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Review

Emerging Role of Biosensors and Chemical Indicators to Monitor the Quality and Safety of Meat and Meat Products

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Abstract: The meat industry requires prompt and effective control measures to guarantee the quality and safety of its products and to avert the incidence of foodborne illnesses and disease outbreaks. Although standard microbiological methods and conventional analytical techniques are employed to monitor the quality and safety, these procedures are tedious and time-consuming, require skilled technicians, and sophisticated instruments. Therefore, there is an urgent need to develop simple, fast, and user-friendly hand-held devices for real-time monitoring of the quality of meat and meat products in the supply chain. Biosensors and chemical indicators, due to their high sensitivity, specificity, reproducibility, and stability, are emerging as promising tools and have the potential for monitoring and controlling the quality (freshness and sensory traits such as tenderness) and safety (metabolites, contaminants, pathogens, drug residues, etc.) of muscle foods. In this review, the application of biosensors in the meat industry and their emerging role in the quantification of key meat quality components are discussed. Furthermore, the role of different biosensors to identify and detect contaminants, adulterants, pathogens, antibiotics, and drug residues in meat and meat products is also summarized.

Keywords: biosensors; meat freshness; quality control; contaminants; pathogens; drug residues

1. Introduction

Meat and meat products are highly perishable and prone to different chemical, enzymatic, microbial, or environmental degradation over time from processing to storage. Emerging microbial hazards directly affecting safety issues of raw and processed meat products are Shiga-toxin-producing *Escherichia coli* O157:H7, *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Campylobacter jejuni*, *Listeria monocytogenes*, and *Yersinia enterocolitica*. These microbial hazards may enter the meat production chain at different processing stages or during storage, resulting in meat-borne outbreaks. Therefore, the quality and safety aspects of meat are a forefront issue to be maintained at each level, from the farm to the fork. This is essential not only to protect the consumer's interest but also to reduce the risk of zoonotic outbreaks or meat-associated food poisoning. Generally, ante- and postmortem inspection, visual observation, palpation, and incision are some of the traditional and time-honored

methods used during meat inspection. But these procedures are not sufficient to offer full protection to consumers and may even increase the chances of cross-contamination of the products. To overcome this problem, many laboratories use conventional analytical techniques such as high-performance liquid chromatography, gas chromatography, real-time polymerase chain reaction (PCR), or enzyme-linked immunosorbent assay (ELISA) to assess the quality of fresh meat and meat products. Although some of the analytical tests and procedures, namely sensory analysis, chemical tests, and microbial culture-based methods are somewhat useful, they are time- and cost-consuming, and require trained personnel and expensive equipment for sample analyses. Moreover, these methods are not sensitive enough to immediately detect quality and safety issues in meat. As food safety control is an urgent matter for public health protection, the development of user-friendly testing devices that can help in the early detection of any kind of traditional or emerging hazards in the supply chain is required for the safety of public health [1]. The drawbacks of conventional quality and safety detection methods for meat and meat products have resulted in recent advancements in the development of quick, user-friendly, and real-time monitoring devices for meat safety and quality assurance. Therefore, research on biosensors is gaining momentum, driven by consumers' needs and preferences regarding meat food safety issues.

In recent years, biosensors or indicator sensors have been used as monitoring devices to trace different hazards, either in raw meat or during different product processing steps that indicate the quality of the product. Research and developments have progressed over time to drive the application of food biosensors at industrial or commercial levels. Freshness indicators, time-temperature integrators, microbial spoilage biosensors, nanosensors, barcodes, RFID (Radio Frequency Identification) tags, etc. are different successful applications of biosensors [2,3]. Moreover, intelligent packaging with different kinds of biosensors or labeling is now available. These smart packaging systems can communicate with the internal or external environment of the packaged meat product. This is beneficial as customers can easily understand and judge the freshness and quality of meat or meat products by real-time optical screening before buying the products.

The basic difference between a sensor and an indicator is that a sensor consists of a receptor and a transducer, whereas an indicator is simple, cost-effective, and communicates through direct visual aid. With recent advancements, biosensors are opening new possibilities to trace different hazards in food safety chains. Biosensors cannot only detect traditional, new, or emerging hazards but also natural toxins and anti-nutrients, pesticides, antibiotics, and the extent of glycolysis in the meat chain system. Moreover, these sensors can also analyze the nutrient content (proteins, fatty acids, vitamin B complex), pH, color parameters or natural drip losses, freshness, tenderness, and more. Novel biosensing technologies are now available as point of care (PoC) devices for pathogen detection by which meat inspection and safety parameters can be monitored from farm to slaughterhouse [1].

Biosensors are analytical devices incorporating biological materials, intimately associated with or within a physiochemical transducer or transducing microsystems, which may be optical, electrochemical, thermometric, piezoelectric or magnetic [4]. These are recognized as functional tools for assessing the quality of meat and meat products due to their specificity, sensitivity, linear response range, reproducibility, short response and recovery time, and ultimately due to their operational stability [5]. Presently, advanced biosensors are being developed with extended applications of nanotechnology to revolutionize the existing quality and safety detection protocols [6]. Furthermore, different types of nanomaterials, such as carbon nanotubes, graphite, and graphene, with a high surface area and several available active sites, may provide added biocompatibility to these sensors. In the near future, these advanced nano biosensors may play a crucial role in the rapid and accurate detection of any kind of physical, chemical, or microbiological hazards in meat preservation systems [7,8]. Although many articles on biosensors have been published recently, an updated and comprehensive review on the usefulness of biosensors in meat and meat products is highly desired. This review aims to give an overview of the

recent advancements in biosensors and their applications in the meat industry (Figure 1). The research developments and further scopes in indicator- or sensor-based smart meat packaging are additionally described. Furthermore, the applicability of biosensors at the commercial level and future possibilities as well as constraints are briefly highlighted.
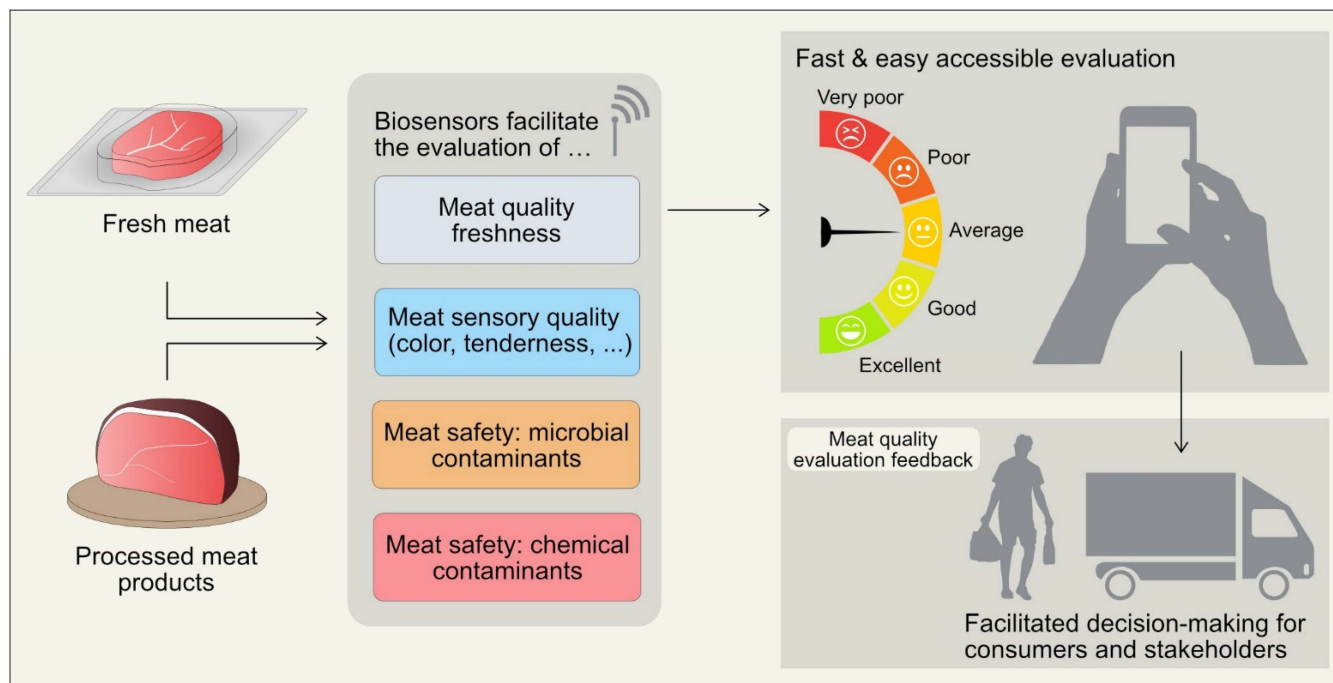

Figure 1. Schematic diagram depicting the use of biosensors to evaluate the quality of meat and meat products with the objective of facilitating decision-making for both consumers and stakeholders.

