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Review

Rapid and quantitative detection of the microbial spoilage of muscle foods: current status and future trends

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The requirement for real-time monitoring in the modern and highly automated food processing environment has stimulated research into rapid microbiological testing. This review will concentrate on the search for a rapid detection system for the microbial spoilage of meats that has been ongoing since at least the 1970s. The metabolic processes and bacteria involved within the microbial spoilage of muscle foods will be outlined prior to a detailed overview of the current methods employed in the industry to quantify levels of spoilage organisms. Despite these detailed microbiological studies there is still a requirement within the food industry for new techniques which would ideally be accurate, non-destructive and give answers in real-time and a range of novel analytical technologies which are currently being developed for the *rapid* assessment of microbial spoilage in muscle foods will be examined.

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Introduction

Muscle foods, which include both meat and poultry, are an integral part of the human diet and have been so for several thousand years. However, within the past two decades public concern, as well as awareness, has been raised due to high profile food safety issues such as the BSE and foot and mouth epidemics centred in the UK (Fox, 2001; Pickrell & Enserink, 2001). These outbreaks, along with concerns over specific pathogenic bacteria within meats (and eggs), namely *Salmonella* spp. (Schlundt, 2001; Stock & Stolle, 2001; White *et al*., 2001; Zhang-Barber, Turner, & Barrow, 1999), *E. coli* O157:H7 (Cassin, Lammerding, Todd, Ross, & McColl, 1998; Mariani-Kurkdjian & Bingen, 1999; Tarr, Besser, Hancock, Keene, & Goldoft, 1997; Tuttle *et al*., 1999) and *Campylobacter* spp. (Altekruse, 1998; Altekruse, Stern, Fields, & Swerdlow, 1999; Frost, 2001; Harris, Weiss, & Nolan, 1986; Hopkins & Scott, 1983), have illustrated the requirement for a rapid and accurate detection system for microbial spoilage of meats within what is a large-scale production industry whose turnover is billions of € and $ per annum. At present no such detection system exists within this industry. Whilst parts of the meat and meat products industry may have suffered some losses due to recent events, such as those dealing in beef and lamb, the converse can be said for the poultry industry. This may be in part concomitant with the issues already mentioned but is far more likely to be related to a more health conscious diet and perversely the huge increase in consumption of convenience foods to which poultry, a relatively inexpensive protein source, is ideally suited. This relatively recent change in eating habits has emphasised the requirement for advances in detection systems whereby those used at present are replaced by methods that are truly rapid and accelerate and enhance the detection of microbial spoilage in muscle foods.

Processes

Microbial spoilage of muscle foods

Muscle foods are described as spoiled if organoleptic changes make them unacceptable to the consumer. These organoleptic characteristics can include changes in appearance (i.e. discoloration), the development of off-odours, slime formation or any other characteristic which makes the food undesirable for human consumption (Jackson, Acuff, & J.S., 1997; Jay, 1996). It is

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known that endogenous enzymatic activity within muscle tissue post-mortem can contribute to changes during storage (Alomirah, Alli, Gibbs, & Konishi, 1998; Jackson *et al.*, 1997; Koohmaraie, 1994; Schreurs, 2000). However, it is generally accepted that detectable organoleptic spoilage is a result of decomposition and the formation of metabolites caused by the growth of microorganisms (Braun, Fehlhaber, Klug, & Kopp, 1999; Kakouri & Nychas, 1994; Nychas & Tassou, 1997; Schmitt & Schmidt-Lorenz, 1992; Stutz, Silverman, Angelini, & Levin, 1991). The organoleptic changes which take place will also vary according to the species of microflora present, the characteristics of the meat, processing methods, product composition and the environment in which the food is stored (García-López, Prieto, & Otero, 1998; Jackson *et al.*, 1997).

