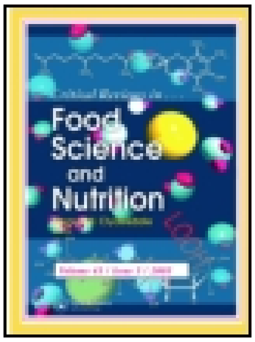

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REVIEW

A comprehensive review of the role of microorganisms on texture change, flavor and biogenic amines formation in fermented meat with their action mechanisms and safety

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ABSTRACT
Meat fermentation ensures its preservation, improved safety and quality. This prominently used traditional process has survived for ages, creating physical, biochemical, and microbial changes, and to significantly affect the functionality, organoleptic property, and nutrition of the fermented products. In some process, the growth of various pathogenic and spoilage microorganisms is inhibited. The production of fermented meat relies on naturally occurring enzymes (in the muscle or the intestinal tract) as well as microbial metabolic activities. In this review, fermented meat types and their health benefits were firstly introduced. This was followed by a description of fermentation conditions vis-à-vis starters, bacterial, yeast and mold cultures, and their role in meat. The review focuses on how microorganisms affect texture change, flavor formation, and biogenic amines (BA) accumulation in fermented meat. In addition, the production conditions and the major biochemical changes in fermented meat products were also introduced to present the best factors influencing the quality of fermented meat. Microorganisms and microbial enzymes in fermented meats were discussed as they could affect organoleptic characteristics of fermented meats. Moreover, safety concerns and prospects for further research of fermented meat were also discussed with emphasis on novel probiotic and starter cultures development; bioinformatics, omics technologies and data modeling to maximize the benefit from fermentation process in meat production.

KEYWORDS
Meat fermentation; biogenic amines; organoleptic and functional properties; health benefits

1. Introduction to fermented meat types and health benefits

Meat is one of the food forms that mainly supply protein and other components such as fat, vitamins, minerals, and other minor nutrients that have various functions. To preserve this form, historical events of various techniques were developed starting with adding simple ingredients, such as sugar and salt, to passing through different steps that included using microorganisms without clear understanding of their role, till developing an advanced recipe that is clearly explained and can be used as an effective method of preservation, fermentation. During fermentation, multiple events occur, such as biochemical, microbial and physical changes as a result of endogenous and microbial enzymatic activities. Although there are some concerns regarding its food safety, the resulted fermented meat has shown number of benefits compared to regular meat products (Leroy, Verluyten, and De Vuyst 2006; Ojha et al. 2015).

Two pathways can be used during meat fermentation; utilization of microflora, which naturally occurs or through the use of starter cultures, which may consist of one or several lactic acid bacterial species, micrococci, and staphylococci. Chemical reactions do occur during this process as well such as the breakdown of sugars by lactic acid bacteria (LAB) and reduction of nitrates and nitrites to nitric oxide by micrococci. These chemical changes mostly take place based on the substrate or meat components such as proteins and lipids, and the ingredients added like spices and condiments before reaching the end of fermentation. The resultant effect is a change in typical flavor and odor of the fermented meat products, which cannot be attributed to volatile substances alone, but to a large number of volatile and nonvolatile compounds present in the product in suitable proportions (Lorenzo, Bedia, and Bañón 2013). Examples of some fermented meat are sausages, where multiple varieties of sausages can be categorized into raw and

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Table 1. Toxic substances against spoilage microorganisms during meat fermentation.

Reference	Toxigenic substance	Microbial source	Microbial strains affected
Urso et al. (2006)	Sakacin	*Lactobacillus sakei*	Activity against *L. monocytogenes, S. aureus, B. thermosphacta, Enterococcus* spp., *Klebsiella* spp., *E. coli, Pseudomonas* spp., and *Campylobacter* spp.
Cocolin and Rantsiou (2007)	Curvacin	*Lactobacillus curvatus*	Activity against *L. monocytogenes, S. aureus, B. thermosphacta, E. coli,* and *Pseudomonas* spp.
Todorov et al. (2010)	Plantaricin	*Lactobacillus plantarum*	Activity against *B. cereus, L. monocytogenes, S. aureus, B. thermosphacta, E. coli, Pseudomonas* spp., *C. tyrobutyricum, C. perfringens, Enterococcus spp.* And *Salmonella* spp.
Parapouli et al. (2013)	Nisin	*Lactococcus lactis*	Activity against *L. monocytogenes, S. aureus,* and *C. tyrobutyricum*
Šušković et al. (2010)	Enterolysin A	*Enterococcus faecalis* and *Enterococcus malodoratus*	Activity against *L. monocytogenes* and *S. aureus*
Šušković et al. (2010)	Lysostaphin	*Staphylococcus simulans*	Activity against *S. aureus*
Šušković et al. (2010) Kęska et al. (2017)	Pediocin	*Pediococcus* spp.	Activity against *L. monocytogenes, Brochothrix* spp., *Clostridium* spp., *Bacillus* spp., *Staphylococcus* spp., and *Enterococcus* spp.

heat processed sausages (Neffe-Skocińska, Wójciak and Zielińska 2016).

1.1. Health benefits of fermented meat

Fermented foods in general possess several health functions owing to their rich microorganism's niche with functional properties such as antimicrobial, antioxidant, peptide and probiotic characters (Ashaolu and Reale 2020). Moreover, fermented products produce some nutritious components, prevent cancer, cardiovascular and gastrointestinal disorders, diabetes, and allergic incidents (Ashaolu 2020). Several examples of functional microorganisms that can be used as a starter for fermentation are found in fermented meat products and are presented in Table 1 together with their functionalities. More studies are though still needed to figure out functional microorganisms, their biological activities, and their various molecules produced during meat fermentation. This would support tailored recipe of a functional fermented meat product.

On the other hand, BAs produced *via* decarboxylation during fermentation are considered a major health issue. High contents of BA in fermented meat products are recognized as unhealthy products (Latorre et al. 2008). For example, human adverse effects can result from high level of BAs like histamine and tyramine at levels exceeding 100 mg/kg (Rauscher-Gabernig et al., 2009).

In the following sections, a description of fermentation conditions vis-à-vis starters, bacterial, yeast and mold cultures, and their role in meat production were discussed. This review focuses on the role of microorganisms on texture change, flavor formation, and biogenic amines (BA) accumulation in fermented meat. In addition, the production conditions and the major biochemical changes in fermented meat products were also introduced to present the best factors influencing the quality of fermented meat. Microorganisms and microbial enzymes in fermented meats were discussed in context to their impact on organoleptic characteristics of fermented meats. Moreover, safety concerns and prospects for further research of fermented meat were also discussed with special interests on novel probiotic and starter cultures development; bioinformatics, omics technologies and data modeling.

2. Fermentation conditions and its role in meats

Fermentation is a way to exploit microorganism's machineries so as to preserve and transform raw materials into high-quality and value-added products (Ashaolu and Reale 2020). It is one of the oldest and most economical processes utilized in the food industry for imparting nutritional value, pleasant flavor, and extending the storage life of food products with the application of beneficial microorganisms, mostly *Lactobacillus, Streptococcus, Lactococcus, Bifidobacterium*, among others (Kumar et al. 2017). Fermentation has thus been employed in food products among, which are prominently dairy, grains, cereals, starchy root crops, herbs, leaves, vegetables, and meat (Simatende et al. 2015; Kumar et al. 2017; Ashaolu 2019, 2020). Of the microorganisms employed in fermentation, starters like LAB became prominent due to the good aroma, improved nutritional qualities, safety, rapid metabolism and organic acids production rate, including its inclusion in the category of Generally Recognized as Safe (GRAS) class. To develop safe and standard meat-derived food products that are highly desirable by consumers across the globe, native starter cultures play crucial role due to their characteristic color, texture, and flavor development in fermented foods (Lorenzo et al. 2016). This has led to the utilization of various screening and characterizing techniques to accurately study native bacteria like LAB and Gram-positive catalase-positive cocci for their applications in the production of fermented meat sausage (dos Santos Cruxen et al. 2019).

