

Electronic noses: Powerful tools in meat quality assessment

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ABSTRACT

Main factors that are considered by consumers when choosing meat products are colour and aroma, of which the latter is a more reliable indicator of quality. However, a simple sensory evaluation of hedonistic qualities is often not sufficient to determine whether protein is past its shelf life, and consumption of spoiled meat can lead to serious health hazards. Some volatile compounds can be used as spoilage indicators, and so a device equipped with a sensor sensitive to particular odorants would prove useful. Unfortunately, no such single compound has yet been identified, as the changes taking place in a sample of meat during storage are contingent on numerous factors. On the other hand, a combination of volatile compounds may form a unique 'fingerprint' which can be analysed pattern recognition algorithms with an electronic nose. It can supplement established techniques of meat quality assessment by providing results that correlate well with hedonic perception in a short time and at a low cost.

1. Introduction

Meat is one of the basic food products. It constitutes an important element of diet due to high content of easily digestible protein, as well as highly caloric fat and vitamins and microelements necessary for the proper functioning of numerous metabolic processes. Experts at OECD estimate that the global meat production rose by nearly 20% over the past decade and will continue to grow another 17% relative to the base period (2012–14) (OECD/Food and Agriculture Organization of the United Nations, 2015). Because of that, meat production and processing industry is looking for novel solutions for the classification and spoilage assessment of meat products, the use of which would provide a rapid result both at the processing plants and at retail level. The quality evaluation of meat products is necessary in order to ensure the consumer's safety, as consumption of spoiled meat can lead to serious health hazards.

Spoilage of meat is a sensory quality and is usually detected when looking for off-odours and discolouration (El Barbri, Llobet, El Bari, Correig, & Bouchikhi, 2008). The off-odours are mostly caused by the activity of spoilage bacteria and their presence is the determining factor in shelf life evaluation, since changes in aroma profile usually occur prior to changes in product's appearance, i.e. discolouration and sliminess. Sensory analysis is often expensive, as it is performed by highly-trained specialists, who cannot work for extended periods of time due to olfactory fatigue – an inability to distinguish a particular

odour after a prolonged exposure to a given airborne compound. For that reason, numerous efforts are under way to develop automated techniques that could be used to ensure the quality and safety of meat products.

Currently, the gold standard in determination of the spoilage status of meat is analysis of the total count of bacteria. However, in that method an incubation period of up to 72 h is required, which means that the product leaves the processing plant long before its shelf life can be established. Additionally, this type of analysis often indicates the count of mesophilic bacteria, and does not provide information regarding the count of psychrotrophic bacteria, which proliferate in meat during cold storage. In general, there is a lack of obvious correlation between the degree of spoilage as perceived during sensory analysis and the total count of bacteria. Despite the fact that bacterial growth on meat has been studied extensively, it is still difficult to use these results to predict the product's shelf life. However, data collected from bacteriological analysis can be used to validate other methods, including techniques that are used to analyse the volatile fraction of meat samples, such as electronic noses (El Barbri et al., 2008). Moreover, spoilage of meat can also be chemical, not just microbial. Autoxidation of lipids and the production of free radicals affect fatty acids and lead to oxidative deterioration of the product (Ghaly, Dave, & Ghaly, 2011). The deterioration of product's quality due to chemical spoilage is difficult to quantify using bacteriological methods. Another method used for evaluation of meat freshness is the measurement of total volatile basic nitrogen (TVB-N), where concentration of nitrogen

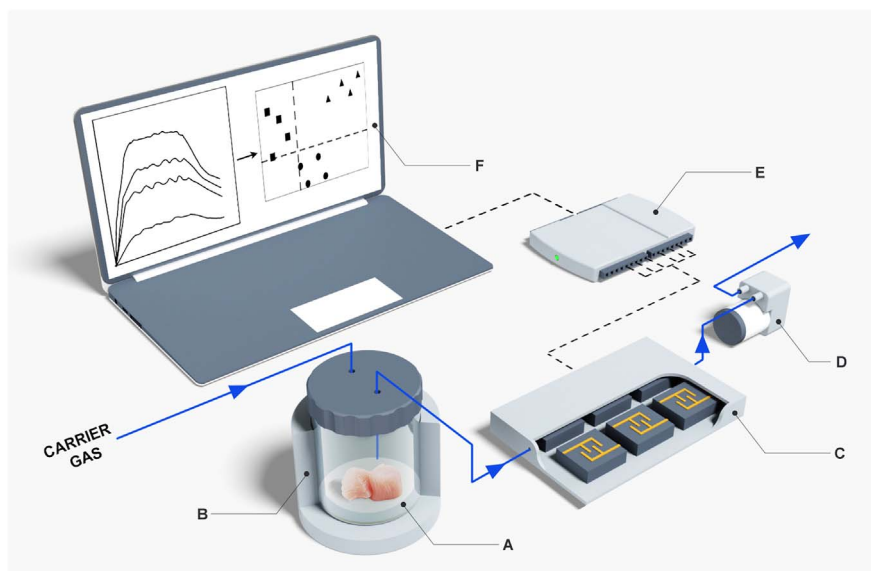


Fig. 1. Main components of an electronic nose, comprised of a sample block (A) with incubation (B), sensor chamber (C), vacuum pump (D), analogue-digital converter (E) and data processing system (F); not to scale.

from volatile nitrogenous bases is measured in mg/100 g of sample. These bases are extracted from the sample using a solution of acid. After alkalisation the extract is distilled and TVB-N concentration is determined through titration (Fishler, 1995). This method is more often used for the evaluation of fish freshness, since aroma of spoiled fish is often associated with the presence of nitrogen compounds.

The most reliable analysis of the volatile fraction of meat samples, both qualitative and quantitative, can be performed using gas chromatography. However, that technique is relatively expensive and its use requires specialised knowledge, and as such is not well-suited for in-line or at-line use in meat processing facilities. Since some volatile compounds could be used as spoilage indicators, a device equipped with a chemical sensor sensitive to a particular odorant would prove very practical. Unfortunately, no single, universal meat spoilage indicator has yet been identified, mostly because the composition of the volatile fraction of a meat sample depends on numerous factors, such as storage temperature, feed composition, bacteria species, the animal's sex and age and even whether or not it experienced stress immediately before slaughter. However, a holistic approach can be taken in which the entire aroma profile of a sample is analysed, and its unique 'fingerprint' is compared with aroma profiles stored in a database using pattern recognition algorithms. A device used for such an analysis is called an electronic nose. It is, according to Gardner and Bartlett (1994), an instrument comprising an array of chemical sensors with partial specificity and an appropriate pattern recognition system, capable of recognizing simple or complex odours. Some applications of the e-nose technique include environmental monitoring (Capelli, Sironi, & Del Rosso, 2014; Monroy, Gonzalez-Jimenez, & Sanchez-Garrido, 2014; Wojnowski, Majchrzak, Gębicki, Dymerski, & Namieśnik, 2016), medical diagnostics (D'Amico et al., 2010; Wilson, 2016), telemedicine (Keller, 1995) and food studies (Śliwińska, Wiśniewska, Dymerski, Namieśnik, & Wardencki, 2014a).

In this article, we describe various applications of electronic olfaction in the area of meat production, processing and distribution. Particular focus was placed on the use of this technique for spoilage assessment, as well as on the detection of various taints and adulterations. The reviewed papers report promising results, with even the early prototypes have performed well in some applications, and some could already be introduced to the market after an adaptation to industrial specifications.

