

Commercially Available (Bio)sensors in the Agrifood Sector

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1. INTRODUCTION

Biosensors were introduced for the first time by Updike and Hicks in 1967 [1], which developed a biosensor based on the immobilization of glucose oxidase enzyme on an oxygen electrode for the measurement of glucose concentration in biological fluids. Then in 1973, Yellow Springs Instruments commercialized the first glucose biosensor [2]. In 1987, Medisense Inc. launched on the market the first pen-sized biosensor for self-monitoring of blood glucose by diabetic patients [3]. Since then, constant research and development efforts have been undertaken in biosensor technology, as demonstrated by the rise in the number of publications and patents (Fig. 1), highlighting the high potential of this technology for in field application in areas as biomedical diagnosis, health care, and biodefence, as well as in agrifood and environmental control. The constant evolution of this technology is leading to the standardisation of

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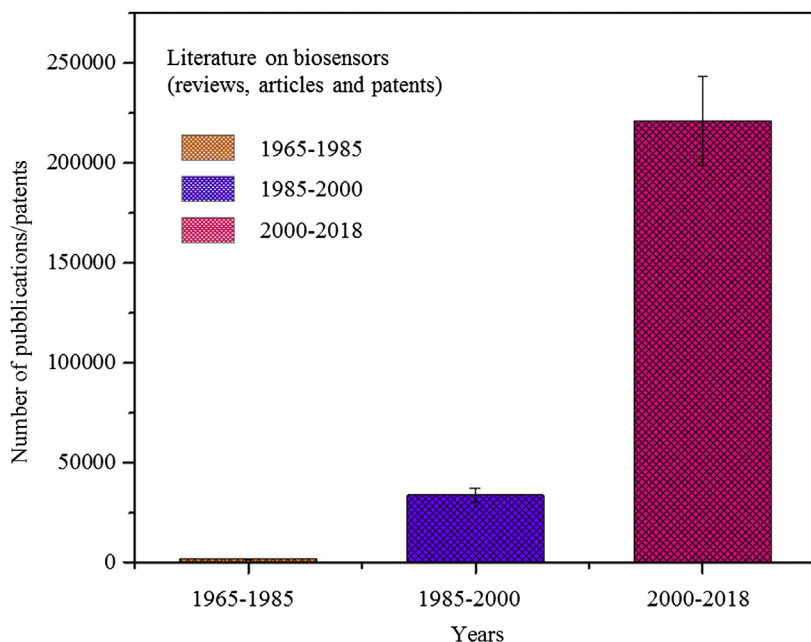


FIGURE 1 Number of publications and patents during the cited period relating to biosensors. Data taken on March 2016 from www.google scholar.com using the term 'biosensor'.

instrumentations and bioelements, as well as to test processes, validation, and scaling-up, resulting in faster and specific applications.

Inline to this trend, the growth for biosensor commercialisation is becoming robust in emerging markets worldwide. The nonmilitary, open market for sensors grew from EUR 81.6 billions in 2006 to EUR 119.4 billions in 2011 and can be expected to grow to EUR 184.1 billions until 2016, according to the new World Report entitled 'Sensors Markets 2016' [4] (Fig. 2). This corresponds to an average annual growth rate of 7.9% between 2006 and 2011, and 9.0% between 2011 and 2016. Among sensors, the global market for biosensors was valued at \$12,963.9 millions in 2014, and it is expected to grow at a compound annual growth rate (CAGR) of 9.7% during 2015–20, to reach \$22,490 millions by 2020. In particular, applications of sensors for biomedical and life science dominate the market, accounting for 99%, followed by agri-food and environmental monitoring, and remediation applications. Fig. 3 shows income percent from worldwide markets in environmental field, security and biodefense, and home diagnostics from 2006 through 2009, and the forecasts up to 2016 suggest that this growing tendency is constant. Although glucose monitoring dominates the market, biosensor use in agrifood, process industries, environmental monitoring, security and biodefence is growing thanks to novel biosensors developed to satisfy specific detection requirements (Fig. 4).

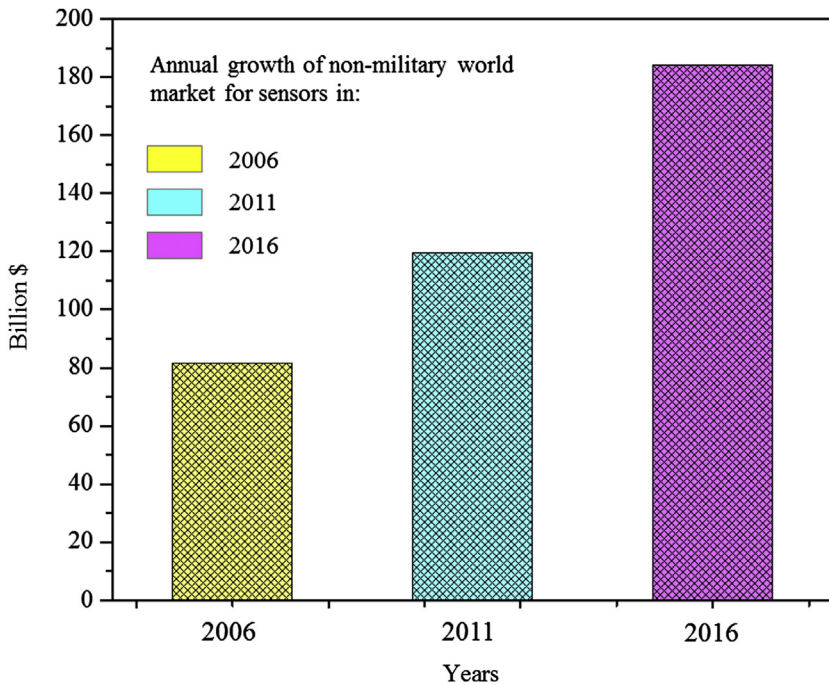


FIGURE 2 Annual growth of nonmilitary world market for sensors.

