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Review

Determining meat freshness using electrochemistry: Are we ready for the fast and furious?

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ABSTRACT

Electrochemistry is providing a variety of sensors at an extremely rapid pace. Many of these sensors offer powerful attributes like a multitude of platforms like voltammetry, impedimetry, amperometry and conductometry, as well as sensor-related gains like high sensitivity, selectivity and low cost. It is natural that their applications to food, especially meat freshness determination, are also increasing. Novel methods for rapidly assessing meat freshness are vital for meeting the increasing worldwide demand for meat products. Therefore, we present a short and succinct review of the most promising electrochemical sensor types, including those based on conductive polymers, nanocomposites and metal nanoparticles. From the wide range of sensors that have been designed to detect microbial pathogens and chemical degradation, we have covered a basic snapshot to yield an impression of recent gains in the research genre of meat freshness.

1. Introduction

Food quality is a growing concern for modern consumers and the food industry worldwide alike as evidenced by numerous excellent works emerging in the literature (Charlebois, Schwab, Henn, & Huck, 2016; Fegan & Jenson, 2018; King, Osmond-McLeod, & Duffy, 2018; Ropodi, Pavlidis, Mohareb, Panagou, & Nychas, 2015). In meat products, food quality can be ensured through robust quality framework. Quality assurance is particularly critical for meat products, with global meat consumption projected to be 72% higher in 2030 when compared to year 2000 levels (Brashears & Chaves, 2017; Fegan & Jenson, 2018; Fiala, 2008; Lentz, Connelly, Mirosa, & Jowett, 2018). Four principal pathways through which food-borne illnesses can occur, as proposed by Fleetwood and co-workers, are food hygiene inspection failures, presence of microbiota, presence of pathogens and the human element (Fleetwood et al., 2019). The resulting illness and harm from inadequacies in the quality process has obvious economic and social effects on health systems and communities (M. Y.-C. Wu et al., 2017). For instance, disease incidents may become widespread as evidenced by the *Listeria* outbreaks reported previously. This episode resulted in over 1056 confirmed cases and 214 deaths, making it the largest *Listeria* outbreak on record (Allam et al., 2018; Manganye, Desai, Daka, & Bismilla, 2018; Sanaeifar, ZakiDizaji, Jafari, & Guardia, 2017). Pathogen outbreaks are also detrimental from a manufacturing perspective. According to Food Standards Australia, 135 recalls were issued for meat products between 2008 and 2017, constituting 22% of all food recalls over this period. In addition to reducing customer confidence, food recalls are also associated with high economic cost (Hussain & Dawson, 2013; Thomas et al., 2015). Therefore, to be able to guarantee consumer safety and avoid costs associated with recalls, the assessment processes for meat freshness need to be robust in all stages.

However, traditional analytical methods of monitoring meat freshness are hindered by cost of chemicals and consumables, lengthy analysis timeframes which affect the relevance of the measurement, elaborate and lengthy sample preparation stages, and the very necessary reliance on highly-skilled personnel (Vazquez-Roig & Picó, 2015). Alternative novel, accurate, high-throughput technologies such as simple electrochemical sensors may be useful avenues for improvements (Albelda, Uzunoglu, Santos, & Stanciu, 2017; Wojnowski, Majchrzak, Dymerski, Gębicki, & Namieśnik, 2017a).

This short review focuses on electrochemical sensors, as they are considered one of the most promising types for detecting pathogens in food (Meng Xu, Wang, & Li, 2017). Our discussion reviews the application of such sensors to meat freshness assessment and provides a perspective on the future of this technology. We begin by listing the various types of degradation pathways for meat. In the second section, we have described the common types of electrochemical detection technologies prevalent to meat degradation. Finally, the review