2.1. Starters

Starters are microbial cultures useful in promoting fermentation of meat products, and include bacteria such coagulase-negative staphylococci (CNS) and LAB, in addition to yeasts and molds (Figure 1). The safety of fermented meat products can be enhanced by starters *via* rapid matrix acidification, and secretion of toxic chemicals or antimicrobial

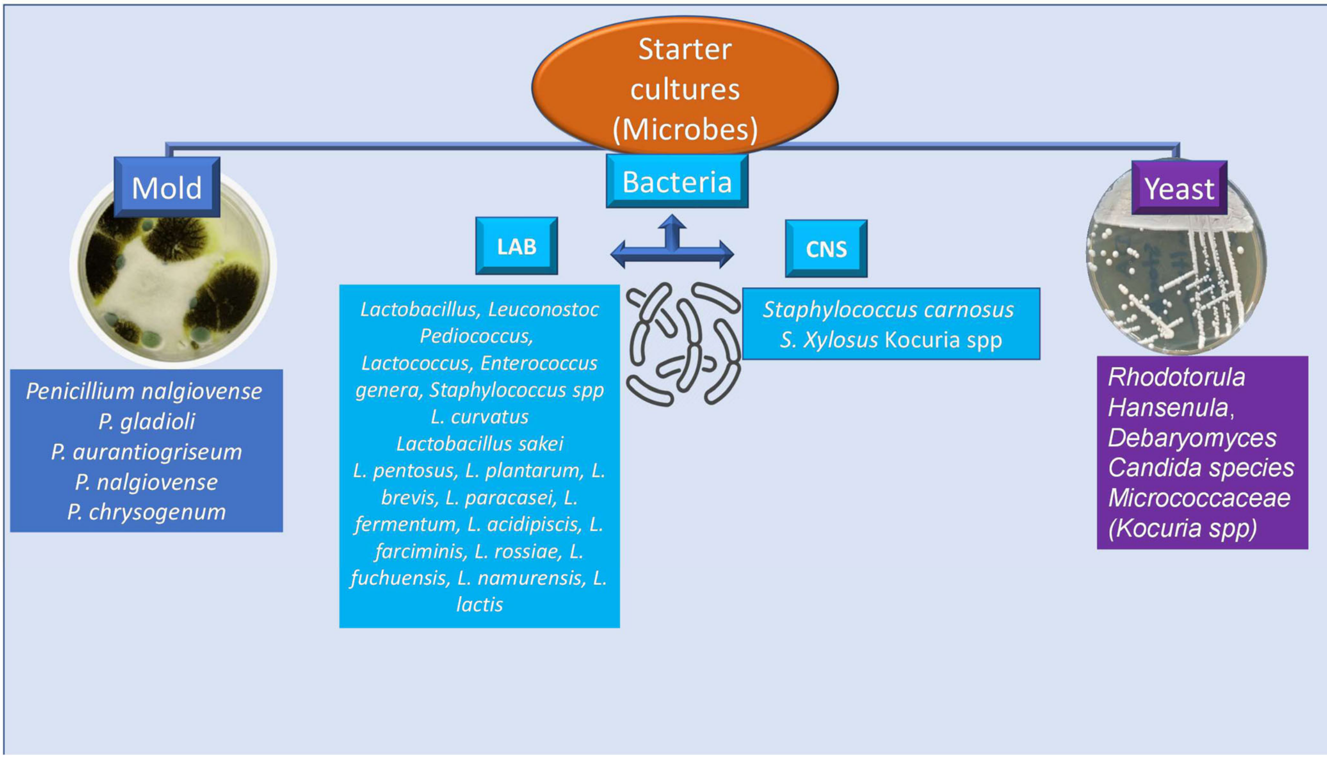

Figure 1. List of the three main starters' categories of bacteria, yeast and mold with examples of each involved in meat fermentation process. M.Os (microorganisms), LAB (lactic acid bacteria), CNS (coagulase-negative staphylococci).

substances (such as bacteriocins), while reducing ripening times of meat products. Table 2 presents some toxic chemicals produced by some starters, which are active against spoilage microorganisms during meat fermentation. If meats are not protected during fermentation, pathogenic organisms like *Listeria* spp., *Escherichia coli*, *Salmonella* spp. among numerous others may colonize leading to the generation of toxic chemicals, especially BAs (decarboxylation of certain amino acids), nitrosamines, polycyclic aromatic hydrocarbons, and mycotoxins. To this effect, starters like *S. xylosus*, *D. hansenii*, *L. sakei* and *L. plantarum* strains could reduce the pH to inhibit microbial induction of amino acid decarboxylation and consequently prevent against BA accumulation, compete with the autochthonous, nonstarter microbiota throughout ripening and storage, in the fermented meat products (Laranjo, Potes, and Elias 2019). Polycyclic aromatic hydrocarbons generated from incomplete organic matter combustion, in this case smoking wood during meat smoking, might be prevented by certain mixed starters such as *Lactobacillus* spp., Gram-positive catalase-positive cocci and yeasts during traditional meat sausages production, except that the starters' effect on PAH reduction have not been properly studied or clearly comprehended. However, some starters generally inhibit or exclude pathogenic organisms. For example, *P. nalgiovense* and *P. acidilactici* were shown to outcompete pathogenic fungi and inhibit *L. monocytogenes* in meat products, respectively (Laranjo, Potes, and Elias 2019).

Weissellin A is a listericidal bacteriocin produced by the sausage-isolated strain of *Weissella paramesenteroides* DX, and has an effective suppressive response to various concentrations of curing agents such as sodium nitrite ($NaNO_2$) at 0.0025, 0.005 and 0.01 g/L (Papagianni and Sergelidis 2013). The purification and characterization of a novel bacteriocin (lactocin MM4) from *Lactobacillus alimentarius* FM-MM4, isolated from a traditional Chinese fermented meat, "Nanx Wudl" (Hu et al. 2017) is of value. It is a thermo-stable class II bacteriocin with the N-terminal sequence QGVGPLGQGHR and molecular mass of 1104.58 Da capable of exhibiting both antifungal and broad-spectrum antimicrobial activity, and thus potentiates lactocin MM4 as valuably important in food preservation (Hu et al. 2017). These bacteriocins play crucial roles in meat fermentation and their final products.

Starter cultures comprise of multifarious bacteria, yeasts and molds that produce acid to preserve the food substrate. The physicochemical and sensorial qualities of fermented food products are enhanced as they follow a seemingly regulated pattern of fermentation. One or more strains of microorganisms can be employed as a starter culture (Macori and Cotter 2018; Ashaolu 2019). Some traditionally fermented foods employ starter cultures that are not though well characterized; however, a lot of the organisms are now being elucidated using 'omics'-based approaches or metagenomics (Ashaolu 2019).

Omics approaches including genomics, transcriptomics, proteomics, and metabolomics have transformed state of the art on microbial fermentation of foods. These novel molecular and biotechnological methods provide speed, accuracy and sensitivity for monitoring changes in cellular products i.e., gene transcripts, proteins and metabolites. They are culture-independent methods used in critical investigation of

Table 2. Microbial enzymes associated with fermented meats, source and effects on meat.

