogen peroxide	Peroxidase, bovine liver
Oxalate	Oxalate oxidase
Tyrosine	Tyrosinase, sugar beet
L-aminoacids	L-amino acids oxidases
Sucrose	Invertase, mutarotase, glucose oxidase
Monoamine	Monoamine oxidase
Inosine phosphate	Nucleoside phosphorilase, xanthine oxidase
Aspartame	Peptidase, aspartate amino transferase, glutamate oxidase
Glutamine	Glutaminase, glutaate oxidase
Lactate	Lactate oxidase, lactate dehydrogenase
Urea	Urease
Phenols	Tyrosinase
Malate	Malate dehydrogenase

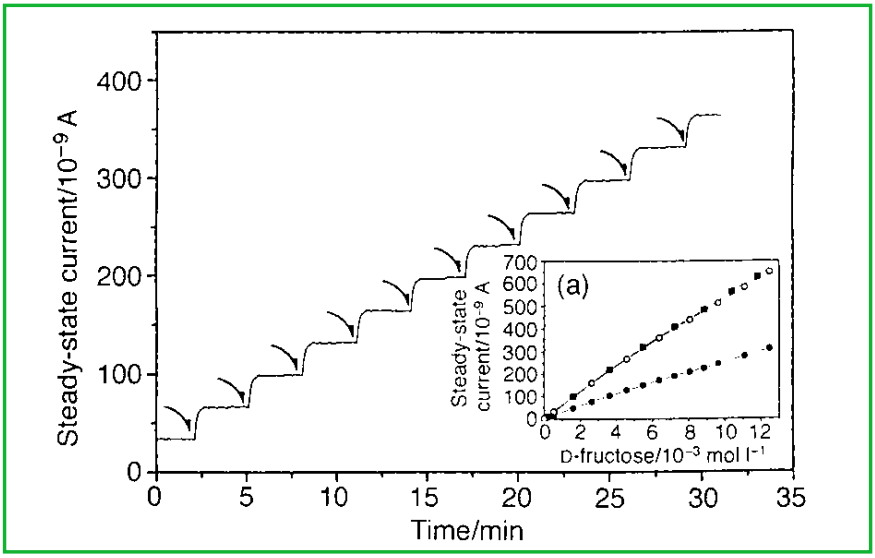

Figure 4 - Electrode response to successive additions of D-fructose. Arrows show the successive addition of 0.5×10^{-3} mol L^{-1} D-fructose. Inset (a): calibration graph for a FDH bulk-modified graphite/SBM composite electrode to D-fructose additions. (■) Newly prepared electrode; (●) after 6 months, without surface renewing; (○) after 6 months, with surface renewing on sand paper. Measurement condition 0.1 mol L^{-1} phosphate buffer, pH 6.7, 2×10^{-3} mol L^{-1} $[Fe(CN)_6]^{3-}$ and 1×10^{-3} mol L^{-1} $CaCl_2$ +0.20 V vs SCE

fructose, ethanol, malate and lactate in wine samples (industrial product: WineChecker 'Perbacco 200X' series licensed by POLYtech SCrl, Trieste, produced by Biofutura Srl, Gorizia, and distributed by Carlo Erba Reagenti, Milano) is already marketed.

After proper interface the POLYtech biosensor system delivers a fast and precise determination of glucose, fructose, reducing sugars, lactate and malate in grapes, must and wine. With such an instrument, it is now easy to perform a real time, cheap and independent monitoring must and of wine production and processing. This oeno-diagnostic package is therefore an interesting product for the wine quality control *in situ,* one of the first successfully commercialized worldwide. Analytical applications of the POLYtech biosensor systems are under development in several fields (Table 4) such as fruit juices, meat, fish, milk and diary products, oil and fats, employing variable transducers set-ups (tip-type, niddle type, screen printed type) [28].