Meat has been described as the most perishable of all important foods and its moist, nutritious surface is conducive to the growth of a wide range of spoilage bacteria (Jay, 1996; Stanbridge & Davies, 1998). The colonization and growth of microorganisms on meat surfaces occurs in stages, the first of which involves the attachment of bacterial cells. This process has been described as a loose and reversible sorption, which may be related to van der Waals forces or other physicochemical factors (Marshall, Stout, & Mitchell, 1971), one of which may be the population of bacteria within the water film present on the surface of the meat (Chung, Dickson, & Crouse, 1989; Firstenberg-Eden, 1981). The second and irreversible stage of attachment involves the production of a glycocalyx by the bacterium that consists of an adhesive extracellular polysaccharide layer (Costerson, Irvin, & Cheng, 1981). Other factors may also influence the attachment of bacteria to meat surfaces and these include, surface morphology, temperature, growth phase, motility and other bacteria already present on the meat surface (Jackson *et al.*, 1997).

In moist atmospheric conditions, a consortium of bacteria is responsible for spoilage of meat stored at between −1 and 25°C. It is agreed that spoilage organisms primarily belong to the genus *Pseudomonas* (and most often *P. fragi, P. fluorescens* and *P. putrefaciens*), which, compared with several other spoilage bacteria, have been observed to attach more rapidly to meat surfaces (García-López *et al.*, 1998; Jackson *et al.*, 1997; Molin & Ternström, 1982; Stanbridge & Davies, 1998). Other major components of the spoilage flora of meat stored aerobically under refrigeration temperatures include the genera *Moraxella*, *Psychrobacter* and *Acinetobacter*. Whilst the dominant spoilage microflora are generally Gram-negative, motile and non-motile aerobic rods and coccobacilli, the initial population may also contain varying levels of Gram-positive genera usually represented by micrococci, then lactic acid bacteria and *Bronchothrix thermosphacta* (Adams & Moss, 2000; Holzapfel, 1998; Stanbridge & Davies, 1998).

Fresh meats generally have a pH range between 5.5 and 5.9 and contain sufficient glucose and other simple carbohydrates to support approximately 10^9 colony forming units per square centimetre (cfu cm^{-2}). The organisms that grow the fastest and utilize glucose at refrigeration temperatures are the pseudomonads (Gill & Newton, 1977; Jay, 1996; Seymour, Cole, & Coote, 1994). At levels of 10^7 cfu cm^{-2} off-odours may become evident in the form of a faint 'dairy' type aroma and once the surface population of bacteria has reached 10^8 cfu cm^{-2} the supply of simple carbohydrates has been exhausted and recognizable off-odours develop leading to what is known as 'sensory' spoilage (Jackson *et al.*, 1997; Jay, 1996; Stanbridge & Davies, 1998). The development of off-odours is dependent upon the extent to which free amino acid utilization has occurred and these odours have been variously described as dairy/buttery/fatty/cheesy at 10^7 cfu cm^{-2} through to a sickly sweet/fruity aroma at 10^8 cfu cm^{-2} and finally putrid odour at 10^9 cfu cm^{-2} (Adams & Moss, 2000; Dainty, Edwards, & Hibbard, 1985).

The surface of the meat will also begin to feel tacky and this is indicative of the first stages of slime formation, attributable to the growth of bacteria and synthesis of polysaccharides which gradually form a layer on the meat surface (Ingram & Dainty, 1971; Jackson *et al.*, 1997). A deterioration in the colour of meat is due to a fall in the partial pressure of oxygen under patches of microorganisms. Once the population of bacteria approaches its carrying capacity ($\sim 10^8$ cfu cm^{-2}) and glucose has been utilized, the diffusion gradient from the underlying tissue of the meat to the surface cannot meet microbial demand and other substrates are used sequentially until nitrogenous compounds lead to the formation of malodorous substances such as ammonia (NH_3), dimethylsulphide (C_2H_6S) and diacetyl ($C_4H_6O_2$) (Stanbridge & Davies, 1998).

Microbial metabolites

Over the last three decades, numerous attempts have been made to associate given metabolites with the microbial spoilage of meat and to utilize this knowledge to provide information about spoilage and possibly determine remaining shelf-life (Alomirah *et al.*, 1998; Braun *et al.*, 1999; Dainty, 1996; Dainty *et al.*, 1985; Dainty, Edwards, Hibbard, & Marnewick, 1988; Dainty, Edwards, Hibbard, & Ramantanis, 1986; Dainty, Shaw, De Boer, & Scheps, 1975; De Castro, Asensio, Sanz, & Ordonez, 1988; De Pablo, Asensio, Sanz, & Ordonez, 1989; Drosinos & Board, 1994; Edwards, Dainty, & Hibbard, 1985; Ingram & Dainty, 1971; Kakouri & Nychas, 1994; Nychas, Drosinos, & Board, 1998; Nychas & Tassou, 1997; Seymour *et al.*, 1994). Further, whilst microbiological changes on, and to a lesser

