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Review Article

Microbial Spoilage of Meat and It's Detection - A Review

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Abstract

Spoilage of meat is a metabolic process which occurs if the meat is left untreated under unsuitable conditions like low oxygen, high water activity & low temperature, hence it becomes unacceptable for human consumption. Meat spoilage is generally caused by unavoidable infections & decomposition of meat by bacteria & fungi. The microorganisms usually come in contact with the meat through the person handling the meat or borne in the animal itself. Unattractive odours & flavours, discolouration, gas & slime are produced by the microbes associated with deterioration of meat. For so long, food industry has been looking for technologies to detect deterioration of meat. Few detection methods are being used in food industry these days to ensure freshness of meat & customer satisfaction such as spectroscopy, use of AI methods, biosensors etc. In the present review article, it has been focused on the detection of meat spoilage by FT-IR spectroscopy with PLS Analysis method, Xanthine Oxidase enzyme biosensor method & Enumeration method.

Keywords: Spoilage of meat; Water activity; Spectroscopy; AI methods; Biosensors; Xanthine; Oxidase enzyme; Enumeration Method.

Introduction

The demand for poultry and poultry products is increasing day by day in both international and domestic markets [1] in order to protect and maintain human-health. Safety and quality are considered as the most important concerns in the food industry as they are directly related to get healthy life. Our human body requires proper and maintained diet comprising of all required nutrition to carry on the daily life-functions [2].

This required diet contains macro - nutrients and micro nutrients which can be found from a number of food source including cereals, meat, legumes, fruits, milk and vegetables [3]. Being nutritious, meat provides energy to the human body to perform daily activities by fulfilling the requirements needed by the body [4]. Meat is considered as a rich source of amino acids, essential fatty acids and vitamins [5] .

Meat is a good source of nutrition and different kinds of meat (e.g., chicken mutton, lamb, beef, fish etc.) has different composition with different nutritional values [6]. Due to increase in concerns of consumer over food quality and food safety, it becomes a very important need for Industries to ensure safe, unspoiled and healthy food to the consumer as food is related to increase in morbidity, mortality, human suffering and also affects economy [7]. To comply with the need a momentous effort made by meat industry has been observed to ensure the quality and safety of meat products which includes food spoilage, food poisoning and food waste [8].

Meat gets spoiled for various reasons but microbiological spoilage in meat occurs with the growth of microorganisms. The spoilage of meat is identified by its organoleptic changes which make the meat unacceptable to eat [9]. These changes include appearance (discoloration), changes in odours, changes in taste, slime formation. This may result from formation of metabolites, decompositions, conversion of organic and inorganic matter and by enzymatic activities which occur within tissues after post-mortem can lead to change during storage time due to the growth of microorganisms [9]. Basically, food gets rejected when it gets spoiled by triggering some characteristics which makes the meat unacceptable to the consumer [10].

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Microorganisms present in meat cause proteins and fats to break down, spoiling the meat, or breed and reach levels by making it unsafe for human consumption. But these organoleptic changes vary with microflora's species, environment, product composition, and characteristics of meat [9].

It has been observed that even with modern preservation techniques, a huge amount of food wastes due to spoilage [11,12] With the standpoint of microbial ecology, microbial spoilage can be defined on basis of three basic mechanisms which are responsible for meat spoilage and they are - microbial growth, enzymatic autolysis and oxidation. Also, lipid oxidation and enzyme reactions play a vital role in meat spoilage and all this criteria breakdown the protein, carbohydrates and fats present in meat and finally they result in off odours, slime formation and off-flavours which makes the meat unacceptable to consume [13-15].

Beyond this, packaging, storage temperature, pH, water activity, product's characteristics, bio preservatives and many factors affects meat spoilage [12,14]. During storage the growth rate, cell number and the lag phase's duration highly depends on the storage temperature [16].

The organoleptic spoilage in meat occurs due to decomposition and formation of metabolites which is the result of microbial growth. This can be detected through FT-IR spectroscopy with PLS Analysis [9]. Along with this another most important biochemical post-mortem reaction in meat occurs due to decomposition of nucleotides where increase in inosine concentration happens due to Rapid degradation of ATP which is then due to microbial metabolism transformed into hypoxanthine and xanthine [17].

