Biosensor: an emerging safety tool for meat industry

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Abstract The meat industry associated with the health hazards like deadly pathogens, veterinary drugs, pesticide residues, toxins and heavy metals is in need of a tool to tackle the awful situation and ensure safer product to consumer. The growth in the industry, global trade scenario, stringent laws and consumer awareness has placed an extra onus on the meat industry to meet out the expectations and demands. Biosensors are the latest tool of detection in the fast growing industries including the food industry. Hence an attempt is envisaged here to review the possibility of harnessing biosensors as tool of safety to safe guard the consumer health and address safety issues in reference to the common threats of concern in the meat industry.

Keywords Biosensors · Meat · Pathogen · Veterinary drug · Pesticides

Introduction

Increase in meat production and the threat of contamination have led the industry to pursue rapid, inexpensive methods of analysis to safeguard the health and safety of consumer. Further, innovation and development in the food industry are guided by the central principles: food safety and quality (Ferreira et al. 2003). Dynamic practices have posed newer threats in terms of physical, chemical and biological hazards

to the product quality and consumer safety. Increasing threats, in addition to pathogens like toxins, drugs, pesticides, heavy metals have been noticed nowadays, due to indiscriminate agriculture and animal husbandry practices having the potential to have serious health implications in terms of causing illness (Kim et al. 2007). The more the quality and safety is challenged, the more stringent tools are needed to produce a product of standard parameters.

Although conventional methods of detection are available but most of the time they are time consuming and require strict protocol with an elaborate procedure and a trained personnel. For example, conventional and standard pathogen detection methods such as culture and colony counting may take up to several days. Further, physicochemical methods such as liquid chromatography–tandem mass spectrometry (LCMS/MS), for the detection of contaminants like toxins, veterinary drug residues, heavy metals tend to be expensive, complicated to perform and time-consuming (McGrath et al. 2012). However, chromatography and spectrometry may provide more accurate and conclusive results but screening tests need a higher throughput with less operator training.

A rapid method of screening these hazards in the bulk handling industry to maintain food safety and quality could only be the answer. In this context, the biosensor can become an attractive alternative to the existing techniques of quality control and safety of meat and meat products in the rapidly growing meat industry. They to offer several advantages like high degree of sensitivity and specificity of detection where minimal sample preparation is there, cost-effectiveness, miniaturization and portability for real time monitoring (Singh et al. 2013). Moreover, HACCP system can be used to verify that a given process is under control, since high biosensor sensitivity allows the detection of pathogenic microorganisms, pesticides and other contaminants in hours or minutes (Luo et al. 2009; Mostafa 2010).

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Biosensors can be defined as an analytical device, which converts a biological response into an electrical signal and consists of two main components: a bioreceptor or biorecognition element, which recognizes the target analyte and a transducer, which converts the recognition event into a measurable electrical signal (Velusamy et al. 2010). Hence, biosensors can be classified on the basis of type of biocomponent involved, mechanism or mode of signal transduction etc (Lavecchia et al. 2010). The recognition elements can be systems containing enzymes, microorganisms, cell receptors, antibodies, antigens and nucleic acids. The transduction elements are generally electrochemical, optical or piezoelectric, and the electrical signals when based on a change in the measured current are amperometric; when change in the measured voltage between the electrodes are potentiometric and when a change in the ability to transport charge, then they are conductometric. The optical sensors are generally based on the principle of absorbance, fluorescence, chemiluminescence, surface plasmon resonance etc. The sensors based on mass produce a mass dependent signal for the analyte that interact with the sensors and give information regarding analyte presence.

Although the initial development in the biosensors was aimed to meet out the medical diagnostic requirements but later on the principles of detection and quantification of biological molecules found its way in the food industry too. The basis of biological recognition in the biosensors helped in analysis of above stated contaminants associated meat food system. Biosensors can be easily applied to meat sample with least processing techniques like mincing or homogenization, however initial enrichment stages may not be required for pathogen or toxin detection, saving the assay time. Although little commercialization of this tool has been done in the meat industry unlike the fields like medical diagnostics but with the increasing load of bulk handling it is going to be difficult to maintain the safety and quality of products without a faster screening tools like biosensor. The present review here envisages to discuss the possible application of this emerging tool in the meat industry to ensure the quality and safety of meat products.