Reference	Enzymes	Microbial source	Biochemical activities/effects	Optimum conditions
Bacteria				
Andersen, Østdal, and Blom (1995), Lopes et al. (2002)	Lipase	*L. plantarum*	Lipolysis; extracellular and thermoresistant; acceleration of dry sausages maturation	pH 9.3; 37 °C
Barriere, Leroy-Sétrin, and Talòn (2001)	Superoxide dismutase	*S. xylosus*	Detoxification of superoxide radicals leading to antioxidant effects	Mn^{2+}
Barriere et al. (2002)	Catalases		Detoxification of hydrogen peroxide radicals leading to antioxidant effects	Two enzymes
Kenneally, Leuschner, and Arendt (1998)	Lipase		Limited lipase activity in fermented sausages; production of free fatty acids	pH 7.0; 30 °C; 5% NaCl
Talòn et al. (1995)	Lipase	*S. warneri*	Lipolytic action; increased free fatty acid in sausages	pH 9.0; 25 °C
Fadda et al. (2002)	Decarboxylase	*S. carnosus*	β-decarboxylation process generates CO_2 and methyl ketones with characteristic aroma	Constitutive enzyme
Engelvin et al. (2000)	β-oxidation and thioesterase activities		Production of methyl ketones via β-oxidation, and the characteristic aroma in fermented sausages	N/A
Barriere, Leroy-Sétrin, and Talòn (2001)	Catalase and superoxide dismutase		Detoxification of hydrogen peroxide and superoxide radicals leading to antioxidant effects; decreased levels of volatile compounds generated from lipid oxidation	Low pH and aeration; NO_2^- and NO_3^-
Hertel et al. (1998)	Catalase	*L. sakei*	Antioxidant; Catalase production	pH 7.0
Sanz, Mulholland, and Toldrá (1998)	Tripeptidase		Degradation of hydrophobic tripeptides; production of exopeptidase	pH 7.0; 40 °C
Sanz and Toldrá (2001)	X-prolyldipeptidylpeptidase		Increased free amino acids to enhance flavor; production of exopeptidase	pH 7.5; 55 °C
Sanz and Toldrá (2002)	Arginine aminopeptidase		Increased basic amino acids to enhance flavor; production of exopeptidase	pH 5.0; 37 °C
Montel et al. (1995)	Dipeptidase		Increase Val, Met and Leu in dry sausages; broad but selectively specific exopeptidase	pH 7.6
Sanz and Toldrá (1997)	Aminopeptidase		Increased free amino acids to enhance flavor; production of exopeptidase	pH 7.5; 37 °C
Fungi/yeasts/molds				
Benito et al. (2002)	Protease EPg222	*Penicillium chrysogenum*	Activity against myofibrillar proteins; enhanced aroma development; reduction of hardness in dry-fermented sausages; production of endoprotease	pH 6.0; 45 ° C
Durá, Flores, and Toldrá (2002)	Glutaminase	*Debaryomyces hansenii*	Acidity neutralization and enhancement of flavor; production of L-glutamine amidohydrolase	pH 8.5; 45 °C
Bolumar et al. (2005) Bolumar et al. (2008)	Protease A, B and D		Production of Endoprotease; sarcoplasmic proteins hydrolysis; proteolysis in acidic and salty meats	pH 5.5, 8.0 and 7.5
Bolumar et al. (2003a)	Arginyl aminopeptidase		Production of exopeptidase; increased free amino acids to enhance flavor	pH 7.0; 37 °C
Bolumar et al. (2003b)	Prolyl aminopeptidase		Production of exopeptidase; increased free amino acids to enhance flavor	pH 7.5; 45 °C

the metagenomic compositions of diverse and complex microbial communities within fermented food products. Available databases serve as match references to microbial DNA sequences, in order to identify the microorganisms and their potentials. When DNA sequencing is combined with bioinformatics, the whole characterization process becomes more rapid and accurate (Ashaolu and Reale 2020). Metagenomics and metatranscriptomics, although still underexploited, promise to uncover the functionality of complex microbial consortia directly in the food matrix, and understand microbial behavior in response to different process conditions (De Filippis, Parente, and Ercolini 2017). In metagenomics or metatranscriptomics studies, no PCR is performed, and total DNA or cDNA is sequenced.

Metagenomics analysis allows the obtaining of the taxonomical composition of the community, and the abundance of all microbial genes. The potential activities of the microbial communities are highlighted, because a specific gene may not be really expressed in that condition, or because DNA may arise from dead or metabolically inactive cells. In order to identify the genes actually expressed in a food sample, RNA sequencing (RNA-seq) is the most appropriate path to take, which is what happens in metatranscriptomics. Metatranscriptomics is currently being considered as an option for doing an in-depth investigation into gene expressions of fermented foods' microbial communities. The results may be coupled with other 'omics'-based approaches, including proteomics and metabolomics, for a much deeper insight into the interactions of the microorganisms in association with the organoleptic and physicochemical properties of fermented foods in context to the ultimate generated products i.e. proteins and or metabolites (De Filippis, Parente, and Ercolini 2017; Ashaolu and Reale 2020). Unlike classical analytical methods targeting a certain class of compounds, metabolomics is an emerging technology that allows for a multi-targeted analysis of large subset of metabolites. Such approach represents a paradigm shift in quality control analysis of fermented food products such as in meat and dairy products.

To appropriately select the right starter cultures, raw materials in use, food safety requirements, quality attributes and other strain properties such as nutritiveness, fermentativeness, bioactivity, protectiveness, and sensory qualities should be accounted for (Holzapfel et al. 2003; Ashaolu 2019). A great deal of attention is being shifted to the use of starter cultures in meat products production worldwide owing to that their application provides a tremendously robust means for meat conservation with high sustainability.

2.1.1. Bacterial cultures

Many studies have reported on the broad use of bacterial starter cultures in the likes of LAB (Gram-positive, catalase-negative cocci or bacilli), Gram-positive, catalase-positive cocci, mainly CNS, and *Micrococcaceae*, molds or yeasts, whose metabolism produces several compounds with antimicrobial action (Montel et al. 1996; Laranjo, Potes, and Elias 2019). Species used from the CNS to ferment meat products mainly include the facultative anaerobes *Staphylococcus carnosus* and *S. xylosus*, while those from *Micrococcaceae* family include the aerobic *Kocuria* spp., commonly utilized in sausage fermentation (Cocconcelli and Fontana 2014; Stavropoulou, Vuyst, and Leroy 2018). These bacterial cultures cause changes in organoleptic characteristics of the fermented meat products. For instance, bacterial growth in fermented sausages together with activity of the meat endogenous enzymes are undoubtedly partially responsible for the development of a number of aromatic and sapid compounds (Bis-Souza et al. 2019).

LAB starters used for meat fermentation are mostly facultative anaerobes, belonging to *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus,* and *Enterococcus* genera, as well as *Staphylococcus* spp. for overall impact on meat products' quality and properties (Casaburi et al. 2007, 2008; Gao, Li, and Liu 2014; Ojha et al. 2015; Fraqueza, Patarata, and Lauková 2017).

The processing conditions used with each starter culture led to different outcomes. For example, the use of *L. sakei, L. curvatus* and *S. xylosus* strains had no remarkable impact on proteolysis and lipolysis (Zuber and Horvat 2007; Casaburi et al. 2007); conversely, in another study, *S. warneri* caused a significant effect on sausage by increasing the rate of lipolysis post inoculation (Montel et al. 1996).

As shown in a study that involved inoculation of *L. sakei*, *S. carnosus* and *S. xylosus* into Spanish sausage (fuet), the amount of BA levels was reduced up to 90% in naturally fermented sausages (Bover-Cid, Izquierdo-Pulido, and Vidal-Carou 2000). The study implies that certain LAB reduces BA concentration in fermented meat products. Other than *L. sakei*, other LAB starters like *L. curvatus* can participate in BA prevention, ripening and every form of competition with nonstarter bacteria during storage (Suzzi and Gardini 2003). The identification of strains that can limit BA production in fermented meat is an ongoing approach while maintaining consumer's preference. A vast number of commercially available LAB cultures are used in sausage fermentations, mostly belonging to the *Lactobacillus* and *Pediococcus* species; thus, some two starters were developed from the *Staphylococcus* genus meant to ferment dry sausages to include *S. xylosus* and *S. carnosus* employed synergistically or separately (Leroy, Verluyten, and De Vuyst 2006; Corbiere Morot-Bizot, Leroy, and Talòn 2007). In another study, a novel coagulase-negative staphylococcal strain as flavor agent for the processing of fermented meat products, *Staphylococcus xylosus* P2, was screened from Chinese bacon (Meizhong et al. 2018). After investigating its proteolytic, lipolytic, and nitrate reductive activities, as well as growth ability at different temperatures, pH, salt concentrations, biogenic amine, and as its potency as a starter culture, *S. xylosus* P2 was selected good starter culture for fermented meat products, in addition to its attractive color, odor, texture, taste and overall acceptability qualities (Meizhong et al. 2018).

2.1.2. Yeast cultures

Although both bacterial and yeast starters are often synergistically used in meat fermentation, *Debaryomyces* spp. and *Candida* spp. are among the most common yeasts used as meat starters due to their aerobic and facultative anaerobic mechanisms (Laranjo, Elias, and Fraqueza 2017; Laranjo, Potes, and Elias 2019). The ability of yeasts to break down protein and lipids plays crucial role in the development of meats and sausage flavor associated with dry-cured sausages (Ojha et al. 2015). The most common yeast cultures associated with sausage fermentation include *Rhodotorula* and *Hansenula*, alongside *Debaryomyces* and *Candida* spp. (Flores et al. 2004; Andrade et al. 2009, 2010).

