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Modern Trends in Detection of Microbial Spoilage of Muscle Foods - A Review

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Abstract

Meat spoilage and detection is very important for meat technologists, quality control agencies, and the meat industry. Thus the microbial spoilage of meat and detection methods is widely studied. For decades microbial metabolites have been used as indicators of organoleptic spoilage of meat. Detection of spoilage by screening for microbial metabolites without identifying specific bacteria is the common approach. The ability to correlate biochemical change with microbial biomass is a complex problem. Current methods for the detection of spoilage in meats are inadequate, time consuming and labour intensive. ATP bioluminescence and fluorescent methods are already in use and are popular due to quick results. Molecular methods like multiplex PCR to detect a group of spoilage bacteria have already been tried. The concept of electronic nose using the odour sensors, conducting organic polymers and metal oxide conductors are promising method. The modified spectroscopy widely studied for detection of meat spoilage. Fourier transform infrared spectroscopy, electrical impedance spectroscopy, near infrared spectroscopy is some of those methods. The use of laser especially the laser speckle imaging is already put into use in this field. Latest technology introduced in the field of meat spoilage detection is the use of smart phone with different attachments. This paper briefly reviews the microbial spoilage of meat and microbial metabolites, then discusses about the currents methods for detection of spoilage and the modern upcoming methods with their potential.

Keywords: Meat Spoilage; Microbial Spoilage; Detection of Spoilage; Modern Technique

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. Meat and meat products are integral part of human diet and any issue pertaining to the acceptability of meat is of great concern. Meat is rich in nutrient and the first-choice of animal protein for people all over the world and the consumption is increasing steadily [1]. On the other hand, a significant portion of meat and meat products are spoiled every year and approximately 3.5 billion kg of poultry and meat were wasted at the consumer, retailer and foodservice levels which have a substantial economic and environmental impact. Significant portion of this loss is due to microbial spoilage [2]. Meat is not only nutritious to consumers, but

is also excellent source of nutrients for microbial growth. The concept of meat deterioration and detection is always an area of interest for the food technologists, food quality control agencies, and the meat industry. Meat spoilage is the result of decomposition and the formation of metabolites by the growth of microorganisms. The microbial spoilage detection thus is very important and direct and indirect methods of detection of spoilage bacteria are widely studied [3].

The spoilage can be also due to autolytic, oxidative and other chemical changes which can happen to meat during storage and supply. At the level of consumer, detection of spoilage can be really challenging. Most of the earlier methods were focussing on the spoilage detection directly on meat; whereas, now in most of the cases consumer is not getting raw meat but mostly ready to eat products [4]. In this situation the detection of spoilage is becoming more challenging as most of the ready to eat foods have contents other than meat. Despite these detailed microbiological studies there is still a requirement within the meat industry for new techniques which would ideally be accurate, non-destructive and give answers in real-time.

This review concentrates on the search for a rapid detection system for the microbial spoilage of meats. The bacteria involved within the microbial spoilage of muscle foods and their metabolic processes is briefly outlined prior to a brief overview of the current methods employed in the industry to quantify levels of spoilage organisms and future trends. A range of novel analytical technologies which are currently being developed for the rapid assessment of microbial spoilage in muscle foods are also highlighted.

Microbial Spoilage of Muscle Foods

Muscle foods are described as spoiled if organoleptic changes make them unacceptable to the consumer [5]. These organoleptic characteristics can include changes in appearance (i.e. discoloration), the development of odours, slime formation or any other characteristics which make the food undesirable for human consumption [6]. It is generally accepted that detectable organoleptic spoilage is a result of decomposition and the formation of metabolites by the growth of microorganisms [7]. The organoleptic changes vary according to the species of microflora present, the characteristics of the meat, processing methods, product composition and the storage conditions [8].

The first stage of colonization and growth of microorganisms is the attachment of bacterial cells on meat surfaces. The second and irreversible stage involves the production of a glycocalyx by the bacterium [9]. Main spoilage organisms belong to the genus *Pseudomonas* and other major spoilage flora of meat stored aerobically under refrigeration include the *Moraxella, Psychrobacter* and *Acinetobacter*.

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 cfu /cm^2. The pseudomonads grow fastest and utilize glucose at refrigeration temperatures [10]. At levels of 10^7 cfu/ cm^2 odours may become evident in the form of a faint 'dairy' type aroma and once it has reached 10^8 cfu /cm^2 the recognizable off odours develop leading to 'sensory' spoilage [11]. The development of off odours depends upon the free amino acid utilization. These odours are described as dairy/ buttery/fatty/cheesy at 10^7 cfu/ cm^2; a sickly sweet/fruity at 10^8 cfu /cm^2 and finally putrid odour at 10^9 cfu/ cm^2 [12]. The surface of the meat will be tacky, indicating the first stages of slime formation due to bacterial growth and synthesis of polysaccharides. Deterioration in the colour of meat is due to a fall in the partial pressure of oxygen. Once the population of bacteria approaches 10^8 cfu/ cm^2, the nitrogenous compounds lead to the formation of malodorous substances such as ammonia (NH_3), dimethylsulphide (C_2H_6S) and diacetyl ($C_4H_6O_2$) [11].