Application of biosensors to detect health hazards in meat and meat products

Pathogen

Biosensor detection has not been limited to the common pathogens like *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, *Clostridium perfringens* and *Staphylococcus* but also cover many others, such as, *Campylobacter*, *Bacillus* and *Shigella* (McGrath et al. 2012). Microbiological methods generally consist of an enrichment stage (pre-enrichment and/

or selective enrichment), culturing in selective or differential agar plates to isolate cultures, followed by phenotype analysis or metabolic fingerprint analysis to confirm the result, which takes 2–10 days which makes the detection system quite cumbersome (Bai et al. 2010). More over conventional pathogen detection methods mainly rely on microbiological and biochemical analysis, which are highly accurate but are time consuming and costly and sometimes it is not feasible to integrate them for on-site detection (Singh et al. 2013). Fast and accurate pathogen detection is the area which has attracted, the researches involved in the quality and safety management of food items throughout the world where biosensors have shown the promising results in comparison to conventional methods. As a consequence several different types of biosensors have been developed by the scientist to test their feasibility in the complex food materials like meat. Some of them are discussed here to develop an understanding of their possible applications.

Optical fibers are an instruments working on the principle of total internal reflection and used extensively in the medical diagnostics as well as other clinical approaches. Biosensors based on optical fiber use a tapered fiber to send excitation laser light signals to the detection surface and receive emitted light. Light propagation through a fiber or waveguide can be very sensitive phenomenon, which makes the optical fibers excellent detectors for a variety of applications in the industry specially the detection of pathogenic organisms. A fiber optic portable biosensor working on the principle of fluorescence resonance energy transfer (FRET) for rapid detection of *Salmonella typhimurium* in pork samples was reported (Ko and Grant 2006). A portable evanescent-wave fiber-optic biosensor to detect *E. coli* O157:H7 in seeded 10 and 25 g ground beef samples was demonstrated by DeMarco and Lim (2002) where hundred percent correct identification of positive samples obtained at 9.0×10^3 CFU/g for 25 g ground beef samples with silica waveguides and at 5.2×10^2 CFU/g for 10 g ground beef samples with polystyrene waveguides. Similarly Ye et al. (2002) developed a biosensor, consisting of a chemiluminescence reaction cell, a fiber-optic light guide, and a luminometer linked to a personal computer, in conjunction with immunomagnetic separation for rapid detection of *E. coli* O157:H7. The chemiluminescence biosensor was selective to *E. coli* O157:H7 even in the presence of other bacteria in the sample, including *S. typhimurium*, *C. jejuni*, and *L. monocytogenes* where they could detect of *E. coli* O157:H7 in ground beef and chicken carcass, with the detection limits of 3.2×10^2 and 4.4×10^2 CFU/g, respectively and the detection was completed within 1.5 h without any enrichment. Valadez et al. (2009) achieved a limit of detection (LOD) of 10^2 CFU/ml for *Salmonella enterica* in egg and chicken after 6 h enrichment by a fibreoptic biosensor when utilising a fluorescently labelled secondary antibody. Ohk et al. (2010) described an alternative, optical (fluorescence) based, fibre-optic approach in which they used a capture

antibody and a fluorescently labelled aptamer to sandwich *Listeria monocytogenes* with a LOD of 10^2 CFU/25 g in ready-to-eat meat samples. Banerjee and Bhunia (2010) used mammalian cells (Ped-2E9), with specific sensitivities for pathogens in a portable optical (absorbance) biosensor that assessed cytotoxicity by measuring the colour change and detected *L. monocytogenes* in ready-to-eat meats at 10^3 – 10^4 CFU/ml.