2. The Potential Use of Biosensors for Muscle Foods

The production of high-quality meat and the supply of safer meat products are now considered essential prerequisites in the meat processing sector. This is due to growing awareness among consumers, requesting quality meat products that are safe and available at an affordable price with a longer shelf-life [9]. Hence, meat producers or processors are putting more emphasis on safe and good manufacturing practices, suitable product packaging and labeling, and uniformity in maintaining the quality control methods to satisfy consumers and regulatory authorities [6]. Although conventional methods for analyzing meat quality are available, such as detection of microbial pathogens, antibiotic and drug residues, adulterants and contaminants, toxins, heavy metals, pesticides, etc., these methods are cost- and time-consuming, and require sophisticated instruments to yield results [5,10]. Hence, meat processors and researchers are in constant search of simple, low-cost, and easy-to-operate analytical techniques or methods that use portable instruments for quantification of various key meat quality components and to efficiently detect contaminants with great accuracy.

In this context, biosensors could play a vital role in meat safety and quality analysis due to their portability, high sensitivity, and specificity. In general, a biosensor is an analytical sensing device that comprises two main elements, like a transducer and a biorecognition element, with supporting components [11]. The main advantages of using biosensors in the food system are that they are inexpensive, easy to operate, and require less time for analysis of samples, which can detect a wide spectrum of food samples, either quantitatively or semi-quantitatively [12]. Hence, when biologically active components (analytes or groups of analytes) such as glucose, lactate, antibodies, drug residues, receptors, bacterial cells, toxins, or enzymes interact with the biorecognition (sensing) element, the attached transducer converts the biological interaction between the sensing element and analyte to meaningful

and measurable electrical signals [13]. The interpretation of the resulting electrical signals is carried out by a signal processor. A schematic diagram depicting the basic principles of a biosensor is presented in Figure 2.
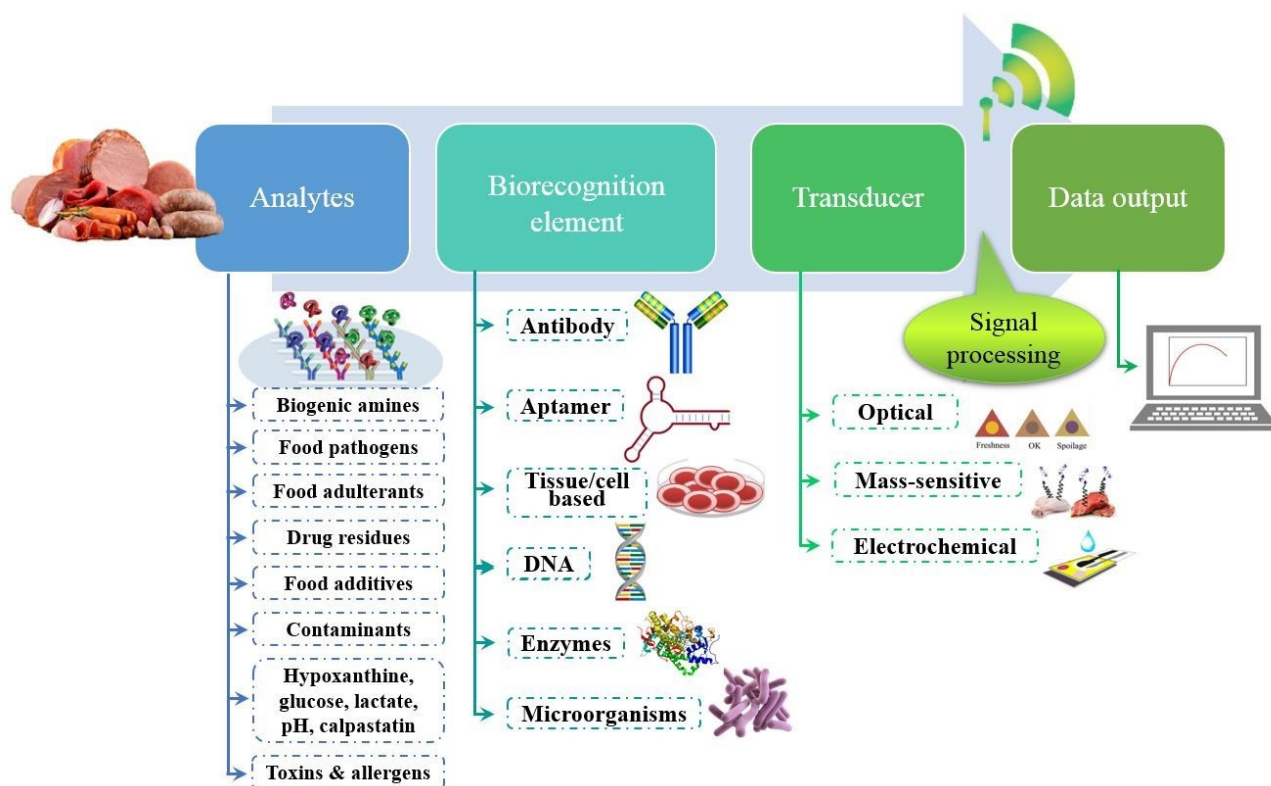

Figure 2. Schematic diagram of the working principle of biosensors for the detection of different analytes from meat and meat products.

The use and choice of any biorecognition element(s) is crucial for real-time analysis of target substrates or biologically active components of interest [14]. As shown in Figure 2, biosensors are classified in diversified ways based on the biorecognition elements (enzyme, aptamer, whole cell, nano, immunosensors, antibody-based), transducers (electrochemical, optical, mass-based/gravimetric) and also based on label-free (surface plasmon resonance-SPR, mass spectrometry, acoustic wave, etc.) and label-based (fluorescence, chemiluminescence, etc.) detection techniques [15]. As this review focuses on the quality and safety of meat and meat products, the advantages and disadvantages of transducer-based biosensors are summarized in Table 1. A good number of research and review articles, elaborately discussing the materials used in biosensors, their stability and stabilization, performance in different environments including validation and calibration, are already available [5,15–19], and hence are not extensively covered in this review.

Table 1. Advantages and disadvantages of transducer-based biosensors.

Biosensor Type	Advantages	Disadvantages
Electrochemical	• Robust, rapid response, cost-effective, saves time • Low detection limits • High sensitivity • Not prone to interferences • Good stability and reproducibility • Wide linear response range • Requires less sample volume	• Susceptible to temperature change • Short shelf life • Sensitive to sample matrix effects • Highly buffered solutions may interfere

Table 1. *Cont.*

Biosensor Type	Advantages	Disadvantages
Optical	• Highly sensitive, specific, and cost effective • Chemically inert • Provide real-time and label-free detection of biological and chemical substances • Allow multiple analytes to be detected • Robust, reliable, small, and lightweight	• Susceptible to interference from environmental effects • Can be costly, fragile • Susceptible to physical damage • High cost of some instrumentation • Requires trained personnel • Fluorescent signal may not be strong enough
Mass-based	• Highly sensitive to minor mass change • Useful for analytes that possess no electrically conducting properties nor the florescent signals • Fast response time • Low cost of fabrication	• Fragile • Mechanically unstable • Size of these equipment large, so unfeasible for field applications

3. Application of Biosensors in Assessing Meat Quality

3.1. Biosensors to Assess Meat Freshness

The fresh appearance of food or its freshness is an important quality attribute that largely influences the purchase decisions of consumers. In the case of meat and meat products, freshness indicates the quality and safety of the product, which is important from both the producers' and consumers' point of view. The most common and popular quantitative and qualitative attributes used to evaluate the freshness of meat and meat products are pH, visual appearance, and meaty aroma [20]. Metabolites (organic acid, biogenic amines, glucose, sulfur, carbon dioxide, etc.) originating from microbial growth and chemical components generated due to oxidative changes (lipid and protein) during storage alter the freshness and quality of muscle foods [21,22]. Microbial metabolites and oxidative chemical compounds react with the indicator, which irreversibly affects the visual indication of the freshness and quality of the product. Therefore, a thorough understanding of the metabolites that can impact specific quality changes of food products over time is an essential prerequisite for the development and customization of indicators [23]. Many laboratory methods, including the measurement of total volatile basic nitrogen (TVB-N), pH, and culture-based bacterial quantification, are available, which are precise and mostly non-destructive but require more time to yield results [24]. Therefore, it is important to find new methods for detection of meat freshness to ensure a supply of quality and safe meat food to consumers [20].

Meat freshness as an indicator of product quality has an inherent relationship with aging as well as spoilage of meat. Therefore, during prolonged preservation, both monitoring of aging and spoilage of meat need to be evaluated. In that sense, the single important marker indicating meat freshness is hypoxanthine (Hx), a degradation product of ATP (adenosine triphosphate) [6].