2. Electronic nose technique

First mechanical device used for flavour classification was described in 1961 (Moncrieff, 1961). Early electronic noses (although the term itself was coined much later) were constructed three years later (Wilkins & Hartman, 1964). The proposed setup was based on reactions of oxidation and reduction of odorous substances on electrode's surface. At the same time changes in conductivity caused by the exposure to volatile organic compounds (VOC) were investigated (Buck, Allen, & Dalton, 1965; Dravnieks & Trotter, 1965). The development of these devices proved that chemical reactions between volatile compounds and gas sensors can be successfully transformed into a digital signal. The concept of an 'intelligent' device equipped with an array of sensors intended for the detection of odours was first described in 1982 (Deisingh, Stone, & Thompson, 2004; Persaud & Dodd, 1982). First commercial e-noses were introduced in 1993. Currently, the researcher's attention is mostly focused on curtailing the time of a single analysis, increasing the sensitivity of the measuring setup, miniaturization and validation of the employed methods (Dymerski, Chmiel, & Wardencki, 2011). The term 'electronic nose' might bring to mind a device with capabilities similar to the human olfactory apparatus, where in fact the main aspect in which e-nose resembles its biological counterpart is its function. According to some, terms like 'gas sensor array' or 'multisensorial system' should be used instead (McEntegart, Penrose, Strathmann, & Stetter, 2000; Santonico, Pennazza, Grasso, D'Amico, & Bizzarri, 2013). However, in the body of scientific literature regarding the subject the term 'electronic nose' or 'e-nose' is already well-established and commonly used, together with the nomenclature regarding other techniques described as 'artificial senses', i.e. 'electronic noses' and 'computer vision' (Kiani, Minaei, & Ghasemi-Varnamkhasi, 2016). The principle of operation of this type of devices is as follows: volatile chemical compounds are introduced into the sensors chamber through the sampling system. The sensor's response signal is subsequently transmitted to a computer, where pattern recognition is carried out. A schematic overview of main e-nose components is shown in Fig. 1.

In the research into possible applications of electronic noses in various industries numerous types of gas sensors were used, such as MOS, MOSFET, semiconducting polymers and piezoelectric sensors such as BAW or SAW. Employing these gas sensors presents several challenges,



caused by their sensitivity to temperature and relative humidity changes, as well as the impact of ambient gas on results of the analysis. The emergence of electronic noses based on mass spectroscopy (MS) provided the possibility to alleviate these problems. Such a device is characterized by high stability of operation, sensitivity and a wider range of potential applications than its analogues equipped with an array of chemical sensors (Mielle, Marquis, & Latrasse, 2000). The use of that technique can resolve some persistent issues like sensor poisoning and drift or nonlinearity of the sensor's response signal. The MS-based e-nose technique is rapidly developing. It might lead to an application in which the analysed substance is introduced directly into the spectroscope's ionisation chamber without a need for prior separation in a chromatographic column (Martí, Busto, Guasch, & Boqué, 2005).

Further development of the electronic nose technique is prompted by the emergence of novel types of sensors. In early applications, where classical chemical sensors were used, it was difficult to analyse complex matrices like food products. These difficulties were often caused by insufficient stability of measurement, the need for frequent calibration and high power consumption. Another challenge is the miniaturization of electronic noses, with an integration with consumer electronics in mind. Focus is also placed on the development of units intended for real-time monitoring of odours (Zhang, Tian, & Pei, 2014). Juxtaposition of electronic noses and other meat spoilage assessment methods is presented in Table 1. One might wonder why the technique is not already widely used in the industry, if it has so many advantages (short time of a single analysis, no sample preparation, possibility for automatization, low cost and possibility of real-time analysis). Part of the reason lies in the two major disadvantages that were listed, namely reproducibility and sensor drift. For a statistical model to correctly classify a sample numerous training data sets are needed, that should ideally be obtained in the conditions as close as possible to the intended deployment. That is to say, the device should be trained with the samples and reference methods used e.g. in a particular meat processing plant, which makes large-scale production of a generic, commercial device difficult. Moreover, the trained method can become less reliable over time, since the characteristic of the chemical sensor's response signal tends to change with continued use. However, these issues are not impossible to overcome, and when they are resolved the technique might prove a useful tool supplementing the currently used methods.

2.1. Sensors used in electronic noses

The taxonomy of e-noses that is most commonly used in the literature is based on the mechanism of detection of chemical compounds (Ampuero & Bosset, 2003). Choice of a type of sensors adequate for a given application should be dictated by conditions in which the measurement will be carried out. Things to consider should be cost,

durability of the sensors and also the composition of the gaseous mixture to be analysed. For example, metal oxide sensors were often used by the researchers because they are readily available on the market in large number of varieties, which enabled the creation of sensor arrays tailored to a specific application. However, the high energy consumption of these sensors makes it difficult to use them in hand-held devices, and alternatives like conducting polymer sensors should be considered instead ("Cyanose 320 Portable Handheld Electronic Nose", 2014). If price and durability of the array is of secondary importance, like in a device intended for laboratory use, then more sensitive sensors can be used instead, e.g. piezoelectric sensors. Below is an overview of the types of sensors most commonly used in electronic noses.

2.1.1. Metal oxide sensors (MOS)

Metal oxide sensors are most commonly a ceramic cylinder coated with a layer of semiconductor oxides (SnO_2 , TiO_2 , ZnO). They are equipped with a platinum coil which heats up to 500°C (Mielle et al., 2000). Their principle of operation is the measurement of changes in voltage caused by the reaction of volatile chemical compounds catalysed by metal oxides (Zhang, Tian, Peng, & Yin, 2014). Selectivity and sensitivity of MOS sensors depends on the type of semiconductor used. They are often modified by doping with precious metals (Pt, Pd, Cu, Au), which has a desirable effect on the sensor's operation, reducing the impact of fluctuations of temperature and humidity on the obtained results (Ampuero & Bosset, 2003). Another method of optimizing the performance of MOS sensors is the adjustment of operating temperature or of the semiconductor layer thickness. Metal oxide sensors are susceptible to poisoning by ethanol and volatile sulphur compounds (Schaller, Bosset, & Escher, 1998). The major drawback of using these sensors is high power consumption which limits their potential application in portable and hand-held devices.

2.1.2. MOSFET sensors

Metal-oxide-semiconductor field-effect transistor (MOSFET) sensors are characterized by a short response time. Their construction is based on a field transistor with an electrode coated with a thin layer of a noble metal (Pt, Pd) (Bedoui, Faleh, Samet, & Kachouri, 2013). Silicon dioxide is most commonly used as an insulator. Varying operation temperatures and metal thicknesses can be used to obtain different selectivity. MOSFET sensors usually operate at $50\text{--}170^\circ\text{C}$ which increases the rate of reactions on a catalytic metal gate and also serves to reduce the impact of relative humidity on the output signal (Kalman, Löfvendahl, Winquist, & Lundström, 2000). The detection mechanism is based on induction of polarization at the catalytically active surface caused by the formation of charged reaction intermediates. Voltage change caused by polarization constitutes the output signal. The relationship between

Table 1
Comparison of selected techniques used for meat freshness evaluation.

Parameter	Sensory panel	Total count of bacteria	TVB-N	Gas chromatography	Electronic nose
Time of a single analysis	Several seconds	24–72 h	Up to several hours	From tens of minutes to a few hours	From several seconds to a few minutes
Cost of a single analysis	High	Low	Low	High	Very low
Reproducibility	Depending on the panellists	Depending on measurement conditions	Reproducible	Reproducible	Depending on build and training set
Sample preparation	None	Extensive (preparing a rinse, stomaching, etc.)	Extensive (grinding, filtration, titration)	Extraction (e.g. SLE, SPE, SPME)	None
Simplicity of measurement	Very simple	Simple	Moderate	Complicated (chromatogram interpretation)	Very simple
Qualitative analysis	Yes	Yes	Yes	Yes	Yes
Quantitative analysis	No	No	Yes	Yes	No
Automatization	No	No	No	Yes	Yes
Real-time analysis	Yes	No	No	No	Yes
Major disadvantage	Olfactory fatigue	Time-consuming	Labour-intensive, solvents needed	Calibration & validation step	Sensor drift



the sensor's response time and the type and concentration of the analyte is linear (Eklöv, Mårtensson, & Lundström, 1997). The use of MOSFET sensors in the construction of electronic noses designed for research purposes is becoming increasingly popular. They are also applied in measuring the concentration of hydrogen, ammonia and volatile organic compounds (Eklöv et al., 1997; Morvan, Talou, & Beziau, 2003).