Fourier transform infrared (FT-IR) spectroscopy is another technique in optical category biosensor which is a non-destructive analytical approach with considerable potential for application in the foodborne pathogen detection and this approach was developed by Yu et al. (2004) for microbial differentiation and quantification. It has been reported by Ellis et al. (2002) that FT-IR technique can be used directly on the surface of food to produce biochemically interpretable “fingerprints” where FT-IR was exploited to measure biochemical changes within the meat substrate, enhancing and accelerating the detection of microbial spoilage; quantitative interpretation of FT-IR spectra was possible using partial least-squares regression and allowed accurate estimates of bacterial loads to be calculated directly from the meat surface in 60 s.

The inherent ability of bacteriophages to selectively bind to their target pathogens has been exploited to design biosensors for application in pathogen detection system. Prokaryotic and eukaryotic luciferase expressing gene (*lux* and *luc*), *E. coli* β -galactosidase (*lacZ*) gene, bacterial ice nucleation (*inaW*) gene and green fluorescent protein (*gfp*) expressing gene have been most commonly used for such applications (Singh et al. 2013). As phages are host dependent for any physiological function they are capable of expressing the reporter gene only after host infection, thereby confirming the presence of the host bacterium upon gene expression. Loessner et al. (1996) successfully employed A511::luxAB recombinant phage for detection of *Listeria* bacterium in microbiologically complex food samples such as minced meat, where 10 bacteria per gram could be detected with 20 h of sample processing time and pre-enrichment steps and the total detection time was estimated to be 24 h compared to 4 days by conventional microbiological methods. In another development a different approach was there to produce optical signals where Cheng et al. (2009) described experiment that measures bioluminescence caused by isolated bacterial ATP from meat juice. Immunoparticles were used to specifically capture and isolate the bacteria before bacterial ATP was released in the presence of luciferin–luciferase system to produce the optical (luminescence) signal.

Su and Li (2005) demonstrated a quartz crystal microbalance (QCM) immunosensor for the detection of *S. typhimurium* with simultaneous measurements of significant changes in the resonance frequency and motional resistance. In the direct detection of *S. typhimurium* in chicken meat sample, resonance

frequency and motional resistance were proportional to the cell concentration in the range of 10^5 – 10^8 and 10^6 – 10^8 cells/mL, respectively where the detection limit was lowered to 10^2 cells/mL by using anti-Salmonella-magnetic beads. In a similar study for identification of *E. coli* O157:H7 from a ground beef sample Chen et al. (2008) immobilised an oligonucleotide specific for gene onto a mass/acoustic (piezoelectric) transducer in their flow-based biosensor. The specific gene was amplified after extraction from the samples by PCR (Polymerase Chain Reaction) using synthetic primers (including a tag sequence) and the amplicons were denatured before hybridising with the immobilized oligonucleotide. A gold-nanoparticle-labelled secondary oligonucleotide that was complementary to the tag was introduced. This gold label amplified the signal, providing a LOD of 5.3×10^2 CFU/ml without enrichment.

The pathogen detection system approached a new height when Bai et al. (2010) described work that facilitates the detection of 11 food-borne pathogens, in buffer and pork meat, using a microarray approach with biospecific DNA probes immobilised on a sensor surface. The PCR products were denatured and allowed to hybridise with the immobilised probes; thus, the primer region on the biotinylated PCR products were able to bind with their relevant probes. Antibiotin IgG conjugated to HRP was then passed over the surface to complete the sandwich, followed by substrate, which facilitated the detection of the PCR product as a concentration dependent colour change on the surface of the optical (absorbance) biosensor.

The concept of an ‘electronic nose’ for pathogen detection is a new development in the field of biosensors. Balasubramanian et al. (2005) used a commercially available Cyranose-320™ electronic nose system to identify *S. typhimurium* in inoculated beef samples. An electronic nose containing an array of 32 conducting polymer sensors was used to obtain the odour patterns of the headspace of the meat samples and the volatile organic compounds emanating from vacuum packaged beef strip was analysed where the electronic nose system was able to identify meat samples contaminated with *S. typhimurium* at a population concentration level $\geq 0.7 \log_{10}$ CFU/g.