For meat freshness analysis, various biosensors are used. For example, Albelda et al. [25] fabricated a graphene-based amperometric Hx sensor with titanium dioxide (TiO_2-G) that was used for the estimation of Hx in pork meat stored under room temperature conditions for 7 days. The authors reported a high correlation coefficient (r = 0.9795) between the conventional enzymatic method and the biosensor, with a detection limit of Hx at 9.5 μM. Therefore, TiO_2-G nanocomposite can be used as a suitable electrode component in biosensors for the evaluation of meat freshness. An innovative amperometric biosensor with Fe_3O_4/polyaniline nanoparticles was developed for the estimation of xanthine in fish and chicken meat samples [26]. This biosensor with good reproducibility was able to detect xanthine within 8 s due to its quick response time, linear range ($R^2 = 0.997$) and high sensitivity (13.58 μA μM^{-1} cm^{-2}). The detection limit of the biosensor was 0.1 μM. Similarly, Hernández-Cázares et al. [27] developed and optimized an enzyme-based (amperometric) biosensor coupled with an oxygen electrode for the measurement of pork meat freshness. However, when using high-performance liquid chromatography (HPLC) and the biosensor, these authors found little variation in Hx concentration. In another study, a simple, easy-to-use, low-cost paper-based biosensor having microfluidic properties was

fabricated for Hx estimation in meat samples [28]. The biosensor could detect Hx in meat samples within 5 min, having a detection limit of 1.8 mg/L and showing a good linear range of 5–40 mg/L, thus it can be used as an alternative to conventional methods with high accuracy. In a recent study by Garg and Verma [29], a fiber-optic biosensor was used for the estimation of xanthine in chicken meat. The authors concluded that the developed technique was very fast and reliable, requiring a minute volume of sample for detection of xanthine. Hence is suitable for mass-scale screening of samples.

Omanovic-Miklicanin and Valzacchi [30] developed two innovative chemiluminescence biosensors based on enzyme oxidase (putrescine and diamine) to detect the concentration of biogenic amine in meat products that were compared to samples evaluated by HPLC. The authors reported that the biosensors can quantify putrescine in the range of 1–2 mg/L with a detection limit of 0.8–1.3 mg/L, and thus may be preferred over HPLC analysis due to their simple nature of operation and the short-time detection of analytes. The results of Ag/AgCl and platinum electrode-based amperometric biosensors developed to quantify the biogenic amines and hypoxanthine of beef were comparable with conventional liquid chromatography methods [31]. Similarly, a non-destructive method was developed to detect the TVB-N as a freshness indicator in pork meat by combing two sensing components like hyperspectral imaging (HSI) and colorimetric sensors [32], which had a coefficient value of $R^2 = 0.932$. The authors stated that integrating two sensors along with back propagation adaptive boosting non-linear data fusion algorithms can potentially monitor the quality and safety of pork meat in real-time. Moreover, an inexpensive novel sensor was developed by applying titanium dioxide-polyaniline composites on the surface of silk fibroin fiber to quantify TVB-N in pork samples [33]. This sensor showed a good correlation with the TVB-N concentration in meat samples ($R^2 = 0.990$).

The use of *Bacillus subtilis*, a Gram-positive bacterium, as a biosensor to assess meat spoilage has also been reported. The volatile compounds released from spoiled meat can activate the specially identified promoter to drive fluorescent protein formation in *B. subtilis* [34]. The researchers explained that cell-based biosensors have immense potential on a commercial scale and could be used as a promising tool to evaluate meat quality traits. The various types of biosensors used for the detection of freshness in meat and meat products are summarized in Table 2.

Table 2. Biosensors used for the detection of freshness of meat and meat products.

Analyte	Biorecognition Element	Electrode Used	Immobilization Technique and Detection	Meat and Meat Products	Detection Limit/Sensitivity or Correlation	Refs
Hypoxanthine	Xanthine oxidase	Amperometric hypoxanthine sensor; Graphene/titanium dioxide nanocomposite	Electro catalytic activity	Pork	LOD:9.5 μM, Sensitivity: 4.1 nA/μM	[25]
Glucose, triglycerides, and lactic acid	Glucose, lactic acid, etc., in drip loss	Strip method; Accutrend Plus apparatus	-	Longissimus muscle of pork	Accuracy level 86.54% (Rc = 0.93, $p < 0.01$)	[7]
Xanthine	Xanthine oxidase	Pencil graphite electrode	Immobilizing by glutaralde-hyde	Chicken meat	LOD: 0.074 M Sensitivity: 124 mA m^{-1}	[35]
Calpastatin	Specific antibody for calpastatin	Tendercheck system; portable electrochemical device	Immunoreaction	Beef meat	Correlation ($R^2 = 0.62$) between calpastatin and WBSF values	[36]
Hypoxanthine	Xanthine oxidase	Paper-based colorimetric biosensor	Dienzyme catalytic reaction	Fresh and processed meat samples	LOD: 1.8 mg L^{-1} Quantitative limit: 6.1 mg L^{-1}	[28]
Cadaverine	Cyclam (1,4,8,11-tetraazacyclotetradecane)	Titanium adhesion layer (3 nm); silicon nitride cantilevers containing piezoelectric layers	Silicon; gold	Beef, chicken, or pork	-	[37]

Table 2. *Cont.*

Analyte	Biorecognition Element	Electrode Used	Immobilization Technique and Detection	Meat and Meat Products	Detection Limit/Sensitivity or Correlation	Refs
Xanthine	Guanine deaminase; Xanthine oxidase	Circular plastic discs as biosensor; Fiber optic probe (Ocean Optics)	Enzyme immobilization by hydro sol-gel method; Co-immobilization of xanthine oxidase and adsorptive dye phenol red	Chicken meat	LOD: 0.5 μM Linear range: 0.5 μM–150 μM	[29]
IMP	5′-nucleotidase and xanthine oxidase	Three-electrode system including a modified GCE electrode, an Ag/AgCl electrode, a platinum wire as an auxiliary electrode. A multilayer film of GCE/Ti3C2TX-Au@Pt nanoflowers/5′-nucle-otidase-xanthine oxidase/BSA	GCE/Ti3C2TX-Au@Pt nanoflowers	Chicken, pork, beef, lamb	LOD: 2.73 ng mL^{-1} Linear range: 0.04~17 g L^{-1} Correlation coefficient: 0.9964	[38]
Calpastatin	Muscle sample, monoclonal anti-calpastatin antibody	The sensor chip	Carboxymethylated dextran layer with N-hydroxysuc cinimidemediated by N-ethyl-N-(3-diethylaminopropyl) carbodiimide	Beef meat	Correlation coefficients: 0.51–0.99 Mean inter-assay CV: 5.8%	[39]
Volatiles in spoiled meat	Bacteria specific genetic material; BioBrick compatible integration plasmid	Promoter PsboA	Transcriptome analysis	Pork/cow minced meat (Ratio: 70%/30%)	-	[34]
Calpastatin	Anti calpastatin antibody	Capillary tube optic biosensor	Silanization covalent immobilization	*Longissimus* muscle from beef	Calpastatin activity ($R^2 = 0.6058$)	[40]
Xanthine	Xanthine oxidase	Hybrid nanocomposite film; Carbon paste electrode	Covalent immobilization by glutaraldehyde	Chicken meat	LOD: 0.1 μM Linear working concentration: 0.2–36.0 μM ($R^2 = 0.997$)	[26]
Putrescine	4-aminobutyraldehyde with putrescine oxidase or diamine oxidase as catalysts	Luminometer microplates with different enzymes	Self-adhesive reinforcement ring	Beef, pork, chicken, turkey meat samples	LOD: 0.8 mg/L–1.3 mg/L Linearity range: 1–2 mg/L	[30]
Glucose	Glucose oxidase	Glassy carbon electrode modified with multi-walled carbon nanotubes and chitosan	Cross-linking with enzyme through glutaraldehyde with BSA	Beef meat	LOD: 0.05 mM Linear range: 0.2–1.2 mmol L^{-1} linearity ($R^2 = 0.9902$)	[41]
TVB-N	Pork meat (derivatives of biogenic total volatile basic nitrogen)	Hyperspectral imaging and colorimetric sensors	Adaptive boosting algorithm for data fusion and modeling	Pork meat	Correlation coefficient ($R^2 = 0.932$)	[32]

BSA: Bovine serum albumin; GA: Glutaraldehyde; Hx: Hypoxanthine; IMP—Inosine monophosphate; LOD: Limit of detection; TVB-N; Total volatile basic nitrogen; WBSF: Warner-Bratzler Shear Force.

3.2. Biosensors to Evaluate Meat Tenderness

Appearance/color, taste, tenderness, juiciness, among other quality attributes, are the most important quality traits of meat [42,43]. Amongst these, tenderness is one of the major quality attributes and an important quality trait for consumer satisfaction and repurchasing meat [6,44,45]. The tenderization of meat largely depends on the degradation of cytoskeletal proteins responsible for the structural integrity of muscle fibers and many other interconnected pathways, as evidenced recently by Gagaoua et al. [46] using an integromics proteomics approach. The post-mortem muscle changes and variations in tenderness are driven by five mechanisms that are: (a) the degradation of muscle myofibrils by endogenous proteolytic systems during ageing, (b) collagen and cross-linking, (c) sarcomere length and its status during the post-mortem period, (d) intramuscular fat content

known as marbling, and (e) the denaturation of muscle proteins during cooking [47]. The activation of the endogenous proteolytic systems (proteases and their inhibitors) is the first key among these mechanisms [48]. Among them, different research studies have proven that calpain proteases are responsible for the changes in muscle/meat that occur during ageing or post-slaughter tenderization. Calpains, cathepsins, caspases, and proteasomes are endogenous proteolytic systems, among many others, that can degrade several myofibrillar proteins, ultimately driving the final tenderness of meat [49,50]. In the skeletal muscles, the calpain system mainly consists of three proteoforms, from which μ-calpain and m-calpain and their specific endogenous inhibitor, calpastatin [51,52], have been extensively studied. Other associated factors like post-mortem glycolysis and pH, which additionally affect meat tenderness, may vary because of different dietary systems, use of growth promoters, and finally due to the stress of animals during transport and slaughter [53–55].

