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**Quality, functionality and shelf life of fermented meat and meat products: A review**

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**Abstract**

Fermentation of meat is a traditional energy efficient preservation method widely used for improving quality, functionality, keeping quality and shelf life of fermented meat products. Fermentation of meat causes number of physical, biochemical and microbial changes, which eventually imparting functional properties, sensory characteristics and nutritional aspects of these products and inhibit the growth of various pathogenic and spoilage microorganisms. Those changes include acidification (carbohydrate catabolism), solubilization and gelation of myofibrillar and sarcoplasmic proteins of muscle, degradation of proteins and lipids, reduction of nitrate into nitrite, formation of nitrosomyoglobin and dehydration. Dry fermented sausages are

increasingly being used as carrier for probiotics. The production of biogenic amines during fermentation can be controlled by selecting proper starter cultures and other preventive measures such as quality of raw materials, hygienic measures, temperature etc.

**Key word**

fermentation, quality attributes, safety, probiotics, biogenic amines

**Fermentation:**

Fermentation is one of the oldest and most economical processes utilized in food industry for imparting nutritional value, pleasant flavour and extending storage life of food products with the application of beneficial microorganisms mostly being *Lactobacillus*, *Streptococcus*, *Lactococcus*, *Bifidobacterium* etc. Fermented meat products have their characteristics aroma and enhanced nutritional value. Lactic acid bacteria are the most commonly used bacteria for fermentation due to their safe metabolic activity, ability to produce organic acids rapidly, long association and acceptance as Generally Recognised As Safe (GRAS).

Fermentation can be defined as energy yielding microbial metabolism in which an organic substrate, usually a carbohydrate, is incompletely oxidized, and an organic carbohydrate acts as the electron acceptor (Adams, 1990). Adam (1990) described three main advantages of fermentation of meat viz. safety, acceptability and stability (Adams, 1990). Campbell-Platt (1987) defined fermented foods as foods that are subjected to action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification to the food. It is basically a metabolic process for obtaining energy by yeast and bacteria cells by converting sugars into acids, gases and alcohol in the absence of oxygen or an electron transport system. Fermentation involves glycolysis and utilizes organic molecules for transportation of electrons. It also occurs in muscle cells during intense exercise or limited supply of oxygen. During fermentation sugars are chief substrate and ethanol, lactic acid being main produce. There are various metabolites produced during fermentation depending upon the types of microorganisms used, raw material, processing conditions etc.

**Meat fermentation:**

The practice of preservation of meat by fermentation is very old and had been practised since time immemorial. The fermented meat products gained popularity at the time of World War-II due to preservation, health benefits, and better keeping quality. At present, 20-40% of the total processed meat products in European countries are fermented meat products majority of them being fermented sausages. The first ever evidence of fermented meat product has been reported in India by adding ghee, containing lactic acid bacteria during preparation of processed meat products (Hamm et al., 2008).

Fermented foods are very popular in Europe and accounts nearly 3-5% of total meat consumed. At present, the total volume of fermented meat products equals the cheese production. There are more than 50 and 350 different types of fermented sausages in Spain and Germany respectively. According to one estimate, more than 600 million kg of fermented sausage are consumed in Germany, Spain, France, and Italy (Hutkins, 2006; Krockel, 2006).

The enhanced nutritive value of fermented food is attributed to the increasing soluble fraction of food, decreasing anti nutritional factors to safe level, synthesis of vitamins (mainly thiamine, B12, folacin and riboflavin), and essential amino acids, reducing the dry matter content of food (Adam, 1990). Fermentation increases the digestibility of food, save transport cost by reducing volume of food and save fuel/ energy for cooking of preserved food (Simango, 1997). Fermentation detoxifies and reduces undesirable substances in raw foods such as phytates, tannins and polyphenols (Gadaga et al., 1999). In addition to improve nutritional and organoleptic properties, lactic acid bacteria of fermented food products including meat products ensure food safety by preventing the growth of harmful microorganisms. Fermentation of meat improves sliceability and support reddening of these products. World Health Organization

(WHO) also recommended the use of fermentation technology for preparation and storage of various food products in order to prevent diarrhoeal diseases that accounts one tenth of total infant death worldwide due to dehydration. Singh et al. (2012) enlisted following benefits of fermented meat products over traditional meat products-

- i. Development of meat products with improved sensory attributes such as flavour, taste, aroma and colour.
- ii. Fermented meat products are tender in nature due to action of proteolytic enzymes produced from starter cultures at the time of ripening/ fermentation.
- iii. The storage life of fermented meat products is longer due to acidification, low water activity and production of bacteriocins.
- iv. Fermented meat products are safe for human consumption due to preventing growth of pathogenic and spoilage microorganisms.
- v. Fermented meat products are easier to prepare and energy efficient.

#### **Starter culture:**

In fermented meat products, fermentation process is carried out by either only on ðhouse floraö for traditional products or on starter cultures for artisanal and industrial sausages which must be competitive to prevent outgrowth of indigenous bacteria (Leroy et al., 2006). Previously, the fermented foods were prepared in artisan way without having any idea about the role of microorganisms involved in it. In middle of nineteenth century, with the industrialization and urbanization, there is concentration of population around urban areas and thus rapid increase in the demand for food resulted in production of food products at large scale. The emergence of microbiology as science in 1850s resulted in better understanding the role of microorganisms in

fermentation and their benefits (Blandino et al., 2003; Kabak and Dobson, 2011). Traditionally, a mixture of native microflora had been used for fermentation of meat and meat products. This has resulted in inconsistent quality and lot of variations in the end products. The process is depending upon the types of food material, natural flora of ingredients, surface contacts as well as ambient temperature. This has been overcome by developing commercial starter culture under controlled conditions, consisting of defined microflora for maintaining the same quality day by day. Moreover, use of commercial starter culture has solved the problem of long ripening period faced by meat industry during preparation of fermented meat and meat products. Inoculation of selected microorganisms was introduced about 100 years ago. The use of starter cultures was commonly used by middle of the 20th century (Talon and Leroy, 2011).

The introduction of starter culture into the meat during preparation of dry sausage significantly reduces the ripening period. A starter culture is a microbiological culture of defined organisms consisting cultivation medium or nutrient liquids colonized by living microorganisms used for fermentation. The protective cultures are the cultures of microorganism that are added into the finished product to protect as well as assess/ indicate the failures of temperature regulations during storage. They do not produce any specific changes and are unable to grow at low temperature.