2.1.3. Conducting polymer sensors

Conducting polymer sensors are built from semiconducting materials, most commonly polymers of aromatic compounds such as polypyrrole, polyaniline, polythiophene or polyacetylene (Persaud & Pelosi, 1992). An active layer obtained through chemical or electrochemical oxidation is situated between two gold-plated electrodes (Janata & Josowicz, 2003). When this layer comes into contact with VOC molecules, the conductivity of the sensor's measuring circuit changes. Conducting polymer sensors were first used for detecting polar volatile compounds. However, doping with ions, introduction of new functional groups and changing the polymer's structure can lead to obtaining highly selective sensors, capable of detecting non-polar compounds. An advantage of this type of sensors is the linearity of response signal. Future development of CP sensors might involve introducing adjuncts like enzymes and antibodies into the polymer (Gebicki & Dymerski, 2016). Conducting polymer sensors are more selective than MOS sensors and are less prone to poisoning by volatile sulphur compounds. Moreover, they operate at room temperature, and thus require less power. Their response time is short and inversely proportional to the thickness of the polymer layer (Dymerski et al., 2011). The main disadvantage of using this type of sensors is their high susceptibility to changes of relative humidity.

2.1.4. Piezoelectric sensors

Two types of piezoelectric sensors are being used in electronic noses. The first is bulk acoustic wave sensors (BAW) including quartz microbalance (QCM), and the second is surface acoustic wave sensors (SAW). Their principle of operation is based on measuring the changes in vibration frequency of a piezoelectric crystal caused by exposure to odorant. Bulk acoustic wave sensors are the least complex piezoelectric sensors. They are comprised of a single quartz crystal, two gold-plated electrodes and a membrane on which volatile substances are adsorbed. The mass of particles adsorbed on the membrane's surface causes a change in the vibration frequency of the quartz crystal (Glatz & Bailey-Hill, 2011). The use of BAW sensors allows to identify the components of a gaseous mixture at ppb concentration levels (v/v) (Escuderos, Sánchez, & Jiménez, 2011). This type of sensors is particularly sensitive to VOC. Their main drawback is susceptibility to ambient temperature changes, which restricts their use in field conditions. QCM sensors are manufactured using the microsystems technology, which allows to produce sensors as small as tens of nanometres (Wilson, 2012). In SAW sensors the acoustic wave travels along the sensor's surface, not through its bulk. They consist of a piezoelectric plate with transducers placed on its surface. Between the transducers there is a membrane, made usually of polymer which can be permeated by volatile substances. The lower the energy of activation of the permeation process, the faster the sensor's response signal can be obtained (Yadava & Chaudhary, 2006). SAW sensors are sensitive to a wide range of analytes and have a low power requirement. They can also be easily miniaturized. Devices utilizing these sensors for on-line monitoring of VOC have already been introduced (Barié, Bücking, & Rapp, 2006). However, the need to use expensive peripheral devices and also susceptibility to changes of temperature and humidity limits the potential applications of these sensors.

2.1.5. Other chemical sensors

It should be noted that other types of sensors, e.g. optical and electrochemical sensors (EC), are also used in electronic noses arrays. Electrochemical sensors are equipped with electrodes placed in dedi-

cated electrolyte (Collier, Baird, Park-Ng, More, & Hart, 2003). Measured volatiles dissolve in the electrolyte where they are subject to reduction-oxidation reactions. Electrolyte can be either liquid or solid, with the former used in sensors operating at room temperatures, and the latter usually at higher temperatures (Dymerski et al., 2011). The most commonly used EC sensors are amperometric and potentiometric sensors (Gebicki, 2016). Their main advantage is insusceptibility to fluctuations of relative humidity (Korel & Balaban, 2009), durability and linearity of response signal over a wide range of concentrations (Śliwińska, Wiśniewska, Dymerski, Namieśnik, & Wardencki, 2014b). Until recently, their application was restricted by their relatively large size and specificity to a narrow range of compounds but newer sensors do not suffer from these drawbacks. Yet another type of sensors used in electronic olfaction is optical sensors, which measure modulation of light properties (Dymerski et al., 2011). Measured variables are polarization, absorbance, fluorescence, colour or optical layer thickness. They are usually built of polymer support doped with reactive material – a chemo-responsive dye (Aernecke & Walt, 2009; Korel & Balaban, 2009). Despite wide availability of different dyes, short response time and high sensitivity these sensors are rarely used in commercial e-nose applications due to complexity and relatively high price of support electronics (Albert et al., 2000; Arshak, Moore, Lyons, Harris, & Clifford, 2004).

2.1.6. MS-based electronic noses

In this type of e-noses mass spectrometers with quadrupole analysers are commonly used, since they are relatively inexpensive compared to other types of analysers. The response signal can be processed using chemometrics. The so-called 'fingerprint' (a unique profile of a given odorant) can be compared with profiles of standards compiled in a database. The MS technique can be successfully coupled with other instrumental techniques, e.g. gas chromatography. Moreover, the results of analysis performed using MS-based e-nose can be compared with the results obtained using GC-MS. The use of this type of devices is cheaper and less labour-intensive compared to traditional instrumental techniques that utilize mass spectroscopy (Cynkar, Cozzolino, Damberg, Janik, & Gishen, 2007). However, MS-based electronic noses are significantly more expensive than their counterparts equipped with an array of gas sensors, and their relatively large size limits their use as portable devices (Burian et al., 2010).

An overview of advantages and disadvantages of different types of detectors used in electronic noses is presented in Table 2.

2.2. Signal processing and data analysis

Since in electronic noses the response signals are generated by an array of sensors, each measurement is a set of multivariate data. It is difficult to interpret multidimensional data of this nature without using statistical methods. Data processing can be divided into three stages: pre-processing, feature extraction and classification. Pre-processing can be limited to centering or modification of distribution (e.g. to achieve Gaussian distribution) but in the case of signals obtained during analysis with electronic noses it is often necessary to also minimize the impact of sensor drift and residues from previous analysis. Since each chemical sensor outputs a signal of a different magnitude, their responses should be normalized (Scott, James, & Ali, 2006). During feature extraction inputs are transformed into new, discrete data points. This is done to reduce the dimensionality, as data is transposed from patterns space to feature space. At this stage unsupervised (e.g. cluster analysis, principal component analysis) or supervised (e.g. discriminant function analysis) pattern recognition techniques are commonly used (Dymerski et al., 2011).

If there are significant differences between analysed samples they can be simply classified by juxtaposing the data on a chart or histogram. In more complex cases however it might be necessary to use statistical methods, such as principal component analysis (PCA), discriminant

Table 2

Advantages and disadvantages of types of detectors commonly used in electronic noses.

Detection type	Advantages	Disadvantages
MOS	Sensitivity to a wide range of different chemicals, low cost	High power consumption, high sensor drift, susceptible to poisoning and humidity
MOSFET	Short response time, good selectivity and sensitivity, linear response	High power consumption, susceptible to poisoning
CP	Sensitivity to a wide range of VOCs, short response and recovery time	Low durability, high sensor drift, susceptible to moisture
BAW (QCM)	High sensitivity, selectivity, relatively small size	Low signal-to-noise ratio, high cost, low reproducibility of measurements
SAW	Short response time, high sensitivity	High cost, limited commercial availability
EC	Low power consumption, resistance to humidity, high durability	Relatively large, high sensitivity only to several gaseous substances
Optic	Short response time, good linearity, resistance to humidity and poisoning	High cost, complex construction, sophisticated peripheral devices needed
MS-based	Short response time, high sensitivity, universal detector, enables qualitative and quantitative analysis	Large size, high power consumption, very complex construction, high cost

function analysis (DFA), linear discriminant analysis (LDA) or partial least squares (PLS). Principal component analysis is used to reduce the dimensionality and to plot the transformed data, usually in a form of a biplot. In this technique a new coordinate system is chosen so that first new coordinate explain as much variance of the original dataset as possible, the second coordinate, orthogonal to the first, explains the majority of remaining variance, and so on (Law & Jolliffe, 1987). The main advantage of PCA is the ease of application when the distribution of processed data is close to linear.