Different conventional techniques like sensory taste panels, Warner-Bratzler Shear Force (WBSF) as an instrumental method, ELISA, proteomics, or chromatography are available to evaluate meat tenderness, but these methods are time-consuming, expensive, and difficult to use on a broad scale [43,56]. Amongst these, the WBSF method has been proven to be more accurate in tenderness evaluation compared to other methods [57]. These techniques evaluate the resistance of the meat during cutting without providing a direct determination of meat tenderness [36,58]. In such a scenario, biosensors may play an immense role and act as a measurement tool that converts biochemical information into an analytical signal by using biological ligands as part of a biotransducer.

Geesink et al. [39] developed an immunological biosensor using the SRP system (Biacore Q) for the detection of calpastatin activity in beef meat. The biosensor showed a linear correlation with a conventional enzymatic assay and the correlation coefficient was in the range of r = 0.51–0.99 in several experiments. Based on their findings, Geesink et al. concluded that the Biacore Q is an effective online screening tool to measure calpastatin activity, and thus, may be used as a predictor of meat tenderness for high-throughput applications in the commercial sector as well as in research. Bratcher et al. [40] used a fluorescence resonance energy transfer (FRET) method to estimate calpastatin in an optical biosensor device in stored meat. They found that the 48 h post-mortem would be the most accurate time for grading and classification of meat using the FRET optical biosensor. Zór et al. [36] developed a multi-channel portable electrochemical immunosensing device known as the Tendercheck system, based on antibody-antigen biorecognition and amperometric detection. The device accurately quantified calpastatin. Furthermore, results from this biosensor showed a similar correlation (R^2 = 0.62) compared to the WBSF method. More studies are required to develop user-friendly biosensing methods for rapid screening of meat tenderness that will help the meat industry in providing high quality meat products to consumers. The types of biosensors used for the detection of tenderness in meat and meat products are summarized in Table 2.

3.3. Biosensors to Detect Microbial Contaminants in Meat

Contamination of food has become a public health concern, seeking worldwide attention as human consumption of such products may cause food poisoning and disease outbreaks. These foodborne outbreaks cause irreparable damage to human health as well as to the world economy and environment. Microbial contamination in any food product may occur via raw materials or by cross contamination at any point of the food processing system [59]. A continuous microbiological environmental surveillance system is therefore necessary for the early detection of pathogenic organisms in the food chain to assure microbiological safety [60]. For detection of microbial contamination, apart from traditional biochemical and microbiological methods, which include bacterial colony counts, staining, and methylene blue reduction tests; various conventional techniques such as ELISA, PCR, and fluorescence detection are also available [11]. However, most of the most advanced analytical methods require well-equipped laboratories, sophisticated and expensive instruments, and skilled technicians. Furthermore, most of these methods necessitate lengthy

sample preparation and processing steps that involve various enrichment and incubation stages, and can take up to 10 days to produce results [61–63]. These limitations have created a demand for novel in situ analysis methods that are more sensitive, accurate, fast, and specific than existing methods [7,14,64]. Recently, analytical tools like biosensors with high specificity and sensitivity have become available, which can detect microbiological safe limits, toxins, or their metabolites in different products.

In fact, biosensors are simple, cost-effective, easy-to-handle devices that can rapidly detect pathogens but do not need pre-enrichment methods, unlike nucleic-acid based and immunological methods [65]. Nowadays, various user-friendly biosensors based on optical, electrochemical, photoelectrochemical, and bioluminescence are available [11]. For instance, optical biosensors facilitate real-time monitoring of microbial activities in the food matrix. These biosensors discriminate against microbes in foods either by changing the level of signal or refractive index, or by detecting the concentration of the microbial cells attached to the biorecognition sensing site on the optical transducer surface [17]. Out of different optical analyzing techniques like colorimetric, fluorescence, localized SPR, and chemiluminescence; SPR is widely used as an optical biosensor [66]. In SPR, bioreceptors are immobilized on a metal surface transducer, where electromagnetic radiation of a specific wavelength interacts with the electron cloud of the transducer and thereby generates a strong resonance. When the bacterial cells (target analyte) interact with the metal surface, an observable alteration in the refractive index can be noticed [61]. Generally, it allows reflectance spectroscopy for target pathogen detection.

There are many reports available on the use of optical biosensors that can detect pathogenic organisms in various meat and meat products. In a study, *L. monocytogenes* in meat was detected up to 3×10^2 CFU/mL by a specially designed fiber optic immunosensor, equipped with a powerful immunomagnetic separation [52]. An aptamer-based fiber-optic biosensor was employed to detect pathogenic *L. monocytogenes* from other non-pathogenic or pathogenic species in artificially contaminated ready-to-eat (RTE) meat products [53]. Oh et al. [54] identified *S. Typhimurium* from pork meat up to 4 log CFU/mL within 30 min using localized SPR. Zhang et al. [55] developed a multi-channel SPR biosensor for specific detection of three different foodborne pathogens, namely *E. coli* O157:H7, *S. Enteritidis*, and *L. monocytogenes*, together in naturally contaminated food. In another study, bacteria of the species *Shigella sonnei* were detected and isolated apart from other enteric organisms such as *E. coli* and *S. Typhimurium*, using fluorescent biosensors with basic aptamers [56]. By combining the lateral flow biosensors with multiple cross displacement amplification, Wang et al. [57] reported high specificity and sensitivity of the sensor that could detect *Shigella* spp. within one hour. However, high costs, quality assurance, stability disputes, sensitivity issues, and instrumentation design are current limitations which need to be addressed prior to the commercialization and wider applications of these optical biosensors.

Based upon the antigen-bioreceptor interactions, different kinds of electrochemical biosensors like amperometric, impedimetric, potentiometric, and conductometric are available [67]. An electrochemical immunosensor prepared with chitosan/gold nanoparticles composite has been reported to provide a wide range of detection limits from 1–5 Log CFU/mL that may be helpful for proper detection of *Salmonella* contamination [68]. Similarly, Morant-Miñana and Elizalde [69] isolated *Campylobacter* spp. from chicken meat using an electrochemical genosensor prepared with thin-film gold electrodes. Che et al. [70] isolated *C. jejuni* from turkey and chicken meat samples using a fluorescence biosensor with a detection limit of 2.1×10^4 CFU/mL. The rapid detection of *C. jejuni* is important, as it is considered as a major food-borne pathogen, causing diarrhea and fever in consumers.

Currently, nano-based sensors are outstanding in detecting different food-borne pathogens and toxins [61]. Using carbon nanotube-based biosensors, Yamada et al. [62] was able to detect *E. coli* within 5 min with a detection limit of 2 log CFU/mL. In another study, Muñoz-Berbel et al. [63] could detect *E. coli* with a detection limit of between 1 and 7 log CFU/mL by the use of impedimetric spectroscopy.

A quartz crystal microbalance (QCM) biosensor is generally characterized by the resonant frequency of quartz crystals with a higher sensitivity for qualification and quantification of microbial whole cells at extremely low levels. By using this QCM technique, *C. jejuni* (LOD: 1.30 log CFU/mL) and *S. Typhimurium* (LOD: <100 CFU/mL) were detected in chicken meat samples [71–73].

Liu et al. [74] developed an impedance based microfluidic biosensor for the detection of *Salmonella* serotypes B and D in a RTE turkey matrix. The study reported the detection of a very low concentration of *Salmonella* (300 cells/mL) within one hour. The selectivity of the sensor was also tested using non-specific binding of different *E. coli* strains. Besides, high concentrations of inactivated *Salmonella* and very low levels of alive *Salmonella* cells can additionally be differentiated by this sensor. Liang et al. [75] prepared a smartphone-based biosensor for preliminary screening of microbial contamination in food matrices. The researchers applied this system, coupled with a digital camera and a gyro sensor of a smartphone, to detect microbial pathogens in ground beef. *E. coli* K-12 contaminated beef was identified by simply taking a picture with a smartphone, which was subsequently analyzed by an implemented procedure. Mie scatter assays were applied at different angles (15°, 30°, 45°, and 60°) out of which the lower limits were detected (1 log CFU/mL) at a 45°angle, and 2 log CFU/mL at angles of 30° and 60°. This biosensor, integrated with a smartphone, is easy to handle, rapid, cost-effective, and does not need antibodies, microbeads, or any other reagents.