The ratio of the quantity of hypoxanthine(Hx) and inosine(HxR) to the total quantity of ATP and related elements, is defined as K-value which is commonly used as index for meat freshness. With this k-value and Nano material mediated, the enzyme xanthine oxidase (XOx) biosensor spoilage of meat can be detected rapidly. These two methods are very useful tools for rapid detection of meat spoilage [17]. Apart from these two another method, which is enumeration method also serves as a great way to detect spoilage in meat.

Spoilage of meat

Meat is categorised into one of the most perishable food products due to the presence of chemical and enzymatic activities. Moreover, meat provides a suitable environment for the growth of a wide variety of microorganisms. The three basic mechanisms responsible for the spoilage of meat is microbial growth, oxidation and enzymatic autolysis [18].

Lipid oxidation is responsible for the chemical detoriation of meat. It starts taking place in the muscles of the living animal and increases after slaughtering takes place. It happens because of the changes that takes place in the environment and loss of intrinsic antioxidant capacity. [19]

The overall basic mechanism of oxidation takes place in three steps:
1. Oxidation substrates such as oxygen and fatty acids starts disappearing.
2. formation of the primary products of oxidation i.e., peroxides and hydroperoxides takes place.
3. Formation of products like aldehydes, alcohols and other volatile and non-volatile compounds which are basically the secondary products of oxidation.

Meat tissues autolyses rapidly in ordinary temperatures. Autolysis of meat basically refers to the decomposition of protein. Generally, autolysis is caused by different set of catalysts. In autolysis, along with protein, fat also decomposes making the meat more acid and rancid as well [20].

Factors responsible for microbial spoilage of meat

There are four different factors on which microbial spoilage of meat or any food products depend upon [21].

Intrinsic factors

Intrinsic factors are basically the parameters that are inherent to the food product itself. It implies the chemical and physical characteristics of the food product. This may include: water activity, pH, moisture content, nutrient content, and physical structure of the food.

Extrinsic factors

Extrinsic factors refer to the environmental factors which generally affects both, the food product as well as the growth of microorganisms

This may include temperature, relative humidity, presence of carbon dioxide and oxygen.

Implicit factors

These factors are the result of mutual interactions that takes place in mixed microbial communities. It includes synergism, antagonism, and growth rate.

Processing factors

Microbial growth also takes place during the processing of the food product which may include bacterial treatment, washing, slaughtering (in case of meat), and packaging.

Microorganisms responsible for meat spoilage

Unacceptable food which termed as spoiled food is result of metabolic process [22]. Meat is an ideal media for microorganisms to reproduce as it provides all required nutrition for growth [23]. It is frequently observed in aerobically stored meat to omit undesirable odours (like cheesy, putrid, sweet, sulphuric and fruity) [24]. Though oxygen is required for microorganisms to survive but there are many microorganisms which can survive without oxygen and some are facultative bacteria [25]. Spores spore forming bacteria and yeasts are the main reason for meat spoilage where spores can survive even in a low oxygenated ambience [26]. As 37°C is the optimal temperature for *E. coli's* growth [27] and as minimum 4°C is required for bacterial growth, meat should be refrigerated below 4°C to avoid spoilage [28].

In meat spoilage it starts with an average of 10^2 to 10^3 cfu/g microbiota [29]. Pseudomonas is one of chief microorganism for meat spoilage and aerobic storage condition is favorable for Pseudomonas growth [30]. Along with Pseudomonas spp., Acinetobacter spp. and Moraxella sp are found to be responsible for meat spoilage in aerobic conditions [18]. *L. sakei, H. alvei, S. putrefaciens* converts the colour into green by producing Hydrogen Sulfide which results in sulphomyoglobin under an anaerobic condition [31]. Meat can turn into green by the presence of Hydrogen Peroxide which is produced by microorganisms like Leuconostoc spp. and by Leuconostoc spp. by oxidizing nitrosomyochromogen due to the exposure of meat to oxygen [32].