As an example of an application of the SBM technology, *D*-fructose biosensor can be illustrated. The principle exploited is based on a typical enzyme-dependent catalytic process that could be expressed as follows [34]:

D-fructose + FDH (PQQ) → 5-keto-*D*-fructose + $FDH(PQQH_2)$

$FDH(PQQH_2) + 2\ [Fe(CN)_6]^{3-} \rightarrow FDH\ (PQQ) + 2\ [Fe(CN)_6]^{4-}$

$2\ [Fe(CN)_6]^{4-} \rightarrow 2\ [Fe(CN)_6]^{3-} + 2e^-$

where FDH is the *D*-fructose dehydrogenase (EC 1.1.99.11), the enzyme that catalyzes the oxidation of *D*-fructose in the presence of a redox mediator [35].

The typical *D*-fructose response (current *vs* time) for *D*-fructose biosensor containing FDH as the biocatalytic element inside of the composite transducer and with $[Fe(CN)_6]^{3/4-}$ as the electron relay system, at an applied potential of +0.20 V *vs* SCE, is shown in Figure 4. From the response curves, the stady state current response at various *D*-fructose concentrations can be readily determined, and the calibration graph can be drawn. Figure 4 , inset (a), shows a calibration graph for a newely prepared biosensor and for a biosensor after prolonged storage (6 months at room temperature) with and without surface renewing. The presented biosensor is highly reproducible and specific [34]. To test the electrode in real sensing applications, the proposed amperometric method was applied to the analysis of different foodstuffs and compared to those obtained from the enzymatic spectrophotometric assay kit (Boehringer Mannheim, Germany). As can be seen form Table 5, the biosensor results obtained for *D*-fructose agree satisfactory with those obtained from the reference test kit.

Other commercially available biosensors for the food industry

Biosensors that have been developed for application in food and beverage analysis have tended to be based on similar technology, involving either an oxygen electrode or an hydrogen peroxide electrode in conjunction with an immobilized oxidase. Amperomeric de-

Table 3 - SBMs based biosensors

Analyte	*Enzyme*	*Mediator*
Glucose	Glucose oxidase	Hexacyanoferrates, TTF, Ferrocene, POPDA
Alcohol	Alcohol oxidase Alcohol dehydrogenase	Hexacyanoferrates, TTF, Ferrocene, Thiazines
Fructose	Fructose dehydrogenase	Hexacyanoferrates
Malate	Malate Dehydrogenase	Hexacyanoferrates, Thiazines
Lactate	Lactate Dehydrogenase	Hexacyanoferrates, Thiazines
Sulphite	Sulphite Oxidase	Hexacyanoferrates
Urea	Urease	Hematein, lauryl gallate
Oxalacetate	Oxalacetate decarboxylase	Hematein
Benzylpenicilline	Penicillinase	Hematein

TTF, Tetrathiafulvalene; POPDA, poly-*o*-phenylendiamine

Table 4 - Fields of potential application of the SBMs based POLYtech Biosensor Systems in food and drink industries

Samples	*Analytes*
Milk and Diary products	Lactose, lipids, casein, Lactic acid, citric acid, phosphates, glycerol, urea, aceton, lactulose, residual pesticides, bioorganic amines, antibiotics, phosphatase activity, hydrogen peroxide
Beer	Ethanol, glycerol, total acidity, reducing sugars, dextrins, diacetyl, starch, biogenic amines, polyphenols, sulphites. a-amylase
Wine	Glucose, fructose, ethanol, lactate, malate, sulphites, carbon dioxide, aldehydes, citrate, glycerol, sucrose, reducing sugars
Fruit and vegetables	Sucrose, starch ethanol, methanol, citric acid, lactic acid, malic acid, reducing sugars, glucose, fructose, residual pesticides
Feeds	Urea, Sucrose, starch, ammonia
Fish	Biogenic amines, ATP, Hipoxanthine, ammonia, ethanol
Soft drinks and concentrated fruit juices	Ethanol, ascorbate, caffeine, citrate, glucose, fructose, malic acid, methanol, sucrose, reducing sugars
Liquors	Ethanol, Sucrose, aldehyde, glycerol, methanol
Honey	fructose, glucose, maltose, sucrose, reducing sugars
Fats and oils	Peroxides, antioxidants, fatty acids, polyphenols, cholesterol
Meat and meat extracts	Lactose, Glucose, Lactate, Creatinine, starch sulphites

vices offer the advantages of being inexpensive and relatively simple to make and use, and can operate over a wide concentration range (10^{-3} - 10^{-9} M) for many practical applications. A description of the activities of selected companies that have been involved in the development and commercialization of biosensors [23,36,37] for use in the food and drink industries is shown in Table 6.