Bioluminescence-based ATP detection is another approach to microbial spoilage detection [11,76]. It is well known that ATP is present in all living microbial cells (bacteria, mold, yeast, and algae) as an activated energy carrier. For the bioluminescence-based ATP detection, ATP is converted to adenosine monophosphate (AMP) with the emission of light. The intensity of light as the result of the breakdown of ATP in a bioluminescence reaction can be quantified using very sensitive photons from light meters placed in an instrument called a luminometer. The more ATP is present, the higher the intensity of light in terms of relative light units will be obtained from the reaction. The ATP bioluminescence assay is a very useful tool, as it takes very little time to give successful results [77]. In a study by Siragusa et al. [78], a rapid ATP assay was developed to measure total bacterial counts in samples obtained from beef and pork carcasses. The results were compared to the conventional bacterial plate count methods, which allowed a positive correlation coefficient of 0.91 for beef and 0.93 for pork carcasses. Cheng et al. [79] combined an ATP bioluminescence assay with functional and suitable magnetic nanoparticles for rapid estimation of *E. coli* from ground beef, which was artificially contaminated. A detection limit of 1.30 log CFU/mL was observed. However, one weakness of this detection method is that ATP is present in all living microorganisms, including meat. Therefore, ATP should be destroyed in meat prior to performing the bioluminescence assay.

In a recent study by Vizzini et al. [80], a paper-based DNA biosensor was used to detect *Campylobacter* spp. in chicken meat. The authors reported that the developed biosensor was very cost-effective, portable, and the level of detection (3 pg/μL of DNA) was comparable to available qPCR kits. The types of biosensors used for the detection of various microorganisms in meat and meat products are summarized in Table 3.

Table 3. Biosensors for the detection of various microbes and toxins in meat and meat products.

Analyte	Biorecognition Element	Electrode Used	Immobilization Technique and Detection	Meat and Meat Products	Limit of Detection/Sensitivity or Correlation	Refs.
Bacteria (*Campylobacter* spp.)	Genomic *Campylobacter* DNA	Paper membrane	Biotinylated silica-nanoparticles	Chicken meat	LOD: 3 pg/μL of DNA	[80]
Salmonella enterica, Listeria monocytogenes, and *Escherichia coli* O157:H7	Alexa Fluor 647-labeled monoclonal antibodies	Streptavidin coated optical waveguides	Biotinylated polyclonal antibodies	Ready-to-eat beef, chicken and turkey breast meat	LOD: 10^3 CFU/mL	[81]

Table 3. *Cont.*

Analyte	Biorecognition Element	Electrode Used	Immobilization Technique and Detection	Meat and Meat Products	Limit of Detection/Sensitivity or Correlation	Refs.
Salmonella Typhimurium and *Staphyloccocus aureus*	Aptamer	The Raman signal probe and the capture probe; gold nanoparticles modified with Raman molecules (Mercaptobenzoic acid and 5,5′-Dithiobis (2-nitrobenzoic acid)	Fe_3O_4 magnetic gold nanoparticles	Pork paste	LOD: 35 CFU/mL for *S. aureus* and 15 CFU/mL for *S.* Typhimurium Recovery rate: 94.12%–108.33%	[82]
L. monocytogenes	Aptamer, a single-stranded oligonucleotide ligand	Fiber-optic sensor	Streptavidin-coated optical waveguide surface; Alexa Fluor 647-conju-gated A8	Ready-to-eat such as sliced beef, chicken, and turkey	LOD: 10^2 CFU 25 g−1	[83]
Whole cell of *S. enterica* serovar Typhimurium	DNA bases of aptamer	Reduced graphene oxide -azophloxine nanocomposite aptasensor	Azophloxine	Chicken meat	LOD: 1 CFU/mL Linear range (detection): 1–8 log CFU/mL	[84]
S. enterica serovar Typhimurium	Phage peptide	Magnetoelastic biosensor	Gold-coated sensor layer	Chicken fillets (boneless and skinless)	LOD: 7.86×10^3 CFU/mm² Analysis time: 2–10 min	[85]
Salmonella pullorum	Anti-*Salmonella* polyclonal antibodies	Screen printed electrode modified with multi-wall carbon nanotubes-chitosan-peroxidase was connected to a portable self-made amperometric sensor	Cellulose nitrate membrane	Chicken sample	LOD: 100 CFU/mL Detection time: 1.5 to 2 h	[86]
Staphylococcal enterotoxin B	Antibody	Electro-optical biosensor	Carboxymethyl-dextran	Potted meat (Hormel)	Sensitivity: 1–10 ng/mL	[87]
E coli K-12	Anti-*E.coli* antibody	Electrochemical impedance spectroscopy and SPR imaging techniques	Gold surface	Frozen chicken meat	LOD: 3 log CFU/mL	[88]
Trichothecene T-2 toxin	Anti-T-2 (toxin) and T-2-bovine serum albumen	Electrochemical immunosensor (GCE)	Gold nanoparti-cles/carboxylic group-functionalized single-walled carbon nanotubes/chitosan composite	Swine meat	LOD: 0.14 µg/L Recovery: 91.42%–100.80%	[89]
Microbial contamination (Meat spoilage)	-	Gyro sensor and the digital camera of a smartphone	-	Ground beef	LOD: 1 log CFU/mL at 45° and 2 log CFU/mL at 30° and 60°	[75]
Salmonella serotypes (B, and D)	Aptamer ssDNA	GCE	Graphene oxide and gold nanoparticles	Ready-to-eat turkey samples	LOD: 300 cells/mL Detection time: <1 h	[74]
Salmonella	Aptamer ssDNA	GCE	Graphene oxide and gold nanoparticles	Pork samples	LOD: 3 CFU/mL	[90]
S. Typhimurium	Aptamer	Plasmonic sensor	Gold nanoparticles	Pork meat sample	LOD: 4 logCFU/mL	[91]
S. Typhimurium	Antibodies	Quartz-crystal microbalance	Functionalized nanoparticles	Chicken meat samples	LOD: 1 CFU/mL	[73]
Salmonella gallinarum and *S. pullorum*	Antibodies	Screen-printed carbon electrode	Gold nanoparticles	Chicken meat	LOD: 3 log CFU/mL	[92]
E. coli O157:H7	Antibody	Integration of bifunctional glucose oxidase–polydopamine based polymeric nanocomposites and Prussian blue modified screen-printed interdigitated microelectrodes	Gold nanoparticles	Ground beef	LOD: 2.05×10^3 CFU/g	[93]
Campylobacter jejuni	Antibodies	Optical biosensor based on SPR	Immunoaffinity	Broiler samples	Good sensitivity: 10^3 CFU/mL against *C. jejuni*	[94]
Salmonella spp.	*Salmonella* spp. monoclonal antibodies	GCE	Grafted ethylene diamine and self-assembled gold nanoparticle monolayer	Pork meat	LOD: 2 log CFU/mL Dection time: 40 min	[95]

Table 3. *Cont.*

Analyte	Biorecognition Element	Electrode Used	Immobilization Technique and Detection	Meat and Meat Products	Limit of Detection/Sensitivity or Correlation	Refs.
L. monocytogenes and *Bacillus cereus*	B-Lymphocyte Ped-2E9 cell-line	Cell based biosensor	Collagen matrix	Ready-to-eat hotdog and salami	LOD for pathogens: 3 log LOD for toxins: 10–40 ng	[96]
E. coli O157:H7	Complementary DNA	Gold electrode surface	Multiwalled carbon nanotubes	Fresh beef	LOD: 1.97×10^{-14} M correlation coefficient: 0.989	[97]
Listeria	Protease	Gold sensor surface	D-amino acid substrate	Meat	LOD: 2 log CFU/g	[98]
S. Typhimurium	Thiolated *S. Typhimurium* aptamer; biotinylated aptamer	Surface-enhanced Raman scattering nano probes	Spiny gold nanoparticles	Pork samples	LOD: 4 CFU/mL	[99]

LOD: Limit of detection; RTE: Ready-to-eat; SPR: Surface plasmon resonance.

Although much progress has been made in the biosensing technologies for the detection of microorganisms in contaminated food to date, more research in this field is needed to address the requirements of industry with more simplified, easy-to-handle, and cost-effective methods. Overall, high sensitivity with good specificity and modern nanotechnology-based biosensors can be an alternative for food-borne pathogen detection.

3.4. Biosensors to Detect Contaminants, Antibiotics, and Drug Residues in Meat and Meat Products

Various contaminants such as toxins, pesticides, antibiotics, veterinary drug residues, and hazardous food additives can enter the food chain system at any processing step and contaminate the whole batch. Many analytical techniques like HPLC, capillary electrophoresis, and mass spectrometry are available for analyzing the samples at the end of the processing steps. However, these procedures are costly, complex, require sophisticated instruments, and the intervention of skilled personnel [100]. Therefore, there is a growing interest in the rapid, reliable, and more sensitive detection of contaminants in the processing steps itself, through the involvement of biosensor technology, which can provide a real-time screening and monitoring of food contaminants, minimizing the unwanted threat to consumers' health. The types of biosensors used for the detection of various antibiotics and drug residues, adulterants, allergens, and additives in meat and meat products are summarized in Table 4.

Table 4. Biosensors for the detection of various adulterants, antibiotics and drug residues, and additives in meat and meat products.