extent, within, the meat substrate have been studied in detail, the physicochemical changes that take place during microbial colonization have not been studied in equivalent detail (Jay, 1996; Nychas *et al.*, 1998). The physicochemical changes during the spoilage process occur within the aqueous phase of meat and this phase contains low molecular weight compounds, such as glucose, lactic acid, certain amino acids, nucleotides, urea and water soluble proteins that are catabolized by the vast majority of the meat microflora (Drosinos & Board, 1994; Nychas *et al.*, 1998). The order in which these compounds are catabolized by the major meat spoilage organisms is summarized in Fig. 1.

Spoilage in meats is most frequently associated with the post-glucose utilization of amino acids by pseudomonads and it has been observed that surface levels of glucose decrease significantly as the first signs of the organoleptic changes associated with spoilage become evident. Borch, Berg, and Holst (1991) concluded that glucose limitation caused a switch from a saccharolytic to an amino acid-degrading metabolism in at least some bacterial species. Extensive studies on the metabolic activities of pseudomonads in an extract of minced lamb (Drosinos & Board, 1994) have illustrated that the oxidation of glucose by this genus caused a transient accumulation of D-gluconate and 6-phosphogluconate which coincided with the exponential growth curve of the microflora.

Once surface levels of glucose have been depleted bacteria will metabolize secondary substrates such as free amino acids and lactate. Many bacteria secrete proteases (endoproteases, proteinases, aminopeptidases and carboxypeptidases) and in general Gram-negative bacteria in chilled meat predominantly secrete aminopeptidases (Nychas *et al.*, 1998). This factor alone has been forwarded as a means of acquiring a rapid estimation of the bacterial quality of meat by the use of enzyme assays (Braun *et al.*, 1999; De Castro *et al.*, 1988). The utilization by bacteria of free amino acids leads to an increase in levels of ammonia and it has been observed that the switch from a saccharolytic to an amino acid-degrading metabolism occurs whilst considerable levels of glucose are still present deep within the muscle tissue (Seymour *et al.*, 1994). In addition to ammonia, the by-products of amino acid utilization include sulphides, indole, scatole and amines, such as the diamines putrescine and cadaverine (Adams & Moss, 2000; Dainty *et al.*, 1986, 1988; Jay, 1996; Kumudavally, Shobha, Vasundhara, & Radhakrishna, 2001). It is the production of these compounds, amongst others, that lead to the characteristic changes associated with spoiled meat, such as malodours and the increase in pH.

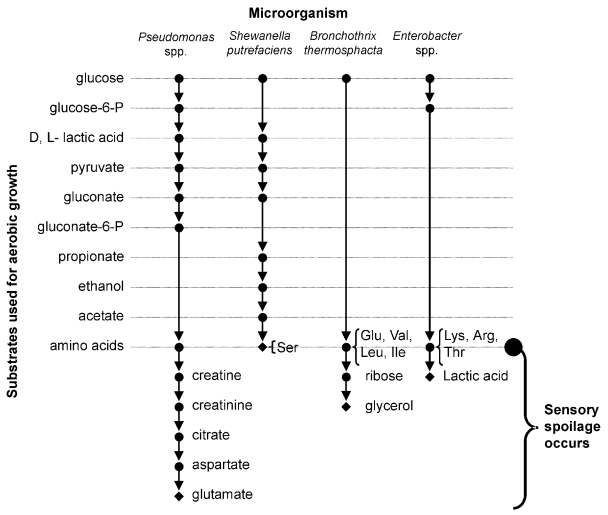

Fig. 1. The order in which muscle food substrates are catabolized by the major meat spoilage organisms. Adapted from aerobic spoilage process data summarized by Nychas *et al.* (1998).

