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Factors affecting microbial growth in livestock products: A review

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Abstract
Livestock products such as milk, meat and eggs are very good source of high quality proteins, essential vitamins, minerals and water. High nutrient density in these products facilitates growth of different microbes resulting in spoilage and food poisoning. The microbial growth is affected by many factors within and surrounding the livestock products**.** These factors can be broadly classified into two categories namely intrinsic and extrinsic factors. The parameters that are inherent part of the food products are referred to as intrinsic factors which include nutrient content, water activity, pH, oxidation-reduction potential, biological structures, antimicrobial constituents and competitive microflora. Extrinsic factors are those properties of the storage environment that affect both the product and microorganisms. They are temperature, relative humidity and presence and concentration of gases. Proper understanding of these factors and their interaction with each other can help us in formulation of strategies for prevention of growth of spoilage organisms and food borne pathogens.

Keywords: Intrinsic, extrinsic, pH, water activity, temperature

Introduction

Consumption of meat is continuously increasing worldwide. An increase in the per capita consumption of meat annually has been observed from 10 kg to 26 kg from 1960 to 2000 (Heinz and Hautzinger, 2007) [6]. The total meat production in India is projected at 7.4 million tons for the year 2016-17 (BAH & FS, 2017) [1]. The worldwide meat consumption is expected to reach 37 kg by 2030 (Heinz and Hautzinger, 2007) [6]. This increase in production raises concern as a significant portion of meat and meat products are spoiled every year. Kantor *et al.* (1997) [10] reported that 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 growth. Microorganisms have great importance and impact on our lives, but not always in a pleasant way. They are fundamental for obtaining some livestock products, but are also the main cause of most cases of food and cultivar deterioration. Aside from this, they also play an important role in food poisoning, as they are the main cause of outbreaks and referenced situations. There are many factors that influence the growth of microorganisms in livestock products. They all must be considered when trying to prevent the occurrence of food intoxications. There are many types of microorganisms of different forms and more or less complex structures. Bacteria, molds, and yeasts are, among all, those that generally have a greater impact on livestock products deterioration. Many factors contribute to the presence of microorganisms. The endogenous presence and cross contaminations are the factors most pointed out as being sources of microorganism in livestock products. The surroundings of the microbes i.e. their physical and chemical nature influence the growth of microorganisms. A better understanding of the various influences can help in preventing the microbial growth and hence, avert spoilage of the meat and meat products. The factors affecting microbial growth in livestock products can be broadly divided into two categories namely intrinsic factor and extrinsic factor.

Intrinsic factors

Intrinsic factors are those factors that are characteristic of the product itself.

Moisture content and water activity

Microorganisms need water in an available form to grow in livestock products.

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Water activity is defined by as the amount of water available in the food or food environment to the microbes for their growth. The good manufacturing practices (GMP) regulations for low-acid canned foods defined water activity as the vapor pressure of the food product divided by the vapor pressure of pure water under identical conditions of pressure and temperature. It can be mathematically expressed as $a_w = \frac{p}{p_o}$ Where, aw = water activity of the product, p = vapor pressure of the solution, p_0 = vapor pressure of the solvent (usually water).

The a_w of pure water is considered as 1.The a_w of a product on this scale from 0.00 to 1.00 is related to the equilibrium relative humidity above the food on a scale of 0 to 100%. It can be mathematically expressed as: Equilibrium Relative Humidity (ERH) = a_w x 100. The a_w of a product describes the degree to which water is "bound" in the food, its avail as cellulose, protein or starch (Mossel *et al*., 1995) [15]. Water activity is inversely related to osmotic pressure; if a solution has high osmotic pressure, it's a_w is low. The ability to adapt to habitats with low water activity is highly variable in different microbes. In a low a_w habitat, the microorganisms must expend extra effort to grow as it should maintain a high solute concentration to retain water. The microorganisms able to tolerate or grow over a greater range of a_w are known as osmotolerant. Usually, the microbes thrive at a_w of 0.98 or more. Different microbes react differently to different a_w due to a number of factors. For instance, gram-negative bacteria are more sensitive to low a_w than gram-positive bacteria. Only *Staphylococcus aureus* can grow and produce a toxin below a_w of 0.90. A_w varies with the choice of solute as well. For instance, with NaCl as the choice of solute, the a_w for *Clostridium botulinum* type A was 0.94 and with glycerol as the choice of solute, the a_w for *Clostridium botulinum* type A was 0.92 (Mossel *et al*., 1995) [15]. Most bacteria fail to grow in a food or other medium where the a_w is lower than 0.94. Moulds tolerate the lowest a_w levels followed by yeast and lastly by bacteria. Thus, any condition that lowers the a_w first inhibits bacteria, then yeasts, and finally molds (Elliott and Michener, 1965). Other growth factors also play a crucial role in survival of the pathogenic or spoilage microbes species-wise in harsh conditions. Some molds can grow in livestock products with a_w 0.62–0.65 (Elliott and Michener, 1965) [5]. At this lower limit, growth is very slow.