Discriminant function analysis is one of the most popular supervised pattern recognition techniques. As is the case with PCA, it can be used to reduce the dimensionality of a data set. Discriminant functions are devised in such a way as to maximize the variance between classes and at the same time reduce the variance within each class. In DFA, first target classes are defined and the subsequent analysis verifies whether this initial classification is in fact statistically valid (Bougrini et al., 2014).

Another technique used for classification is linear discriminant analysis (LDA). In it, similarly to DFA, variance within classes is minimized and variance between classes maximized. A necessary condition is for the groups to be linearly separated, distribution of objects in each class should be close to normal and the number of objects in each class should be no less than three times the variance. For that reason, LDA is usually preceded by pre-processing.

In order to compare various samples and sensor response signals and to graphically represent relationships between them PLC technique is frequently used. In it, a picture of the entire set of data is created in a multidimensional space created by a matrix of objects and variables (Borjesson, Eklov, Jonsson, Sundgren, & Schnurer, 1996). Partial least squares method is particularly useful, since it has minimal requirements regarding residual distribution and sample size (Chin, 1998). The algorithm is optimized in such a way as to increase the capability to predict the value of dependent variable based on independent variables.

When the data distribution is entirely unpredictable artificial neural networks (ANN) can be used with good results. These systems are based on a network of interconnected nodes, the significance of which changes during machine learning (Dayhoff & DeLeo, 2001). In a process of training ANN gain the ability to classify various objects, even without supervision. Neural networks are comprised of several layers. It is assumed that a three-layer network is already sophisticated enough to properly process most of the input signal (Giordani, Siqueira, Silva, Oliveira, & Castro, 2008). However, in order to obtain best results, it is necessary to train ANN on relatively large datasets.

Some other methods used for data processing and pattern recognition include hierarchical clustering analysis, spectral clustering analysis, Sammon mapping, Kohonen self-organizing maps; support vector machines; soft independent modelling class analogy (SIMCA) and clustering algorithms such as k-means (Kodogiannis, 2013; Scott et al., 2006; Tominaga, 1999).

3. Areas of e-nose applications for meat quality assessment

The application of e-nose technique in meat quality evaluation is one of the main application areas of these types of devices in food industry. A large number of studies on this subject has been reported over the last two decades, including a review paper, in which particular emphasis is placed on issues regarding signal processing and data analysis (Ghasemi-Varnamkhashti, Mohtasebi, Siadat, & Balasubramanian, 2009). The main applications of e-noses with respect to meat are in spoilage detection, estimation of shelf life, detection of off-flavours and in classification.

3.1. Spoilage and shelf life

There are numerous factors that determine the shelf life of a meat product, including the type of meat, packaging conditions, storage temperature, processing procedure, exposure to certain strains of bacteria or the meat's pH after the onset of *rigor mortis*. Furthermore, there are three principal mechanisms of meat spoilage, namely microbial spoilage, lipid oxidation and autolytic enzymatic spoilage (Ghaly et al., 2011). This can lead to a change in the composition of the volatile fraction of a meat sample, which in turn can lead to the identification of potential meat spoilage markers. Information regarding some chemical compounds identified as potential meat spoilage indicators is listed in Table 3. Chemical sensors chosen for electronic noses intended for meat quality assessment should display at least partial sensitivity to potential spoilage indicators.

One of the first papers concerning the quality estimation of meat using an electronic nose was published as early as 1993 (Winquist, Hornsten, Sundgren, & Lundstrom, 1993). In it, a prototype electronic nose which would later be introduced to the market as NST 3210 was used to monitor the spoilage of ground pork over a period of 4 days, and of ground beef over a period of 8 days. The results of storage time predictions using ANN were deemed satisfactory by the authors. This early study, although performed on a relatively small number of samples, substantiated the possibility of using e-noses for meat quality evaluation. Other applications of electronic olfaction in pork quality assessment are listed in Table 4.

The same electronic nose was later used to determine the spoilage of vacuum-packed beef strip loins (Blixt & Borch, 1999). The vacuum pouches containing meat were refrigerated for up to 8 weeks. Together with VOC analysis, sensorial and bacterial tests were also performed. The degree of spoilage was quantified by means of a model describing the relationships between the degree of spoilage, as determined by a sensory panel, and the signal magnitudes of the electronic nose's sensors. The authors reported best prediction results when signals from only two sensors were considered (R^2 value of regression = 0.94, root mean error of prediction = 0.41). In mid-1990's came first investigations of application of a QCM-based electronic nose to analysis of foods,



Table 3

Potential volatile spoilage indicators of selected types of meat.

Type of meat	Potential volatile spoilage markers	Sensory descriptor	Ref.		
Pork	2,3-Butadienol	Fruity, onion	(Mayr et al., 2003; Xu, Cheung, Winder, & Goodacre, 2010)		
	Dimethyl sulphide	Cabbage, gasoline, mouldy, sulphurous			
	Dimethyl disulphide	Cabbage, onion, putrid, ripened cheese, sulphurous			
	Dimethyl trisulphide	Alliaceous, cabbage, cauliflower, fishy, onion, rotten food, sulphurous			
	2,5-Dimethylpyrazine	chocolate, medicinal, roastbeef, woody			
	2-Heptanone	Cheese, cured ham, gravy, nutty, soapy			
	Methanol	Pungent			
	Methoxybenzene	Fragrant, phenolic, sweet			
	5-Methylpyrimidine	n.a.			
	2-Octanone	Cheese, earthy, gasoline, soap, stew			
	2-Octenal	Burnt, mushroom, nutty, waxy			
	Phenol	Medicinal, phenolic			
	Phenylethyl alcohol	Floral, honey, lilac, rose			
	Toluene	Caramelized, paint, rubber, pungent			
	Beef	2,3-Butadienol		Fruity, onion	(Mayr et al., 2003)
		Dimethyl sulphide		Cabbage, gasoline, mouldy, sulphurous	
Dimethyl trisulphide		Alliaceous, cabbage, cauliflower, fishy, onion, rotten food, sulphurous			
Isoamyl acetate		Banana, fresh, pear, sweet			
Methanethiol		Cheese, cooked cabbage, fishy, rotten egg, sulphurous			
Methanol		Pungent			
1-Octanol		Burnt matches, fatty, green, sulphurous, toasted bread			
Toluene		Caramelized, paint, rubber, pungent			

including veal (C. Di Natale et al., 1997). Satisfactory results were reported, as the measurements have shown an intrinsic sensor classification which was in agreement with the duration of storage of meat.

The potential application of another commercial e-nose developed in the early 2000's, the Cyranose 320 (Sensigent, USA), was also investigated (Balasubramanian, Logue, & Marchello, 2004). The main objective of the study was to evaluate a novel method of validation called the bootstrap analysis, in which multiple surrogate groups are used for resampling of the subjects in the population (Bellec, Rosa-Neto, Lyttelton, Benali, & Evans, 2010). However, the study also demonstrated that an electronic nose based on CP sensors can be successfully used to discriminate between spoiled and unspoiled samples of beef, if not to correctly identify the day of refrigerated storage. The maximum classification accuracies obtained for the unspoiled and spoiled samples by QDA were 87.88% and 100%, respectively, and using LDA 99.83% and 83.63%, respectively. The authors note however that the validity of the results may have been impacted by the relatively small number of samples. The same device was used to identify salmonella-inoculated beef (Balasubramanian, Panigrahi, Logue, Marchello, & Serwood, 2005). Meat samples were classified based on the microbial population. The results have shown that it was possible to identify meat samples contaminated with *S. Typhimurium* at a population concentration level of 0.7 log₁₀ cfu/g with classification accuracy of 87.3%.