Detection methods

Current status

The conventional microbiological approach to food sampling has changed little over the last half century and it has been estimated that there are currently in excess of 40 methods to measure and detect bacterial spoilage in meats (Betts, 1999; Jay, 1996; Nychas *et al.*, 1998). The development of rapid microbiological test procedures over the last two decades can be divided into two main groups; enumeration and presence/absence tests.

Enumeration methods

Current rapid enumeration methods are generally based on microscopy, ATP bioluminescence or the measurement of electrical phenomena. In the case of microscopic methods sophisticated techniques have been developed where microorganisms are stained with fluorescent dyes and viewed with an epifluorescent microscope. Whilst initial problems such as staining of both viable and non-viable cells were overcome with the introduction of the direct epifluorescent filter technique (DEFT), the procedure is both time consuming and laborious (Pyle, Broadaway, & McFeters, 1999; Restaino, Castillo, Stewart, & Tortorello, 1996; Shaw, Harding, Hudson, & Farr, 1987; Wang & Sharpe, 1998). This process has been aided with the development of fully automated systems and the use of flow cytometry (Rattanasomboon *et al.*, 1999), but results from low levels of microorganisms in food samples can still take 18–20 h to obtain (Betts, 1999) and the spoilage organism has to be disaggregated from the meat surface which, with some organisms forming a glycocalyx layer, is necessarily difficult.

ATP bioluminescence acts by measuring ATP levels in bacterial cells in culture in order to calculate the number of cells present in that culture (Champiat, Matas, Monfort, & Fraass, 2001; de Boer & Beumer, 1999; D'Souza,

2001; Siragusa, Dorsa, Cutter, Perino, & Koohmaraie, 1996). The problem with this method is that ATP is the primary energy source of *all* living cells and the food samples themselves will also contain large amounts of this chemical which have to be destroyed before microbial ATP can be measured. Therefore, the measurement of ATP bioluminescence is probably best suited to detection of contaminated surfaces on equipment and machinery associated with food production and preparation. Electrical measuring methods are based on the detection of electrical current during microbial growth, as changes are caused by bacteria that metabolize uncharged particles in any growth medium, thereby increasing the conductivity of that medium. Commercially available instruments include the Bactometer, Malthus Analyser, Rabit and Bactrac (Betts, 1999; Jay, 1996).

Detection methods

Current detection methods are based on immunological or nucleic acid-based procedures. Immunological methods employ antibodies that are raised to react to surface antigens of specific microorganisms (Betts, 1999; Jay, 1996). The most common form of these methods is the enzyme linked immunosorbent assays (ELISAs) and these are based on the use of an enzyme label. Those in use are currently aimed at the detection of food-borne pathogens such as *Salmonella*, *Listeria*, *E. coli* O157:H7 as well as toxins produced by *Staphylococcus aureus* and proteases from species belonging to the food spoilage genus *Pseudomonas* (Jabbar & Joishy, 1999). Nucleic acid-based procedures utilize probes that are small segments of single-stranded complementary nucleic acid that are used to detect specific genetic sequences in test samples. Nucleic acid probes can be used to detect either DNA or RNA sequences in order to identify accurately a specific microorganism (Alexandre, Prado, Ulloa, Arellano, & Rios, 2001; Venkitanarayanan, Khan, & Faustman, 1996)

The most widely applied nucleic acid detection method at present utilizes the polymerase chain reaction (PCR) (Mullis & Faloona, 1987). This method has been reported to allow for rapid and selective identification and/or detection of microorganisms in different matrices by amplifying specific gene fragments and detecting the PCR amplicons by gel electrophoresis (Cloak, Duffy, Sheridan, Blair, & McDowell, 2001; Gutierrez *et al*., 1998; Scheu, Berghof, & Stahl, 1998; Yost & Nattress, 2000) and thus, like for nucleic acid probes, the DNA sequence of the target organism must be known prior to the analysis. Nevertheless, this method also has inherent limitations for as long as intact nucleic acid sequences are present in a sample they will be amplified by PCR. Therefore, DNA from non-viable microorganisms can lead to false positive results being obtained. Other major problems likely to be encountered when using PCR methods with food are the presence of PCR inhibitors, such as those present within the matrix of cheeses (Jay, 1996; Scheu *et al*., 1998).