By a technological point of view, the sensor market can be distributed in electrochemical, optical, thermal and piezoelectric transducers, according to P&S Market Research that releases in 2015 a new research report on ‘Global Biosensors Market’ during 2015–20 [5]. Among them, electrochemical has a larger segment in the (bio)sensor market, followed by the optical technology.

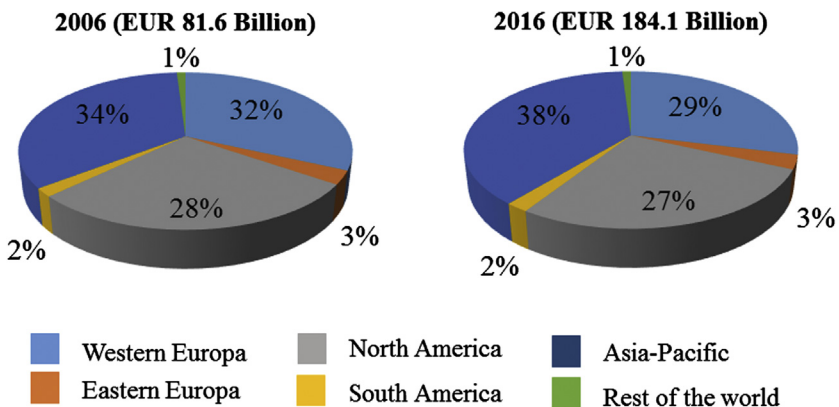


FIGURE 3 Overview of nonmilitary world market for sensing systems, and its growth from 2006 to 2016.

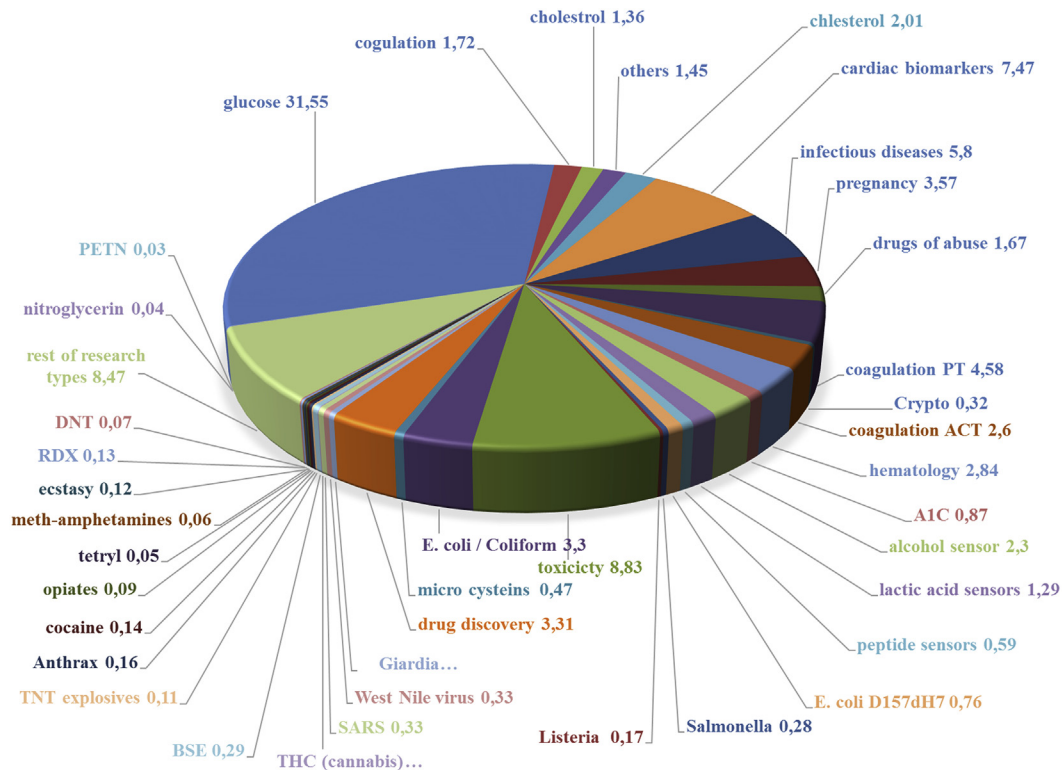


FIGURE 4 The global biosensor market incomes from specific detection application (2009).

These statistical calculations were provided on a list of 225 companies engaged in research and development, equipment manufacturing, supplies and distribution of biosensors [4]. However, there are well over 500 companies worldwide working on biosensors and bioelectronics employed in biosensor fabrication/marketing; design and development of materials, reagents, and instruments; integration of the components and launch into the market [6]. Among all these companies, few multinational companies with huge finance sources for technology acquisition and validation dominate the whole biosensor industry. The main companies involved worldwide in biosensor technology are listed in Table 1, mainly working in biomedical diagnostics, besides to biodefence, food analysis, industrial control, microbiology and proteomics [7].

TABLE 1 Main Companies Involved in Biosensor Technology Worldwide.

Company	Website	Main Expertise
Abbott Laboratories	www.abbottdiagnostics.com	Diagnosis and treatment of diseases/illness; in vitro diagnostic instrument systems and tests for hospitals, reference labs, blood banks, physician offices and clinics
ABTECH Scientific Inc.	www.abtechsci.com	Biosensor systems for the biomedical research community and for point-of-concern biomedical diagnostic applications
Bayer AG	www.bayerdiag.com	Gene chip development for the detection of genetic diseases
Biacore International AB	www.biacore.com/lifesciences/index.html	Health care and medical products industry
Affymetrix	www.affymetrix.com/index.affx	Analytical systems for antibody characterization, immunogenicity, biotherapeutic development/production and proteomics
Cygnus, Inc.	www.cygn.com/homepage.html	Glucose monitoring
DiagnoSwiss	www.dignoswiss.com	Development and commercialization of biochip technologies for medical diagnostics, microbiology, food, warfare's, pharmaceutical research, proteomics, industrial control
Lifescan	www.lifescan.com	Glucose monitoring