Table 1: The water activity (a_w) limits for growth of principal food-borne pathogens.

Microorganism	Minimal a_w for growth	Reference
Salmonella	0.945	Christian and Scott (1953) [3]
Clostridium botulinum	0.95	Scott (1957) [16]
Clostridium perfringens	0.93	Kang, *et al*. (1969) [9]
Staphylococcus aureus	0.86**	Scott (1957) [16]
Vibrio parahaemolyticus	0.94	Beuchat (1974) [1]

*These limits are the lowest reported, with all other growth conditions optimal. If other conditions are less than optimal, the minimal a_w will be higher.

**Troller and Stinson (1975) [17] have shown that minimal a_w for toxin production is higher than that for growth (0.93) in their experiments.

pH

It refers to the acidity or alkalinity of a solution. It is defined as the negative logarithm of the hydrogen ion concentration and is a measure of the hydrogen ion activity of a solution.

$pH = -\log [H^+] = \log (1/H^+)$

The pH scale ranges from 1.0 to 14.0. The pH influences the growth of microbes and species-wise a definite pH growth optimum and pH growth ranges exist. Acidophiles have their growth optimum between pH 0 and 5.5; neutrophils between 5.5 and 8.0 and alkalophiles prefer pH range of 8.5 to 11.5. Most bacteria and protozoans are neutrophiles, fungi prefer acid surroundings about pH 4 to 6; algae also seem to favour slight acidity. Drastic changes in cytoplasmic pH can harm microorganisms by disrupting the plasma membrane or inhibiting the activity of enzymes and membrane transport proteins. Death results if internal pH drops much below 5.0 to 5.5 in prokaryotes. Ionization and reduction in availability of nutrients may occur due to external pH changes. The microorganism needs to maintain a neutral cytoplasmic pH and for this the plasma membrane may be relatively impermeable to protons. It appears that neutrophiles exchange potassium for protons via an antiport transport system. Maintenance of internal pH closer to neutral by extreme alkalophiles is done by exchanging internal sodium ions for external protons. Small variations in pH are probably corrected by the antiport systems. In case of too much acidity (below 5.5 to 6.0) *E. coli* and *S. typhimurium* synthesise a wide range of new proteins as their acid tolerance response. If the external pH decreases to 4.5 or lower, chaperones such as acid shock proteins and heat shock proteins are synthesized. By the production of basic or acidic metabolic waste products the microorganisms can change the pH of their own habitat.

Increasing the acidity of livestock products, either through fermentation or the addition of weak acids, has been used as a preservation method since ancient times. In their natural state, most livestock products such as meat and milk are slightly acidic. Certain livestock products like egg whites are alkaline. Like other factors, pH usually acts together with other factors in the food to stall growth. While using acidity as a control mechanism the buffering capacity of food should be taken into account. It is the ability of food to resist changes in pH. The pH changes quickly in foods with a low buffering capacity due to acidic or alkaline compounds formed by bacteria as they develop. Meats have a higher buffering capacity in comparison to vegetables because of different proteins. At pH level less than 4.6, pathogens do not grow, or grow very slowly with certain exceptions. Numerous pathogens can stay alive in foodstuff at pH levels lower than their growth minima.