The degree of inhibition is entirely dependent on the type of food being sampled and whilst procedures exist to circumvent inhibition, such as dilution of food samples, this also decreases the sensitivity of the test (Scheu *et al*., 1998). The final limitation of PCR is yet again the time factor, as this can be a time-consuming method especially as regards large-scale testing and the tedious and exacting nature of the reaction set-up (Barbour & Tice, 1997).

However, PCR is at present one of the most rapid procedures available for the detection of pathogens in foods with test times for *Salmonella* spp., for example, of approximately 18 h (Warneck, 2001).

Future trends

It is apparent that the range of protocols currently undertaken to determine the presence, type and enumeration of microorganisms and their metabolic products all have inherent limitations. Whilst some methods are superior to others and most give adequate results, the major drawback at present is the time taken to obtain results, which because they are so slow give retrospective information. This can be a major drawback within the food industry as monitoring procedures, such as the Hazard Analysis Critical Control Point (HACCP) system, need to give results in real-time to enable corrective action to be taken as soon as possible within busy and highly automated processing environments. The ideal method for the on-line microbiological analysis of meat would be rapid, non-destructive, reagentless, quantitative and relatively inexpensive and at present no such method exists within the meat industry. The majority of studies within the literature have concentrated on refinement of current methods and in particular immunological (Jabbar & Joishy, 1999) and nucleic acid-based approaches (Cloak *et al*., 2001; Warneck, 2001), whilst others have made significant improvements in their technique in terms of rapidity by targeting *specific* metabolites with accurate chromatographic separation and relate the levels of the spoilage indicator cadaverine to the bacterial numbers within 1.5–2 h (Kumudavally *et al*., 2001).

Some of the most interesting analytical approaches being forwarded for the rapid and quantitative detection of microbial spoilage in meats could fall under the generic heading of biosensors. These include most notably enzymatic reactor systems with amperometric electrodes for the determination of the quality of chicken by sensing diamine levels (Okuma, Okazaki, Usami, & Horikoshi, 2000; Suzuki, Usami, Horikoshi, & Okuma, 2001; Yano, Yokoyama, Tamiya, & Karube, 1996). It has been reported that accurate results were possible within 5 min from one of these studies (Suzuki

et al., 2001) however, this was preceded by 10 min sample preparation for the enzyme reactor system and would therefore not be conducive to non-invasive on-line monitoring. However, this is a significant and desirable improvement in rapidity in comparison to current techniques.

Electronic noses were first developed in the mid 1980s and are essentially an instrument comprised of an array of electronic chemical sensors with partial specificity and an appropriate pattern recognition system capable of recognizing simple or complex odours (Craven, Gardner, & Bartlett, 1996; Gardner & Bartlett, 1994, 1999) (the details of the electronic nose system are given in Fig. 2). These instruments contain an array of sensors that utilize a variety of different sensor technologies including organic polymers, metal oxides and microbalances (Harper, 2001). Whilst these instruments have only recently become available commercially and are still in the developmental phase they are likely to have many potential applications in the future including rapid and non-invasive detection of spoilage and a range of quality attributes in foods, including muscle foods.

The rapid and quantitative detection of microbial volatiles associated with muscle food spoilage would seem a logical route to follow since this mirrors our own organoleptic olfactory interpretation of sensory spoilage, and indeed this approach has been attempted already in terms of analysis of both meat and fish (Di Natale *et al*., 1997, 2001; Haugen, 2001; Schaller, Bosset, & Escher, 1998; Ziegler *et al*., 1998). However, there are several *severe* weaknesses to overcome including; loss of sensitivity in humid conditions or high concentrations of alcohol (for example); very significant instrumental drift, even within a day, and the inability to provide absolute calibration; sensor life-span and the incapability to provide quantitative data for aroma differences (Harper, 2001). Despite the current limitations associated with electronic noses they have stimulated a great deal of research activity and it is anticipated that they will find a range of applications within the food industry within the next decade, provided the above limitations are adequately addressed. Many of the drift problems are associated with the use of chemical sensors and whilst this could be overcome by suitable mathematical transformation routines as employed for other analytical approaches (Goodacre & Kell, 1996; Goodacre *et al*., 1997), the utilization of a mass spectrometer detector for headspace analysis may greatly improve detection.