Analyte	Biorecognition Element	Electrode Used	Immodilization Techniques and Detection	Meat and Meat Products	Detection Limit/Sensitivity or Correlation	Refs.
DNA (Donkey meat)	DNA	Multi-parameter SPR device with gold chips	A boiling solution of NH3 (30%), H_2O_2 (30%), and Milli-Q water in ratio 1:1:5 for 10 min at 95 °C, drying by N_2 stream; incubated in thiolated capture probe	Beef sausages	LOD: 1.0 nM	[101]
DNA (Pork meat)	DNA probe with gold nanoparticles bioconjugate	Gold-modified screen-printed carbon electrode	MPA (3 -me rcaptop ropionic acid), EDC (1-ethyl-3-(3-dimethylamino propyl) carbodiimide), and NHS (N-Hydroxysuccinimide)	Food products	LOD: 0.58 µg/mL Recovery rate: 101.74%	[102]

Table 4. *Cont.*

Analyte	Biorecognition Element	Electrode Used	Immodilization Techniques and Detection	Meat and Meat Products	Detection Limit/Sensitivity or Correlation	Refs.
DNA (Horse meat)	Antibody specific to RNA/DNA duplexes and a bacterial protein conjugated with a horseradish peroxidase homopolymer	Disposable screen-printed carbon electrodes	Immobilization at magnetic microcarriers	Beef containing horse meat	Can detect beef meat adulterated with even 0.5% (w/w) of horse meat within 1 h	[103]
Nitrate (Contaminants)	Nitrate reductase	Ag/AgCl reference electrode, platinum auxiliary electrode, GCE	Bloom gelatin	Meat samples	LOD: 2.2×10^{-9} M LOQ: $5.0–90.0 \times 10^{-9}$ M response time: 10 s	[104]
Porcine serum albumin	Anti-T-2 (toxin) and T-2-bovine serum albumen	GCE	Gold nanoparticles/carboxylic group-functionalized single-walled carbon nanotubes/chitosan composite	Pork and its products	LOD: 19.81 ng/mL Linear range: 1.0–450 ng/mL	[105]
Dopamine Adulterant	Anti-dopamine substance	Colorimetric sensor	CuS-BSA-Cu$_3$(PO$_4$)$_2$ nanoparticles-copper sulfide encapsulated within bovine serum albumin functionalized with copper phosphate	Beef meat	LOD: 0.13 mM Linear range: 0.05–100 mM	[106]
DNA	Enzymes	Optical thin-film biosensor chip	Silicon	Meat from deer, rabbit, duck, beef, horse, sheep, and pork	LOD: 0.5 pg	[107]
Tetracyclines	Lyophilized reconstituted sensor cells	Cell-biosensor	Solution based	Poultry muscle samples	Sensitivity: 10 µg/kg	[108]
Chloramphenicol (CAP)	CAP derivative and antibody	Immunochemical screening assays using SPR	Regeneration solution	Poultry muscle	Detection capabilities: 0.02 µg/kg	[109]
CAP and tetracycline (TET)	ss-DNA fragment coordinately controlling gold nanoparticles aggregation	Colorimetric aptasensor	Gold nanoparticles	Chicken	LOD: 32.9 nM (TET) and 7.0 nM (CAP) Linear range: 0.05–3.0 µM	[110]
Sulfadiazine (SDZ) and acetaminophen (AP)	Molecularly imprinted polymer	Electrochemical sensor; GCE	A graphene oxide@covalent organic framework nanocomposite for signal amplification.	Pork and chicken samples	LOD: 0.160 µM (SDZ) and 0.032 µM (AP)	[111]
Kanamycin	Antibody of kanamycin	Thionine mixed graphene sheet	Silver hybridized mesoporous ferro-ferric oxide nanoparticles	Pork meat sample	LOD: 15 pg/mL, Linear range: 0.05–16 ng/mL Recovery: 96.7–102%	[112]
Chloramphenicol (CAP)	Monoclonal antibody to CAP (anti-CAP)	Electrochemical immunosensor; Electrochemical impedance spectroscopy technique	Entrapped into gold nanospheres/chitosan composite modified on a GCE	Beef and pork meat samples	LOD: 0.06 ng/mL Linear range: 0.1–1000 ng/mL	[113]
Kanamycin	Anti-kanamycin antibody	Amperometric immunosensor based on graphene sheet - nafion/thionine/platinum nanoparticles	Electrostatic adsorption	Chicken liver	LOD: (5.74 pg/mL) Linear range: 0.01–12.0 ng/mL	[114]
Quinoxaline-2-carboxylic acid	Molecularly imprinted polymer	Modified GCE and differential pulse voltammetry	Multi-walled carbon nanotubes-chitosan functional composite	Pork products	LOD: 4.4×10^{-7} mol/L Linear range: $2.0 \times 10^{-6}–1.0 \times 10^{-3}$ mol/L	[115]
Ractopamine	Ractopamine derivative	SPR biosensor inhibition immunoassay	SPR-2004 biosensor chip	Pork	LOD: 0.6 µg/kg pork sample	[116]
Benzimidazoles	Molecularly imprinted polymer	Chemiluminescence sensor on 96 -well microplate	Horseradish peroxidase-labeled hapten as binding agent	Beef and mutton samples	LOD: 1.5–21 pg/mL Rate of recovery: 65.8%–91.2%	[117]

BSA: Bovine serum albumin; GCE: Glassy carbon electrode; LOD: Limit of detection; SPR: Surface plasmon resonance.

According to Erofeeva et al. [118], almost half of all produced antibiotics worldwide are used in the livestock sector. In fact, a wide range of broad-spectrum antibiotics are used in the livestock sector that are effective against both Gram-positive and Gram-negative bacteria. Furthermore, the combined use of veterinary antibiotics for metaphylactic and prophylactic purposes, or as in-feed supplements for growth promotion, outweighs their therapeutic usage by far [119]. The extensive and non-therapeutic use of antimicrobials in animals, either in therapeutic or sub-therapeutic doses, not only aids the development and spread of antimicrobial resistance, but may also result in the accumulation of residual amounts of antimicrobials or their metabolites in food producing animals, which may then be consumed by humans via meat, milk, and eggs [120]. The presence of antimicrobial residues in animal-derived food products beyond maximum permissible limits has become an intensely debated topic and has received much attention in recent years. The growing concern is due to their adverse public health effects, causing hypersensitivity reactions, antimicrobial resistance, disruption of the intestinal flora, and even neurological disorders [121]. It is therefore crucial to monitor the presence of high concentrations of antimicrobial residues in meat products to guarantee food safety [122,123]. In this context, SPR is a widely accepted technique in biosensors to detect such drug residues. By using the SPR technique, chloramphenicol and sulphonamides have been quantified in different meat species such as pork, beef, and chicken [124–126]. Cai et al. [117] developed a chemiluminescence based-sensor to check the presence of benzimidazole residues in beef and mutton. This sensor acquires ultrahigh sensitivity (range of detection: 1.5–21 pg/mL) and can detect residues in a short time (18 min).

Mohammad-Razdari et al. [127] designed an aptamer-based electrochemical biosensor to detect sulfadimethoxine (SDM) in beef and chicken meat. The sensor offered adequate sensitivity and better stability with a LOD of 3.7×10^{-16} M. Moreover, the percentage recovery of SDM was comparable with the results of the HPLC analysis. In a study conducted by Stevenson et al. [128], an affinity-based electrochemical biosensor was used to detect ceftiofur residues in turkey meat samples. The biosensor was cost-effective and rapidly detected the antibiotics within 15 min, even at low concentrations (10 ng/mL). A luminescent bacterial biosensor designed by Pikkemaat et al. [108] was able to screen a large number of poultry muscle samples for tetracycline (TET) within three hours, for which approximately 12 h were needed using conventional microbiological assays. Additionally, the sensor was cost-effective and highly sensitive. Comparing the performance of the SPR biosensor with that of LC-MS/MS and GC-MS/MS to detect chloramphenicol (CAP) and its glucuronide residue in poultry muscle, Ferguson et al. [109] showed that the performance of the biosensor was better with detection capabilities of 0.02 μg/kg. In another study, a novel colorimetric aptasensor used for the detection of multiplex antibiotics, namely CAP and TET, was able to detect these antibiotics in chicken meat with a LOD of 7.0 nM and 32.9 nM, respectively [110]. This method is very simple and does not require high-end instruments, so it could be used for on-site screening of samples. The detection of sulfadiazine and acetaminophen in pork and chicken samples has been examined by Sun et al. [111]. This study used an electrochemical sensor that exhibited great accuracy and stability for concurrent determination of both antibiotics, with results comparable to those obtained through HPLC analysis. By using biosensing technology, aminoglycosides, lincosamides, quinolones, and tetracyclines have also been detected in meat samples from different animal species [6,13,112,114].

For identifying bacterial or fungal toxins present in meat or meat products, electrochemical biosensors are beneficial. These contaminants may occur at any stage of the food processing chain, or even during transportation and storage. Toxins are not only harmful from a public health point of view but are also associated with severe economic losses. Several reports/findings are available in this regard. For instance, Staphylococcal enterotoxin B was identified using an electrochemical biosensor in pork and milk [129] and trichothecene (T-2 toxin) in swine meat [89]. In another study, an SPR biosensor was used as a tool for the rapid and real-time analysis of Staphylococcal enterotoxin B in potted meat [87].