Microbial Metabolites

Numerous attempts have been made to associate metabolites with the microbial spoilage of meat and to provide information about spoilage and possibly determine remaining shelf-life [7]. The physico-chemical 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 [13]. Once surface levels of glucose have been depleted bacteria will metabolize secondary substrates such as free amino acids and lactate. Borch, et al. [14] concluded that glucose limitation caused a switch from a saccharolytic to an amino acid degrading metabolism in at least some bacterial species. Many bacteria secrete proteases and in general Gram-negative bacteria in chilled meat predominantly secrete amino-peptidases [13]. In addition to ammonia, the by-products of amino acid utilization include sulphides, indole, scatole and amines, such as the diamines putrescine and cadaverine [12]. 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.

Current Status of Detection Methods

The conventional microbiological approach to food sampling has changed little over the last half century and it has been estimated that there are currently more than 40 methods to measure and detect bacterial spoilage in meats [13,15]. 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. The problems such as staining of both viable and non-viable cells were overcome with the introduction of the direct epifluorescent filter technique (DEFT), but the procedure is time consuming and laborious [16]. Though fully automated systems with the use of flow cytometry was developed [17], but results from low levels of microorganisms can still take 18-20 hours [15] and disaggregation of the spoilage organism from the meat is 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 [18,19]. The problem with this method is that ATP present in meat has to be destroyed before microbial ATP can be measured. 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 [15].

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 [15]. 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 food spoilage genus *Pseudomonas* [20]. 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 [5].

The most widely applied nucleic acid detection method at present utilizes the polymerase chain reaction (PCR) [21]. 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 [22,23]. Thus, for nucleic acid probes, the DNA sequence of the target organism must be known prior to the analysis. This method has 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 micro-organisms can lead to false positive results. The final limitation of PCR is yet again the time factor, as this can be a time-consuming method especially for large-scale testing and the tedious and exacting nature of the reaction set-up [24]. 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 [5,25].

Modern Trends

Measuring microorganisms in food products is a critical issue for food safety and human health. Although various approaches for detecting low-levels of microorganisms in food have been developed, they require high cost, complex equipment, invasive procedures, and skilled technicians which limit their widespread use in the food industry. 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. While some methods are superior to others and most give adequate results, the major drawback at present is the time taken to obtain results. This can be a major drawback within the food industry as monitoring procedures, such as the Hazard Analysis Critical Control Point (HACCP) system. The HACCP 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 requirement for real-time monitoring

in the modern and highly automated food processing environment has stimulated research into rapid microbiological testing [5].

The ideal method for the online microbiological analysis of meat would be rapid, non-destructive, reagent less, 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 [20] and nucleic acid-based approaches [20,25]. There is significant improvements in other 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 [26].

Biosensors

Biosensors are defined as indicators of biological compound that can be as simple as temperature sensitive paints or as complex as DNA-RNA probes. The potential application of biosensor technology to food testing offers several attractive features. Many of the system are portable, can be used on site in time, rapid and are capable of testing multiple analyses simultaneously. Biosensor is an analytical approach for the rapid and quantitative detection of microbial spoilage in meats. These include most notably enzymatic reactor systems with amperometric electrodes for the determination of the quality of chicken by sensing diamine levels [27,28]. It has been reported that accurate results were possible within 5 min from one of these studies [28]. However, this was preceded by 10 min sample preparation for the enzyme reactor system and would therefore not be conducive to non-invasive online monitoring. However, this is a significant and desirable improvement in rapidity in comparison to current techniques [5].

Electronic Noses

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 [29,30]. These instruments contain an array of sensors that utilize a variety of different sensor technologies including organic polymers, metal oxides and micro-balances [31]. 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 reflects our own organoleptic and olfactory interpretation of sensory spoilage of both meat and fish [32,33]. However, there are several weaknesses to overcome including; loss of sensitivity in humid conditions; very significant instrumental drift, and the inability to provide absolute calibration; sensor life-span and the incapability to provide quantitative data for aroma differences [31]. 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 this could be overcome by suitable mathematical transformation routines [34,35], the utilization of a mass spectrometer detector for headspace analysis may greatly improve detection.