Interesting results were obtained during a study that compared several techniques, namely: dynamic headspace GC-MS, electronic nose, and sensory analysis, among others, as applied to the analysis of early stages of lipid oxidation of freeze-stored pork back fat, mechanically recovered meat and sausages (Olsen, Vogt, Ekeberg, et al., 2005; Olsen, Vogt, Veberg, Ekeberg, & Nilsson, 2005). The results obtained using instrumental methods showed high correlation with data from sensory analysis. The e-nose detected changes in the meat samples at the stage as the panel, whilst with the use of dynamic headspace GC-MS it was possible to detect changes in the sample's headspace at an earlier stage. This does not necessarily mean that electronic olfaction is a less valid technique for detection of spoilage in freeze-stored meat trimmings, as it is less time-consuming than gas chromatography, and cheaper and less labour-intensive than employing a sensory panel. Other factors, other than the duration of freeze-storage, which may impact the deterioration of pork scratchings, are the effects of oxygen availability and exposure to light (Jensen, Bertelsen, & Van

Den Berg, 2005). An experiment, in which scratchings were stored over the period of 24 weeks packed with varying levels of oxygen. The electronic nose prototype based on 6 metal oxide sensors was able to discern samples with regard to storage time, exposure to oxygen and light when PCA was used. However, no reliable model of prediction was constructed (R² values of 0.62–0.87 for PLS).

An electronic nose based on metal oxide sensors was developed and employed for spoilage classification of beef loin, using microbial count as classifier (< 6 log₁₀ cfu/g for “unspoiled” and ≥ 6 log₁₀ cfu/g for “spoiled”) (Panigrahi, Balasubramanian, Gu, Logue, & Marchello, 2006). The sensor array included 8 MOS as well as humidity and temperature sensors. Beef headspace was sampled directly from meat packed via a needle. Samples were stored at 4 °C and 10 °C for a period of 15 and 7 days, respectively. The highest accuracy of classification was obtained with QDA and bootstrapping – 96.0% for samples stored at 4 °C and 93.2% for the ones stored at 10 °C. The same prototype device was later used to develop neural networks for beef spoilage classification (Balasubramanian, Panigrahi, Logue, Gu, & Marchello, 2009), with the experiment set up in a way similar to the above-mentioned study. Here, the microbial population in samples stored at 10 °C was predicted with greater accuracy. Again, the total maximum classification accuracy exceeded 90%. However, as the authors have noted, the accuracy of prediction using neural networks generally increases with the sample size used for training, so these results do not necessarily mean that the data processing method used has no significant impact on the final outcome of the analysis using electronic olfaction. Yet another study that employed the same electronic nose and different pattern recognition algorithms was carried out, aiming at monitoring the changes in the headspace of beef inoculated with *Salmonella typhimurium* and stored at 20 °C (Balasubramanian et al., 2008). A prediction accuracy of 82.99% was obtained using independent components analysis (ICA), as opposed to 69.64% using PCA. The inoculated samples were packed using a polystyrene base tray and stretch wrap in order to emulate the storage conditions in retail distribution centres. Some other investigations into applications of electronic noses in quality assessment of beef can be referenced in Table 5.

Modified atmosphere packaging (MAP) is considered an effective method of prolonging the shelf life of both fresh meat often with high level of O₂ and meat products often without O₂. The effectiveness of



Table 4
Applications of electronic noses in pork quality assessment.

Application	Product	Data Processing	E-nose model	Manufacturer/scientific institution	Sensors	Ref.
Classification	Sausage, ham	DFA	Fox 2000	Alpha M.O.S.	6 MOS	(Vernat-Rossi et al., 1996)
Classification	Ground meat	LDA, SIMCA	Fox 2000	Alpha M.O.S.	6 MOS	(González-Martín et al., 2000)
Classification	Sausage (salami)	PCA	Prototype	IMM-CNR, Lecce	5 MOS	(Taurino et al., 2003)
Classification	Processed meat	PCA	Moses II	Lennartz Electronic	7 QCM, 8 MOS, 7 EC	(Pardo et al., 2005)
Classification	Ham	PCA, ANN	Prototype	CSIC, Madrid	16 MOS	(García et al., 2006)
Classification	Meat	PCA, DFA, PLS	FOX 4000	Alpha M.O.S.	18 MOS	(Jia et al., 2011)
Classification	Meat	PCA	zNose	Electronic Sensor Technology	SAW	(Nurjuliana, Che Man, Mat Hashim, et al., 2011)
Classification	Ground meat	PCA, LDA, LR, ANN	PEN 2	Airsense Analytics	10 MOS	(X. Tian et al., 2013)
Classification, processing	Ham	PCA	Prototype	CSIC, Madrid	12 MOS	(Otero et al., 2003)
Off-flavour and taints	Boar fat	DFA	Prototype	INP-ENSIACET	14 MOS	(Bourrounet et al., 1995)
		MDA	NOSE	Neotronics Scientific Ltd	12 CP	(Annor-Frempong et al., 1998)
Off-flavour and taints	Fat	PLS	Prototype	Univ. Rome-Tor Vergata	4 QCM	(Corrado Di Natale et al., 2003)
Off-flavour and taints	Meat	PCA, PLS	MGD-1	EnviroNics Ltd	Ion mobility cell	(Vestergaard et al., 2006)
Off-flavour and taints	Fat	PCA, DFA	SMart Nose 151	LDZ	MS - based	(Ampuero et al., 2006)
Off-flavour and taints, spoilage and shelf life	Meatballs	PLS	NST-3320	AppliedSensor AB	12 MOS, 10 MOSFET, 1 IR	(Tikk et al., 2008)
Off-flavour and taints, processing	Ground meat	PCA	Fox 3000	Alpha M.O.S.	12 MOS	(Kim et al., 2008)
Processing, sensory quality	Sausage	PCA, ANN	NST 3210	AppliedSensor AB	10 MOSFET, 4 MOS, 1 IR	(Eklöv, Johansson, & Winquist, 1998)
Sensory quality	Cooked meat	ANOVA, PLS	MGD-1	EnviroNics Ltd.	Ion mobility cell, 1 MOS	(O'Sullivan, Byrne, Jensen, Andersen, & Vestergaard, 2003)
Sensory quality	Meat loaf	PLS, PCA	DOSS	Royal Vet. and Agric. Univ., Frederiksberg	16 MOS	(Hansen, Petersen, & Byrne, 2005)
Sensory quality, spoilage and shelf life	Pizza topping	PCA, PLS	MGD-1	EnviroNics Ltd.	Ion mobility cell, 1 MOS	(Vestergaard et al., 2007a, 2007b)
Sensory quality, Spoilage and shelf life	Salted meat	PLS	Prototype	Jiangsu Univ.	Colorimetric sensor array	(X. Huang et al., 2014; Xiaowei et al., 2015)
Spoilage and shelf life	Meat	PCA, LDA, ANN				(Li, Chen, Zhao, & Ouyang, 2014; Li, Chen, Zhao, & Wu, 2015)
Spoilage and shelf life	Meat	PCA, ANN	Prototype		11 MOS	(L. Huang, Zhao, Chen, & Zhang, 2014)
Spoilage and shelf life	Ground meat	ANN	NST 3210	AppliedSensor AB	10 MOSFET, 4 MOS, 1 IR	(Winquist et al., 1993)
Spoilage and shelf life	Back fat, MRPM, sausage	PCA	NST 3220	AppliedSensor AB	12 MOS, 8 MOSFET	(Olsen, Vogt, Ekeberg, et al., 2005; Olsen, Vogt, Veberg, et al., 2005)
Spoilage and shelf life	Scratchings	PCA, PLS	Prototype	PBI-Dansensor	6 MOS	(Jensen et al., 2005)
Spoilage and shelf life	Ground meat	LDA	KAMINA	Research Center Karlsruhe	MOS divided into 38 elements	(Musatov et al., 2010)
Spoilage and shelf life	Ground meat	PCA, SVM	Libra Nose	TechnoBioChip	8 QCM	(Papadopoulou et al., 2011)
Spoilage and shelf life	Meat	PCA	Prototype	Tsinghua Univ.	8 MOS	(X. Y. Tian et al., 2012)
Spoilage and shelf life	Meat	LDA, ANN, LR	PEN 2	Airsense Analytics	10 MOS	(Hong & Wang, 2012)
Spoilage and shelf life	Meat	SVM, PCA, PLS	FOX 4000	Alpha M.O.S.	18 MOS	(Wang et al., 2012)
		PCA				(Tang et al., 2013)
Spoilage and shelf life	Sausage	PCA, PLS	Prototype	Valencia Univ.	Colorimetric sensor array	(Salinas et al., 2014)