Origin of bacterial organisms are mainly from animal skin, fleece, and intestines, and bacteria repeatedly found on fresh meat owned by a range of various genera, counting to *Achromobacter, Bacillus, Campyloyobacter, Carnobacterium, Enterobacter, Citrobacter, Clostridium, Hafnia, Flavobacterium, Kocuria , Listeria, Microbacterium, Micrococcus, Pantoea, Proteus, Providencia , Pseudomonas , Vibrio* and many more [33].

This review article focuses on microbial spoilage of meat, so a brief idea about microbial spoilage of meat under various conditions is summarized below in the given table 1 [34].

Detection methods

Xanthine oxidase biosensor

This is an electrochemical biosensor which helps to detect the meat spoilage for real time. The most important biochemical reaction on post mortem meat is the degradation of tissues or muscles [35]. During this biochemical reaction of meat, the ATP down gradation results in increasing the concentration inosine. Subsequently from inosine, hypoxanthine and xanthine is formed as a result of bacterial metabolism. These products are known to correlate well with the degree of spoilage or more significantly they are the two important biomarkers of nucleotides degradation by spoilage microorganisms. The ratio of the quantity of inosine (HxR) and hypoxanthine (Hx) to the total quantity of ATP and related substances is defined as the *K*-value. The *K*-value is commonly used an index for meat freshness. [35]. The K-value is generally expressed as eq. (1).

$$K_i \text{ value } (\%) = \frac{HxR + Hx}{ATP\ HxR\ Hx} * 100 \qquad (1)$$

In order to maintain a healthy & hygienic supply of meat-based food stuffs, a rapid detection method is very much needed. The most efficient way to approach a rapid detection method is by developing a biosensor that can identify the toxins which is present in the spoiled meat products & by products. Surface of platinum (pt) electrode is introduced to the food sample which immobilize enzyme xanthine oxidase & helps to measure the presence of Xanthine & Hypoxanthine in the sample.

Table 1. Microbial spoilage of meat under various conditions

Conditions	Spoilage	Microorganism Responsible	Changes taking place
AEROBIC CONDITIONS	Surface spoilage	*Pseudomonas, Acenatobacter, Streptococcus, Leuconostoc, Bacillus* and *Micrococcus.* (BACTERIA)	Slime texture
	Change in colour of meat	*Lactobacillus* and *Leuconostoc* cause greening of sausage. (BACTERIA)	Red colour of meat may be changed into green brown or grey due to production of oxidising agent, H_2S, etc. by microorganisms.
	Change in fat	*Pseudomonas* and *Achromobacter.* (BACTERIA)	Fat of meat may become rancid due to lipase producing microorganisms
	Surface colour due to pigmented bacteria	*Serratia marcescens, Pseudomonas syncyanea, Chromobacterium lividum,* (BACTERIA)	▪ *Serratia marcescens* give red spots. ▪ *Pseudomonas syncyanea* give blue color, *Chromobacterium lividum* gives greenish blue to brownish black color
	Phosphorescence	luminous bacteria e.g., *Photobacterium*	Discolorations
	Off odours and off taste	*Actinomycetes*	Undesirable odour and taste called taint are caused by many bacteria due to production of volatile acids such as formic acid, acetic acid, butyric acid etc.
	Stickiness	*Thamnidium, Mucor mucedo, Mucor racemosus*	Whiskers: when meat is kept at temperature near freezing, mould grow slowly without sporulation on surface producing while cottony growth.
	Black spot	*Cladosporium herbarum.*	Appearance of black spots
	White spot	*Sporotrichum carnis*	Appearance of white spots
	Green spot	*Penicillium species.*	Appearance of Green spots
	Change in fat	*Molds*	Hydrolytic rancidity of fat.
	Off odour and off taste		Musty flavour to meat in the vicinity of their growth.
	Sliminess, rancidity of fat, off odour and taste and discolorations like white, pink, brown spots.	*yeasts*	Yeast grows on surface of meat causing sliminess, rancidity of fat, off odour and taste and discolorations like white, pink, brown spots.
ANAEROBIC CONDITIONS	Souring	*Lactic acid bacteria*	Stale flavour.
	Putrefaction	*Clostridium species, Pseudomonas proteus and Alkaligens*	Anaerobic decomposition of protein with production of offensive smelling compounds such as H_2S, mercapatans, indole, skatole etc.