Nutrient content

Microorganisms need certain fundamental nutrients for development and maintenance of metabolic functions. These are water, a source of energy, nitrogen, vitamins, and minerals (Mossel *et al*., 1995) [13] and (Jay, 2000) [8]. Varying amounts of these nutrients are present in livestock products. Meats have abundant protein, lipids, minerals, and vitamins. Most muscle foods have low levels of carbohydrates. Livestock products such as milk and milk products and eggs are rich in nutrients. Carbohydrates, alcohols, and amino acids may serve as source of energy for food-borne microbes. Many of them are able to metabolize simple sugars such as glucose. Others can metabolize more complex carbohydrates such as glycogen found in muscle foods. Fats are utilized as an energy source by some microorganisms. Most microorganisms use amino acids as a source of nitrogen and energy. Some microorganisms are able to metabolize peptides and more complex proteins. Other sources of nitrogen include urea, ammonia, creatinine and methylamines. Examples of minerals required for microbial growth include phosphorus, iron, magnesium, sulfur, manganese, calcium and potassium. Generally, the gram-positive bacteria are considered to be more particular in their nutritional requirements and are unable to produce certain nutrients required for growth (Jay, 2000) [8]. For instance, amino acids, thiamine and nicotinic acid are required by the gram positive food-borne pathogen *S. aureus* for growth (Jay, 2000) [8]. On the other hand, the gram negative bacteria are usually capable of obtaining their basic nutritional requirements from the existing carbohydrates, proteins, lipids, minerals and vitamins that are easily found in an array of livestock (Jay, 2000) [8]. Another pathogen, *Salmonella* Enteritidis has very specific nutrient requirements especially iron which limits its growth. Egg albumin contains antimicrobial agents that prevent the growth of *Salmonella* Enteritidis to high levels. It was demonstrated by Clay and Board (1991) [4] that an increased growth of *Salmonella* Enteritidis was registered due to addition of iron to an inoculum of *Salmonella* Enteritidis in comparison to the control where no iron was supplemented to the inoculums. Usually the simple nutrients like carbohydrates and amino acids are amongst the first to be utilized, followed by more complex nutrients. Availability of essential nutrients limits the growth rate of microbes. The abundance of nutrients in most livestock products is sufficient to support the growth of a wide range of food-borne pathogens. Thus, it is very difficult and impractical to predict the pathogen growth or toxin production based on the nutrient composition of the livestock product.

Biological structure

Biological structures may prevent the entry and growth of pathogenic microorganisms. Examples of such physical barriers include animal hide, egg cuticle, shell and membranes. Livestock products may have pathogenic microorganisms attached to the surface. An important role is portrayed by the intact biological structures in comparison to others in prevention and growth of invading microbes. Several factors may influence penetration of these barriers. The availability of nutrients and moisture is restricted by outer barriers. The egg is a good example of an effective biological structure that, when intact, will prevent external microbial contamination of the perishable yolk. In order to be contaminated by microbes present on the egg surface, the shell and the shell membranes need to be penetrated. When there are cracks through the inner membrane, microorganisms penetrate into the egg.

Oxidation-reduction potential (Eh)

The oxidation-reduction or redox potential of a substance is defined in terms of the ratio of the total oxidizing (electron accepting) power to the total reducing (electron donating) power of the substance. In effect, redox potential (Eh) is a measurement of the ease by which a substance gains or loses electrons. It is measured in milivolts. The Eh is dependent on the pH of the substrate. Normally the Eh is taken at pH 7.0 (Jay, 2000) [8]. The microorganisms can be classified into aerobes, anaerobes, facultative aerobes and microaerophiles based on their relationship to Eh for growth. Examples of foodborne pathogens for each of these classifications include *Aeromonas hydrophila*, *Clostridium botulinum*, *Escherichia coli* O157:H7 and *Campylobacter jejuni* respectively. Generally, the ranges at which different microorganisms can grow are as follows: aerobes +500 to +300 mV; facultative anaerobes +300 to –100 mV and anaerobes +100 to less than –250 mV (Ray 1996, p 69–70). For example, *C. botulinum* is a strict anaerobe that requires an Eh of less than +60 mV for growth; however, slower growth can occur at higher Eh values. Presence of salt and other food constituents greatly affects the relationship of growth to Eh.