The starter culture to be used in meat industry for fermentation should have following characteristics-

- i. It should not contain any harmful or pathogenic microorganism or producing any harmful metabolites.
- ii. Should have positive effect on consumer's health.

- iii. Have ability to form same quality end product each time.
- iv. Have high rate of growth and multiplication.
- v. Should be able to grow on cheap substrates.
- vi. Protect meat products against growth of spoilage and pathogenic organisms.
- vii. Helps in extending the shelf life of food products.
- viii. Starter culture is typical for the food product such as formation of specific flavour compounds.
- ix. Should be easily detected.
- x. Recently meat industry prefers the use of functional starter cultures (De Vuyst et al., 2003).

Niven et al. (1995) developed *Pediococcus cerevisae* culture for meat fermentation in 1995. It has been the first LAB starter culture to be used for meat. Later on several other starter cultures such as *Micrococcus M 53*, *Lactobacilli spp.*, *Pediococcus acidilacti*, *P. pentosaceus*, *Staphylococcus xylosus*, and *S. carosus* have been used. Besides bacteria, several moulds are also used for fermentation of meat and meat products such as *Aspergillus*, *Rhizopus*, *Mucor*, *Actinomucor*, *Amylomyces*, *Neurospora* *Monascus* and *Penicillium spp.* Jessen (1995) grouped the starter cultures in two groups viz. lactic acid bacteria predominantly responsible for acidification process (*Lactobacillus*, *Pediococcus*) and flavouring microorganisms capable of reducing nitrite (*Staphylococcus*, *Kocuria*, *Debaryomyces*, *Penicillium*). *Debaryomyces hansenii* is the dominant yeast species widely present in naturally fermented sausages and available as starter culture (Encinas et al., 2000; Metaxopoulos et al., 1996; Jessen, 1995). It needs ample amount of oxygen for growth, thus maximum growth observed in periphery of sausages. This



delays rancidity and reduces the risks associated with drying of sausages (Lucke, 1998). It helps in development of colour, degrades lactic and acetic acid and produces ammonia (Geisen et al., 1992; Gehlen et al., 1991) in sausages. However, it does not have any significant influence on sensory attributes. In fermented sausages, yeasts are preferably found internally whereas molds reside on surfaces due to availability of oxygen (Santos et al., 2001). Molds has the ability to produce lipase and proteases, thereby helps in improving organoleptic properties as well facilitating dehydration by forming micro-pores in casing (Incze, 2004).

LAB and CNS (coagulase negative staphylococci) are the two groups of bacteria predominating fermented meat. During fermentation, there has been sharp increase in LAB from initial 3 to 4 log CFU/g in raw meat to 8 log CFU/g (Comi et al., 2005; Greco et al., 2007) and upto 5-7 CFU/g of CNS in traditional sausages (Corbiere et al., 2006; Iacumin et al., 2006). LAB plays an important role in development of sensory attributes of sausages through acidification (Leroy et al., 2006; Weckx et al., 2009), whereas CNS stabilize colour and modify flavour by antioxidant properties and amino acid catabolism (Leroy et al., 2006; Talon et al., 2002).

In fermented dry sausages, *Lactobacillus*, *Pediococcus*, *Leuconoctoc*, *Weissella* and *Enterococcus* predominant LAB genera (Albano et al., 2009; Ammor and Mayo, 2007). *Lactobacillus* species are most prevalent amongst all LAB genera (Talon and Leroy, 2011) with *Lactobacillus sakei*, *Lactobacillus curvatus* and *Lactobacillus plantarum* constituting the most common microbiota in traditional sausage (Bonomo et al., 2008; Cocolin et al., 2009; Lebert et al., 2007). Other species of *Lactobacillus* that have been reported are *Lactobacillus pentosus*, *Lactobacillus paracasei*, *Lactobacillus casei* and *Lactobacillus alimentarius* (Aymerich et al.,

2006; Comi et al., 2005). Different genera of LAB with different biodiversity have been reported from different parts of world such as *Enterococcus faecium* and *Enterococcus faecalis* in fermented Spanish sausages (Martin et al., 2005), *Pediococci* in Iberian dry fermented sausages, Chorizo (Benito et al., 2007; Benito et al., 2008) and in certain Italian sausage (Bonomo et al., 2008), *Pediococcus acidilactici* and *Pediococcus pentosaceus* as starter culture in fermented sausages in USA (Leroy et al., 2006) and *L. sakei*, *L. curvatus*, *L. plantarum* and *L. pentosus* in starter culture in EU (Ammor and Mayo, 2007; Talon et al., 2002). Enterococci have the paradoxical place in meat fermentation with the beneficial effect in fermentation but at the same time posing potential risk (Hugas et al., 2003; Ogier and Serror, 2008). Among CNS, only two species, *S. carnosus* and *S. xylosum* are used as starter cultures in preparation of dry sausages in the EU (Corbiere et al., 2007). CNS is the natural habitat of skin and mucous membrane and comes in contact with food unintentionally or added as starter culture for fermentation (Iringer, 2008; Leroy et al., 2010; Martin et al., 2006).

#### **Effect of fermentation on quality attributes of meat products:**

In meat fermentation, several types of microorganisms of different genera, species and strains owing different biochemical potential modify the organoleptic profile, enhance nutritional value, keeping quality as well as stability of end product (Leroy et al., 2006). There are several biochemical and physical reactions takes place during fermentation resulting in improved functionality of end products such as fermented sausages with characteristics aroma (Flores et al., 2004; Schmidt and Berger, 1998), dry fermented sausages with improved texture (Ordóñez et al., 1999), semi-dry fermented sausages with improved texture and flavor etc. The acid

production during fermentation leads to improvement in quality and safety of fermented sausages (Lucke, 1998).

**a) Stability and Keeping quality:**

During fermentation several biochemical and physical reactions occurs in the meat leading to improve stability and keeping quality. The natural microbial load in fermented sausages ranges from  $10^5$  to  $10^6$  cfu/g consisting of very varied and diverse microflora, closely resembling to the natural microflora of fresh meat such as lactobacilli, micrococci, enterbacteria, *Pseudomonas spp.*, *Achromobacter spp.*, *Flavobacterium spp.*, *Bacillus spp.*, etc. molds and yeasts. With the addition of salt and other ingredients accompanied by low oxygen tension, the growth of spoilage and pathogenic microorganisms is controlled and typical natural microflora of dry fermented sausage is established (LAB and *Micrococcaceae*). This boosts the activity of starter cultures.