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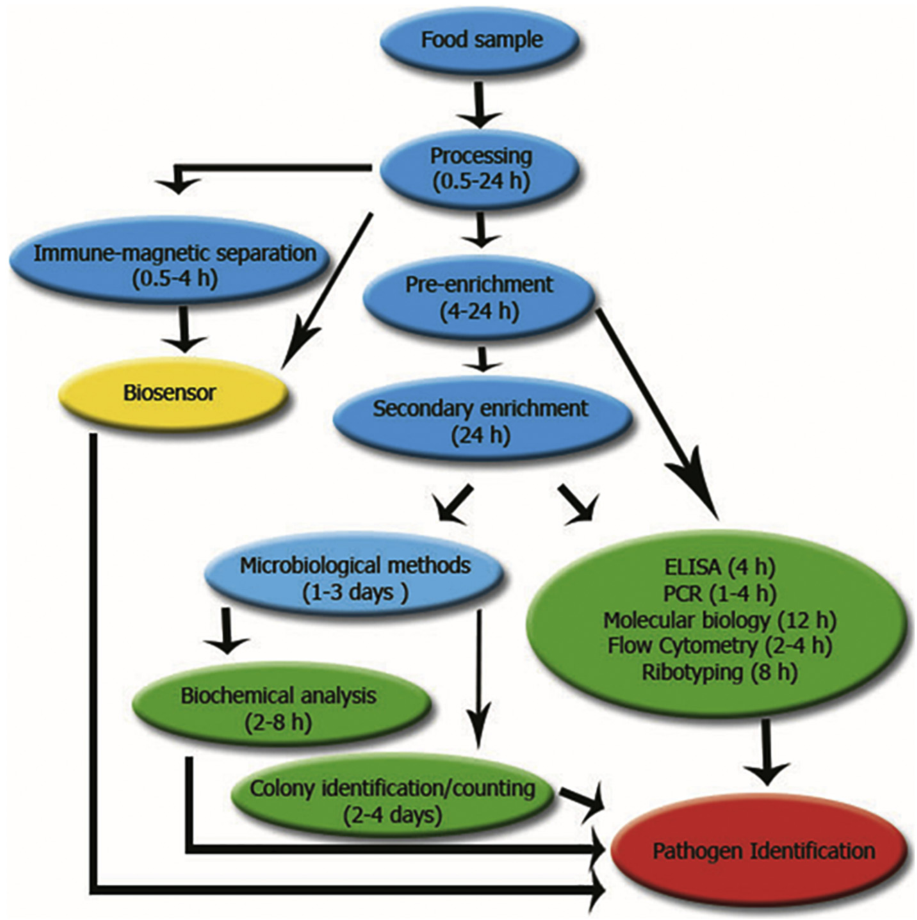

Fig. 1. Flowchart illustrating the timeframes involved in pathogen identification in a food sample by traditional total viable bacteria counts compared with a biosensor. Reproduced under Creative Commons Attribution license (Singh et al., 2013).

concludes with a summary of findings and future outlooks.

1.1. *Assessment of meat freshness*

Meat freshness can be defined in various contexts. At consumer level, it can be classed as being of acceptable organoleptic properties of tenderness, color, juiciness and flavor to meet consumers demands (Liu, Warner, Zhou, & Zhang, 2018). In a scientific context, this can be classified as the chemical, microbiological, sensory and technological attributes (Xiong, Sun, Zeng, & Xie, 2014).

Traditional methods for evaluating meat freshness have, among others, included total bacterial counts (Masoumbeigi, Tavakoli, Koohdar, Mashak, & Qanizadeh, 2017), and an examination of its organoleptic properties (Ruiz-Cano, López-Jiménez, Frutos, Zamora, & Pérez-Llamas, 2016). Both methods are often time-consuming, with the bacterial counts taking up to 72 h for incubation (Wojnowski et al., 2017a). On the other hand, alternatives such as electrochemical biosensors, may offer dramatically decreased analysis times as shown in Fig. 1.

In the next section, we present common contaminants that are increasingly being isolated from various meats. We have categorised these into contamination by microbiological, chemical and pharma residue contamination.