The model 2700 industrial analyzer marketed by YSI, is generally accepted within the sugar molasses, and confectionary industry as providing a standard method for determining level of glucose and sucrose. The system consists of a platinum electrode poised at +0.7 V (with respect to a silver/silver chloride electrode) for the detection of hydrogen peroxide in conjunction with various membranes containing appropriate immobilization oxidases. The company also sells enzymes for the detection of total starch, fructose, dextrose, lactose, ethanol, glycerol and *L*-lactate. Several companies in Japan (Fuji Electric Co., Kyoto Daichi Karaku and Omron Toyoba), France (Solea- Tacussel), and Germany (VEB-MLW Prufgerate-Medigen) also offer biosensors for glucose assays.

A comparable amperometric biosensor system (platinum *versus* silver/silver chloride at +0.7 V), designed for determination of fish freshness, is made by Pegasus Biotechnology (Agincourt, Ontario, Canada). In this device, immobilized nucleotidase, nucleoside phosphorylase, and xanthine oxidase convert the degradation products of ATP into uric acid and hydrogen peroxide. Similarly, the Oriental Electric Co. (Niiza Saitama, Japan) markets the KV101 freshness meter, a system containing soluble enzymes together with an oxygen electrode (a platinum cathode poised at -0.7V *versus* silver/silver chloride).

A system containing various oxidases and an oxygen electrode to detect alcohol, glucose, lactose and lactic acid was, until recently, marketed as the Multipurpose Bioanalyzer (Provesta Corp., Bartlesville, Oklahoma Usa).

A flow injection analysis (FIA) amperometric biosensor with a hydrogen peroxide electrode was introduced by the Control Equipment Co. of Princeton, New Jersey, Usa. It could handle up 300 glucose samples per hour but did not last long in the market. Another FIA system, equipped with a Clark-type hydrogen peroxide electrode, is currently marketed by Eppendorf North America (Madison, Wisconsin, Usa).

Development trends and prospects in the biosensing elements

Oxidase systems used with amperometric electrodes will continue to dominate the technology of commercial biosensors [23,38-42]. The search will continue for oxidases that could be used in these devices; examples include the recent development of glutamate oxidase (Yamasa Shuyu, Choshi, Japan) [43], NADH oxidase (Nippon Paint Ltd., Tokyo, Japan) [44] and phenylalanine oxidase [45]. Multiple enzyme systems could be improved by incorporating new enzymes. In one of these systems, an aspartame biosensor with a hydrogen peroxide electrode uses peptidase, glutamate oxaloacetate transaminase (GOT) and glutamate oxidase (GO). The recent discovery of the purification of phenylalanine oxidase may allow the GOT and GO enzymes to be replaced.

The increase of the purity of the used enzymes may allow a reduction in analysis times, as there would be more molecules of enzyme per unit area of membrane surface. Improvements in immobilization technologies should also result in the possibility of increased enzyme loading. For example, the number of binding sites on the surface could be increased using avidin and biotynilated enzymes.

To make biosensors more affordable for the food and drink industries, their long-term stability must be increased; the enhanced stability of the used enzymes is most important, as their replacement accounts for most of the operating costs. This could be achieved using enzymes from thermophilic microorganisms that are stable at high temperatures. Chemical oxidation of enzymes to make them more stable and specific

Table 5 - Determination of *D*-fructose in foods

	D-fructose found (%, ±RSD calculated based on five measurements)	
Sample	*Biosensor*	*Enzymatic test kit*
Cherry jam	17.47±0.48[a]	18.04±0.71
Floral honey	38.57±1.16[a]	38.01±0.80
Milk chocolate	1.82±0.05[a]	1.71±0.06
Whole biscuit	13.04±0.31[a]	13.98±0.59
Orange juice	5.57±0.15[b]	5.78±0.26
Pear juice	6.35±0.18[b]	6.41±0.26
Wine 1	1.45±0.02[b]	1.47±0.07
Wine 2	3.24±0.11[b]	3.00±0.14