Posing capacity of food is another important factor. It is defined as an extent to which a food offers resistance to the external change in the redox potential. It is analogous to buffering capacity. The oxidizing and reducing components in the food and the active respiratory enzyme systems in the food affect this property. The muscle foods, meats continue to respire due to active respiratory enzyme systems in them resulting in low Eh values (Morris, 2000) [10]. The measurement of redox potential of a product is done rather easily, either for single or multicomponent livestock products.

Naturally occurring antimicrobials

Some livestock products intrinsically contain naturally occurring antimicrobial compounds that convey some level of microbiological stability to them. The usual concentration of these compounds in livestock products is relatively low, so that the antimicrobial effect alone is slight. A greater stability is produced by these compounds when they combine with other factors. Examples include Lactoferin, conglutinin and the lacto peroxidase system in cow’s milk, lysozyme in eggs and milk and other factors in fresh meat, poultry and seafood (Mossel *et al*., 1995) [15]. In bovine milk, the lacto peroxidase system has three components namely, lacto peroxidase, thiocyanate and hydrogen peroxide. Gram negative psychographs such as the pseudomonads have been shown to be very sensitive to the lacto peroxidase system. Consequently, this system, in an enhanced form, has been suggested to improve the keeping quality of raw milk in developing countries where adequate refrigeration is scarce (Mossel *et al*., 1995) [15]. It is also known that some types of processing result in the formation of antimicrobial compounds in the livestock products. The smoking of fish and meat can result in the deposition of antimicrobial substances onto the product surface. Some amount of antimicrobial effect is exhibited by the maillard browning which is a resultant of reaction between the carboxyl group of sugar and amino group of peptide (Mossel *et al*., 1995) [15]. Smoke condensate includes phenol which is not only an antimicrobial but also lowers the surface pH. Antimicrobial substances like bacteriocins, antibiotics etc can be produced naturally by some kind of fermentation. Bacteriocins are antimicrobial substances which are proteins in nature, produced by some bacteria to inhibit other closely related bacteria (Lück and Jager 1997) [12]. The most commonly characterized bacteriocins are those produced by the lactic acid bacteria. Nisin produced by *Lactococcus lactis*is one of the best known bacteriocins permissible in food applications. It is chemically polypeptide. Mostly Gram positive bacteria are inhibited by it but against Gram negative organisms and fungi can grow in its presence. There are a number of other bacteriocins and natural antimicrobials that have been described.

Competitive Microflora

Within the microbial flora in a product, there are many important biological attributes of individual organisms that influence the species that predominates. The important attributes include growth rates of the different microbial strains, their mutual interactions and inhibition of each other (ICMSF 1980) [7]. Accumulation of metabolic products generated by one species may limit the growth of other species. If the limiting metabolic product can be used as a substrate by other species, these may take over (partly or wholly), creating an association or succession (ICMSF 1980)[7]. Due to the complex of continuing interactions between environmental factors and microorganisms, a livestock product at any one point in time has a characteristic flora known as its association. Succession is the continuous changing of one association to another association. This phenomenon has been observed in the microbial deterioration and spoilage of many livestock products (ICMSF 1980) [7]. Based on their growth-enhancing or inhibiting nature, the interactions are either antagonistic or synergistic. In food systems, antagonistic processes usually include competition for nutrients, competition for attachment or adhesion sites (space), unfavorable alterations of the environment and a combination of these factors. An example of this phenomenon is raw ground beef. Even though *S. aureus* is often found in low numbers in this product, staphylococcal enterotoxin is not produced. Microorganisms having high metabolic rate utilize many required nutrients and thereby inhibit the growth of other organisms. Depletion of oxygen or accumulation of carbon dioxide favors facultative obligate anaerobes which occur in vacuum-packaged fresh meats held under refrigeration (ICMSF 1980) [7]. *Staphylococci* are very sensitive to nutrient deficiency. Coliforms and *Pseudomonas* spp. may consume the amino acids necessary for staphylococcal growth. *Streptococci* utilise the supply of nicotinamide or niacin and biotin and thus prevent staphylococcal growth (ICMSF 1980) [7]. Changes in the composition of the food as well as changes in intrinsic or extrinsic factors may either stimulate or decrease competitive effects. The buildup of a typical flora is significantly influenced by changes in growth stimulation which has been noticed among several organisms like yeasts, *Micrococci*, *Streptococci*, *Lactobacilli* and Enterobacteriaceae family (ICMSF 1980) [7].