1.1.1. *Microbiological contamination*

The main ways in which meat degrades post-slaughter include microbial decay, lipid oxidation and enzymatic action (Iulietto, Sechi, Borgogni, & Cenci-Goga, 2015; Wojnowski, Majchrzak, Dymerski, Gębicki, & Namieśnik, 2017b). There are over 40 bacterial genera commonly associated with raw meat (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015). However, the most common aetiological agents of meat contamination are *Escherichia*, *Clostridium*, *Salmonella*, *Staphylococcus* and *Listeria* (Painter et al., 2013). Bacterial presence in freshly-harvested carcasses is not uncommon as evidenced by a study that found at the end of the processing phase, *Salmonella* and *Escherichia* (especially *E. coli*) contaminated approximately 0.34% and 14.8% of Australian beef carcasses, respectively (J. Liu, 2017).

Broadly, microbial detection methods fall under traditional or rapid detection methods (Law, Ab Mutalib, Chan, & Lee, 2014). Traditional methods are typically culture-dependent and take 2–7 days for results, compared to rapid detection, which are generally classed as nucleic acid-based (genosensors), biosensor-based or immunological-based (Zhao, Lin, Wang, & Oh, 2014). Of these, we have focused our discussion on electrochemical-based methods and the reader is referred to the works of others on alternative technologies, for example using computer vision (Jackman, Sun, & Allen, 2011), proteomics and mass spectroscopy (Mora, Gallego, & Toldrá, 2018), Raman spectroscopy (Herrero, 2008) and chromatography among many (Marques et al., 2011).

1.1.2. *Chemical contamination*

Meat spoilage can also be driven by self-oxidative fat rancidity that arises from autooxidation of unsaturated fatty acids and enzymatic activity arising in conjunction with microbial presence (Caldera et al., 2016; Echegaray, Domínguez, Franco, Lorenzo, & Carballo, 2018). There are elaborate chemical pathways in which these processes follow, resulting in many volatile organic compounds being released into the headspace of the meat. All these pathways need to be considered for

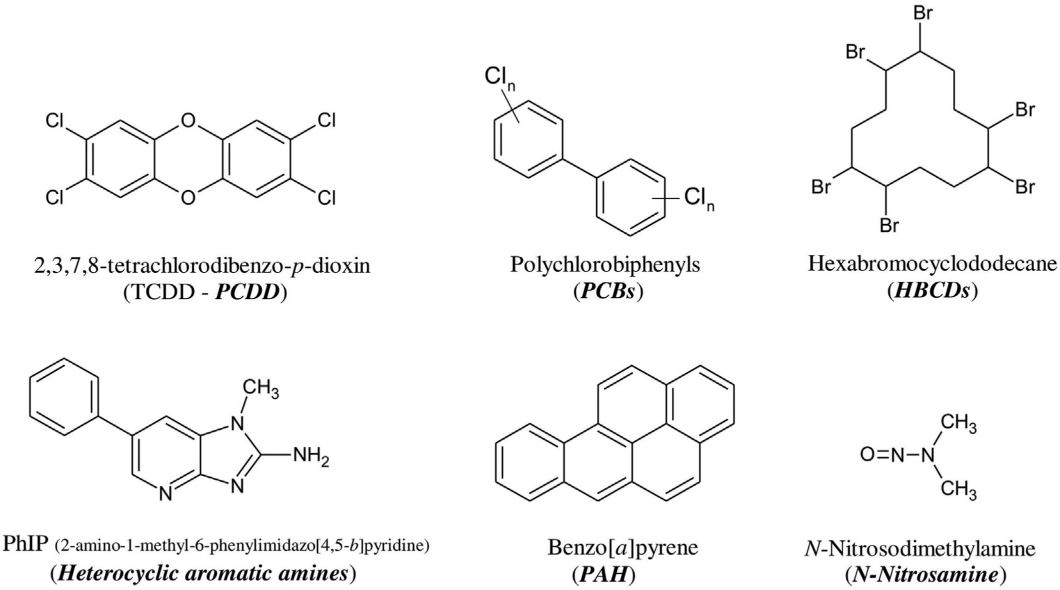

Fig. 2. Some chemical toxins from the major micropollutant categories normally encountered in meat products (Meurillon et al., 2018).

determining the extent of degradation in the meat. Fig. 2 lists common toxins from the major micropollutant families that are encountered in meat products (Meurillon, Ratel, & Engel, 2018).