During fermentation, lactic acid is produced from sugars, leading to rapid reduction in pH of meat leading to hostile environment for growth of pathogenic and spoilage microorganisms (Ammor and Mayo, 2007). LAB used as starter cultures are generally homofermentative and produces lactic acid. About 1.8 mol lactic acid is produced from one mol of metabolized hexose making approximately 10% of all byproducts (Gottschalk, 1986). Inhibitory action of lactic acid is exerted in uncharged and undissociate form, most efficient at low pH (Nazer et al., 2005; Aslim et al., 2005). The undissociate form easily cross plasma membrane of bacteria and fungi and once inside, these molecules dissociated due to high pH inside the cell and resulted in formation of charged protons and anions. These charged ions could not cross the cell membrane and accumulated inside cell. This leads to inhibition of cell growth and other toxic effects (Brul

et al., 1999; Russel et al., 1992). Incorporation of salt (sodium chloride) in foods shows preservative effect due to osmotic and chloride ions. The presence of salt in fermented meat products increases pH and decrease the efficiency of lactic acid to inhibit *E. coli* O157: H7. This forces to take necessary precautions to control it in fermented sausages (Casey and Condon, 2002).

The keeping quality and stability of fermented meat is enhanced by the bacteriocins produced by LAB during fermentation/ripening. These bacteriocins are proteinaceous compounds exerting antibacterial activity against closely related bacteria, including pathogens. Bacteriocins lack toxicity towards eukaryotic cells and are tolerant to heat treatment, salt and bactericidal in nature. Their anti-microbial spectrum is limited to closely related LAB but some bacteriocins have broad inhibitory spectrum against *Listeria monocytogens* and *Staphylococcus aureus*. Bacteriocins increase the competitiveness of the producer cells and the safety of the final products (De Vuyst and Leroy, 2007).

Lactic acid bacteria secrete bacteriocins exerting antibacterial properties to the closely related organisms. This has significant effect upon increasing functional value of fermented meat products. These are peptides in nature and have ability to reduce or inhibit the growth of other Gram-positive bacteria (Cleveland et al., 2001) and also known to control growth of food borne pathogens such as *L. monocytogenes* in food products. *Pediococcus acidilactici* isolated from Spanish dry fermented sausages documented to exert strong inhibitory effect against gram-positive genera. Hugas et al. (1995) recorded the inhibition of *Listeria spp.* in fermented sausages by *Lactobacillus sakei*, *Lactobacillus curvatus*, *L. plantarum*. Lactocin 705, a

bacteriocin produced by *L. casei*, showing antibacterial effects against *L. plantarum*, *L. monocytogenes*, *S. aureus* and a wide range of Gram-negative bacteria.

The safety and shelf life of dry sausages are attributed to the lowering of water activity of meat by salt before entering the microbes, anti-microbial and antioxidant role of nitrite and nitrate, removal of oxygen during chopping, low pH and water activity and smoke compounds. The most commonly LAB isolated from conventional dry-fermented sausages or those used in starter cultures have been *Lactobacillus sake*, *Lb. curvatus* and *Lb. plantarum*, *Pediococcus pentosaceus*, and *P. acidilacti* (Hammes et al., 1985). The growth of lactic acid bacteria is favoured by anaerobic conditions prevailing, low initial pH, higher curing salt and sugar. A low pH of less than 5.3 is reported to inhibit the growth of *Staphylococcus aureus* and *Salmonella* at temperature less than 18°C (Schillinger and Luecke, 1989) with the application of suitable culture. In products which should not taste sour such as Italian-type unsmoked raw sausages, the rate of lactic acid formation is adjusted to the level as sufficient to control unwanted microorganisms with taking certain measures such as limiting the added levels of sugar and lowering water activity below 0.91 by drying. The occurrence of *Listeria monocytogenes* is low in fermented sausage (Farber et al., 1993) and ICMSF (International Commission on Microbiological Specifications for Foods, 1994) recommends the tolerance limit of 100/ gm of meat. This can be controlled in sausages by bacteriocins produced by LAB. LAB also helps in controlling EHEC (enterohaemorrhagic *Escherichia coli*) by lowering pH and lowering water activity.

Occasionally some outbreaks of *Salmonella enterica* and *Listeria monocytogenes* in fermented meat products have been reported. These pathogens survives processing conditions

such as pH, water activity, temperature, storage conditions (Drosinos et al., 2006) and even survive under fermentation (Martin et al., 2011). *L. monocytogenes* contamination occurs during slicing and/or packaging due to its ubiquitous nature (Martin et al., 2011). Martgas et al. (2015) documented better control of *Salmonella enterica* and *L. monocytogenes* in Italian fermented sausages Cacciatore and Felino and reported that the environmental conditions prevailing in the first 48 h are critical with regard to the growth and subsequent survival rate of these pathogens. Comi et al. (2005) studied the characterization of naturally fermented sausages produced in Friuli Venezia Giulia region North East of Italy and observed accentuated acidity, slight sourness and elastic, semi-hard consistency with final pH of 5.6-5.7 at the end of ripening period with high numbers of faecal enterococci playing important role in the development of sensory properties of these sausages. *Listeria monocytogenes*, *Salmonella spp.* and *Staphylococcus aureus* were absent in fermented sausages during the maturation.

Zhao et al. (2011) documented the physico-chemical characteristics and free fatty acid composition of dry fermented mutton sausages under various combinations of starter cultures and spices and noted the safety of products by inhibiting pathogenic and spoilage microorganisms due to high LAB count ( $10^8$ CFU/g), sudden fall in pH within 15 days ripening, lower water activity and better nutritional advantages of dry fermented mutton sausage with lower SFA and higher MUFA, PUFA and MUFA+PUFA/SFA compared to the control at the end of ripening.

#### **b) Organoleptic and nutritional characteristics:**

The flavour of fermented meat products improved significantly (Flores et al., 2004) due to production of several aromatic volatile compounds (Rantsiou and Luca, 2008). The

development of characteristics flavour of dry fermented sausages is due to the balance of several volatile (alcohol, ketones, aldehydes and furans) and non volatile compounds (amino acids, peptides, sugars and nucleotides) produced from raw ingredients or as metabolites of fermentation (Flores et al., 2002; Lopez et al., 2015). Lactic acid bacteria and CNS plays important role in development of distinct flavour due to production of several volatile and aromatic compounds as a result of proteolysis and lipolysis (Toldra, 2008). Yeasts and molds are also associated to the development of flavour due to lipolytic and proteolytic activities. Contribution of yeasts in flavour development is attributed to their powerful proteolytic effect in salchichon had been reported by Santos et al. (2001) and Andrade et al. (2010).