2.1.3. Mold cultures

Mold starters such as *Penicillium nalgiovense* and *Penicillium gladioli* are surface inoculated, exhibiting characteristics of strict aerobes (Berni 2014; Laranjo, Potes, and Elias 2019). The contributions of molds to the ripening of some dry fermented meat products are critical, as they influence the development of typical sausage flavor *via* proteolytic, β-oxidative and lipolytic activities (Sunesen and Stahnke 2003; Ojha et al. 2015). In this regard, *P. aurantiogriseum, P. nalgiovense* and *P. chrysogenum* have proven to enhance organoleptic attributes, concurrent with an overall acceptability or sensory characteristics fermented meat products (Benito et al. 2004, 2005).

The strict aerobic characteristic of molds offers antioxidative potential on dry-cured meat products, thus reducing the amounts of light and oxygen that could penetrate into the cured or fermented meat product, and thereby resulting in taste and color stability, concurrent with antimicrobial activity against pathogenic or spoilage microorganisms *via* competitive exclusion (Ludemann et al. 2004).

2.2. Fermentation as a tool of improving meat products

Using starter cultures in fermentation speed up lactic acid production from sugars, and thus create unfavorable conditions that inhibits the growth of pathogenic organisms. Other than sugars, starter cultures may use up other substrates as well, such as protein as found in meat and its products. Also, apart from lactic acid, acetic and propionic acids, ethanol, hydrogen peroxide, reuterin, antimicrobial peptides, and bacteriocins may be produced during fermentation, which exert inhibitory or antimicrobial action against spoilage microbes including *Pseudomonas* spp., *Clostridium tyrobutyricum*, *Brochothrix thermosphacta*, *E. coli*, *Y. enterocolitica*, *L. monocytogenes*, *C. perfringens* and enterobacteria (Casquete et al. 2012; Pragalaki, Bloukas, and Kotzekidou 2013; Gänzle 2015; Di Gioia et al. 2016; Laranjo, Elias, and Fraqueza 2017).

Some strains of *L. sakei* have some inhibitory effects on major food borne pathogens i.e., *E. coli* O157:H7*, L. monocytogenes, Salmonella spp., L. monocytogenes* and *S. aureus* in vitro (Pragalaki, Bloukas, and Kotzekidou 2013; Díez and Patarata 2013), and whether such effect is demonstrated in meat product should be carefully examined. Both *L. monocytogenes* and *E. coli* O157:H7 titers decreased at days 7 and 15, and the first 4 days of ripening, respectively, whilst maintaining survival rate at 2.0 log cfu/g after 26 days (Pragalaki, Bloukas, and Kotzekidou 2013). Similar in vitro observations were reported on the inhibitory effect of *L. sakei, L. plantarum* and *L. curvatus* on enterobacteria in low pH fermented sausage (3.5 log cfu/g decreased to 1.0 log cfu/g) in 16 days, as well as *L. plantarum* and *L. delbrueckii* on *C. perfringens* and *Clostridium* spp. (Di Gioia et al. 2016). When ground pork was used in the preparation of fermented salami for 9 days, artificially inoculated *Clostridium* spp. and *C. perfringens* at concentrations of 2.0 log cfu/g and 1.5 log cfu/g, respectively, were inhibited by *L. Plantarum* (9.0 log cfu/g) (Di Gioia et al. 2016).

In the study of Schlafmann et al. (2002), North European cured raw hams inoculated with *Tetragenococcus halophilus* and *S. equorum* remarkably improved the product's color while reducing the concentrations of *S. aureus*. South to the continent, *L. acidophilus* was introduced into the Romanian traditional dry sausage at 8.0 log cfu/g count, and was reported to increase the product's acidity thereby reducing the concentrations of Gram-negative microorganisms (enterobacteria) during the ripening period (Simion et al. 2014). Indeed, acidification is a major effect of fermentation on meat products, which then leads to inhibition of the growth of undesired microorganisms in the derived/fermented products. More examples could be found in the studies of Wang et al. (2013), and Casquete et al. (2012) whereby an increase in sausage and Iberian dry-sausage (Salchichón) acidity due to the inoculation of *L. sakei*, and *P. acidilactici* and *Staphylococcus vitulinus*, respectively led to the inhibition of *E. coli* and enterobacteria, and coliforms, respectively throughout the ripening time. These works however, did not show acidification as the only mechanism of modification, other compounds were also implicated to affect both fermentation process and the products thereof mitigating ultimately against bacterial pathogens growth.

3. Biochemical characteristics and changes during meat fermentation

Biochemical changes occurring during meat fermentation are meant to improve the stability, quality, safety and sensory attributes of the fermented products. Fermentation involves the accumulation of energy by yeast and bacteria cells when they convert sugars into acids, gases and alcohol in an anaerobic or electron transport system, using organic molecules for electrons transport (Kumar et al. 2017). The end products of fermentation are improved and become more functional due to series of biochemical and physical reactions that occur. The breakdown of meat protein as major meat component represents a major biochemical event that is brought about or speed up by meat enzymes, the endogenous enzymes or microorganisms. Once meats are fermented, the non-protein nitrogen fractions contain both low molecular weight peptides and free amino acids, which directly or indirectly provide the needed mechanisms for the production of volatile and nonvolatile flavor compounds in dry and semi-dry sausages (López et al. 2015).

Biochemical characteristics during meat fermentation are summarized in Figure 2. Chiefly, the safety and sensory properties of fermented meat products are improved based on this transformative process. For instance, bacteriocins are produced by some LAB members such as Gram-positive *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Propionibacterium, Enterococcus,* and Gram-negative *Escherichia coli* (colicins) and *Enterobacteriaceae* (microcins), in the fermentation process (Kęska et al. 2017). The bacteriocins may have narrow, moderate or broad spectra in their range of action, and exert biochemical changes based on their characteristics. Thus, in a study by de Carvalho et al. (2010), *L. sakei* subsp. sakei 2a that was isolated from

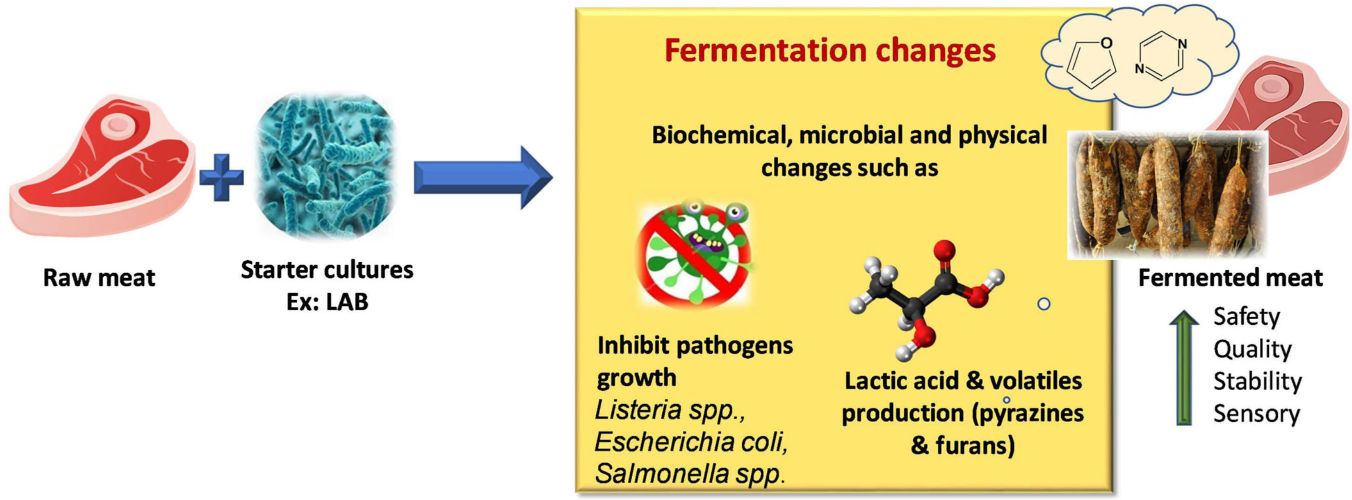

Figure 2. Diagrammatic sketch of the meat fermentation showing main steps starting from starter cultures, associated biochemical changes leading to fermented meat with higher qualities.