Continued

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TABLE 1 Main Companies Involved in Biosensor Technology Worldwide.—cont'd

Company	Website	Main Expertise
Neogen Corporation	www.neogen.com	Products dedicated to food and animal safety for the detection of pathogens in raw ingredients, finished food products, and environmental samples; rapid diagnostic Test kits to detect foodborne bacteria, spoilage organisms, mycotoxins, food allergens, genetic modifications, drug residues, plant diseases, and sanitation concerns
Panbio	www.alere.com	Tests for diagnosis of infectious disease
Roche Diagnostics AG	www.rochediagnostics.com	Biosensors used for analysing blood samples; kits and reagents for genomics and proteomics research, bioreagents for industrial use, products for food safety and pharmaceutical testing
Pelikan Technologies, Inc.	www.pelikantechnologies.com	Hand-held diagnostic and monitoring devices for the testing and management of a variety of medical conditions
Applied Biophysics	www.biophysics.com	Real-time, label-free, impedance-based method to study the activities of cells grown in tissue culture

Among the different application fields, (bio)sensor technology is achieving importance also in the agrifood sector, due to the frequent occurrence of food contamination and the growing apprehension about public safety. Indeed, a huge number of chemical contaminants represents a significant source of foodborne illness. For example, there have been long-standing concerns about chemical safety of food due to a misuse of pesticides during food production and storage, resulting in the occurrence of undesirable residues. More than 2.5 million tons of herbicides valued over US\$30 billions are being used in agriculture all over the world increasing the toxic effect on human health. According to the World Health Organization, between 1 million and 25 million people suffer from herbicide poisoning each year. It is estimated that as many as 20,000 people in the United States will develop cancer each year from the residue of herbicides on their food, but the number is much higher when we include the enormous number of people unknowingly suffering from herbicide symptoms [8].

For this reason, the demand for (bio)sensors for food control is estimated to raise in the near future. Undeniably, the total market potential for food diagnostics is gaining momentum due to these pervasiveness of foodborne diseases worldwide, being projected to reach a value of US\$16.1 billions by 2020, at a CAGR of 7.4% from 2015. Fig. 5 shows data from the Report ‘Food Safety Testing Market by Contaminant, Technology, Food Type and Region – Global Trends & Forecast to 2020’ about the food safety testing market revenues in the period from 2015 to 2020, as well as the global size of food safety testing market by region (data have been omitted by the company but provide indications on market volumes) [9].

This food diagnostic industry demands for rapid, accurate and easy-to-use devices, especially detection kits for food safety and quality testing in processed foods, which show essential advantages over the traditional methods that are time consuming and labour intensive, and require laboratory set-up instrumentations. Key features for food analysis include competitive detection in a short time (eg, US Food Safety Inspection Service admits zero-tolerance threshold for *Escherichia coli* O157:H7 contamination in raw meat products; while Environmental Protection Agency infectious admits 10 cells of *E. coli* O157:H7), reliability, integration and portability for in-field analysis, sample

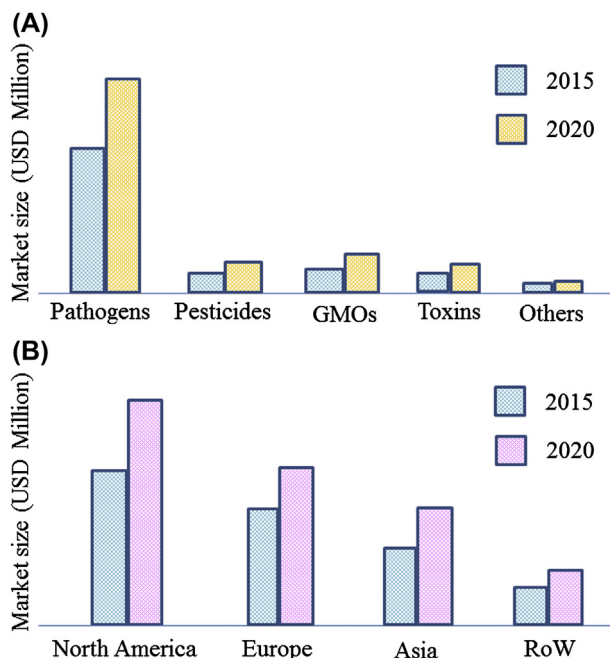


FIGURE 5 (A) Food safety testing market revenues in the period from 2015 to 2020. (B) Global market size of food safety testing market by region.

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collection and treatment, real-time and multiplexing detection, low cost, and minimized consumables.

Despite the high number of research studies using different (bio)sensors for the food industry, an open dialogue between academic and industrial professionals still lacks to acquire a reciprocal know-how and to adapt this technology according to the market requirements. This is due to a series of technical challenges that constrain biosensing technology to reach a commercial maturity. For instance, tests of prototypes in real samples have still critical drawbacks owing to immobilization procedures of the bioreceptors, requirement of specific conditions of temperature and pH to preserve the bioreceptor activity and stability, sample preparation, sensor fouling and presence of interfering species. Moreover, very few (bio)sensors are exploited in agrifood especially in online analysis to monitor raw materials, product quality or manufacturing processes, although they can be combined with several available flow injection systems. As stated by Luong et al. [10] ‘successful biosensors must be versatile to support interchangeable biorecognition elements, and in addition miniaturization must be feasible to allow automation for parallel sensing with ease of operation at a competitive cost’.

Table 2 reports the main challenges to overcome and that still restrain biosensor awareness and use.