Degradation of proteins during the fermentation process has been considered as key factors involved in the improvement of functional value of meat products (Zhang et al., 2010). The development of unique taste and aroma of fermented meat products is due to the production of lactic acids and low molecular weights flavour compounds such as peptides and free amino acids, aldehydes, organic acids and amines resulted from microbial as well as endogenous proteolysis of meat (Naes et al., 1995). The lactic acid is considered as the main flavouring agent in the fermented meat products, whereas in fully dried meat products, acetic acid also plays an important role. Acetic acid has higher antimicrobial effect than lactic acid on same pH and concentration. Acetic acid imparts less pure or sour flavour to the product. These organic acids are produced as a result of fermentation of carbohydrates. The desirable lactate: acetate ratio for development of proper flavour should be in the range of 7:1 to 20:1 in dried meat products (Erkkila et al., 2001; Hamm et al., 2008).

The improved texture of dry fermented sausages (Ordenez et al., 1999) and improved flavour and texture of semi-dry fermented sausages have been reported due to dehydration.

The characteristic taste of fermented meat products is attributed to production of lactic acids and low molecular weights flavour compounds such as peptides and free amino acids, aldehydes, organic acids and amines released during proteolysis of meat (Naes et al., 1995). The characteristic tangy taste of fermented meat sausages are attributed to the production of organic acid, peptides, free amino acids, aldehydes, organic acid and amines during fermentation. The degradation of amino acids resulted into production of volatile compounds, responsible for characteristic flavors of dry sausages such as valine, leucine and isoleucine degradation resulted in production of 2-methylpropanal, 2- and 3-methylbutanal, 2-methylpropanol, 2- and 3-methylbutanol, 2-methylpropanoic, and 2- and 3-methylbutyric acids imparting sweet odour to Spanish dry fermented sausages (Montel et al., 1996). The endogenous as well as microbial enzymatic activities during fermentation of meat products are responsible for production of acid due to carbohydrate catabolism, solubilization and gelation of myofibrillar and sarcoplasmic proteins of muscle tissue, degradation of proteins and lipids, reduction of nitrate into nitrite, formation of nitrosomyoglobin and dehydration (Hamm et al., 2008). Lipase and proteases are considered important for aroma formation.

Lipids are the major constituents (25-55%) of fermented sausages and their breakdown during fermentation has important role in sensory characteristics. There are broadly two types of lipid breakdown viz. hydrolysis and oxidation, producing free fatty acids. These free fatty acids (FFA) play important role in the development of unique flavour in fermented meat products (Samelis et al., 1993; Galgano et al., 2003) by easily undergoing to oxidation and producing



alcohols, aldehydes, ketones, esters and lactones (Viallon et al., 1996) due to release of lipase by microorganisms. *Micrococcaeae* are considered as chief organisms responsible for breakdown of fat in dry fermented sausages by producing bacterial lipase. The extracellular lipase plays more active role in lipid breakdown as micrococci load start decreasing after 15-20 days of ripening (Selgas et al., 1988). The extracellular lipase remains in the medium and can catalyze hydrolysis of the long chain triglycerides, including those with 16 and 18 carbon atoms (Selgas et al., 1993). Some moulds are also reported to have lipolytic effect. Carbonyls are reported to be the most abundant fraction and important volatile compounds imparting sapid and aromatic properties of dry-fermented sausages (Berdague et al., 1993). The sausages with smaller diameter (õfuetö type with diameter of 20-40 mm) had been reported to contain higher amount of aldehydes and ketones than sausages with greater diameter (õsalchichonö with diameter of 40-60 mm). This could be due to the greater diffusion of atmospheric oxygen by õfuetö to inside (Edward et al., 1999).

Fermentation helps in development of stable red colour as some microorganisms such as coagulase-negative cocci (CNC) reduces nitrate into nitrite by nitrate reductase and leads to formation of nitrosomyoglobin. Nitrite acts as antioxidant and prevents lipid oxidation (Talon et al., 1999). The functional value of meat improves due to the degradation of sarcoplasmic protein by endogenous proteinase and myofibrillar protein during fermentation by both endogenous and bacterial proteinase. During fermentation and ripening period, the amounts of free amino acids increased as a result of degradation of peptides by bacterial proteinase. The proteolysis of meat by endogenous enzymes such as cathepsin D-like enzymes produces peptides during the fermentation process (Hierro, et al., 1999; Molly et al., 1997). Further degradation of peptides by

microorganisms results in the production of amino acids especially hydrophobic amino acids and aromatic substances. Further degradation of free amino acids produces volatile compounds, important for the production of characteristic aroma of dry sausages.

#### **Fermented sausage:**

On the basis of acid produced during fermentation, fermented meat products can be grouped into two types viz. low acid fermented meat products ( with pH- 6- 5.3, preservation by drying at low temperature, salt- 2.3-3.0%) and high acid fermented food (pH- 5.3- 4.8, preservation by inoculation and drying, temperature playing less critical role in preservation (Pearson and Gillet, 1997). Common salt (2.5- 3.0%) plays critical role in preservation of fermented sausage by preventing microbial growth in these products by decreasing the water content and inhibitory effect of chloride ions of salt. The other potential factors checking the microbial growth are low pH, lower water activity due to acidification and drying in the fermented meat products.

Sausage is one of the oldest, popular, nutritious processed meat products, consumed worldwide for its characteristics flavour and nutritious characters. The term sausage has been derived from latin word *salsus* meaning salt. The sausages are prepared from minced or chopped meat preserved by salting. The preparation and consumption of sausage had been recorded as back as by as 1500 BC in Babylonian and China. The wide popularity of sausage is attributed to its simple preparation, easier transport, variety, reasonable cost due to uses of low value meat or trimmings and high nutritional value with good quality animal protein. During preparation, sausage mix is prepared from chopped or emulsified meat, animal fat, binders, salts, spices and seasoning ingredients. Sugar is added as substrate for fermentation to get the desired

level of acidity. This raw sausage material is stuffed into casing (either natural/collagen/artificial) of various diameters and hung vertically in the ripening chambers for a fixed time. To improve the flavour and increase in the shelf life, the sausages are smoked during first few days of fermentation. Based on the processing conditions and composition, the fermented sausage can be divided into 3 types viz. dry sausage, semi-dry sausage and moist/ undried/spreadable sausage.