Brazilian pork sausage, produced Sakacin P bacteriocin, which can inhibit pathogenic *L. monocytogenes* growth *via* membrane pores formation, mediation of concentration-dependent efflux of 5(6)-carboxyfluorescein from *L. monocytogenes* liposomes, reduction of proton motive force and the intracellular ATP concentration (de Carvalho et al. 2010). This mechanism could possibly become more pronounced if a well-designed fermentation is involved or optimized. Indeed, bacteriocins like Sakacin P cause integrity damage to the bacterial cytoplasmic membrane through pores formation, dovetailing the weakened membrane with passive efflux of small metabolites like ions (potassium, phosphate, etc.), amino acids, nucleotides and other cytoplasmic solutes (Kęska et al. 2017). Consequentially, all biosynthetic processes of macromolecules become terminated and cell death occurs.

A lot of changes occur during meat fermentation based on varying factors like pH, salt concentrations, temperature, and time among others. In this regard, Sulaiman, Arief, and Budiman (2016) introduced *L. plantarum* IIA-2C12 or *L. acidophilus* IIA-2B4 that were isolated from local cattle, into lamb sausage before fermentation. The study showed that the pH value, protein, and cholesterol contents of the sausages were affected as the product with *L. acidophilus* IIA-2B4 had them in higher values than that of *L. plantarum* IIA-2C12. Whereas the latter possessed a much more titratable acid value, texture, and contents of fat, carbohydrate, tyrosine, lysine, myristoleic, pentadecanoic, heneicosanoic and cis-11-eicosatrienoic than that of the former. The authors though stated that there were differences between the physicochemical characteristics and microbiological quality of the fermented sausages by both *L. plantarum* IIA-2C12 and *L. acidophilus* IIA-2B4, and suggestive that fermentation at cold temperature (4 degrees) for 21 days could extend shelf life and maintain quality of fermented sausages (Sulaiman, Arief, and Budiman 2016). Similar results were observed in the same starter (*L. plantarum*) where its acidifying capacity, proteolytic activity, and antimicrobial and antibiotic resistance in fermented sausages were investigated (Mejri and Hassouna 2016). All these changes were confirmed as the pH of the fermented product was reduced to below 4.3 just after three days at either 15 or 25 °C. These changes are among many biochemical and physicochemical activities that could occur in fermented meat products.

In another study of physicochemical, rheological and microbiological changes occurring throughout the ripening period of dry fermented poultry sausages, Zinina et al. (2018) reported no difference in the protein, fat, moisture, salt, ash and nitrite contents of both inoculated (with *L. curvatus, S. carnosus,* and *P. pentosaceus* starters) and control sausages, but lower pH levels, growth of viable microbes, increased critical stress, and accumulation of amine nitrogen during ripening was established in the inoculated sausages. Studies like this show the potentials of starter cultures in speeding up biochemical processes during meat fermentation, which in turn accord them with necessary functional and technological properties.

The factors that contribute to the biochemical changes that occur in fermented meat products other than fermentation itself, should not be undermined. For example, the volatile constituents of fermented meat sauce could generate biochemical products, including potent odor-active components like 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon), 4-hydroxy-2(or 5)-ethyl- 5(or 2)-methyl-3(2H)-furanone (ethyl furaneol), 4-hydroxy-2,5-dimethyl-3(2H)-furanone, and 3- methylbutanoic acid (Ohata et al. 2017). The conditions associated with this product involved the mixture of ground pork, koji, and salt for 12 months. In this case, salt concentration among other compounds in the fermenting meat could lead to different biochemical changes, such as aroma and flavor.

The pH level, amount of microbial metabolites, contents of moisture, fat, protein, and carbohydrate among other conditions do affect the biochemical status of fermented meat products. In this regard, proximate analyses of the chemical contents of Indonesian traditional fermented rabbit meat "bekasam" for 7 days had a decrease in pH level, reduced contents in moisture, fat, and carbohydrate, but increased protein and lactic acid contents (Wulandari et al. 2020). In addition, sodium chloride (NaCl) remains a significant challenge for the food industry, as its influence on the oxidative damage to meat proteins during processing of experimental fermented sausages was reported (Lobo et al. 2016). It was observed that NaCl reduction led to ca. 30% decreased protein oxidation, and lower detection of Strecker aldehydes, but enhanced proteolysis, protein carbonylation, formation of Schiff bases, and remarkably increase hardness in fermented sausages (Lobo et al. 2016). These unfavorable quality indicators were indeed affected by the red-ox state of the meat proteins orchestrated by exchange of ions, volatile compounds and protein hydrolysis.

It is expected that the most influencing parameters on the growth of fungal or yeast starter cultures in fermented meat products would be water activity, temperature and salt concentrations i.e., NaCl, $NaNO_2$, and potassium nitrate (KNO_3) among others. In a study conducted on various factors and compounds used in the manufacturing of 'Cantimpalos chorizo', Spanish fermented meat sausage, certain *Penicillium olsonii* and *P. nalgiovense* strains were capable of growing at 10 °C, and the lowest water activity tested (0.86), but pH, nitrates, nitrites, and the spices tested had very little or no effect on their growth (Dı́az et al. 2002). The experiment showed that water activity, temperature and sodium chloride are the main factors that could stimulate changes in the fermented meat based on the growth of the yeast cultures used.

If curing agents like ascorbate and nitrite are used, the oxidative and nitrosative damage to fermented meat proteins would cause reactions leading to color and textural changes. Nitrite has a pro-oxidant effect on tryptophan depletion and can promote protein carbonyls and Schiff bases formation (Villaverde, Morcuende, and Estévez 2014). Nitrite concentration could predetermine the degree of fermentation in sausages, and ascorbate may act as an efficient inhibitor of the oxidative and nitrosative damage to meat proteins, in order to favor red color development (Villaverde, Morcuende, and Estévez 2014).

Furthermore, series of processes that occur in fermented meat obviously result in their breakdown into smaller and low molecular weight peptides. Sometimes it could be as low as <2 kDa as shown in the study of López et al. (2015) where commercial Argentinean fermented sausages were analyzed peptidomically. Of the 36 small peptides identified from sarcoplasmic and myofibrillar proteins, which fall into 1–2 kDa range, α-actin, myoglobin, and creatine kinase M-type were described. In this regard, muscle enzymes exerted a major role on peptidogenesis, but microbial contribution cannot be excluded (López et al. 2015). When beef and fallow deer meat were fermented without curing, high amounts of peptides, such as glutathione were attained, with beef possessing greater amounts than fallow deer (22.91–25.28 mg/100 g of fermented beef sausage and 10.04–11.59 mg/100 g of fermented fallow deer sausage) (Kononiuk and Karwowska 2020). Consequently, the nutritional status and antioxidant properties of fermented meat products may be increased as proteolysis that occurs during fermentation.

4. Microorganisms and microbial enzymes in fermented meats

Microbial enzymes commonly associated with fermentation of meat are usually derived from LAB members such as Gram-positive *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Propionibacterium, Enterococcus,* colicins from Gram-negative *Escherichia coli* and microcins from *Enterobacteriaceae* (Francesca et al. 2013; Kęska et al. 2017). They can include α-actin, myoglobin, and creatine kinase M-type (López et al. 2015). The meat proteins undergo proteolysis to be transformed into polypeptides by endogenous muscle enzymes, such as cathepsins and calpains. The polypeptides then release shorter peptides and amino acids under the action of peptidases and aminopeptidases from meat muscle and microbes. Table 2 lists major enzymes that are associated with meat fermentation, including lipases, superoxide dismutases, catalases, decarboxylase, thioesterase, tripeptidase and others.

For the microorganisms involved in fermented dry sausages, *Lactobacillus, Pediococcus, Leuconoctoc, Weissella* and *Enterococcus*—all predominantly LAB have been reported, while *Lactobacillus* species are the most pronounced of them all including mostly *Lactobacillus sakei, L. curvatus, L. pentosus, L. paracasei, L. casei, L. alimentarius* and *L. plantarum,* among others (Cocolin et al. 2009; Talòn and Leroy 2011; Kumar et al. 2017).