Moreover, food additives are widely used components in the food industry to maintain and improve physicochemical, sensory and rheological properties, and to extend the shelf-life of products. Commonly used food additives are nitrite, benzoic acid, monosodium glutamate (MSG), propyl gallate, and food colorants, which are hazardous and undesirable if used beyond the maximum permissible limits. Batra et al. [96] developed an amperometric biosensor and enzyme biosensor (glutamate dehydrogenase and glutamate oxidase) to detect the presence of excessive amounts of MSG in food, which can pose a significant health risk to consumers. Wang et al. [97] discovered a biosensor for the determination of food colorants, especially Amaranth (E123) and Ponceau 4R (E124) in processed food products, based on the use of carbon nanotubes and a polypyrrole (ppy-) composite modified electrode. Another amperometric biosensor was made by Shan et al. [98] to screen for the presence of benzoic acids in processed foods using mushroom tissue, tyrosinase, and polyphenol oxidase as biological recognition compounds. By employing the amperometric biosensor, Dinçkaya et al. [99] estimated the nitrate levels in meat and demonstrated that this method is simple and inexpensive with high accuracy and sensitivity. Moreover, this method could estimate nitrate with an LOD of 2.2×10^{-9} M and a response time of 10 seconds. According to the aforementioned reports the meat processing industry should focus on using biosensing technologies, which are simple but rapid in response with high sensitivity, to reduce hazards or contaminations in the meat processing chain, to produce safe and quality meat and to gain consumer trust and confidence.

Adulteration of meat is mostly done by the fraudulent addition of cheaper/low quality meat species or non-meat ingredients or by mislabeling actual constituents of commercial products for economic gain. The authenticity of meat and meat products is required to safeguard religious sentiments, comply with the norms of regulation authorities and, above all, to protect the interests of consumers [105,130]. Analytical techniques based on protein and DNA analysis to discriminate meat from different species are not only expensive but also time-consuming and require skilled personnel.

Several reports indicate that the use of SPR and electrochemical biosensors allows discrimination against adulterated meat samples or processed meat products. For example, SPR-based DNA biosensors were employed to identify donkey meat samples in beef sausages. The sensor was specific and had a high sensitivity with a detection LOD of 1.0 nM [101]. Likewise, in another study, an electrochemical DNA biosensor detected pork in food products when added up to 10% in biological samples [102]. Biosensors are also capable of differentiating adulteration of meat from closely related animal species. Using an amperometric PCR-free electrochemical biosensor, beef meat that was adulterated with horse meat (0.5 % w/w) could be obtained within one hour without the involvement of any extraction or amplification of genetic material [103].

Food allergies caused by different allergens are regarded as an emerging public health problem. For example, porcine serum albumin (PSA) is a major allergen in pork and its products, which can cause allergic reactions. Although conventional methods like PCR, ELISA, and mass-spectroscopy are employed for the analysis of food allergens, SPR can rapidly and accurately detect PSA with an LOD of 19.81 ng/mL, similar to the ELISA-based method [105].

4. Sensors and Indicators for Smart Packaging of Meat and Meat Products

Currently, there is no standard and reliable method available to satisfactorily confirm the freshness of meat, except for sensory analysis, chemical, and microbiological tests. Consumers have no other option but to check the 'use by' or 'sell by' date on printed packaging material [131]. Conventional food packaging systems with expiration dates can protect the food from external environments and delay the spoilage of the product, for a stipulated period. The ever-increasing incidences of meat-borne outbreaks is an indication that expiration dates displayed on packaging material alone are not sufficient in protecting consumers from the threat of spoiled meat and meat products.

To overcome this problem, research on the integration of intelligent materials with traditional packaging systems is gaining interest. This kind of smart packaging system

with different indicators or sensors can sense, track, detect, and record the external or internal environment of products and turns out to be a promising tool for the freshness detection of food products [132–135]. As far as meat and meat products are concerned, intelligent packaging systems can monitor the quality in real-time throughout the supply chain and detect any quality deterioration with the help of chromogenic substances. Several indicators or sensor-based packaging systems, including freshness indicators, gas sensors, time-temperature indicators, pH indicators, barcodes, and RFID tags, are available for the assessment of meat freshness using changing color patterns of the indicator. Out of these, freshness indicators based on pH sensitivity have gained popularity due to their simple applicability and authentic results [136]. In fact, during microbial spoilage, acidic or alkaline metabolites such as NH_3, CO_2, H_2S, TVB-N, including dimethyl amine and trimethyl amine, are released after the decomposition of nutritive components of meat. When these metabolites come into contact with intelligent chromogenic material in the packaged headspace, a chromatic change occurs due to their pH sensitivity. Thus, the chromogenic indicator reacts with microbial metabolites and exhibits color changes as a result of changes in pH [132,134].

Various chemical, and natural chromogenic substances are currently used as freshness indicators of food products by various studies because of their rapid response, color-changing ability, and good stability. Commonly used chemical indicators are methyl, phenol, and bromophenol red, bromophenol blue, bromocresol violet, and bromocresol green. These can be used as non-contact indicators to avoid direct contact between chemical reagents and food, considering the problem of migration of chemical reagents [134]. In a study by Shukla et al. [137], a colorimetric indicator sensor was used with bromophenol blue to examine the quality deterioration in buffalo meat cuts stored for 9 days in a refrigerated condition. The study reported a correlated color response of the indicator sensor from yellow to blue at different levels of spoilage based on the increase in concentrations of TVB-N, indicating the deterioration in meat quality. In a different study, Kuswandi et al. [138] designed a litmus paper-based (red to blue from pH 5.7 to 6.0) sticker type indicator and attached it to the inner side of a plastic-film wrapped polyethylene tray containing beef stored at a conventional temperature. The study reported that the color change of the indicator sensor was driven by the production of biogenic amines, which were produced by microbes inside the packaged product. Although single chemical dye indicators are used, composite or mixed indicators are advantageous compared to the former in regard to detecting the spoilage threshold and freshness levels of the food accurately [139]. Rukchon et al. [140] developed two mixed pH sensitive indicator-based smart packaging containing bromothymol blue and methyl red, and a mixture of bromothymol blue, bromocresol green, and phenol red for observing spoilage of skinless chicken breast stored under refrigerated and modified atmospheric packaging (MAP) conditions, respectively. These chemical indicators responded well to produced CO_2 and other microbial metabolites by changing their colors. Chen et al. [141] developed a colorimetric sensor array containing three pH indicators (bromocresol green, bromocresol purple, and neutral red) and nine metalloporphyrins fabricated on a C2 reverse silica-gel flat plate. This array provided an excellent color fingerprint for the detection of biogenic amines from chicken during spoilage. Another prototype freshness indicator was developed by Lee and Shin [131] with a methyl red-cellulose acetate mixer to trace the freshness of beef stored at 20°C for 24 h. The authors observed a positive color change from red to yellow, indicating the production of microbial metabolites [123,131]. Although the chemical chromogenic sensors respond quickly with an assured color change and good stability, the migration of chemical reagents into food can be disadvantageous [134]. Conversely, the natural chromogenic sensors are safe to use and inexpensive compared to chemical sensors.

The commonly used natural chromogenic indicators that can be safely used in smart packaging are anthocyanin, carotenoids, chlorophyll, curcumin, and betaine [142]. Out of these natural freshness indicators, anthocyanin has drawn much attention for its wider spectrum and vibrant color-changing patterns at different pH ranges [143,144]. Anthocyanin is

one of the most important flavonoids and can be readily extracted from purple cabbage, roses, pomegranates, blueberries, black grapes, eggplant, and black raspberry. Apart from having a rich matrix, anthocyanins are hydrophilic, non-toxic, and odorless. The color stability of anthocyanin is affected by pH, light, temperature, metal ions, enzymes, UV radiation, gases, and also different chemical forms of anthocyanidin [133,135]. The use of anthocyanin in packaging systems has been reported in different studies. Shukla et al. [145] extracted anthocyanin from rose and red cabbage, which was printed on filter paper to prepare colorimetric sensor-based intelligent packaging. The natural sensor was capable of detecting the release of ammonia, indicated by a color change from red to green at a higher pH. Golasz et al. [146] developed a smart film containing cassava starch and grape anthocyanin, which was applied to pork loin and stored under refrigerated conditions for up to 14 days. A positive correlation was observed between the color change of the film and microbial spoilage. Choi et al. [147] developed another type of intelligent packaging with agar, potato starch, and natural dyes (anthocyanins) extracted from purple sweet potatoes. The study reported a change in the color of the dye from red to green, indicating the spoilage of pork samples.

However, it is important to mention that dye-based indicator sensors have limited practical applications because of their poor mechanical and gas barrier properties and high polarity [133,144]. Making composite films with more than one biopolymer by casting, compression molding, or extrusion method is, therefore, becoming more popular nowadays [135,148,149]. This natural pH-sensitive color changing indicator is incorporated with different biodegradable, biocompatible, and eco-friendly biopolymers, which are also edible, for the preparation of smart packaging to track the freshness of food [135,142]. The selection of appropriate nanomaterials and their applications in biodegradable films, which may improve the structural morphology, including mechanical barrier and thermal properties of the film, plays a crucial role [150]. In this regard, polysaccharide-based biopolymers are more preferable over protein- or lipid-based due to their ability to form cohesive networks with other polymers through covalent or non-covalent bonds [151,152].