Fourier transform infrared (FT–IR) spectroscopy is a non-destructive analytical technique with considerable potential for application in the food and related industries (van Kempen, 2001). For FT–IR a particular bond *absorbs* light (or electromagnetic (EM) radiation) at a specific wavelength (for example, the infrared spectra of proteins exhibit strong amide I absorption bands at 1653 cm^{-1} associated with the characteristic stretching of C=O and C–N and the bending of the N–H bond (Stuart, 1997)), therefore, by interrogating a food sample with EM radiation of many wavelengths in the mid-IR range (usually defined as 4000–600 cm^{-1}) one can construct an infrared absorbance spectrum which can be considered as a 'fingerprint' which is characteristic of any (bio)chemical substance (Gillie, Hochlowski, & Arbuckle-Keil, 2000; Schmitt & Flemming, 1998; Stuart, 1997). This technique is very rapid (taking seconds) and has been shown to be a valuable tool for the rapid and accurate characterization of *axenically* cultured bacteria (Goodacre, Rooney, & Kell, 1998; Goodacre, Timmins, *et al*., 1998; Goodacre, Timmins, Rooney, Rowland, & Kell, 1996; Lang & Sang, 1997; Naumann, Helm, & Labischinski, 1991; Naumann, Helm, & Schultz, 1994; Naumann, Schultz, & Helm, 1996; Timmins, Howell, Alsberg, Noble, & Goodacre, 1998), including antibiotic resistance profiling (Goodacre, Rooney, *et al*., 1998; Goodacre, Timmins, *et al*., 1998) and single gene knockout strains (Oliver, Winson, Kell, & Baganz, 1998).

Whilst a number of studies have applied this technique to the discrimination and adulteration of meats (Al-Jowder, Defernez, Kemsley, & Wilson, 1999; Al-Jowder, Kemsley, & Wilson, 1997; Downey, McElhinney, & Fearn, 2000; Rannou & Downey, 1997), its application in terms of rapidly detecting microbial spoilage in meats is only recently under investigation in our laboratory (Ellis, Broadhurst, Kell, Rowland, & Goodacre, 2002). A particularly robust and reproducible form of this method is attenuated total reflectance (ATR) where the food sample is placed in intimate contact with a crystal of high refractive index, such as diamond, germanium, zinc selenide or thallium iodide (as illustrated in Fig. 3) and an IR absorbance spectrum collected in just a few seconds. In the form of an on-line fibre optic probe and in combination with the appropriate statistical methods and calibration, we believe it could have the potential for rapid and quantitative enumeration of the total viable counts of bacteria on the surface of meat. We have already discussed that the spoilage in meat is the result of the decomposition and formation of metabolites caused by the growth and enzymatic activity of microorganisms. With the FT–IR approach one is able to acquire a *metabolic snapshot* of the meat thus this information is exploited and rather than detecting the presence of bacteria *per se* on the meat surface, FT–IR can be used to measure biochemical changes within the meat substrate, enhancing and accelerating the detection of microbial spoilage (Ellis *et al*., 2002).

Machine learning

The main hurdle that needs to be overcome when exploiting the advanced analytical technologies detailed