In this context, the last advances accomplished in recent years in biomimetic chemistry, new material design (biohybrids), nanotechnology, micro/nanofluidics and ICT will be addressed in this chapter to highlight the main

TABLE 2 Main Factors Limiting Biosensor Awareness and Use

Key Unmet End Users’ Needs

High sensitivity and specificity
Fast readout times
Bioreceptor storage stability
Simple sample pretreatment
Miniaturization
Low costs for mass production
Migration to lab-on-chip biosensors
Availability of wireless connection
Self-configuring biosensor
Multitest detection and monitoring
Integrated biosensing platforms

limitations hindering the transition from the bench to the market. In particular, emerging technologies devoted to improve the stability, sensitivity and reliability of the biosensing/biohybrid elements, as well as miniaturisation and integration, will be discussed in view of their possible commercialization. In addition, an overview of the main commercial biosensors exploited in the agrifood sector, their key limitations and the last trends in biosensing technology will be reported, fostering (bio)sensors to reach a commercial success.

2. COMMERCIAL BIOSENSORS IN THE AGRIFOOD SECTOR

Different kinds of sensing configurations are available on the market for the food industry, including autoanalysers, manual laboratory instruments and hand-held devices [11]. In the following sections, a brief overview of the commercial (bio)sensors for the agrifood sector, which can find main applications in different compartments including food analysis, process control, intelligent packaging and smart agriculture, has been reported.

2.1 Biosensors for Quality and Safety

Innovation and development in the agrifood sector and the recent globalisation of agroindustrial markets are pointed on two fundamental beliefs regarding food safety and quality, which have become a great concern in the last years [12]. Food quality and safety concepts are related to the production of foodstuffs that do not represent a risk for the health of the consumers. The term food quality is related to appearance, taste, nutritional value content, functional ingredient, freshness, flavour, texture and chemical, and to the presence of compounds able to enrich the food. The analysis of food composition allows characterising and attesting if the food contains all the desired constituents such as natural components (sugars, amino acid, alcohol) and additives (vitamins and minerals). The food safety concept entails the production and the commercialisation of safe foods that are free from allergenic or toxic substances, including pesticides, fertilizers, heavy metals, organic compounds, toxins and pathogens.

These requirements of quality and safety can be guaranteed only when the whole food supply chain undergoes to a strict control of a series of links ranging from crop cultivation to production and processing, sales and consuming. The availability of procedures, inspections and records is required to minimise the possibilities causing unsafe or off-quality end products. Therefore, the development of control systems for the analysis of a wide range of compounds that could affect the food performance became a crucial demand. The market for diagnostics for agrifood application grows each year, taking in consideration that millions of assays are performed on milk, fish and meat to evaluate the freshness and presence of contaminants. Indeed, the diagnostic industry claims several commercial analytical systems for water

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and food control including both multi-analysers, bench-top portable instruments and one-shot disposable tests. These devices can provide specific, sensitive, rapid and inexpensive analyses of food components and contaminants, both under laboratory conditions but also at on-site locations. In Table 3, a selection of companies involved in the last 20 years in the commercialisation of (bio)sensors and their components for food quality control is reported together with the target analytes.

The determination of sugars is the most performed, as depicted in Table 3, due to its high occurrence in a wide number of food products. In particular, the most used biosensor configuration for glucose detection entails glucose oxidase as biocomponent and an amperometric electrode as transducer. In combination with other enzymes, a similar configuration allows the determination of other sugars, using oxidases, dehydrogenases and microorganisms in

TABLE 3 Companies Commercialising Biosensors for Food Quality Control

Company	Country	Target Analyte
Fuji Electric Co.	Japan	Glucose
IBA GmbH	Germany	Sucrose, glucose, alcohol
GeneScan Europe AG/Scil Diagnostic GmbH	Germany	NutriChip, DNA detection with array technology
Oriental Electric Co.	Japan	Freshness metre: Degradation products of ATP
Pegasus Biotechnology	Canada	Degradation products of ATP
Biosentec	France	Lactate
Analox Instruments	UK–USA	Ethanol, methanol, glucose, ethanol, lactate, glycerol
Sensolytics	Germany	Glucose, lactate, sucrose, ethanol, glutamate
BioFutura s.r.l.	Italy	Glucose, lactate, malate
Tectronik	Italy	Glucose, lactate, malate
Chemel AB	Sweden	Glucose, sucrose, lactate, ethanol, methanol
YSI Inc.	USA	Glucose, sucrose, lactate, lactose, ethanol, methanol, glutamate
Inventus BioTec	Germany	Ascorbic acid in juice, fruits and vegetables

combination with different types of transducers (eg, optical or potentiometric), and with sampling systems, such as flow injection analysis.

Among companies listed in Table 3, some of them rearranged their R&D strategies in the last years by commercialising different products, while other companies are nowadays widely involved in (bio)sensor development for food and beverage analysis. For example, *Biosentec* commercialises an electrochemical biosensor within the Microzym analyser for lactate determination in collaboration with the Laboratoire de Genie Chimique et d'Electrochimie (Toulouse), GIP Exercice (C.H.U. Hopital de Bellevue, Saint Etienne), and the Laboratoire de Recherche 'Transfert industrielle en Biotechnologie' (I.N.S.E.R.M. Toulouse), demonstrating how the collaboration between academics, industries and laboratories involved in a technology transfer is a crucial assumption for marketable successes. The Microzym instrument is able to perform 100 analyses with each electrode showing high suitability in agrifood application, as demonstrated by its good analytical performances: (1) detection threshold of 0.05 mM, (2) linear range between 0.05 and 1.5 mM, and (3) short time response between 5 and 10 s. The analysis is also cost-effective (0.5 Euro for each test considering only the cost of the electrode without reagents). However, this biosensor is not characterised by a high robustness in term of stability, since a storage stability of 15 days at 4°C is indicated by the company.

Similarly, *Tectronik* Company claims collaboration with academic partners, including the Universities of Padua and Venice (Italy), and won the Innovation Award granted by the Veneto Region, in the development of a novel biosensor (Senzytec) to measure ethanol in the agrifood sector with specific application in wine, oranges and apples. The Senzytec instrument is a reliable and quick system which, thanks to the enzymatic-based technology, allows the user to run low cost tests in a very short time (3–6 min) and on-site, on a small sample volume (0.1 mL). Senzytec2 system is the evolution of Senzytec, allowing for the detection of a wide range of analytes (ethanol, malic acid, lactic acid, glucose and fructose) (Fig. 6).