From across the globe, different biologically diverse LAB genera have been recorded to actively participate in the fermentation of meat products, as found in fermented Croatian wild boar and deer meat sausages (*L. sakei* and *L. curvatus*), Spanish sausages (*E. faecium* and *E. faecalis*), in fermented sausages in USA (*P. acidilactici* and *P. pentosaceus*), in EU meat products (*L. sakei, L. curvatus, L. plantarum* and *L. pentosus*), and in Iberian dry fermented sausages, Chorizo and certain Italian sausage (*Pediococcus* spp.) (Benito et al. 2007; Bonomo et al. 2008; Leroy et al. 2010; Martin, Garriga, and Aymerich 2011; Mrkonjic Fuka et al. 2020). *L. Plantarum* and *L. sakei* were identified in Cantonese sausage and Taiwanese fermented ham, respectively while Pepperoni and Nham or "musom" are largely fermented by LAB species and *Micrococcus* spp., and *Pediococcus cerevisiae*, *L. plantarum*, and *L. brevis*, respectively (Hussain 2018). In Sicilian swine breed "Suino Nero Dei Nebrodi," *Lactobacillus sakei* and enterococci were recognized as starters found thereof (Francesca et al. 2013), while diverse LAB members like *L. pentosus, L. plantarum, L. brevis, L. paracasei, L. fermentum, L. acidipiscis, L. farciminis, L. rossiae, L. fuchuensis, L. namurensis, L. lactis, Leu. citreum, Leu. fallax, P. acidilactici, P. pentosaceus, P. stilesii, W. cibaria and W. paramesenteroides* were associated with "nem chua," a popular traditional Vietnamese uncooked fermented meat *via* both culture-dependent and culture-independent methods (Nguyen et al. 2013). *Lactobacilli* in their diverse forms are also reportedly predominant in llama sausages of the South American region (Fontana et al. 2016). The traditionally fermented Chinese meat products have *Lactobacillus plantarum* strains such as *L. plantarum* BM-1 as starters (Zhang et al. 2013), while three *L. sakei* strains and their bacteriocins were associated with "salpicao," a fermented meat product from North-West of Portugal (Todorov et al. 2013). *Weissella paramesenteroides* grown in meat simulated model suggested that bacteriocin (weissellin A) and lactate production when LAB species are involved are potentially released by fermented sausages (Papagianni and Sergelidis 2013). In Tunisia, a polyphasic study involving phenotypic tests and ribosomal DNA-based techniques, showed that *Lactococcus lactis, Enterococcus faecium, Enterococcus faecalis, Enterococcus sanguinicola, Enterococcus hawaiiensis, Lactobacillus sakei, Lactobacillus curvatus, Lactobacillus plantarum, Lactobacillus alimentarius, Pediococcus pentosaceus*, and *Weissella confusa* were the predominant LAB members associated with Tunisian fermented meat products (Belgacem et al. 2009). *L. sakei* and *L. curvatus* dominated Swiss spontaneously fermented meat products followed by *Staphylococcus* species (88.2% Gram-positive, catalase-positive cocci) and *S. equorum* during maturation and in the end products, suggestive of potential starter species for meat fermentation (Marty et al. 2012). Needful to say though that this particular experiment provided substantial reasons to consider the importance of safety and hygiene of fermented meat products as pathogenic strains were abundantly identified too. Development of Hazard analysis and critical control points (HACCAP) plans to prevent against pathogen inclusion or growth in fermented meat should be thus implemented at production facilities (Adeyeye and Ashaolu 2020).

The processing techniques/methodologies used before, during or after fermentation of meats will considerably impact the type or levels of the microorganisms that could be found in the fermented meat products. For example, a microstructural analysis showed that dry fermented poultry sausages ripened with the starter bacteria (*L. curvatus, S. carnosus*, and *P. pentosaceus*) differ from the control sausage formed by drying, smoking and maturation, indicating a much more uniform moisture removal (Zinina et al. 2018), and suggestive of how fermentation improved meat organoleptic characters. Interestingly, enhanced texture and flavor of dry and semi-dry fermented sausages have been reported due to dehydration (Lorenzo et al. 2016).

5. Organoleptic characteristics of fermented meats

The flavor of fermented meat-stuffs are enhanced meaningfully (Flores et al. 2004) owing to the formation of numerous aromatic volatile components (Skovgard 2008). The development of distinguishing flavor of dry-fermented sausages is for example, owing to the equilibrium of many volatile (aldehydes, alcohols, ketones, and furans) and nonvolatile components (peptides, amino acids, nucleotides, and sugars) formed from raw constituents and/or as fermentation metabolites (Flores et al. 2004; López et al. 2015). Lactic acid bacteria and CNS play vital roles in the development of noticeable flavor owing to the formation of many aromatic and volatile components as a consequence of lipolysis and proteolysis (Toldrá 2008). Yeast and molds are also related with the development of flavor owing to proteolytic and lipolytic effects. The involvement of yeasts in flavor development is ascribed to their influential proteolytic activity in "salchichón" (a Spanish summer sausage) as noted by Andrade et al. (2010) and Santos et al. (2001). For example, the 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon), 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone (ethyl furaneol), 4-hydroxy-2,5-dimethyl-3(2H)-furanone, and 3-methylbutanoic acid are the main odor of fermented meat sauce prepared from ground pork, koji, and salt for 12 months (Ohata et al. 2017). Another example, 9–14 key aroma compounds with high odor-active value, including 3-methyl-1-butanal, octanal, 1-octen-3-ol, nonanal, cis-4-decenal, ethyl caproate, (E)-2-octenal, (Z)-2-nonenal decanal, 3-methyl-1-butanol, 1-heptanol, 3-octanone, 2-octanol, and 6-methyl-5-hepten-2-one, were identified as the key aroma contributors in dry-cured Spanish mackerel (*Scomberomorus niphonius*) (Wu et al. 2021). Likewise, 3-ethylphenol, 2,6-dimethoxyphenol, 3,4-dimethylphenol, 4-ethylguaiacol, 4-methylguaiacol, 3-methylphenol, and 2-acetyl-1-pyrroline, (E)-2-nonenal, 2-methoxy-4-vinylphenol, guaiacol, 3-ethylphenol, 2,6-dimethylphenol, 2-acetyl-1-pyrroline, and methional were key odorants in smoke-cured pork leg (Pu et al. 2020).

The degradation of proteins during the fermentation process has been measured as a crucial factor tangled in the improvement of meat products functions (Zhang et al. 2010). The development of exclusive aroma and taste in fermented meat stuffs leads to the creation of lactic acids and flavor components of low-molecular weight compounds like free amino acids, peptides, organic acids, amines, and aldehydes ensuing from microbial and endogenic proteolysis of meats (Domínguez et al. 2019). The features (like tangy taste) of fermented meat sausages are ascribed to the creation of organic acids, amines, and aldehydes during fermentation. Moreover, the deprivation of amino acids, such as leucine, valine, and isoleucine could cause the generation of volatile components usually accountable for the characteristic flavor of dry sausages, and thus results in the production of 2-methylpropanal, 2- and 3-methylbutanal, 2-methylpropanol, 2- and 3-methylbutanol, 2-methylpropanoic, and 2- and 3-methylbutyric acids; which convey sweet aroma as found in Spanish dry-fermented sausages (Montel et al. 1996).

Lactic acid is measured as the key flavoring ingredient in fermented meat stuffs, while acetic aids play key role in the fully dried meat products. Acetic acid has better antimicrobial activity than lactic acid at same level and pH-values. Acetic acid conveys sour flavor or low purity to meat products. These organic acids are formed because of carbohydrates fermentation. Notably, the required lactate:acetate levels for development of appropriate flavor ought to be in the level of 7:1 to 20:1 in the dried meat-stuffs (Erkkilä and Petäjä 2000; Farnworth 2008).