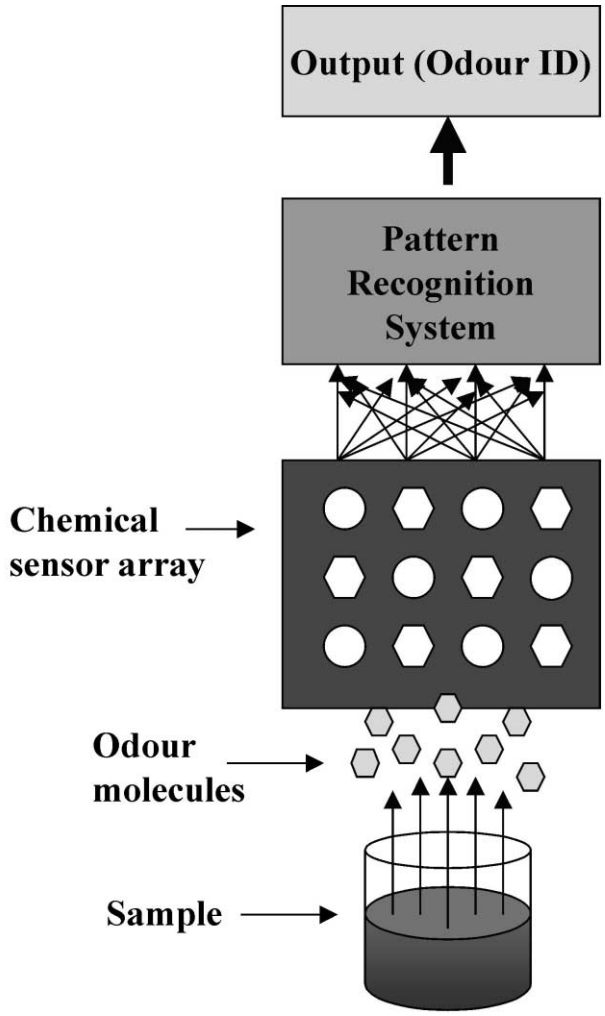

Fig. 2. Generalised schematic of an electronic nose employing chemical sensors. For details of the pattern-recognition system, which to date are based on neural networks please refer to the machine learning section.

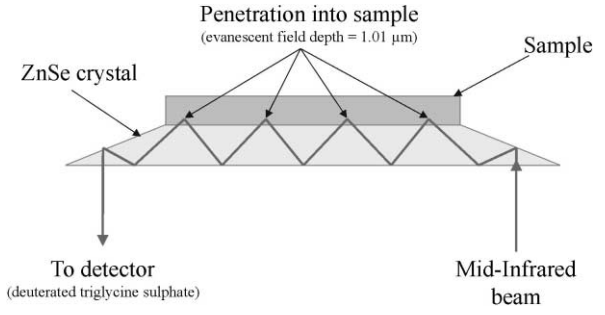

Fig. 3. A cartoon depicting the analysis of a (meat) sample by Fourier transform infrared (FT–IR) spectroscopy using horizontal attenuated total reflectance (HATR).

above is that the floods of data produced by these methods may seem unmanageable. This is particularly true when one is measuring the metabolome (Oliver *et al*., 1998) and generating 'fingerprint'-like metabolite profiles (Fiehn *et al*., 2000; Kell & Mendes, 2000; Raamsdonk *et al*., 2001; Trethewey, 2001). These data are complex and since 100s to 1000s of different variables are collected are multidimensional in nature. Each variable may be regarded as constituting a different dimension, such that if there are *n* variables each object (thing measured) may be said to reside at a unique position in an abstract entity referred to as *n*-dimensional hyperspace. This does not easily lend itself to simple visual interpretation!

Conventionally the reduction of the multivariate data generated has normally been carried out using principal components analysis [PCA; (Joliffe, 1986)] or clustering algorithms [discriminant analyses and hierarchical clustering (Manly, 1994)]. These are *unsupervised* learning methods in which the relevant multivariate algorithms seek "clusters" in the data (Everitt, 1993). This allows the investigator to group objects together on the basis of their perceived closeness in the *n*-dimensional hyperspace referred to above. Such methods, although in some sense quantitative, are better seen as qualitative since their chief purpose is merely to *distinguish* objects or populations. However, with the advent of modern machine learning approaches, which employed *supervised* learning algorithms (Beavis *et al*., 2000; Goodacre, 2000; Lavine, 1998; Massart *et al*., 1997; Shaw *et al*., 1999), the opportunity now exists to analyse such complex high dimensional spectral patterns and form a model (mathematical transformation) that correctly associates the multivariate inputs with a specific target answer to some pre-determined question (the so-called 'gold' standard) of biological interest which has much-lower dimensionality. Of particular interest to the food spoilage areas will be, for example, a qualitative question like "What is the contaminating organism?" and a quantitative one like "What is the bacterial load on the meat surface?".