[a] g per 100g sampl; [b] g per 100mL sample

Table 6 - A selection of companies that have been involved in the development and commercialization of biosensors

Company	*Activity*
POLYtech/Biofutura/Carlo Erba (Italy)	SBMs biosensors technology. Biosensors for glucose, fructose, lactate, malate, ethanol
Yellow Spring Instruments, YSI (Yellow Springs, OH, Usa)	Hydrogen peroxide is detected electrochemically. Biosensors for glucose, lactate, ethanol, sucrose, lactose, glutamate
Integrated Genetics (MA, Usa)	DNA probe for detection of microbial contamination such as Salmonella in food
Solea-Tacussel (Villeurbanne, France)	The detection principle is similar to the YSI analysers
Ajinomoto C. (Kawasaki, Japan)	Biosensors based on whole cells and FET
Mitsubishi Co. (Tokyo, Japan)	FET biosensor for glucose
Oriental Freshness Sensors (Niiza, Saitama, Japan)	Fish freshness determination (ATP degradation monitoring), using soluble enzymes and an oxygen electrode
NEC (Kawasaki, Japan)	FET biosensor for glucose
Universal Sensors (LA, Usa)	Potentiometric electrodes for glucose, alcohol, lactate, and glycerine, lactose, cholesterol, and a wide range of *L*-amino acids
Wolverine Medical Inc. (MI, Usa)	Lactate biosensor
Toyo Jozo (Tokyo, Japan)	Biosensors for glucose, lactate, lipids
ENZO Biochem (NY, Usa)	DNA probe for the bacterium chlamydia
Hybritech Inc. (CA, Usa)	DNA probe for bacteria
Cambridge Life Sciences (Cambridge, UK)	Biosensor for glucose
Cranfield Insitute of technology (Bedford, UK)	Biosensors for glucose, microbial contamination, and methanol
Eppendorf (Germany)	Biosensor for glucose

is another interesting approach, and modified enzymes are likely to become important in the next generation of biosensors.
Microbial biosensors and tissue-based biosensors deserve a brief mention here because do not require the purification of biological compounds. Several microbial biosensors have been developed so far for glucose [46], acetate [47], ethanol [48], sucrose [49] and glycerol [50]. The main drawbacks of microbial biosensors are their poor selectivity, insufficient storage stability and sluggish response times. This type of biosensor can only be used for well defined samples and will remain in the laboratory as a useful research tool and possibly for in-house applications. Some tissue based biosensors have been developed using several sources such as cucumber [51], potato [52], coconut [53] and mushroom [54] for the determination of ascorbate, organic solvents, phenols and mycotoxins, respectively.
Although still subject to much speculation, some recent work has focused on the production of taste and smell receptors arrays (so called electronic nose, artificial tongue, etc.) as biosensors of this type could have broad applications in the food and drink industries. For instance, a piezoelectric biosensor recently introduced to the market by Sogo Pharmaceutical of Japan contains a synthetic bilayer film that functions in a similar way to human olfactory cells for the determination of substances that release odours [55].

Conclusions

Several key issues need to be solved before biosensors will find widespread applications in the food and drink industries, despite the range of devices being in development so far. The first step appears to be the development of better methods for the industrial production and reliable sources of biological materials for the recovery of the biorecognition systems. Improvements in the engineered solutions that make the technological contourn of the biosensing device (electronic apparatus, micro- and nano-fabricated hardwares and other accessories) and after-sale technical support and service are also required.
Increasing the stability of immobilized material is another important area. The discovery of thermophilic enzymes and/or new stabilizers would help but improved immobilization methods are still needed to enhance the loading activity, and stability of immobilized materials particularly for membrane-bound receptor proteins.
Other efforts are aimed at producing portable, cheap reagentless biosensors other than those using amperometric electrodes. Fiber optic sensors, thermal sensors and ion-selective field effect transistors are potential candidates for development as they can easily be miniaturized and integrated for simultaneous determination of different target compounds in food samples. This challenging feature is vital for the successful commercialization of biosensors in this conservative and competitive sector.

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