this procedure is based on the antimicrobial properties of CO₂, the presence of which in meat packages inhibits microbial growth and causes a shift in the dominant microflora to bacteria with a lesser potential for causing spoilage (Koutsoumanis, Stamatiou, Drosinos, & Nychas, 2008; Limbo, Torri, Sinelli, Franzetti, & Casiraghi, 2010). However, MAP makes it difficult to use electronic noses which were not previously trained to analyse that particular mixture of ambient gasses. Researchers also investigated the application of an electronic nose in measuring and modelling changes in a pizza topping, consisting mostly of pork sausage mix, during storage (Vestergaard, Martens, & Turkki, 2007a, 2007b). A method involving a minimum of sample preparation and a short sampling period was developed in order to emulate an on-line application. Samples were stored at 7 °C over a period of 19 days in modified atmosphere packages (25% CO₂, 75% N₂). The e-nose used in this study was based on the principle of ion mobility spectroscopy (IMS), with ion mobility cells as analogues of individual sensors in a matrix. The device was also equipped with a single MOS sensor. Prediction of storage time by the electronic nose data using PLS algorithm had a correlation of R² = 0.96. It should be noted, that in

order to bring the conditions of the experiment close to the ones anticipated during potential deployment of the technology in processing plants, the sample cycle was curtailed to approximately 5 min, and the authors underlined the possibility of further reducing it to 2–3 min. The application of electronic olfaction, among other techniques, to evaluation of shelf life of meat stored in a modified atmosphere was also examined in a different study (Limbo et al., 2010). The headspace of packaged minced beef consisted of 30% CO₂ and 70% O₂. A clear discrimination between fresh and spoiled samples was obtained using PCA and CA. Furthermore, the results obtained using the e-nose overlapped with the ones obtained using traditional methods of meat freshness assessment.

Shelf life of meat stored in MAP was also investigated using an electronic nose based on QCM sensors (Papadopoulou, Tassou, Schiavo, Nychas, & Panagou, 2011). Samples of ground pork were stored at different temperatures (0, 5, 10, 15, and 20 °C), both aerobically and in a mixture of 60% CO₂, 20% O₂ and 20% N₂ using SVM, the overall correct classification in the sensory classes was 81%, whereas correct classification for fresh, semi-fresh and spoiled samples amounted to 76,



Table 5
Applications of electronic noses in quality assessment of beef.

Application	Product	Data processing	E-nose model	Manufacturer/scientific institution	Sensors	Ref.
Classification	Processed meat	PCA	Moses II	Lennartz Electronic	7 QCM, 8 MOS, 7 EC	(Pardo et al., 2005)
Classification	Meat	PCA, DFA, PLS	FOX 4000	Alpha M.O.S.	18 MOS	(Jia et al., 2011)
Classification	Fat	PCA	zNose	Electronic Sensor Technology	SAW	(Nurjuliana, Che Man, & Mat Hashim, 2011)
	Meat					(Nurjuliana, Che Man, Mat Hashim, et al., 2011)
Processing	Cooked meat	PCA	Prototype	Mannheim Univ.	Gradient MOS with 40 elements	(Ehrmann, Jüngst, & Goschnick, 2000)
Processing, spoilage and shelf life	Cooked meat	ANN	AromaScan	Osmtech PLC	32 CP	(Grigioni et al., 2000)
Processing, sensory quality	Sausage	PCA, ANN	NST 3210	AppliedSensor	10 MOSFET, 4 MOS,	(Eklöv et al., 1998)
Spoilage and shelf life	Ground meat	ANN		AB	1 IR	(Winquist et al., 1993)
Spoilage and shelf life	Meat	PLS				(Blixt & Borch, 1999)
Spoilage and shelf life	Meat	LDA, QDA	Cyranose 320	Sensigent	32 CP	(Balasubramanian et al., 2004)
Spoilage and shelf life	Meat	LDA, QDA	Cyranose 320	Sensigent	32 CP	(Balasubramanian et al., 2005)
Spoilage and shelf life	Meat	PCA, SVM, PLS	Prototype	Moulay Ismail Univ.	6 MOS	(El Barbri et al., 2008)
Spoilage and shelf life	Meat	n.a.	Prototype	Jilin Univ.	6 MOS	(Zhang, Tong, Chen, & Lan, 2008)
Spoilage and shelf life	Meat	PCA	PEN 2	Airsense Analytics	10 MOS	(Limbo et al., 2010)
Spoilage and shelf life	Meat	PCA, LDA, ANN	PEN 2	Airsense Analytics	10 MOS	(Hong et al., 2012)
Spoilage and shelf life, classification	Meat	LDA, QDA	M-Module	N. Dakota State Univ.	7 MOS	(Panigrahi et al., 2006)
		PCA, ICA				(Balasubramanian et al., 2008)
		PCA, ANN				(Balasubramanian et al., 2009)
Spoilage and shelf life, classification	Meat	SVM, ANN, KNN	Prototype	Sejong Univ.	8 MOS	(ul Hasan et al., 2012)
Spoilage and shelf life, sensory quality	Meat	PCA, DFA, SVM	Libra Nose	TechnoBioChip	8 QCM	(Papadopoulou, Panagou, Mohareb, & Nychas, 2013)

87, and 78%, respectively. Colorimetric sensor arrays have also been used to monitor the spoilage of pork sausage packed in MAP (Salinas et al., 2014).

A study on the use of a MOS-based electronic nose prototype for spoilage classification of red meat, namely beef and mutton was carried out by (El Barbri et al., 2008). Samples were stored at 4 °C for up to 15 days, and PCA and SVM was used for data processing. Similarly to several previously described studies, the spoilage threshold for the purpose of identification was set at the microbial count of 6 log₁₀ cfu/g, and in order to investigate whether the results obtained using e-nose correlated well with the results of the bacteriological analysis, PLS calibration models were built and validated. The results obtained using bacteriological analysis suggested that the shelf-life of beef and mutton stored at 4 °C are 7 and 5 days, respectively. Using the sensor array system, it was possible to discriminate between spoiled and unspoiled meat with a success rate of 98.87% in the case of beef, and 96.43% in the case of mutton.

The real extent of e-nose application in meat quality control, despite being very promising and actual, is limited by the complexity of the samples' headspace composition. It was hypothesized that this state of affairs is mostly due to the following issues remaining uncertain: the amount of training of the e-nose required in order to obtain reliable recognition; the impact of variations of input parameters, e.g. storage conditions or product suppliers; the length of time in which the model retains its usefulness despite sensor drift (Musatov, Sysoev, Sommer, & Kiselev, 2010). These issues were addressed in an experiment performed using 3 commercial electronic noses of the same model and specification based on an array chip carrying a thin metal oxide film segmented by parallel electrodes on minced pork sourced from different vendors and stored at two different temperatures, namely 4 °C and 25 °C. The researchers concluded that one or two exposures of the sensor array to the samples were enough for the device to recognize with 100% probability the unspoiled meat sourced from the same supplier. However, 3–4 training cycles of exposure to meat from different suppliers were necessary for the e-nose to build a reliable model that accounted for the supplier factor. Furthermore, the samples stored at 4 °C and 25 °C were mutually recognized at early stages of

decay. That is a clear indication that a properly set-up MOS electronic nose can be in fact currently utilised for evaluation of meat freshness.

A prototype MOS-based electronic nose for classification of pork freshness was developed and employed, after a period of training in the laboratory, in field conditions, i.e. by placing it on supermarket shelves (Tian, Cai, & Zhang, 2012). The storage conditions of samples (pork) were similar to those of products available to customers. The meat was stored in a refrigerator until spoilage occurred. The field measurements of pork, after classification based on a model developed using PCA in laboratory conditions, yielded an overall 91.7% success rate, and a 100% success rate when analysing spoiled samples. This demonstrated that even an e-nose based on commercially available sensors that are relatively inexpensive, coupled with a rudimentary data processing technique can be, under certain conditions, deployed in the field and used for monitoring of shelf life of meat products. A comparable success rate (88%) was achieved in a study in which a commercial MOS-based electronic nose was employed (Wang, Wang, Liu, & Liu, 2012). In it, SVM method was used to predict the TVC in chilled pork. Bacterial counts were determined by plate counts on agar, and correlated with e-nose measurements.