In machine learning there are a variety of algorithms that can be employed depending on whether the analysis is quantitative or qualitative in nature (for excellent introductory texts see Cartwright, 2000; Cawsey, 1998; Massart *et al*., 1997; Rich & Knight, 1991). Table 1 gives a list with a brief summary of the salient features of the most common supervised learning methods that are employed for the analysis of multivariate data. Over the last decade artificial neural networks (ANNs) (Bishop, 1995; Ripley, 1996; Wasserman, 1989) have been highly popular because of the availability of powerful desktop PCs in conjunction with the development of several user-friendly packages which can simulate such ANNs *in silico* (Goodacre, 2000). However the mathematical transformation from multivariate data to the target question of interest is largely inaccessible and ANNs are often perceived as a 'black box' approach to modelling spectra. It is known from the statistical literature that better predictions can often be obtained when only the most relevant input

Table 1. Features of common supervised learning algorithms

Method	Significant features	Qualitative or quantitative	References
Discriminant function analysis (DFA)	Cluster analysis based method. Involves projection of test data into cluster space	Qualitative	(Manly, 1994)
Partial least squares (PLS)	Linear regression based method	Quantitative	(Martens & Næs, 1989)
Discriminant partial least squares (DPLS)	Linear regression based method	Qualitative	(Martens & Næs, 1989)
Artificial neural networks (ANNs)	Can learn non-linear as well as linear mappings Most popular varieties are multilayer perceptrons (MLPs) and radial basis functions (RBFs)	Both	MLPs (Rumelhart *et al.*, 1986; Werbos, 1994) RBFs (Saha & Keller, 1990)
Rule induction	Often produces interpretable rules includes classification and regression trees (CART) and fuzzy rule-building expert system (FuRES).	Qualitative	CART (Breiman, Friedman, Olshen, & Stone, 1984) FuRES (Harrington, 1991)
Inductive logic programming (ILP)	Constructs general rules by inductive inference	More qualitative than quantitative	(Lloyd, 1987)
Evolutionary computation (EC)	Often produces interpretable rules/genetic code/parse trees. Includes genetic algorithms (GAs), genetic programming (GP) and genetic computing (GC)	Both	EC (Bäck, Fogel, & Michalewicz, 1997) GAs (Holland, 1992) GP (Koza, 1992) GC (Kell *et al.*, 2001)

variables are considered (Kell & Sonnleitner, 1995; Miller, 1990; Ripley, 1996; Seasholtz & Kowalski, 1993). Thus the best machine learning techniques should not only give the correct answer(s), but also identifying a subset of the variables with the maximal explanatory power thereby providing an interpretable description of what, in biological terms, is the basis for that answer (Kell, Darby, & Draper, 2001). Such explanatory modelling methods do exist and are based on rule induction, inductive logic programming, and most recently evolutionary computation (see Table 1 for details).

Evolutionary computational-based methods are currently particularly popular inductive reasoning methods based on the concepts of Darwinian selection to generate and to optimize a desired computational function or mathematical expression to produce so called explanatory 'rules'. These techniques include genetic algorithms (GAs), genetic programming (GP) and genomic computing (GC), and because the models are in English and, by penalizing complex expressions, may be made to be comparatively simple. These methods have been employed to deconvolve and interpret multivariate metabolome data in chemical terms (Broadhurst, Goodacre, Jones, Rowland, & Kell, 1997; Goodacre *et al.*, 2000; Johnson *et al.*, 2000; Kell *et al.*, 2001; McGovern *et al.*, in press), and with particular relevance to food spoilage it has been shown (Ellis *et al.*, 2002) that GP can be used to derive rules showing that at levels of 10^7 bacteria cm^{-2} the main biochemical indicator of spoilage as measured by FT–IR was the onset of proteolysis.

Conclusion

Current methods for the rapid detection of spoilage in meats are inadequate and all have the same recurring theme in that they are time consuming, labour intensive and, therefore, give retrospective information. The processes involved in the microbial spoilage of meats are well established and for three decades microbial metabolites have been forwarded as potential indicators of organoleptic spoilage and remaining shelf-life. Despite this knowledge the ability to correlate biochemical change with microbial biomass is a complex problem, and perhaps only very recently surmountable. With continuous advances in analytical instrumentation coupled with the realization that miniaturization instrumentation is assuming increasing importance (McClennen, Arnold, & Meuzelaar, 1994), as computers processing speeds get more powerful, as our understanding of complex multivariate spectroscopic data and their machine learning interpretation deepens, it will not be long before the so-called 'rapid' detection methods used at present are replaced by those which are truly rapid and detect quantitatively microbial spoilage in meats within seconds as opposed to hours.

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