Glucose detection is also provided by *Analox Instruments* that sells a series of analysers (GM8, GM10 and GL6) to be applied in beverages, based on a very fast electrochemical enzymatic assay, in the very short time of 20 s, and a working range of 0.05–20% W/V (for GM8, GL6) and 0.20–40% W/V (for GM10). In the technical bulletin of the instruments, the company highlights the advantages of the electrochemical detection rather than spectrophotometric, being able to work in complex matrices with high opacity or turbidity.

In Table 4, a selection of companies involved in the last 20 years in the commercialisation of biosensors and their components for food safety control is reported, together with the target contaminants. As for food quality testing, the market for food safety, concerning detection of pathogen, GMOs, toxins and pesticides, is increasing as well, being projected to reach a value of US\$16.1 billions by 2020, at a CAGR of 7.4% from 2015 [13]. Traditional

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FIGURE 6 Senzytec instrument by Tectronik. Photograph courtesy of Tectronik Company (<http://www.tectronik.it>).

methods of food safety testing embrace visual inspection, microbial assays and culturing methods, which require complex procedures and extended time (ie, days) to provide results. In particular, the response time is decisive, due to the short shelf life of some foods, such as milk and ready-to-eat vegetables and fruits. Thus, the technological focus from conventional food safety testing methods has been shifting to rapid testing methods able to ensure timely analysis of a larger sample size. Among the wide variety of contaminants, the total microbial detection is one of the most promising areas for biosensor application in food analysis. There are major players in pathogen identification and treatment market including *Abbott Laboratories*, *Achaogen Inc.*, *Alnylam Pharmaceuticals Inc.*, *Becton, Dickinson and Company*, *Biomérieux Sa*, *Charles River Laboratories Inc.*, *Siemens Healthcare* (subsidiary of Siemens AG), *Sigma–Aldrich Corporation*, *Qiagen N.V.*, *Thermo Fisher Scientific Inc.*, *Bio-Imaging Technologies Inc.*, *Bio-Reference Laboratories Inc.*, *Quest Diagnostics*, *Neogenomics*, *RadNet Inc.* [14].

Several other companies developed in the last decades a number of sensing systems for food contaminants. *Molecular Circuitry Inc.* launched, after more than 1-year development, a novel biosensor (Detex Pathogen Detection System) able to simultaneously test multiple pathogens, including *E. coli* O157

TABLE 4 Companies Commercialising Biosensors for Food Safety Control

Company	Country	Target Analyte
Molecular Circuitry, Inc.	USA	<i>Escherichia coli</i> O157, <i>Salmonella</i> , <i>Listeria</i> and <i>Campylobacter</i> in foodstuffs within 24 h
Ambri Ltd	USA	<i>Salmonella</i> and <i>Enterococcus</i>
Biosensor Systems Design	USA	Microorganisms and toxic substances
Biosensores S.L.	Spain	Online monitoring of the concentration of total microorganisms in water samples in 15 min
Motorola	Japan	Microorganisms and genetically modified organisms
Biacore GE	Worldwide	Folic acid, biotin, antibiotics in cereals, meat, milk, infant food, honey
Texas Instruments Inc.	USA	Sensors for gas and pH determination in food industry
Don Whitley Scientific Ltd.	UK	Sensors and components for microorganism detection
Integrated Genetics	USA	DNA probes for detection of microbial contamination (actually, the company has changed its R&D strategies towards other research products)
Molecular Devices Corporation	USA	Sensors and components for microbial determination

(including H7), *Salmonella*, *Campylobacter* and *Listeria* in food samples. This system has received AOAC Research Institute's performance tested method approval for the Detex 24 h *E. coli* O157 (including H7) assay. The system is a patented electro-immunoassay biosensor that shows high accuracy by utilizing unique mixtures of antibodies, as well as enhanced sensitivity and improved specificity thanks to the lower cross reactivity compared to most commercial antibodies. In addition, this instrument provides fast results on individual tests and also offers simultaneous testing for multiple pathogens. *Ambri Ltd.* obtained a worldwide exclusive licence for sensing applications for the Ion Channel Switch (ICS) technology from the Cooperative Research Centre for Molecular Engineering and Technology. This device was the first purpose-built nanomachine operating with moving parts that are nanometres in dimension, and has application in the on-site, fast detection of bacteriological

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contamination, with potential use in food testing to quickly identify dangerous organisms such as *Salmonella* and *Enterococcus*. This Australian company is also working with United States-based Biosensor Enterprises LLC (BEL), a joint venture between Dow Corning Corporation and Genencor International, to increase the range of applications for this platform technology.

More recently, *Biacore GE* proposes, among several products, Qflex kits (Fig. 7) to run specific assays with a Biacore Q system delivering rapid, reliable and automated quantitation of vitamin content (biotin, folic acid, pantothenic acid, vitamin B12) in food matrices, including coloured or opaque samples. Sample preparation and analysis times are significantly reduced compared to traditional techniques, also providing high flexibility required for routine assays. However, this instrumentation needs a laboratory set-up, and it is not useful for in field analysis.

2.2 Biosensors for Process Control

Continuous monitoring systems of industrial processes to detect in real-time possible errors in the production chain are a crucial requirement to address decision-making. Among these processes, food fermentation is used worldwide to produce a broad variety of beverages (wine, beer, cider and kefir) and



FIGURE 7 Qflex kit Biotin for quantitative determination in food products using Biacore Q by Biacore GE. Photograph courtesy of GE Healthcare (www.biacore.com).

food products (cheese, yogurt, bread, sausage, vinegar, pickled cucumbers and soy sauce olives).