The work of XOx is to catalyse the oxidation of Hypoxanthine/ Xanthine with the aid of molecular oxygen as the acceptor of electron which ultimately results in the formation of hydrogen peroxide. The limit for determining Hx molecule of the prepared biosensor was determined as 5×10^{-6} M, and the linear working range was determined as 5×10^{-6} – 5×10^{-3} M [36]. Finally, hydrogen peroxide further gets decomposed at the anode of the platinum electrode polarized at 0.5 V.

FTIR spectroscopy

Fourier Transform Infrared Spectroscopy method along with an appropriate machine learning strategy is an effective method to detect meat spoilage [37]. FTIR is a rapid as well as inexpensive and sensitive technology that has been extensively used in quality control of various foodstuffs since it gives information from complex spectra about the composition of food components [9]. FTIR spectroscopy provides information on fundamental vibration and stretching of molecules exhibited under infrared light in the spectral region between 4000 and 700 cm^{-1} [38]. In this process, IR radiation is passed through a sample and some of it is transmitted. The light was absorbed by a particular bond at a specific wavelength. [39]. strong amide I absorption bands at 1653 cm^{-1} is exhibit by IR spectra of protein which is associated with the bending of N-H bond & stretching of C=O & C-N bond. [40] The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample [41]. FTIR technique is very fast as it takes only a few seconds to accurate characterization of cultured bacteria [42]. To detect discrimination & adulteration of meat, various studies have applied this technique [43]. In the last few years this technique is being used in many food industries. Presence of Omega-6 & Omega-3 fatty acid in pork tissues can be determined by this process [44].

Enumeration methods

This method is based on the microscopy, ATP bioluminescence or electrical measurement. In the microscopy method, staining is done to the microorganism with the help of fluorescent dyes thus it can be seen with an epifluorescent microscope. The major disadvantage of this technique is the time as it takes 18-20 h [45]. Though the problem of staining viable & non-viable cells can be overcome by the introduction of DEFT method, that is direct epifluorescent filter technique; Still the procedure is laborious. [46] Apart from this, fully automated systems with the use of flow cytometry were developed but results from low level of microorganisms was still time consuming. Bedsides this disaggregation of the spoilage organism from the meat is also difficult [47]. In the ATP bioluminescence process, the number of cells present in the culture can be calculated by measuring ATP levels in bacterial cells. [48] [49]. This process has a serious drawback. ATP is the primary energy source of cells so it is normal for every food samples to contain a huge amount of ATP. In order to use this technique, this ATP must be removed before measuring the microbial ATP [39].

Conclusions

The Even after three decades have passed since the establishment of the microbial spoilage process of meats, no such ideal rapid detection method has found to detect the meat spoilage. The ideal detection method would be inexpensive, rapid, reagent less & mostly non-destructive. With the advancement of machine learning in computer technology, it is a hopeful thought that maybe within next fifty years an ideal detection method may arrive which will be able to detect the meat spoilage within seconds [39][42]. The enzyme Xanthine Oxidase (XOx) biosensor spoilage detection method used to detect and monitor the presence of microbial spoilage present in meat product by demonstrating sensitivity, time of response, durability and the lower limit of the detection for Xanthine/ Hypoxanthine monitoring. Similarly, FT-IR spectroscopy is another most effective instrument to detect spoilage of meat during aerobic storage at different temperature by collecting spectra. Enumeration method also plays an important role as a detection method with the usage of conventional methods on selective or non-selective agar plates. Understanding of the intrinsic factors and extrinsic factors at every meat processing stage (pre slaughtering to meat product development) is necessary before developing proper handling, pre-treatment and preservation techniques for meat. The conventional microbiological approach to food sampling has changed little

over the last half century and it has been estimated that there are more than 40 theoretical methods that are being studied all over the world but the search of ideal rapid detection methods is not over yet. The objective of this review article is to make people aware about the various meat spoilage and its detection methods.

Conflict of interest

Authors declared no conflict of interests.

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