Pesticides

The presence of pesticide residues and metabolites in food, water and soil currently represents one of the major issues in environmental chemistry research (Mostafa 2010). The existing analytical methods for the determination of organophosphate pesticides and N-methyl carbamates are complex or not existent for some compounds however High Performance Liquid Chromatography (HPLC) is an appropriate technique for the determination of these compounds but for the adequate sensitivity of the method, several pretreatment steps are required, adding time and cost (Hiemstra and De Kok 1994).

Enzymes like cholinesterase (AChE), organophosphorus-hydrolase (OPH), and urease are used in the design of biosensors for pesticides detection. A large number of biosensors are based on the enzyme acetylcholinesterase (AChE); action of this enzyme is inhibited by pesticides (Sun and Wang 2010). Pesticides such as organophosphates and carbamates bind to a serine moiety within the active site of the enzyme, thus preventing the deacetylation of acetylcholine (Roepcke et al. 2010). Analytical devices, based on OPH and cholinesterase inhibition, have been widely used for the detection of carbamates and organophosphate (OP) compounds (Pavlov et al. 2005). In a different approach Hildebrandt et al. (2008) developed biosensors for the detection of pesticides in food samples based on the formation of products from the enzyme activity on the substrate affecting the electrochemical activity where pesticide present in the sample caused a decrease in the activity of the biosensor. Similarly Lee et al. (2010) described a bienzyme approach for the detection of organophosphates whereby the choline product from the AChE reaction in solution acts as a product for the immobilised choline oxidase enzyme in their electrochemical (amperometric) biosensor. To detect parathion whole cells of *Flavobacterium sp.* were immobilized by trapping in glass fiber filter and were used as biocomponents along with optic fiber system. *Flavobacterium sp.* has the enzyme organophosphorus hydrolase, which hydrolyzes the methyl parathion into detectable product p-nitrophenol (Kumar et al. 2006). As pesticides and drug residues are generally small molecules they do not produce immune response, hence they are combined to an immunogenic molecules like proteins (haptens), keeping intact the chemical composition of the compound to be analyzed and the design of the specific hapten is decisive in the development of immunoassays, since it is responsible for determining the recognition characteristics of the antibody (Haasnoot et al. 2000).

Drug residues

For decades, antibiotics have been used abundantly worldwide in animal production, so antibiotic resistance may spread to other microbial populations, as reflected by the emergence of infectious diseases that have become resistant to standard antimicrobial treatments (Sapkota et al. 2007). As a result, fast, sensitive methodologies are being developed and used by food-safety control laboratories to ensure the control of antibiotic residues in live animals and animal products (Chafer-Pericas et al. 2010). Ferguson et al. in 2002 reported immunosensor inhibition assay for the detection of streptomycin and dihydrostreptomycin residues in whole bovine milk, honey, porcine kidney, and porcine muscle. Huet et al. (2008) developed an optical SPR-biosensor assay for quinolones in three matrices (i.e. egg, fish, and poultry meat). An amine derivative of the quinolone was immobilized on a sensor chip to detect the bi-active polyclonal antibody

produced by an immunogen prepared after combining features of two complexes (i.e. norfloxacin-BSA and flumequine-BSA). A range of 0.1–100 µg/kg in fish and 0.1–10 µg/kg in egg or poultry meat was achieved for the reference molecule i.e. norfloxacin. Another finding describes an SPR-biosensor assay for the accurate determination of chloramphenicol and chloramphenicol-glucuronide residues in various samples where the method detected 0.05, 0.02, 0.02 and 0.07 µg/kg in milk, poultry muscle, honey and prawn, respectively (Ferguson et al. 2005).