Several analytical methodologies are involved to analyse the quality and the freshness of the produced food, controlling parameters such as pH, temperature, pressure, but also in online determining and quantifying various compounds of great importance to control the productivity and the quality of the process. Typically, central analyses entail the detection of contaminants (pesticides, heavy metals and nitrites), pathogenic microorganisms, toxins, antibiotics, allergens, and hormones, and the determination of food composition (sugars, amino acids, vitamins, fermentation products), including:

1. sugars, which are carbon sources and limiting factors of yeast growth involved in fermentative processes (glucose); or they can indicate excessive thermal treatment of milk during pasteurization processes (lactulose);
2. alcohols, which can be limiting factors in alcoholic fermentation of beer and wine, inhibiting enzymatic reactions when the amount exceeds 14% (ethanol);
3. amino acids, which can be used as supplements in animal feed (lysine), or to control the acidity and formation of cheese crust (lactic acid).

Table 5 reports the main companies commercialising biosensors for process control.

Among other analytical methods, (bio)sensors are of high relevance in process control being able to avoid productivity and economic losses. Furthermore, sensing devices can be integrated into the HACCP (Hazard Analysis Critical Control Points) to verify the successfulness of the processes. However, a limited exploitation of (bio)sensors occurs in process control, due to the short lifetime of enzyme sensors, the need for frequent calibration, the lack of reliable responses to different concentrations and changing physico-chemical conditions.

As listed in Table 5, a number of companies realised biosensing systems for online detection of several analytes of interest in process control. *Yellow Springs Instruments* is the foremost company in the development of systems for monitoring and optimization of bioprocesses. The company constructed an integrated system consisting of the YSI 2900M Online Monitoring/Control System featuring the YSI 2960 Online Sampler, which automatically draws fluids from the bioreactor and directly delivers samples to the analyser for testing. This integrated system provides accurate results in less than 1 min, assuring sterility and detecting a broad range of analytes within the typical concentration range of bioprocesses (eg, glucose, lactate, glutamate, glutamine, glycerol, xylose, choline, hydrogen peroxide, sucrose, ethanol, methanol, lactose and galactose) (Fig. 8).

Gwent Sensor Ltd. provides an assortment of one-shot disposable biosensors (Fig. 9) for agrifood analysis, including biosensors for:

TABLE 5 Commercial Biosensors for Process Control

Company	Country	Target Analyte
Universal Sensors	USA	Ethanol, methanol, glucose, sucrose, lactose, L-amino acids, glutamine, ascorbic acid, oxalate
Yellow Springs Instruments	USA	Glucose, sucrose, lactose, L-lactate, galactose, L-glutamate, ethanol, H ₂ O ₂ , starch, glutamine, choline
BioFuture Srl	Italy	Glucose, fructose, malic acid, lactic acid
Chemel AB	Sweden	Glucose, saccharose, ethanol, methanol, lactose
Analox Instruments	UK, USA	Ethanol, methanol, glucose, lactate, glycerol
Nova Biomedical	USA	Glucose, L-lactate, L-glutamate, L-glutamine, alcohol, sucrose, methanol, ammonia
Gwent Sensor Ltd.	UK	Sucrose, L-glutamate, alcohol
York Scientific technology	USA	Glucose, lactose, sucrose, galactose, lactate, ethanol/methanol, L-glutamate, L-amino acids
Biometra	Germany	Glucose, ethanol
Colora Messtechnik GmbH	Germany	Glucose, lactate, ethanol
NEC	Japan	Glucose, alcohol, L-lactate, glycerine
Provesta Corporation	USA	Glucose, lactate, lactose, alcohol

1. ammonia (based on glutamate dehydrogenase and NADH drop-coated onto sensors, giving response in water solutions in the range 1–10 ppm in water samples),
2. lactate (based on lactate oxidase and peroxidase, measuring concentrations between 0.04 and 0.5 mM in water samples),
3. glucose (based on glucose oxidase and an electrochemical mediator, measuring concentrations between 1 and 30 mM in food samples),
4. pyruvate (based on lactate dehydrogenase and NADH drop-coated onto sensors, giving response in the range 1–10 mM in onion).

2.3 Biosensors for Intelligent Food Packaging

A new application of (bio)sensors in the food sector is represented by monitoring systems integrated in food packaging to analyse and preserve the products



FIGURE 8 YSI 2900M Online Monitoring/Control System by Yellow Springs Instruments. Photograph courtesy of YSI Company (<https://www.ysi.com/lifesciences>).

along the food supply chain. Indeed, one of the most important issues to be considered regarding food sustainability is the management of food excess. Nowadays, about 30% of the total world food production is wasted [15].

Intelligent or smart packaging refers to packaging that monitors the conditions of food, senses freshness properties, and checks the integrity of the packages by exploiting biosensors during transport, storage and displays in markets [16]. Many intelligent packagings involve (bio)sensors as monitoring systems to measure physical parameters (humidity, pH, temperature, light exposure), to reveal gas mixtures (eg, oxygen and carbon dioxide), to detect pathogens and toxins, or to control freshness (eg, ethanol, lactic acid, acetic acid) and decomposition (eg, putrescine, cadaverine).

Sensors for food packaging are demanded to operate in specific physico-chemical conditions; thus their application in the food chain is restricted by a number of limitations. As an example, these devices work in a wide temperature range (from -20 to $+30^{\circ}\text{C}$) and/or high exposure to light (eg, UV/retail

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FIGURE 9 One-shot disposable biosensors by Gwent Sensor Ltd. Photograph courtesy of The Gwent Group (<http://www.gwent.org>).



display lighting). For this reason, many research and development efforts have been spent to ensure their effectiveness and stability under all food storage and distribution conditions. Fast responses are also required (in some case as low as tenths of milliseconds) for real-time, online, and high-throughput control of food packages, to rapidly identify improperly sealed units and provide to their removal.

Several sensing systems have been realised for their integration in intelligent packaging, but few of them have been commercialised with the aim to improve the performance of the entire food supply chain and consequently to contribute to a worldwide food waste reduction.