Extrinsic factors

Extrinsic factors are those factors that refer to the environment surrounding the food.

Presence and concentration of gases

Obligate aerobes are organisms that grow only in oxygen concentration equivalent or more than the atmospheric oxygen concentration. It acts as the terminal electron acceptor for the electron transport chain in aerobic respiration and employs it in the synthesis of sterols and unsaturated fatty acids. Facultative anaerobes are the organisms that are not dependent on oxygen concentration but grow better in its presence**.** Aero tolerant anaerobes such as *Enterococcus faecalis* simply ignore oxygen and grow equally well whether it is present or not. Obligate anaerobes like *Bacteroides, Fusobacterium, Clostridium pasteurianum, Methanococcus, Neocallimastix* etc. cannot bear with oxygen and succumb in its presence. Organisms which grow in oxygen concentration between the ranges of 2% to 16% but are damaged by the normal atmospheric levels of oxygen (20%) are microaerophilic**.** Generally the aerobes grow faster than anaerobes. Therefore, in products where both conditions exist, such as in fresh meat, the surface growth is pronounced. Fungi are generally aerobic but most yeasts are normally facultative anaerobic in nature. Microorganisms use enzymes which can inactivate toxic oxygen products. Obligate aerobes and facultative anaerobes usually contain the enzymes superoxide dismutase (SOD) and catalase which catalyse the destruction of superoxide radical and hydrogen peroxide respectively. Aerotolerant microorganisms also protect themselves by the enzyme, superoxide dismutase. Many scientific studies have demonstrated the antimicrobial activity of gases at ambient and subambient pressures on microorganisms important in livestock products (Loss and Hotchkiss, 2002). Carbon dioxide (CO_2), ozone (O_3) and oxygen (O_2) are gases that are directly toxic to certain microorganisms. Carbon dioxide dissolves in the food and lowers the pH of the food. Carbon dioxide is effective against obligate aerobes and at high levels can deter other microorganisms. In general, the inhibitory effects of carbon dioxide increase with decreasing temperature due to the increased its solubility at lower temperatures (Jay 2000) [8]. Oxidizing radicals generated by ozone and oxygen are highly toxic to anaerobic bacteria and can have an inhibitory effect on aerobes depending on their concentration? Nitrogen, being an inert gas, has no direct antimicrobial properties. It is typically used to displace oxygen in the food package either alone or in combination with carbon dioxide, thus having an indirect inhibitory effect on aerobic microorganisms (Loss and Hotchkiss 2002) [11].

Temperature

All microorganisms grow in a particular range of temperature which includes a minimum, maximum and optimum temperature. The generation time and lag period of an organism is highly dependent on temperature. Over a defined temperature range, the growth rate of an organism is classically defined as an Arrhenius relationship (Mossel *et al*., 1995) [15]. The log growth rate constant is found to be proportional to the reciprocal of the absolute temperature. Mathematically, $G = -m / 2.303\ RT$ where, G = log growth rate constant, m = temperature characteristic (constant for a particular microbe), R = gas constant and T = temperature (°K). This relationship more accurately predicts the growth over the linear portion of the Arrhenius plot. However, when temperatures approach the maxima for a specific microorganism, the growth rate declines more rapidly than when temperatures approach the minima for that same microorganism. At low temperatures the microbial growth obeys the following equation (Jay 2000) [8]: $r = b\ (T - To)$ where, r = growth rate, b = slope of the regression line, T = temperature (°K), To = conceptual temperature of no metabolic significance. At low temperatures, two factors govern the point at which growth stops: (1) reaction rates for the individual enzymes in the organism become much slower and (2) low temperatures reduce the fluidity of the cytoplasmic membrane, thus interfering with transport mechanisms (Mossel *et al*., 1995) [13]. At high temperatures, structural cell components become denatured and inactivation of heat-sensitive enzymes occurs. While the growth rate increases with increasing temperature, the rate tends to decline rapidly thereafter, until the temperature maximum is reached. The microorganisms are classified into four groups according to their temperature ranges for growth. They are thermophiles, mesophiles, psychrophiles and psychrotrophs.