Fermented sausages are prepared by stuffing seasoned raw meat with suitable cultures into casings and kept for fermentation, maturation and drying (Campbell-Platt and Cook, 1995; Lucke, 1998). There should be proper checks to control the growth of spoilage and pathogenic bacteria such as *Clostridium*, *Bacillus* etc. During maturation and drying phase, there should be proper precautions to be taken to prevent the growth of harmful and unwanted mesophilic bacteria. The amount of lactic acid produced depends on the rate and action, strain, type of meat, ripening temperature, diameter and type of the sausage and water activity of the meat. Under normal conditions, the growth of pathogenic and spoilage microorganisms are prevented due to production of sufficient organic acid during fermentation process by lactic acid bacteria. The production of less acids results favours the growth of these organisms. The excessive production of acid during uncontrolled fermentation of meat resulted in fermented meat products with poor sensory quality of the (Ray, 2004).

The fermenting microorganisms can be added in the meat preparation of fermented sausage either by adding the bacteria in meat from previous batch (backslopping) or by adding commercial starter culture of defined microflora. Fermentation of sausage by commercial starter culture is considered as a quick and rapid with high safety margins whereas in the backslopping,

end products are formed with lot of variations in nutritive value and flavour. Thus at present most of the meat industries are following commercial starter culture for fermentation of sausages. Sometimes the accidental inoculation or bacteria present in meat plant may lead to the fermentation of meat (Pearson and Gillet, 1997). The selection of suitable strain for each product is very important for maintaining the quality of end products. The ripening/maturation/ drying temperature determines the suitability of microflora. *Lactobacillus plantarum* exhibits optimum growth between 30- 35<sup>0</sup>C, whereas *Pediococcus acidilacici* grows at more than 40<sup>0</sup>C. *Lactobacillus sakei* and *Lactobacillus curvatus* are able to grow even at lower temperature.

Dry-fermented sausages:

Dry sausages are known as Ne-plus Ultra of meat industry. Dry-fermented sausages can be defined as meat products that are manufactured by selecting, chopping, and mincing meat and fat, with or without offal, adding condiments, spices and certain authorized additives, and ripened, cured, and in some cases smoked (Juan et al., 1999). The process of preparation of dry sausage is very lengthy and very experience. Mostly dry sausages are only dried but not cooked and sometimes smoked mildly. Dry fermented sausages are less tangy than semi-dry sausages. The incubation and ripening period for traditional dry fermented sausage preparation is about 90 days. Such prolong period makes it vulnerable quality deterioration. So the rigorous control of ripening is very important critical for preparation of good quality dry fermented sausage. During preparation, all the ingredients are first refrigerated before stuffing to establish medium for bacterial culture. In the drying chamber, the air velocity is maintained at 15-20 cycle per hour to lose 25- 30% weight. Ripening is done at 15- 35<sup>0</sup>C to get the desired level of acidity (0.5- 1% lactic acid) and pH (4.8- 5.3) and final moisture content in product is maintained between 25 ó

50%. With this level of drying the moisture: protein ratio (M: P ratio) becomes less than 2.3: 1. To hasten the process, the fermentation is carried out at high temperature (30- 45°C) for 24hrs in US. In this rapid method, *Pediococci* perform better than *Lactobacilli* in starter cultures. In Europe, the temperature is generally kept at 20 to 26°C and at that temperature, *Lactobacilli* are generally used. Rapid production of acid by *Pediococci* prevents the growth of other harmful microorganisms at higher temperature (above 30°C). At slower rate of acid production at lower temperature does not lead to inhibition of *Micrococcaceae*. *Micrococcaceae* play an important role in colour development during ripening by reducing nitrates to nitrites. Salami, mortadella, geneo, pepperoni and cervelat are the examples of some common dry fermented sausages. As these sausages are not undergoing any heat processing, so these are best suited for inclusion of probiotics or starter culture.

*Sucuk/sujuk* is a dry fermented spicy sausage prepared from sheep/ goat/ cattle meat. It is a traditional meat product originated in Turkey and became very popular in Central Asia and Middle East. It is prepared by kneading with tail fat, salts and spices such as dry garlic, cumin, black pepper, paprika etc. The kneaded meat (*sucuk* dough) is stuffed into casing and fermented and dried for several weeks at ambient temperature (Aksu and Kaya, 2004; Senoz et al., 2000). Fat content in *sucuk* ranges from 25-50% and causes loosen texture, thus facilitating proper mixing of the contents (Soyer, 2005; Kayaard, and Gok, 2003). Raw *sucuk* prepared without any heat treatment are very hard and stiff and generally being fried before eaten. There is increasingly use of heat processing of *sucuk* with the objective to shorten preparation time as well as food safety by heating *sucuk* to internal temperature of 45-70°C after a short fermentation period. The heat processed *sucuk* had different flavour and taste profile due to

inactivation of the desirable microflora (Dalmis and Soyer, 2008; Kabak and Dobson, 2011). *Sucuk* has long shelf life due to ingredients used (salt, nitrite, spices), low pH (as low as 4.20) caused by acidification due to fermentation, competitive exclusion of harmful microorganism by lactic acid bacteria and production of bacteriocins during fermentation process (Hampikyan and Ugur, 2007).

Lactic acid bacteria are form principal microflora in traditional *Sucuk* comprising *L. plantarum*, *L. curvatus*, *L. fermentum*, *Pedococcus pentosaceus*, *Pedococcus acidilactici* (Kaban and Kaya, 2009), *L. sake* (Ozdemir et al., 1996). The lactic acid bacteria counts has been reported upto  $4 \times 10^8$  CFU/g in some market samples from Turkey (Con and Gokalp, 1998). There have been reports of presence of *Enterobacters*, *Staphylococcus xylosus*, *Staphylococcus saprophyticus*, *S. equorum*, *S. carnosus*, and *Kocuria rosea* (Kaban and Kaya, 2009) and *Debaryomyces hansenii* yeast (Erginkaya and Hammes, 1997). The lack of proper heat treatment to *sucuk* dough has resulted in presence of food borne pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus* in *sucuk* (Colak et al., 2007; Con et al., 2002). Depending upon the hygienic conditions, processing techniques, quality of raw material or microflora, several biogenic amines production such as histamine, tyramine, putrescine and spermine have been reported in *sucuk* (Senoz et al., 2000; Erkmén and Bozkurt, 2004).

*Nham* is a traditional Thai sausage prepared by mixing pork with salts and garlic. The mixture is wrapped in banana leaf or stuffed in cellulose casing followed by fermentation at 30°C for 3 to 4 days. It is eaten as raw and *Lactococcus* and *Pedococcus* being predominant microflora.