The correlation of electronic nose measurements with the more established meat freshness indexes (sensory scores, TVB-N and microbial population) was also established in the case of beef (Hong, Wang, & Hai, 2012). In all three cases the use of neural network-based prediction models showed promising results, although the square error of the prediction of storage time was 1.36 days.

When considering prospective deployment of a portable electronic nose as a tool for meat freshness assessment, e.g. in the hands of a sanitary inspector who gauges the product quality on a butcher shop, the ambient odours need to be taken into consideration. In such an environment, the volatile spoilage indicators cannot be detected in isolation, as is the case in the majority of laboratory studies. For instance, the aroma of spoiled pork could well be masked by the smell of fresh beef stored in the same refrigerator. A study was reported, in which fresh beef was stored with decayed fish, and fresh fish with decayed beef (ul Hasan, Ejaz, Ejaz, & Kim, 2012). The results of this experiment were promising; using KNN it was possible to achieve a



100% specificity and a reasonable sensitivity of 93.42%. However, it remains to be seen how an e-nose device would perform in an environment, in which dozens of interfering aromas are present, as would be the case for instance in a supermarket's meat section. For that reason, it still seems most practical to place the sample in an enclosed sampling chamber, in which the headspace would be isolated.

In general, it seems that researchers envision two main possible applications of electronic noses in the assessment of spoilage and shelf-life estimation. One is the periodic evaluation of the current state of particular meat product, which is generally performed either by sanitary inspectors or by consumers. In this case, where only a binary information is necessary ('fresh' vs. 'suspect'), the research results demonstrate that electronic noses equipped with an array of commercially available chemical sensors can already be successfully used, even in a form of a hand-held device. The other is more of an industrial application, where the results obtained using electronic noses could be correlated with bacterial analysis to develop a model for prediction of shelf-life (Limbo et al., 2010). This would require not only a prolonged period of training at the site of prospective deployment, but also highly sensitive devices which can be situated on-line and which are not affected by sensor drift or changes in ambient conditions. In such a case, where portability or unit cost are of secondary concern, it would seem that the use of MS-based e-noses should be considered.

3.2. Detection of off-flavours and taints

Undesirable flavour characteristics of meat are not always caused by bacterial spoilage. Sometimes taints develop during processing, e.g. pre-cooking, and subsequent storage, as is the case with the so-called warmed-over flavour.

3.2.1. Detection of boar taint

In most countries, pork production from non-castrated males is discouraged, since the meat often exhibits a distinct, unpleasant odour known as boar taint. The chemical compounds that contribute significantly to boar taint are skatole and androstenone, with the latter exhibiting an intense, urine-like odour (Bonneau, 1982). Other substances have also been suggested to play a role in the perception of boar taint (Haugen, 2006), however several studies have indicated that androstenone and skatole concentrations in pork do not always match the sensory sensation perceived by panellists, and so their concentrations alone are not sufficient to predict the consumer response (Haugen, 2006; Xue et al., 1996). Early investigations of the possibility of using electronic nose technology for this purpose were conducted using devices with arrays of chemical sensors, i.e. MOS (Bourrounet, Talou, & Gaset, 1995), QCM (Di Natale et al., 2003) and CP sensors (Annor-Frempong, Nute, Wood, Whittington, & West, 1998). However, the highest overall successful classification in correlation with sensory panel scores reported in these studies did not exceed 85%.

More recently, the potential to detect boar taint using an e-nose based on mass spectrometry with SPME sampling mode was examined (Ampuero, Bee, & Hansen-Møller, 2006). With this technique 65% of unknown pork back fat samples were correctly classified as either low level (1.11 µg/g) or high level (> 1.11 µg/g) of androstenone, the concentration of which was first determined using HPLC, and 100% of boar meat samples were correctly discriminated against the samples from the castrates. It should be noted that the SPME extraction time was 1 h, which is relatively long considering that one of the main advantages of electronic noses is the short time of a single analysis.

The main difficulty in using the electronic nose technique to detect the presence of skatole and androstenone is the fact that both compounds have a relatively high molecular weight compared to other odorants, which results in lower volatility. For that reason their presence in the sample's headspace is low relative to the concentration in the sample (Vestergaard, Haugen, & Byrne, 2006). In order to overcome that issue an extended period of incubation or more elaborate

sampling methods can be used, which would however make it difficult to employ the device on-line.

3.2.2. Detection of warmed-over-flavour

The development of warmed-over-flavour (WOF) is attributed to the auto-oxidation of lipids which in turn decompose to various volatile compounds. It is the main cause of rancidity of meat products that have been pre-cooked, refrigerated and re-heated (Byrne, Bak, Bredie, Bertelsen, & Martens, 1999). The presence of these oxidation products is undesirable not only because of the unpleasant flavour, but also because they can have adverse effects on the human health (Jayathilakan, Sharma, Radhakrishna, & Bawa, 2007). The increase in the market demand for 'ready-to-eat' meat products leads to an increasing demand for tools the use of which would enable the detection of WOF and facilitate the control of its formation (Tikk, Haugen, Andersen, & Aaslyng, 2008).

A study of the development of warmed-over flavour in cooked meat during refrigeration performed using the AromaScan electronic nose on beef cooked sous-vide has led to sample classification into two distinct classes, namely up to 20 days of storage and over 34 days of storage, with a recognition confidence > 70% (Grigioni, Margaría, Pensel, Sánchez, & Vaudagna, 2000). The correlation between the signals of metal oxide gas sensors and the sensory attributes associated with WOF, as well as with the presence of its potential indicators, namely hexanal, pentanal, pentanol and nonanal has been established (Tikk et al., 2008).

The results reported by the researchers suggest, that there use of electronic olfaction in this area is not as promising as in the case of the detection of bacterial spoilage. In the case of boar taint, due to the relatively low volatility of the chemical indicators other techniques, e.g. liquid chromatography might yield better results (Haugen, Brunius, & Zamaratskaia, 2012).

3.3. Classification

Food adulteration, including adulteration of meat products is a global issue. Analysts are continuously trying to develop methods of verification of both the quality and authenticity of food products. Electronic noses can be used to classify products based on numerous parameters, such as the composition of feed given to animals, processing conditions or meat product composition.

One of the earliest studies specifically targeting the use of electronic noses for classification of various processed meat products was reported by (Vernat-Rossi, Garcia, Talon, Denoyer, & Berdagué, 1996). In it, a commercial e-nose was used to distinguish between 6 distinct types of French dry sausage, and also Iberian hams separated based on their sensory quality into two groups, one of which contained hams in which a defective aroma called *cala* was recognized. It should be noted, that in the case of Iberian hams, the presence of certain bacterial strains, i.e. *Staphylococcus carnosus*, *Staphylococcus xylosum*, *Micrococcus varians* and *Staphylococcus warneri* is considered desirable, as it aids in aroma development. Of the sausages, 94% were correctly classified. A proper classification of hams proved more difficult, with 87% success rate. The cause of that may well lay in the fact that the *cala* aroma is difficult to characterize and its perception may be subjective. The same e-nose model was later used to differentiate between samples of pork based on the animals' diet (González-Martín, Pérez-Pavón, González-Pérez, Hernández-Méndez, & Álvarez-García, 2000). The pigs were given standard feed, acorns, or a combination of the two. The diet of the animals is an important factor contributing to the flavour of the meat, and products from Iberian breed swine are sold at a premium price because of their unique sensory qualities. Processing of the data obtained during the analysis resulted in correct classification of all the samples using LDA. When SIMCA was used, the correct classification was obtained in 72–96% of the cases (depending on the class).