The optimum temperature for growth of thermophiles is between 55 to 65 °C (131 to 149 °F) with the maximum as high as 90 °C (194 °F) and a minimum of around 40 °C (104 °F). Mesophiles which include virtually all human pathogens, have an optimum growth range of between 30 °C (86 °F) and 45 °C (113 °F) and a minimum growth temperature ranging from 5 to 10 °C (41 to 50 °F). Psychrophilic organisms grow better between 12 °C (54 °F) to 15 °C (59 °F). Psychrotrophs such as *L. monocytogenes* and *C. botulinum* type E are capable of growing at low temperatures (minimum of –0.4 °C [31 °F] and 3.3 °C [38 °F] respectively) but have a higher growth optimum range (37 °C [99 °F] and 30 °C [86 °F], respectively) than true psychrophiles. Psychrotrophic organisms are much more relevant to livestock products and include spoilage bacteria, spoilage yeast and molds, as well as certain foodborne pathogens. Growth temperature is known to regulate the expression of virulence genes in certain foodborne pathogens (Montville and Matthews 2001). For example, the expression of proteins governed by the *Yersinia enterocolitica* virulence plasmid is high at 37 °C (99 °F), low at 22 °C (72 °F) and not detectable at 4 °C (39 °F). Growth temperature also impacts an organism's thermal sensitivity. *Listeria monocytogenes,* when held at 48 °C (118 °F) in inoculated sausages, has an increase of 2.4-fold in its D value at 64 °C (147 °F). The intrinsic factors of the food product, however, have been shown to impact the ability of salmonellae to grow at low temperatures. *Staphylococcus aureus* has been shown to grow at temperatures as low as 7 °C (45 °F) but the lower limit for enterotoxin production has been shown to be 10 °C (50 °F).

Temperature profoundly affects microorganisms as the most important factor influencing the effect is temperature sensitivity of enzyme-catalyzed reactions. The microbial growth slows down beyond a maximum temperature. The plasma membrane also is disrupted as lipid bilayer simply melts and the damage is such an extent that it cannot be repaired. At very low temperature, membranes solidify and enzymes don't work rapidly. The cardinal temperatures vary greatly between microorganisms.

Relative humidity (RH)

There is a relationship between relative humidity and temperature. The higher the temperature, the lower is the relative humidity and vice versa. Livestock products that undergo surface spoilage from moulds, yeasts and certain bacteria should be stored under condition of low relative humidity. Surface of improperly packaged and noncovered meats like beef cuts and whole chickens spoils quickly in the refrigerator before deep spoilage occurs. One of the main reasons of this surface spoilage is high relative humidity in refrigerator beside the aerobic nature of surface spoilage organisms.

Conclusion

The interplay between the above listed factors finally determines the growth of a microorganism in a given livestock product. Often, the results of such interplay are unpredictable, as poorly understood synergism or antagonism may occur. Traditional food preservation techniques have used combinations of pH, a_w, atmosphere and other inhibitory factors. Microbiologists have often referred to this phenomenon as the "hurdle effect". For example, certain processed meat products may use the salt-to-moisture ratio (brine ratio) to control pathogens. USDA recognizes this strategy in designating as shelf-stable semi-dry sausages with a moisture protein ratio of less than or equal to 3.1:1 and pH less than or equal to 5.0. It is the interaction of these factors that controls the ability of pathogens to proliferate in livestock products. Despite this long-standing recognition of the concept of hurdle technology (the possible synergistic effect of combining different inhibitory factors), the current definition of potentially hazardous foods only considers pH and a_w independently and does not address their interaction. Models that address the interaction of other factors (for example, atmosphere, preservatives) have been published but are not nearly as numerous as models using pH and a_w. Individual companies have shown, however, that in-house models incorporating preservative effects can be useful tools in reducing the need for extensive challenge testing and assessing risk. However, a general model for foods to cover all interactions of atmospheric gases and/or preservative combinations with pH and a_w does not currently exist. In order to design effective combinations of factors, understandings of the pathogen (vegetative or spore-forming) and of the mechanisms by which individual factors exert their impact are necessary.

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