Meat flavor, and hence volatilome composition often depend on numerous factors, including the meat type and breed (Garcia-Gonzalez, Aparicio, & Aparicio-Ruiz, 2013), storage temperature (Argyri, Mallouchos, Panagou, & Nychas, 2015) and feed composition (Lorenzo, Franco, & Carballo, 2014; Wojtasik-Kalinowska et al., 2016). Animal stress associated with pre-slaughter handling (which can include transport and feedlot dynamics) is also known to affect muscle glycogen metabolism and therefore increases the risk of decreasing meat freshness, though not flavor directly (Yalcintan et al., 2018). In contrast, the age of the animal does not play a direct role in meat flavor (Aaslyng & Meinert, 2017; Jaborek et al., 2018). There also appears to be little volatilome variation between meat stored in regular and modified atmospheric packaging (Lyte et al., 2016). In beef, over 50 volatile compounds have been identified to be in association with the raw meat (Saraiva et al., 2015). Most of these volatile compounds, particularly in the form of alkanes, are produced from autooxidation of the lipid fraction (W. Wu, Tao, & Gu, 2014).

Several non-volatile compounds, such as the purine bases hypoxanthine and xanthine (Zheng et al., 2018), are also associated with meat spoilage. Both are intermediates in purine metabolism, starting with the degradation of adenosine triphosphate to hypoxanthine, which is then transformed to xanthine and ultimately into uric acid. This mechanism is the well-known pathway of adenosine triphosphate degradation in fish meat after death (Pierini et al., 2018). Despite the well-known pathway and route involving hypoxanthine and xanthine, surprisingly, the volume of research devoted to developing electrochemical sensors towards their detection in meat studies remains comparatively low.

2. Electrochemical sensors in meat freshness assessment

This section discusses advances in electrochemical technologies applied to meat freshness determination. We are cognisant of the huge volume of work reported in this research space by dedicated research laboratories. To cover analysis of each type of meat and quality index, or sensor type is simply overwhelming. Yet, we have attempted to capture a representative snapshot of recent cutting edge works that was felt as being encouraging and inspiring.

The most significant electrochemical detection platforms used for the meat detection include voltametric, amperometric and conductometric (Śliwińska, Wiśniewska, Dymerski, Namieśnik, & Wardencki, 2014). In addition to these, impedimetric analysis, which is the assessment of impedance across an electrochemical surface in response to a chemical change has also been applied but in fewer applications. Both the amperometric and voltametric sensors use an electrochemical cell and measure the current between the working and reference electrodes which is related to the concentration of electroactive compounds (Paniel, Baudart, Hayat, & Barthelmebs, 2013; Śliwińska et al., 2014). In conductometric sensors, the transduction is achieved via measuring changes in conductivity across the sensing electrode and the material to be examined. As it works without a reference electrode, is insensitive to light and can be miniaturized as planar interdigitated electrodes and integrated easily by the use of cheap thin-film standard technology, conductometric sensors are finding quite an appeal (Braiek, Djebbi, Chateaux, & Jaffrezic-Renault, 2016; Soldatkina et al., 2018).

In recent times, the use of biosensors has enabled rapid gains in meat evaluation. A biosensor can be defined as an analytical device that combines a biologically sensitive recognition element (such as an enzyme) immobilized on a physicochemical transducer, and connected to a detector to identify the presence of one or more specific analytes, their concentrations, and kinetics in a sample (Bunney et al., 2017; Perumal & Hashim, 2014). Biosensors are helping address many challenges in the analysis of food. Converting biological or chemical responses into electrical output signals, these analytical devices are designed to resist physical parameters, including temperature and pH (Mehrotra, 2016). Biosensor devices have emerged among first relevant diagnostic techniques for food, clinical, and environmental monitoring due to their rapidity, specificity, ease of mass fabrication, economics, and field applicability (Chandra, Chapman, Power, Roberts, & Cozzolino, 2017).