Researchers have also described a membrane-based immunosensor for the detection of chloramphenicol by chemiluminescence phenomenon in samples of pork, beef, chicken, and shrimp (Park and Kim 2006). It exploits competition between chloramphenicol and a chloramphenicol-horseradish peroxidase conjugate for binding to an anti-chloramphenicol antibody immobilized on the membrane where addition of peroxidase substrate luminol, the peroxidase on the antibody-bound tracer catalyzes a light-emitting reaction which is measured for the quantification. In 2007, Haasnoot et al. developed a fast, specific biosensor immunoassay for flumequine in broiler serum and muscle. This quantitative screening assay offered simplified sample preparation and suitable measurement ranges (15–800 ng/mL in serum and 24–4000 ng/g in muscle). Haasnoot et al. (2003) again developed a rapid biosensor immunoassay for detecting eight sulfonamides in chicken serum. They used a monoclonal antibody exhibiting 50–149 % cross-reactivity towards eight sulfonamides and lower cross-reactivity towards six others. Another SPR-based assay described can detect at least 19 sulfonamides in porcine muscle (McGrath et al. 2005). According to the developers, the risk of false positives is reduced because the method does not recognize the acetylated metabolites of the drugs.

Toxins

Bacterial toxins such as botulinum neurotoxins, listerial toxins and enterotoxins from *Staphylococcus* as well as *Bacillus spp.* are of concern owing to their association with meat and other food materials. The mouse or other lab animal bioassay is the most widely used test for toxins, but it has associated ethical concerns (Vilarino et al. 2010). Chromatography-based methods such as high-performance liquid chromatography with fluorimetric detection and LCMS/MS are available, but these are time-consuming and costly (Campas et al. 2007).

Pauly et al. (2009) described the simultaneous detection of botulinum neurotoxins type A and type B and staphylococcal enterotoxin B using a suspension-bead-array-based optical (fluorescence) biosensor. Flex-Alert is the R & D division of Scheelite Technologies, known for the development of wireless biosensors for the food industry. The core of Flex-Alert technology is the deposition of anti-toxins on plastic material and biosensors are connected via a wireless network for real

time monitoring where early detection allows preventative measures by manufacturers.

Beef can also be considered as an important pathway of dioxin exposure of humans where beef originating from extensive production systems may contain higher levels of dioxins than beef from intensive production systems. Similar findings were observed for pigs reared in outdoor systems (Hess and Geinoz 2011; Brambilla et al. 2011). This is probably due to animals are allowed to graze in pastures in extensive systems, that can be contaminated with toxins from atmospheric deposition.

A multi-well plate-based biosensor containing B-cell hybridoma, Ped-2E9, encapsulated in type I collagen matrix, was demonstrated for the rapid detection of viable cells of foodborne pathogens like *Listeria*, the toxin listeriolysin O, and the enterotoxin from *Bacillus* species. These pathogenic micro-organisms or toxins can infect and produce detectable cytotoxicity to Ped-2E9 cells, which release alkaline phosphatase when infected. It has been reported that, the pathogenic *L. monocytogenes* cells and toxin preparations from *L. monocytogenes* or *B. cereus* showed cytotoxicity ranging from 24 to 98 % at 3–6 h postinfection. In contrast, nonpathogenic *L. innocua* (F4247) and *B. subtilis* induced minimal cytotoxicity, ranging only 0.4–7.6 % (Banerjee et al. 2008).

Carlyle et al. (2002) demonstrated the application of chromatophore cell-based biosensor assay for the detection of foodborne microbial pathogens and their toxins where chromatophore cells were exposed to purified bacterial toxins and live toxin producing bacterial strains to test the effect of bacterial toxins. Toxin producing strains of *E. coli*, *Salmonella*, *Shigella*, *Vibrio*, *B. cereus* and *Clostridium* induced aggregation of the pigmented organelles in the chromatophore cells and the non-toxin producing strains of *E. coli*, *Bacillus subtilis* and *Lactococcus lactis* did not induce aggregation. Ruan et al. (2004) have used Magneto Elastic (ME) sensors to detect staphylococcal enterotoxin Type B (SEB), they examined the response of the sensor by linearly varying the concentrations of SEB in the range of 0.5–5 ng/mL and demonstrated the sensitivity of the sensors. In a study to detect Listeriolysin O (LLO), the liposomes were encapsulated in porous silica using alcohol-free sol–gel synthesis methods. The immobilized liposomes act as a cellular compartment containing the fluorescent dyes and the dye release due to the pore formation by LLO indicated the presence of the toxin (Zhao et al. 2006).