Kerry et al. [17] have accurately reported some examples:

1. OxySense (Fig. 10) is an optical sensor system for a noninvasive oxygen measurement for sealed packages, tailored for the needs of the food, beverage and pharmaceutical industries. This system is able to measure oxygen with sensitive, rapid (5 s) and not-destructive measurements, both in headspace as well as in dissolved liquids. Its optical system can measure oxygen concentration within packages that are transparent, semitransparent and translucent [18].
2. ToxinGuard (Toxin Alert, Ontario, Canada) is a biosensing system able to detect pathogens (*Salmonella* sp., *Campylobacter* sp., *E. coli* 0517 and



FIGURE 10 OxySense Portable Oxygen Analyzer by OxySense. Photograph courtesy of OxySense Company (<http://www.oxySense.com>).

Listeria sp.) by the use of antibodies directly applied in a polyethylene-based plastic wrap in which food is packaged [19]. Any contaminant binds to a detector antibody that is suspended in a nutrient gel; this antibody is set on the packaging film in an easily recognized icon such as an X. As the contaminant collects in the icon area, a coloured or luminescent chemical, also attached to the detector antibody, makes the X visible on the plastic, warning of the presence of a toxin. A global market of \$24 billion has been indicated by the company for their Toxin Guard technology, in targeting *Salmonella*, *Listeria*, *Campylobacter* and *E. coli*.

3. Food Sentinel System (SIRA Technologies, California, USA) is an immuno-biosensor system patented on 2000 (EP 1018013 A1), occurring in part of a barcode, able to continuously detect contaminating bacteria. As shown in Fig. 11, a barcode can be used on refrigerated food products which incorporate an ink that is invisible when safe conditions exist (*left*), while the ink will turn red in contaminated conditions (*right*) and the barcode will be rendered incapable of transmitting data when scanned. This indicator ink includes a first layer impermeable support, a second capillary layer and a third impermeable cover film layer. The second layer includes an absorbent material providing for the directional flow of juices, an immunobead solution pad and a detection area [20].

An alternative approach to preserve food packages and thus assure quality is the use of noninvasive indicator systems, which are able to provide qualitative or semiquantitative information about, for example, marker metabolites for freshness, or deliver data on time and temperature.

Through visual colorimetric changes or comparison with standard references, indicators are promising tools in ensuring package integrity throughout



FIGURE 11 Food Sentinel System by SIRA Technologies (<http://www.google.com/patents/EP1018013A1?cl=en>).

the production and distribution chain. Several companies have commercialised indicators to sense oxygen in packs, including *Ageless Eye*, *Vitalon* and *Samso-Checker*. Their high sensitivity (indicators are susceptible to oxygen concentration, as low as 0.1%, thus their response could depend from residual oxygen) and reversibility (undesirable where an increased oxygen content, due to a leak, is consumed during subsequent microbial growth) are some of the disadvantage of these devices, which restrain their wide commercialisation.

Table 6 reports the main companies commercialising indicators for food packaging.

Indicators for gas measurement may employ specialized pigments inserted into plastic packaging, showing colour changes if a modified atmosphere occurs that can damage food. These kinds of systems allow advising manufacturers on possible breakages of package integrity to remove damaged foods from the supply chain before reaching supermarkets.

Colour indicator sensings are also employed as freshness indicators, detecting the production of volatile amines, which generate the typical 'fishy odour' common to all seafood, or indicating fruit ripeness by reacting to particular produced aromas. Time–temperature indicators estimate how long a product remained above a certain threshold temperature, within typical ranges between 2 and 8°C or 15–31°C. Moreover, certain sensitive labels indicate time–temperature data adapted to toxin formation of *Clostridium botulinum*.

TABLE 6 Commercial Indicators

Company	Country	Target Analyte
Novas Insignia Technologies	UK	Gas – modified atmosphere
O2Sense	Switzerland	Gas – amount of oxygen
FreshTag	The Netherlands	Freshness – volatile amines
RipeSense	New Zeland	Freshness – aromas released by the fruit as it ripens
3M MonitorMark	USA	Time–temperature – to estimate how long a product was above a threshold temperature (ranging between 15 and 31°C)
Timestrip Complete	UK	Time–temperature – to monitor high and low threshold temperature breaches outside selected ranges (2–8°C)
Fresh-Check	USA	Time–temperature – to monitor temperatures higher or lower than selected ones
CheckPoint	Palestine	Time–temperature – to monitor temperature ranges adapted to toxin formation of <i>Clostridium botulinum</i>
CoolVu Food	Switzerland	Time–temperature – to monitor the storage temperature
Innolabel Timestrip	Belgium	Time–temperature – to provide accurate shelf life guide for fresh fruit and vegetables
Chromatic Technologies, Inc.	USA	Thermochromic inks Reversible colour changes depending on temperature
Matsui International Company, Inc.	USA	Thermochromic inks Reversible colour changes depending on temperature

2.4 Biosensors for Smart Agriculture

Smart agriculture concept entails the integration of cross-disciplinary technologies, including crop grow control, soil analysis, remote sensing, yield mapping and positioning systems, to optimize the sustainability of agricultural

processes and reduce their environmental impacts. Among these technologies, (bio)sensors for dynamic measurement of soil physical (ie, pH) and chemical (ie, nutrients) parameters, as well as for the determination of residual pesticides in crop and soil, are decisive to foster a sustainable farming.

For example, several optical sensors and laser scanning devices have been realised for real time in situ sensing of photosynthetic activity of plants. Nitrogen uptake prediction can be also provided using laser-induced chlorophyll fluorescence measurements. Sensor systems have been developed to monitor the presence of pests, pathogens, or pesticides to tune the amount of insecticides to be employed for crop productivity management. In addition, nanosensors integrated in platforms can monitor the controlled release of fertilizers and pesticides via nanoscale carriers avoiding their overdose, improving productivity and reducing waste. Networks of nanosensors located throughout cultivated fields will promise a real-time and comprehensive monitoring of crop growth, furnishing effective high-quality data for best management practices [21].