The taste of fermented meat stuffs is ascribed to the making of lactic acids and flavor components of low-molecular weight compounds unrestricted during the meat proteolysis (Andersen, Østdal, and Blom 1995). The endogenic and microbial enzymatic effects during fermentation of meat

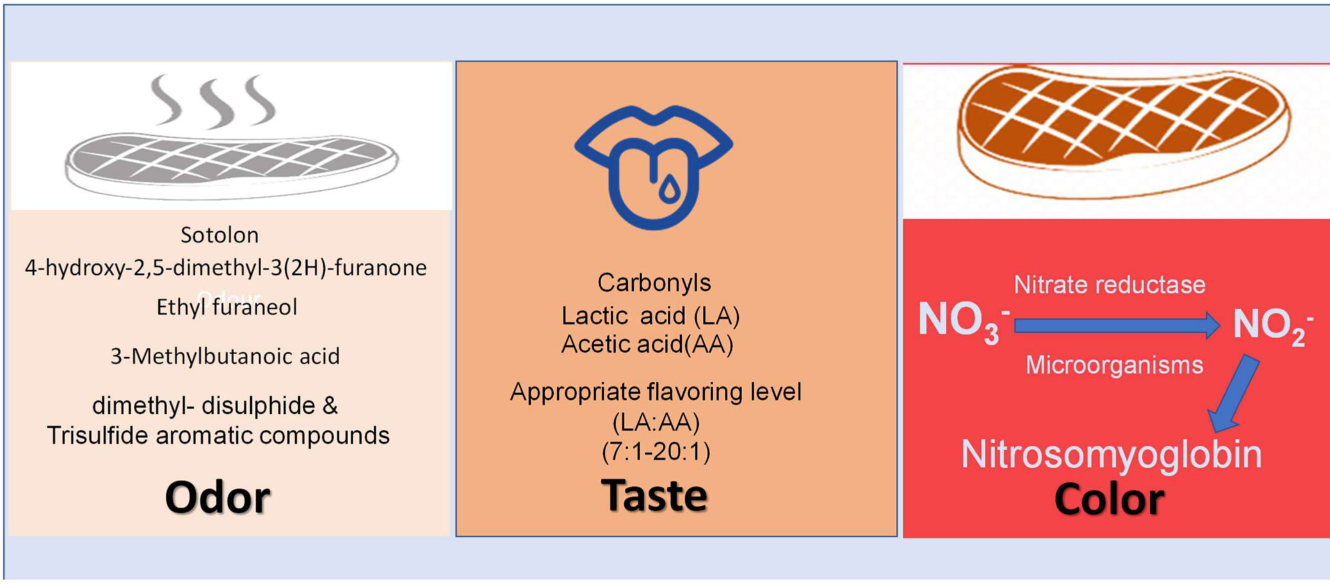

Figure 3. Main chemical components imparting odor, flavor & color of fermented meat products.

stuffs are accountable for acid production owing to carbohydrate catabolism, solubilization, and gelation of myofibrillar and sarcoplasmic proteins of muscle tissue, deprivation of lipids and proteins, reduction of nitrate into nitrite, production of nitrosomyoglobin and dehydration (Farnworth 2008). Lipase and proteases seem to be the main keys for aroma formation. The ability of seven *Debaryomyces hansenii* strains to produce aroma compounds in a fermented sausage was evaluated. Ester (i.e., ethyl acetate, ethyl propanoate, and propyl acetate) and sulfur (i.e., methanethiol) production were strongly dependent on the strain inoculated. Sulfur was mainly produced by *D. hansenii P2* and *M6* leading to dimethyl-disulfide and trisulfide aromatic compounds (Cano-García et al. 2014). Figure 3 summarizes the chief chemical components imparting odor, flavor & color of fermented meat products.

Lipids are the main constituents (25–55%) of fermented sausages, and their interruption during fermentation has vital role in the sensory features. There are sketchily two kinds of lipid degradation, *viz.* oxidation and hydrolysis, which lead to free fatty acids production. These free fatty acids show a key role in the development of single flavor in fermented meat-stuffs (Galgano et al. 2003) being oxidized and further converted to ketones, alcohols, esters, lactones, and aldehydes (Domínguez et al. 2019) due to the release of lipase by microorganisms. The extracellular lipase offers a more effective role in lipid breakdown when organisms like *Micrococci* spp. are involved during aging. The extracellular lipase serves as the intermediate and can catalyze hydrolysis of long-chain triglycerides, plus those with 16 and 18 carbon atoms. Carbonyls are stated to be the abundant and chief volatile composites giving flavorful and scented assets to dry-fermented sausages. Sausages with small width ("fuet" type with a diameter of 20–40 mm) are stated to cover higher amounts of ketones and aldehydes than sausages with greater length ("salchichón" with a width of 40–60 mm). This could be attributed to the better dispersal of atmospheric oxygen inside "fuet" (Edwards et al. 1999).

Fermentation aids in the expansion of steady red color as approximately microorganisms condense nitrate into nitrite by nitrate reductase owing to the formation of nitrosomyoglobin (Tiso and Schechter 2015). Nitrite is typically added in meat as an antioxidant and to mitigate against lipid oxidation. The functional value of meat recovers owing to the sarcoplasmic protein degradation by endogenic proteinase and myofibrillar protein throughout fermentation by both endogenic and bacterial proteinase. During fermentation and ripening retro, the amounts of free amino acids amplify as an outcome of peptides degradation by bacterial proteinase. The meat proteolysis by endogenic enzymes, like cathepsin D-like enzymes, produces peptides through the fermentation procedure (Hierro, de la Hoz, and Ordónez 1999). For instance, *Lactococcus lactis, Leuconostoc mesenteroides*, and *Enterococcus faecium* produced brighter red color, and fluorescence intensity in salt-added minced meat than the control in a recent study (Asaduzzaman et al. 2020). Therefore, the color of fermented meat products could be altered using these promising ZnPP-forming edible bacteria in the absence of nitrite or nitrate.

6. Safety and challenges of fermented meats, and how to prevent them

BA are low-molecular weight, anti-nutritional nitrogen-bearing components shaped by the microbial decarboxylases action on free amino acids and showing biological effects. These cause food poisoning, as tyramine and histamine BA are allied with "scombroid poisoning" from *Scombridae* family fishes (Stadnik and Dolatowski 2010). Alongside food poisoning, these amines influence the freshness and other organoleptic characteristics of meats and their products. The making of biogenic amines poses a challenge due the production of amino acids by *Micrococci, Enterococci, Pseudomonads*, and *Enterobacter* spp. decarboxylation in the incidence of low-acid and temperature in most of the fermented meat-stuffs, which increases upon storage. The