Semi-dry sausages:

The semi-dry sausages are prepared from ground pork or beef or a combination of beef and pork. The fermentation is regulated by salts, time and temperature combinations. These sausages are packaged after fermentation or heating. Whole process is completed within 1-4 weeks. The taste of these sausages is sharp and tangy due to high acidity. The fermentation is carried out to get the final pH in the finish products between 4.7- 5.3 with acidity 0.5- 1.3 % lactic acid, 3.5% salt along with 30-50% water content. The long shelf life of the semi-dry sausage is attributed to heat treatment, high salt and moisture ratio and low pH. To get the desired level of acidity, about 6-12 gram dextrose as substrate for fermentation is added in 100 lb meat. Thuringer, summer sausage and cervelat are the types of some common semi- dry sausage

#### **Fermented sausage as vehicle for Probiotics:**

The basic concept of probiotics was first postulated by Elie Metchnikoff in 1907 by explaining good health status and longevity of Asian and Bulgarian farmers due to consumption of fermented milk products containing lactic acid bacteria. Lilley and Stiwell (1965) reported the origin of probiotics term back to 1965, when it was referred to any microbiologically derived substance that helps in maintaining the proper microbial balance in the intestinal of farm animals. Probiotics are functional food products that exert health benefits to humans. Meat products containing probiotics have been developed for promoting health benefits (Ahira et al., 1998; Hammes et al., 2003). LAB and bifidobacteria have been widely used as probiotic cultures either as dietary supplement or directly added into food products. These helps in maintaining intestinal health by establishing beneficial microflora, thus reducing gastrointestinal infections (McFarland and Elmer, 2006; Korbekandi et al., 2010), helps in reducing cholesterol, alleviating lactose intolerance, immune modulators, controlling blood pressure, anticarcinogenic,

antimutagenic (Jahreis et al., 2002; Shah, 2007; Agrawal, 2009), improving skin health (Krutman, 2009), prevent cold and flu (Leyer et al., 2009; Rouhi et al., 2013). The viability of probiotic cultures is very critical to exert the above mentioned health effects. Thus probiotics should be consumed in sufficient numbers in live in food products (viability) (Korbekandi et al., 2010) to maintain the minimum levels of  $10^6$  to  $10^8$  cfu/ml needed for exerting beneficial effects (Korbekandi et al., 2010; Tamime et al., 2005).

In fermented meat products, viability has been a major concerns due to harsh conditions prevailing in gastro-intestinal tract such as pH, titratable acidity, molecular oxygen, hydrogen peroxides, bacteriocins, microbial competitions and additives (Tamime et al., 2005; Champagne and Rastall, 2009; Korbekandi et al., 2010; Ranadheera et al., 2010). In intestine, these cultures should tolerate acidic conditions and bile acid (Salminen et al., 1996; Molly et al., 1996). Dry fermented sausages are manufactured by fermentation without undergoing heat processing and thus proves suitable vehicle for beneficial live bacterial culture. In addition sausage matrix and fat protects the live probiotic cells during processing (Klinberg et al., 2005).

Probiotic meat products are first developed by the German and Japanese producers containing human intestinal Lactic acid bacteria. In 1998, a German producer prepared a salami product containing three intestinal live lactic acid bacteria strains viz. *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium spp.*). The probiotic fermented meat spread by using *Lactobacillus rhamnosus* FERM P-15120 (Sameshima et al., 1998) were developed in Japan. The fermentation of meat is generally carried out below  $20^{\circ}\text{C}$  in the presence of nitrite (200ppm) and sodium chloride (3.3%) for preventing growth of pathogens and food safety in Japan. Bunte et al. (2002) developed fermented moist type sausage by incorporating *Lactobacillus paracasei*



cultures. The ingestion of probiotic sausage resulted in increases value of CD4, T helper cells, bioactive compounds, CLA (conjugated linoleic acid), antibodies against oxidized LDL and phagocytosis index. A decrease the expression of CD54 (glycoprotein responding to inflammatory regulators) has also been documented upon intake of probiotic fermented sausages.

Dry fermented sausages with their harsh conditions such as low  $a_w$ , pH in addition to curing salts and competing organisms creates challenging environment for the survival of probiotics during processing. *Lactobacillus sakei* and *Pediococcus acidilactici* have been reported better survival under harsh conditions prevailing in intestine. The probiotic fermented sausage prepared with *Bifidobacterium* culture showed poor survival rate during ripening. This can be overcome by very high inoculums at the time of preparation so that minimum level of probiotic bacterial population (6 log cfu/g) can be obtained in the final product (Lucke, 2000). Another approach is microencapsulation of probiotic cultures by enclosing live bacterial cells in protective covering to increase the survival rate of probiotics during the meat fermentation. The incorporation of microencapsulated probiotic cultures does not affect the organoleptic properties of meat products. *L. casei*, *L. paracasei*, *L. rhamnosus* and *L. sakei* isolated from traditional Italian dry fermented sausage showed antibacterial activity against *E. coli* and *Salmonella enterica* ssp. *Enterica* (serovar Typhimurium). The non starter culture of *L. plantarum* and *L. pentosus* obtained from fermented meat products of Scandinavian type fermented sausage possess probiotics properties. These cultures are able to survive and multiply in human intestinal tract and showed to inhibit potential pathogenic bacteria. The presence of these non-culture bacterial populations does not compromise the sensory attribute (Klingberg et al., 2005).

For safety purposes, the growing emphasis is on selection of bacterial isolates from human origin as potential health promoting microorganisms in starter cultures (Klingberg et al., 2005; Elsser and Budde., 2005; Papamanoli et al., 2003; Pennacchia et al., 2004; Pennachia et al., 2006; Rebucci et al., 2007; Villani et al., 2005) or commercially available meat starter cultures showing probiotics properties and ability to survive under artificially controlled gastrointestinal conditions (Erkkila and Petaja, 2000). The sausage isolates such as *L. casei*, *L. paracasei*, *L. rhamnosus* have performed better in terms of adhesion, acid production and pathogen inactivation in in-vitro trials. Pidcock et al. (2002) did not observed any change in the sensory attributes by adding the intestinal isolates such as *L. paracasei* L26 and *Bifidobacterium lactis* B 94 in the fermented meat products. The microencapsulation of *L. reuteri* ATCC 55730 did not cause any reduction in the sensory attributes in the fermented meat products (Muthukumaraswamy and Holley, 2006). This hampers the growth and market of probiotic meat products. Jahreis et al. (2002) documented improved immunity, blood cholesterol and triglyceride levels after feeding 50 gram of probiotic sausage containing *L. paracasei* LTH 2579 daily for several weeks.