Moreover, data remotely sensed through satellites or drones can provide information on soil conditions, plant growth and weed infestation, with cost-effective and reliable measurements of crop emittance and reflectance [22]. The automation of irrigation systems is also a crucial requirement of smart agriculture. In this regard, sensor technology has the potential to maximize the efficiency of water use.

Several companies invested their resources in the development of sensing systems for sustainable farming. Among others, *PrecisionHawk* [23] is a leader company in the construction of open platform sensors designed on the requirements of end users, capable of plant health measurement, water quality assessment, vegetation index calculation, and plant counting, in addition to aerial mapping and imaging, and water temperature detection. Many companies have invested on the realisation of unmanned aerial systems (UAVs) or drones to support farmers to adopt an effective use of products for plant protection and fertilisation, as well as to provide data on soil parameters, to increase productivity, reduce inputs, and maximize yield potential.

Among others, *Ascending Technologies* [24] develops, in collaboration with *e-volo* (<http://e-volo.com>), systems based on precision positioning technology; *Lehmann Aviation* [25] utilises imagery from drones equipped with camera and sensors to provide crop information (Fig. 12); *MicroDrones* designs micro-UAVs equipped with several sensors and microcontrollers [26].

3. CONCLUSIONS AND FUTURE PERSPECTIVES

Nowadays, advances in (bio)sensor development are reasonably increasing with the aim to accomplish fast, sensitive and cost-effective food analysis. However, despite the straightforward investments to support R&D efforts, very low success rates are achieved in biosensor commercialisation. Indeed, there is



FIGURE 12 UAV/drone by Lehmann Aviation. Photograph courtesy of Lehmann Aviation (www.lehmannaviation.com).

not a huge market for (bio)sensors, except for glucose sensors, since the market entry of a new device is very difficult because of some drawbacks inherent to sensing technology. Limited lifetime of the bioreceptors, integration of the components, mass production as well as practicability in handling are some of the main obstacles to tackle with.

Last and future trends in biotechnology, biomimetic chemistry, nanotechnology, and material science guarantee innovation in biosensors and thus allowing their penetration into new application fields including agrifood, environmental monitoring, process industry, and security/biodefense, besides the consolidated biomedical field. The recent advances in synthetic biology and biomimetic chemistry resulted in tailor-made biosensors with desired features of robustness, sensitivity and specificity, by producing synthetic entities mimicking key properties of natural one, or engineering them to provide new desired features. Indeed, the design of artificial molecules, such as aptamers, biomimetics, molecular imprinting polymers, peptide nucleic acids and ribozymes, provided a trustworthy support to biosensing technology in solving some of the problems related to specificity, sensitivity and stability [27]. Associated to them, progress made in the development of new protocols for bioreceptor immobilisation helped to provide an enhanced robustness of the biosensors, in terms of both storage and working stability. Many physical and chemical methods for immobilizing bioreceptors, such as adsorption and entrapment within membranes, deliver complications to the conformational structure affecting the functional activity and result in less sensitivity and short lifetime. Thus, new strategies for the immobilization, orientation and molecular organization of biomolecules have been conceived by designing vanguard materials to construct well-defined microenvironments, mimicking biological membranes for a better orientation of the bioreceptors, and thus for enhancing their stability and activity.

To this regard, nanotechnology emerged in the last years helped to improve the biosensor performance thanks to the special physical, chemical, mechanical, magnetic and optical features of nanomaterials (eg, strong absorption band in the visible region, high electrical conductivity and good mechanical characteristics). Indeed, the use of nanomaterials to functionalise and immobilise the bioelements can improve their stability and specificity, yielding also reproducibility and reliability [28,29]. As an example, carbon nanotubes together with nanoparticles (gold, platinum, copper) have been widely exploited to boost detection sensitivity and facilitate biomolecule immobilization, as well as to promote electron-transfer reactions between bioreceptors and electrodes.

Transfer of (bio)sensor production from the research laboratory bench to large-scale manufacture is also crucial for their marketability. Production of high quantities and at low cost demands high levels of automation, while sensor development burdens a high requirement for manual processing. Among the alternatives to adapt sensors for commercial application, there is the possibility to develop multiplexed sensing systems through microfluidics and lab-on-chip technologies, which hold promise for automation of sampling and screening of a wide range of analytes with minimal consumption of reagents in portable devices. Moreover, progresses in microscale engineering plays an essential role in design (bio)sensors by scaling down systems and providing controllable microenvironments, offering also the possibility of economic mass production [30]. Indeed, novel miniaturised electrodes, micro-field effect transistors and micro-transducers obtained by the last advances in microengineering technology allow (bio)sensors to be integrated into disposable chips, capable of simultaneous determination of multiple food components/contaminants as well as of on-site monitoring of food processes.

The simultaneous detection of analytes in complex matrices is also a fundamental requisite in food analysis, since the main threat of food safety involves hazards caused by both biological and chemical contaminants. The recent results in material science highlight the potential of several materials (eg, silicon, glass, paper, plastic, other polymer) in multiplexed (bio)sensor design because of their miniaturization, integration and automation. Among these materials, paper has attracted significant attention in the last years for the development of simple, easily fabricated, disposable and low-cost devices. In addition, the combination of paper with microfluidics provides a mechanism for multiple detection, separating particulates from fluids and avoiding interference among different chemical species [31,32].

Finally, sample treatment in food analysis is also a critical concern to develop (bio)sensors for in field use. Generally, treatment procedures require several steps, including sampling, extraction of targets from the samples, clean-up and analysis. Considering the high complexity of food matrices and the presence of interfering species, it is hard to provide a simple extraction procedure to be accomplished on site without affecting recovery of the target

analytes. For this reason, further research efforts are required to design (bio) sensor configurations for real application, entailing easy and effective procedures of sample treatment.

Actually, these above-mentioned technologies, including biomimetic chemistry, material science, nanotechnology, and microengineering, are still in their infancy, but they have the highest probability of making an impact in (bio)sensor design in the near future. Surely, their cross-cut disciplinary character will lead (bio)sensors to be commercialised and extensively used in several application field, including agrifood, in the next 20 years.

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