Shi et al. have proposed a low-cost micro-sensor with a diameter based on titanium dioxide-based conducting polymers. The sensor was successfully applied to evaluate pork freshness, where it showed good correlation with the total volatile basic nitrogen levels in the meat (Shi et al., 2018). Elsewhere, Koskela et al. proposed printed and low-cost copper acetate-based sensors for monitoring the freshness of raw broiler meat. The sensor operated by detecting hydrogen sulfide gas as an end product of the microbial metabolism. The authors highlighted the sensor's suitability for large-scale production to enable it to be integrated in a low-cost retail food package (Koskela et al., 2015).

Another type of biosensor, but with more complex recognition/

coupling entity is a genosensor. A genosensor can be described as a microfabricated device containing arrays of nucleic acid hybridization sites, developed for a variety of uses in genomic analysis (Beattie et al., 1995). Various DNA hybridization schemes have since been extended to include their affixation onto conventional biosensors using a myriad of binding mechanisms and assemblies too complex to discuss here. However, this powerful union marries the highly-selective hybridization technique immobilized on a solid substrate producing/fabricating a rapid detection platform, thus providing the analyst with a superior sensor (Asghary, Raoof, Hamidi-Asl, & Ojani, 2015). Electrochemistry then further enhances this fabrication process of modern genosensors, by allowing the analyst to monitor this hybridization via redox markers or labels. Similar in design and function to genosensors, electrochemical immunosensors are biosensors whose recognition entity is based on interactions between an antibody and antigen on a transducer surface (Felix & Angnes, 2018). Many biosensors fall into this category and have targeted *Escherichia* (Chan et al., 2013; Guner, Cevik, Senel, & Alpsoy, 2017; Hassan, de la Escosura-Muniz, & Merkoci, 2015; Pandey, Gurbuz, Ozguz, Niazi, & Qureshi, 2017; M. Xu, Wang, & Li, 2016), *Listeria* and *Salmonella* species (Afonso et al., 2013; Hu, Dou, & Zhao, 2014). A scan of the literature however reveals that sensors for bacterial screening in meats rate very few mentions, but this is more likely to be due to such sensors being recognized as having widespread applicability, and not specifically tagged for meat testing alone. Chapman et al. have recently published an excellent review on bacterial detection using genosensors, which undoubtedly would find application to meat evaluation, and therefore will not be discussed here (Chapman, Power, Kiran, & Chandra, 2017).

Dervisevic et al. have reported their xanthine biosensor based on a hybrid bio-nanocomposite platform in the evaluation of meat freshness. Chitosan–polypyrrole–gold nanoparticles on a glassy carbon electrode surface was the basis of the transducer. The study reported very good performance when the sensor was applied to xanthine detection in fish, beef, and chicken real-sample measurements, and more so in that it also did not suffer from the significant interference from uric acid, ascorbic acid, glucose, and sodium benzoate (Dervisevic, Custiuc, Cevik, and Senel, 2015, Dervisevic, Dervisevic, Azak, Çevik, Şenel, and Yildiz, 2016). In another work, Dervisevic et al. have proposed an amperometric xanthine biosensor based on xanthine oxidase immobilized on a pencil graphite electrode using glutaraldehyde on a conducting polymer film. The biosensor was applied to xanthine determination in chicken meat samples and delivered promising results with high selectivity and sensitivity (Dervisevic et al., 2016). The group has also proposed a xanthine biosensor based on a nanocomposite film of reduced expanded graphene oxide sheets decorated with iron oxide nanoparticles and by covalently immobilizing the xanthine oxidase enzyme on the nanocomposite film surface. The resulting bio-nanocomposite xanthine biosensor was successfully subjected to fish real samples as a freshness measurement technique (Dervisevic, Custiuc, et al., 2015, Dervisevic, Dervisevic, et al., 2016).

Similarly, Sadhegi et al. have proposed an amperometric xanthine biosensor based on xanthine oxidase with a Fe_3O_4/polyaniline nanoparticles nanocomposite. The biosensor exhibited a fast response time to xanthine within 8 s and was successfully applied for measurement of fish and chicken meat freshness (Sadeghi, Fooladi, & Malekaneh, 2014). Devi et al. have recently proposed a successful amerometric biosensor for the measurement of hypoxanthine in fish and meat samples, capable of 200 detections before eventual sensor degradation (Devi, Yadav, Nehra, & Pundir, 2013).