Fourier Transform Infrared (FTIR) Spectroscopy

Fourier transform infrared (FTIR) spectroscopy is a non-destructive analytical technique with considerable potential for application in the food and related industries [36]. For FTIR a particular bond absorbs light at a specific wavelength, therefore, by interrogating a food sample with EM radiation of many wavelengths in the mid-IR range, one can construct an infrared absorbance spectrum which can be considered as a 'fingerprint' which is characteristic of any chemical substance [37]. 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 [38,39].

A number of studies have applied this technique to the discrimination and adulteration of meats [40]. In attenuated total reflectance (ATR) the food sample is placed in intimate contact with a crystal of high refractive index, and an IR absorbance spectrum collected in just a few seconds [41]. An online fibre optic probe in combination with the appropriate statistical methods, it can enumerate the total viable counts of bacteria on the meat surface. With the FTIR approach the metabolic snapshot of the meat can be acquired. Thus rather than detecting the presence of bacteria in the meat, FTIR can be used to measure biochemical changes within the meat, enhancing and accelerating the detection of microbial spoilage [22,41]. Rapid monitoring of the spoilage of minced beef stored under conventionally and active packaging conditions, using FTIR spectroscopy in tandem with chemometrics using video meter has been reported.

They concluded that the color and surface chemistry changes during meat spoilage may be monitored using Video meter Lab and heterogeneity of changes may be measured by a canonical discriminant function (CDF).

Laser Speckle Decorrelation

A simple, non-destructive, non-contact, and rapid optical method for measuring living microorganisms in meat products using laser speckle decorrelation reported [42]. By simply measuring dynamic speckle intensity patterns reflected from samples and analyzing the temporal correlation time, the presence of living microorganisms could be non-invasively detected with high sensitivity. They reported demonstrations for detecting *E. coli* and *B. cereus* in chicken breast tissues. Although it could be used for the detection of bacterial activity, it is not able to identify different pathogenic bacterial strains, such as *Salmonella*, *Listeria*, *B. cereus*, *E. coli*, and *Campylobacter*. Nonetheless, the method can be potentially used for the detection of bacterial activity to avoid food toxicity or to perform prescreening tests.

Electrochemical Impedance Spectroscopy (EIS)

This method offers a simple, rapid and in situ measurement of the onset of spoilage. The determination of fish freshness by EIS using a home-made needle type electrode (NTE) has been described [43]. In situ tissue freshness measurements were carried out simply by inserting the NTE into the fish body and starting the EIS. Determinations from 3 fish species using the NTE EIS system confirmed that the dielectric properties of fish tissue are spoilage dependent. Good correlations between the extent of fish spoilage and several EIS parameters were found, suggesting the usefulness of this approach as a rapid and effective detection of fish freshness. The phase angle and admittance changes are the best freshness indicators, from which four classifications of freshness may be defined for all of the tested fish species. Four categories of freshness: fresh, semi-fresh, semi-deteriorated and deteriorated, could be easily established by inserting the NTE into the fish body and measuring the tissue dielectric properties by EIS. The NTE-EIS system is a prompt and simple in-situ procedure and can easily be adopted for automation in practical applications.

Smartphone-Based Technologies

A new generation of mobile sensing approaches offers significant advantages over traditional platforms in terms of test speed, control, low cost, ease-of-operation, and data management, and requires minimal equipment and user involvement. This novel field of research represents a promising area that has high scientific and commercial impact. Advancements in chemistry, biotechnology and engineering have led to new diagnostic platforms which are more portable, economical and easier for food analysis than lab-based assays. Further, the universal presence of mobile phones makes it suitable for on-site testing.

Recent developments in the field of smartphone-based food diagnostic technologies, are reviewed [44]. These devices typically comprise multiple components such as detectors, sample processors, disposable chips, batteries and software, which are integrated with a commercial smartphone. One of the most important aspects of developing these systems is the integration of these components onto a compact and light weight platform that requires minimal power. To date, researchers have demonstrated several promising approaches employing various sensing techniques and device configurations. The food scanning devices that are increasingly populating the Internet of Things (IoT) market, demonstrating how this field is indeed promising, as the research outputs are quickly capitalized on new start-up companies.

Conclusion

The processes involved in the microbial spoilage of meats are well established and for three decades microbial metabolites have been used as potential indicators of organoleptic spoilage and remaining shelf life. With advances in analytical instrumentation coupled with miniaturization instrumentation is assuming increasing importance. Application of biosensors, electrochemical impedance spectroscopy, fourier transform infra-red spectroscopy, laser technology, smart phone based techniques are some of the promising trends for the rapid detection of spoiled meat. As computers processing speeds becoming faster with our understanding of complex multivariate spectroscopic data, the so called 'rapid' detection methods used at present are likely to be replaced by truly rapid methods which can detect microbial spoilage in meats quantitatively within seconds as opposed to hours.

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