In another study biologically active cell membrane components were incorporated into conjugated polymers with desirable optical properties; polydiacetylenic membrane-mimicking materials that mimic the cell membrane and conveniently report the presence of target with a colour change were used for the colorimetric detection of bacterial toxins (Song et al. 2002). An optical (SPR) biosensor method was

developed by Fonfria et al. (2007) for the detection of paralytic shellfish poisoning toxins in mussels, clams, cockles, scallops and oysters with LODs between 2 and 50 ng/mL. Stevens et al. (2007) developed a six-channel portable optical (SPR) biosensor with integrated fluidics for the detection of domoic acid, with a LOD around 3 µg/kg, in clams using methanol extraction and solid-phase-extraction sample clean-up. For diarrhoeic shellfish poisoning toxins Llamas et al. (2007) developed an optical (SPR) antibody-based competitive method for okadaic acid detection in mussel (LOD 126 ng/g) following homogenisation, methanol extraction, evaporation and reconstitution in buffer. It was revealed that piezoelectric is very sensitive method, noting that a detection limit of 8.6 pg/l was obtained for cholera toxin detection (Chen et al. 2010).

Heavy metals

We may accumulate heavy metals while eating foods of animal origin, since animals have greater contact with poorly treated water, moreover they graze close to areas drained by industries. To determine the concentration of heavy metals such as arsenic, cadmium, mercury, and lead devices have been developed that incorporate genetically modified micro-organisms and enzymes such as urease, cholinesterase, glucose oxidase, alkaline phosphatase etc (Tsai et al. 2003). Biosensor based on optical transduction can detect the inhibition of the enzyme urease and can sense down to 100 ppm of cadmium (Verma et al. 2010). Among the optical biosensors the following have been reported: devices using fluorescence microscopy and fluorescence quenching applied to siderophore bacteria biomediators (Adam et al. 2005) or using FRET and SPR applied to chelating fluorescent protein biomediators and albumin-based biosensors (Chung et al. 2006; Gupta et al. 2008).

The increasing number of published papers has revealed the use of biosensors based on enzymes, DNA, or whole cells—as an easy technique for detections of heavy metals (HMs) and is in continuous improvement in a wide range of areas and also in the environmental market. Particularly, although the DNA and whole-cell-based biosensors have demonstrated their efficacy in gene discovery and genomics research, in the HMs detection they are more laboratory oriented and engage expensive equipment (Graziella 2011). The study conducted showed that the Sol–gel-immobilized-urease conductometric biosensor on a thick film interdigitated electrode can be used as a reliable sensor for heavy metal ion determination in samples (Ilangoan et al. 2006). The optimization and the characterization of a new amperometric biosensor based on acetylcholinesterase (AChE), immobilized on a graphite electrode modified with Pt-nanoparticles (PtNP), are reported for heavy metal detection (<http://dx.doi.org>).

Conclusions

Biosensor technology has received heightened interest over the past decade, since it is a promising tool for lower detection limit with rapid analysis time at relatively low cost. Though conventional detection methods are sensitive, they lag behind the analytical methods by detection time. Biosensor utilization in rapid detection of contaminants can lead to release of products within hours or even minutes, rather than holding them for several days. It can also be a good tool for screening a big thorough put of samples and going for confirmation only for small number. The increased use of array and multiple-channel systems have given to the multiplexing a new and more advanced approach with the potential of detecting a number of pathogens, toxins, pesticides etc. However more research is needed for the development of detection technique which is more reliable, rapid, accurate, simple, sensitive, selective and cost effective.

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