Rebucci et al. (2007) reported higher lactobacilli count ( $30 \pm 20 \times 10^7$  CFU/g) consisting *L. casei* (38% of total), *L. paracasei* (32%), *L. rhamnosus* (21%), and *L. sakei sakei* (9%) than original inoculated numbers on 60 days of ripening period in salami. Burdychova et al. (2008) developed fermented sausages with probiotic bacteria *L. casei* in the mixture with two different starter cultures (*Staphylococcus carnosus* and *L. curvatus*; or *Pediococcus acidilactici*), and reported  $10^4$  CFU/g *L. casei* count during 28 days of the ripening at 11 $\pm$ 13 C and 75% relative humidity. The initial LAB count was recorded higher for probiotic batches as compared to

control due to inoculation at initial phase. There was increase in LAB count during first 14 days of ripening, afterwards a significant decreasing count for LAB as well as other bacteria were noticed in all treatments from  $10^7$  CFU/g to  $10^6$  CFU/gm. LAB has been observed as dominant microflora in fermented sausages after completion of fermentation and ripening stage (Papamanoli et al., 2003). Erkkil et al. (2001) also reported the similar increase in LAB probiotic *L. rhamnosus* strains (GG, E-97800 or LC-705) from  $10^7$  to  $10^8$  CFU/g during first 7 days of ripening followed by slightly lower LAB count at the end of 28 days ripening period. Erkkila et al. (2000) prepared dry fermented sausage by incorporating probiotic LAB (*L. rhamnosus* GG, LC-705 or E-97800) or *P. pentosaceus* (commercial control) as co-cultures with *Staphylococcus xylosus*. During first 3 days of fermentation, rapid growth of LAB was recorded upto  $10^8$  CFU/g followed by decreasing trend. There has been decrease in staphylococci count. Martin et al. (2007) observed the growth of LAB in Iberian dry fermented sausages closely related to their ability to tolerate the salt concentration (NaCl 2.563.5% w/w), pH values (4.765.6), and water activity (0.8460.85) of these products during the ripening process. Macedo et al. (2008) recorded satisfactory growth of probiotics during manufacturing and remained high until the end of ripening. Benito et al. (2007) reported greater than 7 log CFU/g of LAB reached in Iberian dry fermented sausages after 2 months of ripening, with the levels being maintained until the end of the process (4 months).

Papamanoli et al. (2003) developed Greek dry fermented sausages under commercial conditions without the use of starter cultures at the end of the ripening process and recorded higher aerobic plate counts slightly above  $10^8$  CFU/g in dry fermented sausage along with lower Enterobacteriaceae and Micrococcaceae counts (less than  $10^3$  CFU/g) correlated with low pH

values of the fermented products. Klingberg et al. (2005) used fast acid producers (*Lactobacillus plantarum* MF1291 and MF1298 and *L. pentosus* MF1300) during manufacturing of Scandinavian-type fermented sausages by putting these for fermentation (3 days at 24<sup>0</sup>C), drying (25 days at 16<sup>0</sup>C) followed by storage (28650 days at 5<sup>0</sup>C) and noted suasages fermented with MF1298 or MF1300 reached high viable counts of *L. plantarum/pentosus* ( $2.6 \times 10^8$  CFU/g to  $2.9 \times 10^8$  CFU/g, respectively) during the fermentation and maintain that level throughout the storage period.

### **Biogenic amines: A Concern for fermented meat industry**

Biogenic amines are low molecular weight, anti-nutritional nitrogenous compounds formed by the action of microbial decarboxylases on free amino acids and exhibiting biological action. These are known to cause food poisoning such as histamine and tyramine biogenic amines are associated with scombroid poisoning from fish of Scombridae family and cheese reaction from cheese consumption respectively (Stadnik and Dolatowski, 2010). Besides food poisoning, these amines affect the freshness and other organoleptic properties of meat and meat products and used as indicator for freshness of these products. These are widely present in fermented meat and meat products due to high protein content and more prone to amine decarboxylation due to prevalent microflora. Fermented meat products contains significant amount of biogenic amines due to poor quality raw materials, contamination, production of NPN (non protein nitrogen) compounds during proteolysis and unsuitable conditions during processing and storage. In addition, fermenting microflora also decarboxylise amino acids, resulted in biogenic amines accumulation ( Latorre-Moratalla et al., 2010; Ali, 2010; Gernah et al., 2011).

In fermented meat products including dry fermented sausages, the production of biogenic amines remains major concerns due to production from the amino acids by microbial decarboxylation by *Micrococci*, *Enterococci*, *Pseudomonads* and *Enterobacters* in the presence of low acid and temperature. Concentration of biogenic amines increases upon storage. The most prevalent biogenic amines are tyramine, cadaverine, putrescine and histamine in dry fermented sausages (Eerola et al. 1996). Kim et al. (2005) documented the decreased tyramine and spermine in fermented pepperoni sausages upon exposure to irradiation (20 kGy) from 0.9 and 9.6 ppm to 0.2 and 4.2 ppm respectively, whereas putrescine was completely destroyed from 2.6 ppm. The biogenic amines production is closely related with the presence of decarboxylating micro-organisms either from ecological contamination or from inoculation culture and the environment supporting their growth and activity, availability of substrates as free amino acids, redox potential, salt concentration, types of meat, pH, size of sausages, temperature, handling conditions etc (Ruiz-Capillas and Jimenez-Colmenero, 2004; Singh et al., 2012). Under the favourable conditions for the growth of bacteria, the biogenic amines are produced in higher amount. The production of biogenic amines is affected by net pH balance (European Food Safety Authority Panel, 2011). Under low pH conditions, the growth of bacteria is inhibited as well as low pH environment; bacteria are more stimulated to produce decarboxylase as a part of their defense mechanisms against the acidity (Bover-Cid et al., 2006) resulted in production of biogenic amines. The size of dry fermented size also affects the production of biogenic amines with the comparatively higher growth of microorganisms due to low salt concentration and higher water activity in mid portion resulting in higher concentration of certain amines such as

such as putrescine and tyramine (Ruiz-Capillas and Jimenez-Colmenero, 2004; Suzzi and Gardini, 2003).