Alizadeh-Sani et al. [153] developed a multifunctional halochromic intelligent pack aging material with anthocyanins extracted from saffron petals. The anthocyanins were fabricated into chitosan nanofibers and methyl-cellulose to examine their pH-sensitivity, to ammonia gas, which is produced during the storage of lamb meat. Apart from offering good mechanical and gas barrier properties, the packaging material showed eligibility as a freshness indicator, again changing the color from reddish/pink to violet to green to yellow when exposed to increasing concentrations of ammonia vapor. In another study, Vedove et al. [154] developed smart packaging with cassava starch sheets, using an extrusion process to incorporate anthocyanin as a color indicator to monitor spoilage of beef meat stored at 4°C for 3 days. The study reported a positive correlation with the color change of the film with the production of biogenic amine. Dudnyk et al. [155] developed a similar kind of smart packaging material, containing pectin and red cabbage extract, which was rich in anthocyanins. Anthocyanin was extracted by using a casting method and was incorporated with plasticizers to strengthen the mechanical properties of the film. The film was attached to the headspace of different meat products containers, which showed a similar color change from purple to yellow when volatile nitrogenous substances were produced in association with microbial growth. Zhai et al. [156] successfully developed biogenic amine-sensing bilayer films by using agar, gellan gum, TiO_2, and anthocyanin for the detection of NH_3, trimethylamine, and dimethylamine while studying the quality deterioration of meat. Zhou et al. [157] developed another double-layered indicator film containing carrageenan, anthocyanin, curcumin, and an emulsified layer of glucomannan, i.e., konjac, and camellia oil to monitor the freshness of chicken meat. This emulsified, intelligent double-layered film performed well in monitoring the freshness of chicken at 25 °C with a noticeable color change.

To monitor the freshness of pork in real-time, a protein-polysaccharide nano-complex colorimetric system was developed by Zhang et al. [148]. In this study, nanocomplexes of

anthocyanin-loaded ovalbumin-propylene glycol alginate were incorporated into polyvinyl alcohol/glycerol matrices to provide improved strength and barrier properties to the film. A color change from purplish-red to dark-blue was observed in response to the presence of volatile ammonia, which indicated a decrease in pork meat freshness. Likewise, Niu et al. [158] created intelligent packaging to ensure the safety of pork consumption, which was done by tracing the presence of biogenic amines and microbes. For this, anthocyanin was extracted from colored potatoes (Black King Kong) and fabricated into starch/glycerol/gelatin, which was used for the development of the packaging. This matrix-rich anthocyanin loaded film showed improved physicochemical and antioxidant properties along with exceptional ammonia- and TVB-N-sensing properties, indicated by color change. For example, Chayavanich et al. [159] used a pH-sensitive biocompatible smart film consisting of starch, gelatin, and extracted anthocyanin from red radish for real-time monitoring of meat spoilage. Due to the basicity of the spoiled medium, the pH-sensitive film changed its color from orange to pink and then to bluish-purple in real time which was visible to the naked eye.

Kuntzler et al. [160] developed a different kind of pH-sensitive chromogenic intelligent film with nanofibers of polylactic acid and polyethylene oxide, combined with the microalga *Spirulina*. *Spirulina* is known for its natural blue-green color, which contains different pigments such as β-carotene, tocopherols, phycocyanin, phycoerythrin, and chlorophylls, and thus offers a promising role as a pH-sensitive color indicator. The microalga biomass could be encapsulated in the polymer nanofiber successfully and was indicated via a color change from red to green, upon exposure to microbial metabolites in pork meat stored at a refrigerated temperature. Sun et al. [143] developed a double-layer indicator film using anthocyanin from raspberry, which was incorporated into low-acyl gellan. The outer layer of the film matrix was shielded by chitosan. This encapsulated film matrix showed improved mechanical strength, tensile property, high opacity, and water barrier property. The double-layer film not only improved the stability of anthocyanin but also showed a visible positive color changing pattern based on pH variances, indicating alteration of meat quality in refrigerated storage conditions. A nanofiber-based film with curcumin, chitosan, and polyethylene oxide was developed to monitor the freshness of chicken by Yildiz et al. [161]. A color change from bright yellow to reddish yellow in the film implied that meat was producing TVB-N, creating a basic environment, thus ultimately heading for deterioration. In another study, Kanatt [162] utilized a pH-sensitive dye, namely betalain, which was more pH-stable than anthocyanin. Betalain was used to make an intelligent film with gelatin and polyvinyl alcohol. Due to their stable pH-sensitivity and other biological functions, betalains are gaining more attention for their usage as colorimetric dyes. When tested for the packaging of chicken and shrimp, the wrapped film changed its color from red to yellow in association with microbial quality deterioration over time with the production of volatile basic nitrogenous substances.

It can be concluded that natural pigment-based smart or intelligent packaging systems play an important role in critically assessing the internal quality of meat or meat products in stored conditions, apart from providing accurate information related to quality and safety of the products. This may be a good alternative to other types of packaging systems, as they are economical, safer, and user-friendly. However, more research is needed to overcome limitations related to the instability of pH-sensitive pigments in biopolymer matrices and their migration properties in food.

5. Factors Influencing the Analytical Performance of Biosensors

As stated earlier, biosensor technology is based on combining advances in biological detecting elements like a sensor system and a transducer. However, the analytical performance of biosensors depends on several interrelated parameters such as accuracy, selectivity, linearity and range, sensitivity, specificity, reproducibility, limit of detection/quantification, etc. [15]. Further, the biological sensing components (DNA probes, enzymes, antibodies, tissue, cell receptors, etc.) can lose their activity within a short period of time, either due

to the biological nature of the molecule or upon exposure to environmental stresses, such as pH, temperature, or ionic strength (these are the main aspects underlying muscle food variability). The performance of biosensors in terms of accuracy, precision, and long-term stability can be improved by standardizing the sensor elements and overcoming the environmental effects. These can be handled, for instance, by strict control of temperature. Maintaining the stability of different bio-elements of biosensor devices should be ensured before commercialization of their application. Application of nanotechnology [163] or their involvement with high-affinity biomolecules can increase the selective and sensitive detection of target analytes. The incorporation of various nanostructured materials, including nanocomposites, nanoparticles, nanotubes and nanowires into sensor structures can improve sensitivity, response time, and efficiency [15,164]. Although nanomaterials have immense potential, the analytical performance (e.g., sensitivity, detection limit, and signal-to-noise ratio) of nanomaterial-based sensors varies considerably due to variation in properties, which needs to be addressed. Strategies such as studying the nanomaterials' characteristics, their operational conditions, and application of appropriate calibration algorithms are suggested to reduce the inconsistency in analytical response and the properties of sensing devices and to improve the performance and practical application of such sensors [165].

6. Concluding Remarks and Future Perspectives

In the meat industry, the use of bio-sensing technology is growing steadily for real-time monitoring of meat quality and safety in the supply chain, including transport and storage. In fact, biosensors are considered efficient and alternative techniques to conventional methods due to their specificity, sensitivity, and cost-effectiveness. These unique technologies can help detect the purity and freshness of raw meat, followed by the evaluation of the glycolysis extent and tenderness, as well as for the detection of pathogens, adulterants, antibiotics, allergens, drug residues, additives, and other contaminants. Likewise, much progress has also been made in utilizing different natural and chemical indicators in developing smart packaging to trace meat quality and safety on a real-time basis. Although various indicators, including time-temperature, pH, freshness, etc., and sensors are available, 3D printing technology offers new possibilities. There is scope for development of intelligent indicator-based smart packaging technology, which combines 3D-printing approach with indicators to check real-time detection and continuous monitoring of meat freshness/degradation. Efforts should also be made in designing sustainable and environmental-friendly packaging solutions by using edible freshness indicators, such as chromogenic indicator-based smart packaging, to assess the freshness of meat products.

Despite extensive research in this innovative field, not many biosensors are yet available for their application in the meat sector, and still there is a long way to go before conventional methods can be substituted commercially. However, with recent advancements in the field of nanotechnology, nanomaterials are being explored for application in emerging fields of science, including bioengineering that deals with biosensors and bioelectronics. Nanomaterials exhibit high surface area to volume ratio, increased mechanical strength, excellent catalytic activity, good stability, enhanced chemical and biological activities, and better electrical and magnetic properties, which can be exploited in improving the analytical performance of biosensors. Different kinds of functional nanomaterials such as metal and metal oxide nanoparticles, carbon-based nanomaterials, magnetic nanoparticles, polymeric nanoparticles, graphene sheets, and other novel nanomaterials are available that can be incorporated into biosensor technology. However, the key lies in understanding the mechanism of interaction between biomolecules and nanomaterials for the fabrication of biosensors. Therefore, future research work should focus on understanding the novel properties of these nanomaterials and their compatibility with biomolecules to design a new generation of nanomaterial-based biosensors that are not only inexpensive but also portable, easy to operate, and reliable enough. Above all, the designed biosensors should offer lower detection limits, higher sensitivity, and faster response time for real-time mon-

itoring of large numbers of samples and rapid detection of the targets (biological and chemical substances/contaminants) of interest for their wider applications in the food industry, including the meat processing sector.

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