A purpose-built e-nose prototype was also developed in order to control the quality of Iberian hams and the type of fodder given to the animals (Santos et al., 2004). Another study has shown, that using an

electronic nose it is possible not only to discriminate between different sausage samples, but also between salami produced from male or female swine (Taurino et al., 2003). It should be noted that some processed meat like sausages or hams contains additives with distinct aromas, e.g. spices. The aroma of these additives constitutes a large part of the overall aroma profile of the sample. This means that the pattern recognition training sets have to be more specific, and the application less general than in the case of spoilage detection. On the other hand, because of the greater variance in the sample headspace a far better prediction rate can be achieved, often 100%. Also, some methods proposed for the discrimination of these products are relatively time-consuming, with a time of a single analysis exceeding 30 min (García, Alexandre, Gutiérrez, & Horrillo, 2006). This goes against the idea of the electronic nose as a device for rapid classification. However, in many cases the process can be expedited, e.g. by overlapping the incubation of consecutive samples.

Researchers were able to discriminate between samples of minced pork with and without the admixture of beef, as well as between samples of yak meat and beef from animals reared in different geographic locations (Jia et al., 2011). The identification of meat species is relevant to consumers for several reasons. In several religions, e.g. in Judaism, Hinduism or Islam there are strong taboos against eating certain types of meat. In the past less expensive animal protein has been fraudulently used to substitute mutton and beef (Tian, Wang, & Cui, 2013). The identification of pork in samples of minced or otherwise processed meat for the purpose of identification of adulterations using electronic nose systems was successfully performed by several research groups (Nurjuliana, Che Man, & Mat Hashim, 2011; Nurjuliana, Che Man, Mat Hashim, & Mohamed, 2011; Tian et al., 2013).

The attempts to classify different meat samples are not limited to the most commonly consumed kinds of meat, i.e. pork, beef or mutton. Electronic noses were used to distinguish between the cooked meats of domestic camelids, namely llama and alpaca. These animals represent an important food resource for the population of the Andean plateau (Neely, Taylor, Prosser, & Hamlyn, 2001). The classification was performed on the basis of species, but also based on the age of animals, and whether or not they were castrated, using LDA. It is unclear whether the same method could be used to distinguish between samples of raw meat, which would make it possible to detect adulterations prior to purchase and cooking. Other applications of electronic olfaction in quality assessment of assorted types of meat are listed in Table 6.

The reported results indicate that the electronic nose technique can be a useful tool for classification of meat products. Not only is it possible to detect adulterations with different types of meat using devices equipped with relatively inexpensive, commercially available sensors like MOS, but even to verify if the animals were in fact given the declared feed based on the headspace of a finished meat product (e.g. Iberian hams) with good prediction rate – up to 100%.

4. Conclusions

In this paper we have outlined the major applications of electronic noses in meat production and processing. This technique is still under development but the benefits of its use are already evident. One of the greatest advantages presented by the use of e-noses is high correlation of obtained results with data from evaluations by sensory panels - data closer in nature to actual hedonic perception of spoilage. The fact that it is practical to express the results of meat analysis using electronic olfaction in the output units of the more established methods which could potentially facilitate its integration with other procedures (Hong et al., 2012) does not necessarily mean that these methods should be used as a reference for the training of pattern recognition algorithms.

A majority of the reviewed papers used the analysis of the total bacterial count as a standard for validation of the proposed methods. However, good correlation with bacteriological analysis doesn't necessarily imply a good prediction of the actual shelf life. If the spoilage is defined by the onset of unpleasant hedonic characteristics like mal-odour, then the results of an assessment by a trained sensory panel provide a more direct measurement of the samples' headspace than does a bacterial count. The reluctance to employ sensory shelf life tests as reference may come from a belief that they are ultimately subjective in nature. However, if a test is conducted in accordance to a well-established procedure and its results are subject to statistical analysis, the impact of the human factor can be greatly minimized (*Shelf life of foods: guidelines for its determination and prediction*, 1993). Another instrumental method of validating the results obtained using electronic noses is the use of GC-MS which allows to obtain not only a complete aroma profile, but also to determine its individual components both qualitatively and quantitatively.

Many of the electronic noses were developed to investigate prospective use in the meat industry, since the potential benefits of their application in this area, i.e. a short time of a single analysis and capability for continuous operation and integration in- or at-line are undeniable. This interest seems to be shared by the industrial partners who in many instances have provided the researchers with samples and access to operating procedures. A question then remains why electronic olfaction isn't widely used in the industry. Some suggest that the reason for that may lie in the long-term unreliability of electronic olfaction caused by sensor drift. Studies carried out over an extended period of time might be necessary to convince the market of the utility of this technique (Loutfi, Coradeschi, Mani, Shankar, & Rayappan, 2015). Another reason may lie in the fact that the devices developed for research purposes are not cost-effective, while commercial electronic noses may lack the specificity needed to obtain reliable results when analysing a matrix as complex as meat. In order to make the method more feasible for use in industry or at retail level there exists a need to develop cheaper, smaller, more sensitive and selective e-noses designed and trained specifically for evaluation of a particular type of meat or product. Such a device would only be equipped with a few sensors most

Table 6
Applications of electronic noses in quality assessment of various types of meat.

Application	Product	Data processing	E-nose model	Manufacturer/scientific institution	Sensors	Ref.
Classification	Cooked llama and alpaca	LDA	Bloodhound BH 114	Scensive Technologies Ltd.	14 CP	(Neely et al., 2001)
Classification	Goat meat	PCA	Cyranose 320	Sensigent	32 CP	(Ding, Lan, & Zheng, 2010)
Classification	Yak meat	PCA, DFA, PLS	FOX 4000	Alpha M.O.S.	18 MOS	(Jia et al., 2011)
Classification	Mutton fat	PCA	zNose	Electronic Sensor Technology	SAW	(Nurjuliana, Che Man, & Mat Hashim, 2011)
Classification	Mutton	PCA	zNose	Electronic Sensor Technology	SAW	(Nurjuliana, Che Man, Mat Hashim, et al., 2011)
Classification	Ground mutton	PCA, LDA, LR, ANN	PEN 2	Airsense Analytics	10 MOS	(X. Tian et al., 2013)
Spoilage and shelf life	Veal	PCA, SOM	Prototype	Univ. Rome-Tor Vergata	8 QCM	(C. Di Natale et al., 1997)
Spoilage and shelf life	Mutton	PCA, SVM, PLS	Prototype	Moulay Ismail Univ.	6 MOS	(El Barbri et al., 2008)

relevant to the application and would thus cost less than electronic noses designed for various applications (Rajamäki et al., 2006).

A need remains to reliably determine the volatile markers of meat spoilage which would aid in selecting adequate sensors for the sensor matrix, or even in creating sensors dedicated to a particular application. Some compounds already identified as potential spoilage indicators, e.g. diethyl disulphide, have a very low odour detection threshold, and so more sensitive and more specific sensors are needed to detect them. These sensors should also be cheap, reliable and drift-free in order to facilitate the introduction of the electronic nose technology to meat industry.

Abbreviations

ANN	artificial neural network
BAW	bulk acoustic wave
CA	cluster analysis
CFU	colony forming units
CP	conducting polymer
DFA	discriminant function analysis
EC	electrochemical
GC-MS	gas chromatography – mass spectroscopy
HPLC	high-performance liquid chromatography
ICA	independent components analysis
IMS	ion mobility spectroscopy
IR	infra-red
KNN	K-nearest neighbour
LDA	linear discriminant analysis
MAP	modified atmosphere packaging
MOS	metal-oxide-semiconductor
MOSFET	metal-oxide-semiconductor field-effect transistor
PCA	principal component analysis
PLS	partial least squares
QCM	quartz crystal microbalance
QDA	quadratic discriminant analysis
SAW	surface acoustic wave
SIMCA	soft independent modelling of class analogies
SLE	solid-liquid extraction
SPE	solid phase extraction
SPME	solid phase microextraction
SVM	support vector machines
TABRS	thiobarbituric-acid-reactive substances
TVB-N	total volatile basic nitrogen
TVC	total viable counts (of bacteria)
VOC	Volatile organic compounds
WOF	Warmed-over-flavour

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