The production of these amines can be controlled by maintaining strict hygiene conditions and carefully selecting pure and suitable starter culture. The quality of raw material is very critical for preventing biogenic amines in fermented meat products (Eerola et al., 1997; Komprda et al., 2004; Maijala et al., 1995). Selecting appropriate culture owing amino oxidase activity in fermentation of meat helps in controlling production of biogenic amines (Karovicova and Kohajdova, 2005; Suzzi and Gardini, 2003; Latorre- Moratalla et al., 2008). The starter cultures should have ability to grow well at the temperature proposed for processing of the manufactured goods and competitiveness in diminishing the development of wild amine producing microflora (Suzzi and Gardini, 2003). Other approaches for controlling biogenic amines are selecting starter cultures with fast acidification ability, having strong competition with the biogenic amines producing microflora, production of bacteriocins (Somda *et al.*, 2011 Suzzi and Gardini, 2003; Latorre-Moratalla et al., 2010). Strains of *Lactobacillus sake* are unable to produce biogenic amines (Koiozyn-Krajewska and Dolatowski, 2009) and thus more appropriate than *Lactobacillus curvatus* for use as a starter culture to stop the development of biogenic amines. Biogenic amine oxidizers microflora include *Micrococcus* (Leuschner and Hammes, 1998a), *Natrinema gari* (Tapingkae et al., 2010), *Brevibacterium linen* (Leuschner and Hammes 1998b) and *Lactococcus sakei*, *Lactobacillus curvatus* (Dapkevicius et al., 2000).

#### **Future Prospects:**

There has been ever increasing demands for the fermented meat products worldwide. Several researches are going on to develop targeted suitable starter cultures having better

acidification, enzymatic activity, amine negative and oxidase positive for improving the quality and nutritive value of fermented meat and meat products. By introducing specific genes in starter cultures it is possible to get the desired end products in sufficient amount such as volatile compounds, desirable metabolites, vitamins, having ability to produce bacteriocins for improving safety. Recently, there is growing interest in developing techniques to ensure adequate viable numbers of probiotic bacteria with proper survival under harsh processing and intestinal conditions throughout the shelf-life of fermented sausages (Farnsworth et al., 2006). All this can be achieved with the holistic approach involving meat technology with microbiology and biotechnology.

### **Conclusion**

The fermented meat and meat products have a bright future potential in development of novel meat products with improved nutritive value, keeping quality, sensory attributes and functionality. The demand for fermented meat and meat products has been increased rapidly over the past few decades and meat industry is looking for functional starter cultures with improve the sensory, nutritional quality, health and microbial safety of meat products.

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**Table 1:** Role of microbial culture in fermentation.

S.No	Microorganisms	Effect	Substances produced	Effect on product
<b>Bacteria</b>				
1.	<i>Lactobacillus spp.</i>	Acidification, production of Bacteriocins	Lactic acid, ethanol, carbon dioxide, Bacteriocins, biogenic amines, volatile compounds	Flavour, taste, safety, preservation
2.	<i>Pediococcus spp.</i>	Acidification, keeping quality	Lactic acid, acetic acid, ethanol, carbon dioxide	Preservation, aroma
3.	<i>Staphylococcus xylosus</i>	Aroma	Methyl ketones from free fatty acids, volatile compounds	Organoleptic properties
4.	<i>S. carnosus</i> , <i>Kocuria varians</i>	Aroma, colour development	degrading free amino acids and inhibiting the oxidation of unsaturated free fatty acids	Colour, control rancidity by peroxide decomposition,
<b>Yeast</b>				
5.	<i>Debaryomyces hansenii</i>	Colour, flavour	Ammonia, acetic acid, ethanol, volatile compounds	Organoleptic properties

Moulds				
6.	<i>Penicillium nalgiovense</i>	Inhibition of fungi growth on surface, inhibit mycotoxins producing fungi	Popcorn smelling compounds as 2-acetyl-1-pyrroline	flavour
7.	<i>P. camemberti</i> , <i>P. crysogenum</i>	Inhibition of fungi growth on surface, inhibit mycotoxins producing fungi	Popcorn smelling compounds as 2-acetyl-1-pyrroline	flavour

(Source: Singh et al., 2012; Stahne, 2000; Olesen and stahnke, 2000; Sondergaard and Stahnke, 2002)

**Table 2:** Importance of LAB metabolites in meat industry.

S N	Metabolites	Importance	Excessive amount
1.	Lactic acid	Check the growth of harmful microorganisms, sensory quality, flavour, storage life, lowers biogenic amines production	Acidification, sour flavour and taste
2.	Acetic acid	Antibacterial effect, Flavour, shelf life	Off-taste
3.	Bacteriocin	Shelf life and safety	Inhibition of beneficial other LAB
4.	Carbon dioxide	Preservation, colour	Flatulence
5.	Hydrogen peroxide	Antibacterial effect	Discolouration
6.	(LMM) and other antimicrobials	antioxidants and antimicrobial	Health and balance of gut microbiota
7.	Biogenic amines	-	Food poisoning

Source- Holzapfel et al., 1995.

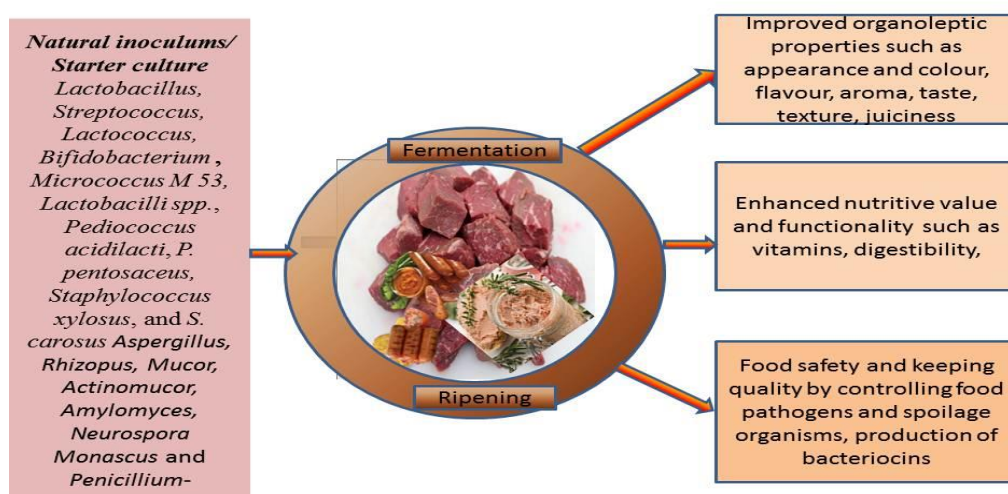


Fig 1: Effect of fermentation on quality of fermented meat and meat products.