Characteristic volatile compounds released by microbiological agents may also be targeted by sensors based on electronic noses (e-noses). Abdallah et al. have found that a commercial e-nose could easily distinguish between fresh meat and meat spoiled by *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Abdallah, Al-Shatti, Alhajraf, Al-Hammad, & Al-Awadi, 2013). Timsorn and co-workers have proposed a novel e-nose based on eight metal oxide sensors for evaluation of chicken meat freshness and bacterial population. Using principal component analysis, chicken meat freshness could be correlated to different storage days and temperatures and the e-nose was successful in predicting bacterial population on chicken (Timsorn, Thoopboochagorn, Lertwattanasakul, & Wongchoosuk, 2016). Huang et al. have used a commercial e-nose to distinguish the odor between fresh and grilled eels. Ten metal oxide semiconductors, distinguished by different chemical compositions and thicknesses, form the sensor array system provided selectivity towards volatile compound classes. From a total of 155 volatiles detected in fresh and grilled eels, 93 were identified (Huang et al., 2018). Lippolis et al. have reported an e-nose based on metal oxide semiconductors to identify ochratoxin A contamination in dry-cured meat samples. Two classes were adopted: samples contaminated by toxin-producing and non-producing *Penicillium* strains. With the aid of discriminant function analysis, calibration models permitted discrimination of dry-cured meat samples after only 5 days of seasoning with mean recognition percentages in calibration and cross-validation of 98 and 88%, respectively (Lippolis et al., 2016).

The performance of a portable quartz microbalance based e-nose has been evaluated in monitoring aerobically packaged beef fillet spoilage at different storage temperatures (0, 4, 8, 12, and 16 °C) (Papadopoulou, Panagou, Mohareb, & Nychas, 2013). Overall classification accuracies of prediction above 89% were obtained for the three sensory classes regardless of storage temperature. For support vector machine regression model development, correlations above 0.96 and 0.86 were obtained between observed and predicted microbial counts for the training and test data sets, respectively (Papadopoulou et al., 2013). Elsewhere, a portable e-nose, equipped with an array of 10 metal oxide sensors has been used for extracting flavor fingerprint map of Chinese-style sausage during processing and storage, in parallel with detection of acid and peroxide values for evaluating lipid oxidation (Gu, Sun, Tu, & Pan, 2017). The e-nose demonstrated acceptable feasibility in evaluating the degree of lipid oxidation of Chinese-style sausage during processing and storage.

An offshoot of electrochemical analysis is the voltammetric tongue, and very limited applications have been published on meat safety. Previous work with voltammetric tongues are rather dated and have mainly focused on determination of salts (Campos et al., 2010; Labrador et al., 2010). Ruiz-Rico and co-workers have evaluated the use of the voltammetric tongue in the shelf-life assessment of fresh cod in cold storage. Based on 8 metallic working electrodes grouped in two electronic tongues, the first tongue was made up of four noble metals (noble e-tongue): iridium, rhodium, platinum and gold and the second of four non-noble metals (non-noble e-tongue): silver, cobalt, copper and nickel. The study reported that the voltammetric tongue was able to differentiate between days 0 and 1 of storage from the other days, confirming the potential usefulness of the voltammetric tongue for assessing cod spoilage (Ruiz-Rico et al., 2013).

A summary of the aforementioned studies is presented in Table 1, with added information of performance evaluation of the sensors alluded to in each work.

3. Conclusion and future directions

Sensory evaluation of meat and meat products is highly complex and subject to considerable variability. Food is facing increasing scrutiny on issues of provenance, adulteration, certification and freshness from consumers, and enabling technology is still catching up with consumer expectations. Electrochemistry has been making giant strides in all these aspects of food freshness, using a multitude of techniques and an ever-expanding girth of sensor modifications to achieve high sensitivity and selectivity and low limits of detection. Yet, challenges are associated with improving electrode robustness, repeatability of performance, analyte poisoning and cost of nanostructures and materials that comprise the transducer assays. Commercialisation of

Table 1
Recent electrochemical methods applied to meat freshness determination.

Analyte/index	Approach	Performance evaluation	Reference
Pork freshness	TiO_2^{-}PANI/SFF micro-sensor	10 s operating time Limit of detection 100 µg/L	(Shi et al., 2018)
Raw broiler meat	Copper acetate-based sensors	Not stated	(Koskela et al., 2015)
Fish	Chitosan–polypyrrole–gold nanoparticles on a glassy carbon electrode	Sensitivity: 16 mA M^{-1} Linear ranges: 2–28 µM, 28–46 µM and 46–86 µM) Limit of detection: 0.12 µM	(Dervisevic, Custiuc, et al., 2015, Dervisevic, Dervisevic, et al., 2016)
Chicken meat	Xanthine oxidase immobilized on pencil graphite electrode using glutaraldehyde on a conducting polymer film	Linear range: 0.3–25 µM Sensitivity: 124 mA M^{-1} Limit of detection: 0.074 µM	(Dervisevic et al., 2016)
Fish	Nanocomposite film of reduced expanded graphene oxide sheets decorated with iron oxide nanoparticles and by covalently immobilizing xanthine oxidase enzyme the nanocomposite film	Linear range: 2–36 µM Sensitivity: 0.17 μAM^{-1} Response time: ~3 s Limit of detection: 0.17 µM	(Dervisevic, Custiuc, et al., 2015, Dervisevic, Dervisevic, et al., 2016)
Fish and chicken meta freshness	Amperometric xanthine biosensor based on xanthine oxidase with a Fe_3O_4/polyaniline nanoparticles nanocomposite	Response time: 8 s Linear range: 0.2–36.0 µM Limit of detection: 0.1 µM Sensitivity: 13.58 µA μM^{-1} cm^{-2}	(Sadeghi et al., 2014)
Fish, chicken, pork and beef	Xanthine oxidase immobilized onto functionalized Au@FeNPs	Linear range: 0.05 µM - 150 µM (hypoxanthine) Limit of detection: 0.05 µM	(Devi et al., 2013)
Bacteria in beef and sausages	Commercial e-nose	Limit of quantitation: 50 - > 350 ppb	(Abdallah et al., 2013)
Chicken meat freshness	Portable e-nose	20–30s operation time Capable of discriminating odors from 0 to 5 days Temperature range: 4.0–30.0 °C	(Timsorn et al., 2016)
Volatiles for odor detection in fresh and grilled eels	Commercial e-nose with metal oxide semiconductor array system	Not stated	(Huang et al., 2018)
Ochratoxin A in cured meats	Commercial e-nose based on metal oxide semiconductors	Mean recognition percentages in calibration and cross-validation of 98 and 88% between toxin producing and non-producing strains	(Lippolis et al., 2016)
Headspace analysis of beef fillet for spoilage	Commercial e-nose implemented with an array of 8 quartz crystal microbalance non-selective sensors coated with different polypyrrole derivatives	Correlations > 0.96 and 0.86 between observed and predicted microbial counts	(Papadopoulou et al., 2013)
Volatiles profiling in Chinese-style sausages	Commercial e-nose equipped with an array of 10 metal oxide sensors	Not stated	(Gu et al., 2017)
Freshness of cod	Metallic voltammetric tongues	Not stated	(Ruiz-Rico et al., 2013)
Fish freshness	Parallel platinum needles applied to electrochemical impedance spectroscopy	Not stated	(Sun et al., 2018)

proposed technologies is also facing a gulf that is hard to bridge, brought about by tightening markets and cost-neutralisation in business.

Our findings reveal an encouraging increase in consumer-level of operability in methods and techniques previously confined to the domains of accredited laboratories. Indeed, we have covered works that have proposed handheld technologies. Portability is perhaps the precursor to household penetration. In a world with greater consumer autonomy, this is indeed the way forward.

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