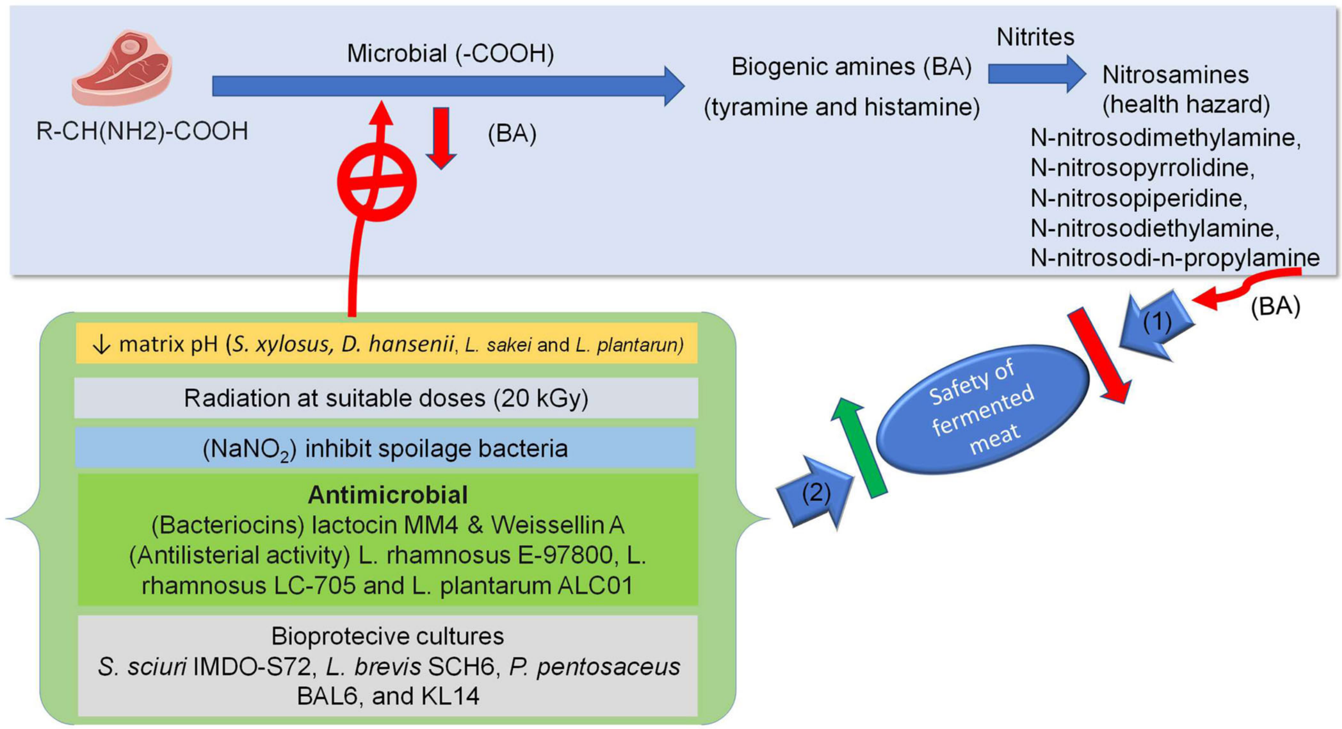

Figure 4. Production of biogenic amines in fermented meat products. The diagrammatic flow presents the two sides: Side (1) with BA negatively compromising meat safety, while side 2 is positively impacting food safety.

common BA in fermented meat-stuffs are tyramine, cadaverine, putrescine, and histamine (Domínguez et al. 2016). The production of BA is strictly linked to the decarboxylating microorganisms and the environment encouraging their activity, accessibility of free amino acids, red-ox reaction, salt levels, meat types, pH, size, temperature, handling conditions, and so on (Singh, Pathak, and Verma 2012). They are common in fermented meats due to their high protein content and are more disposed to amine decarboxylation owing to predominant microflora. Fermented meat products comprise high-level of BA owing to the poor quality of raw ingredients, uncleanness, making of non-protein nitrogen components during proteolysis, inappropriate settings during processing and storage, fermenting microflora, and decarboxylase amino acids (Gernah, Ariahu, and Ingbian 2011).

Harmful compounds found in fermented meat products are illustrated in Figure 4. Biogenic amines are not uncommonly associated with spoilage during fermentation. The amines transform into nitrosamines when nitrites are present. The nitrite becomes converted into nitric oxide, which is a nitrosating agent capable of repeating the reaction cycle with amines to produce nitrosamines. The nitric oxide may also react with secondary amines to generate carcinogenic nitrosamines, which are much more stable than those formed from primary amines. In most fermented meat products, *N-nitrosodimethylamine, N-nitrosopyrrolidine, N-nitrosopiperidine, N-nitrosodiethylamine, N-nitrosodi-n-propylamine, N-nitrosomorpholine*, and *N-nitrosoethylmethylamine* are the main nitrosamines present (Laranjo, Potes, and Elias 2019). These substances may be noxious to the consumers of fermented meat products.

Papavergou, Savvaidis, and Ambrosiadis (2012) suggested that the amounts of BA in dry fermented sausages could lead to health hazards for sensitive individuals or for those undergoing classical monoamine oxidase inhibiting drug therapy. This is due to the high levels of tyramine, putrescine, histamine and cadaverine (197.7 mg/kg, 96.5 mg/kg, 7.0 mg/kg and 3.6 mg/kg, respectively) attained during their qualitative and quantitative profiling of BA in 50 samples of dry fermented sausages sold in Greek retail markets. However, Van der Veken et al. (2020) stated that low incidence of BA production could be possible, especially tyramine and β-phenylethylamine. In their study of more than 300 strains of *Staphylococcus* spp. isolated from meat, 18 strains showed antibacterial activity against same genius species. The concentrations of BA also remained low (<52 mg/L) after a prolonged incubation period, with little or no safety risks. They concluded that *S. sciuri* IMDO-S72 could serve as an interesting candidate for the bioprotection of fermented meats as it showed promising antibacterial activity as well as absence of BA production (Van der Veken et al. 2020).

Meanwhile, great efforts have been dedicated to control the levels of BA in fermented meats and their products. For example, radiation at suitable doses (20 kGy) decreased the levels of tyramine, spermine, and putrescine in fermented pepperoni sausages (Kim et al. 2005). Under low-pH levels, the bacteria growth is repressed, as they are more enthused to create decarboxylase as a portion of their security machines versus acidity (Cid et al. 2008), leading to the generation of BA. The extent of dry fermentation of sausages also disturbs the production of BA, with relatively advanced growth rate of microorganisms owing to low-salt levels and higher water-activity, and thus resulting in higher levels of some amines like putrescine and tyramine (Ruiz-Capillas and Jiménez-Colmenero 2005).

The invention of these amines could be measured by keeping firm hygiene situations and sensibly choosing pure and appropriate starters. The raw material quality is also critical for averting the production of biogenic amines in fermented meat-stuffs (Komprda et al. 2004). Selection of appropriate starter cultures based on amino oxidase effects in meat fermentation could assist with reduction of BA (Latorre-Moratalla et al. 2008). The starter cultures must have capacity to rise well at the temperature planned for dispensation of man-made goods and reduce the growth of rough amine-producing microflora (Suzzi and Gardini 2003). Other tactics for controlling BA are picking starters with wild-acidification capacity, having solid rivalry with the BA-producing and bacteriocin-producing microorganisms (Somda et al. 2011). *Lactobacillus sakei* is inept to make BA (Kołożyn-Krajewska and Dolatowski 2009), and thus are extra suitable than *Lactobacillus curvatus* for utilizing starters to halt BA development. Biogenic amine oxidizing microflora includes *Micrococcus*, *Natrinema gari*, *Brevibacterium linen*, *Lactococcus sakei*, and *Lactobacillus curvatus* (Kołożyn-Krajewska and Dolatowski 2009). Fermented sausages are measured as microbiologically firm; but, in cases where corruption of fresh material is originally high or where insufficient regulation during production arises, safety is traded. Infrequently, fermented meat goods have been associated with food-borne rashes caused by bacteria like *Clostridium botulinum*, *Staphylococcus aureus*, *Salmonella*, *E. coli*, *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Campylobacter*. Intolerable records of *E. coli*, *Enterobacteriaceae*, *Bacillus cereus*, and coliforms are often found (33% of rough boar and stag fermented sausages impulsively incited with no starter nations and ended without nitrites). Albeit *Staphylococcus aureus*, *Salmonella*, and *Listeria* spp. could be noticed only during fermentation.

From another viewpoint, bioprotective cultures may serve a remedial purpose to solve safety issues associated with fermented meat products. For instance, *L. brevis* SCH6, *P. pentosaceus* BAL6, and KL14 revealed selected superior characteristics among other LAB strains investigated on their effects on polish raw fermented meat products, making them a viable bioprotective culture that can be inoculated in raw fermented meat products as starter cultures (Rzepkowska et al. 2017). They were resistant to the harsh gastrointestinal conditions and possessed antimicrobial properties. In addition, dry sausages safety may be based on different factors "the hurdle-concept," albeit *Listeria monocytogenes*, a pathogen particularly harmful to young, old, pregnant and immunocompromized people, is known to survive the commercial dry sausage manufacturing process (Työppönen et al. 2003). In a study however, *L. rhamnosus* E-97800, *L. rhamnosus* LC-705 and *L. plantarum* ALC01 were reported to express antilisterial activity in North European type sausages at an early stage in the ripening process (Työppönen et al. 2003). In addition, *L. sakei* is reportedly very efficient in reducing BA production based on its quick pH drop during horse meat sausage fermentation (El Malti and Amarouch 2008). Moreover, nitrites reduction by combinatorial use of a protective cultures could be a feasible approach to control *L. monocytogenes* growth in fermented meat foods (Nikodinoska et al. 2019), thus enhancing their safety. In pork ground meat meant for fermented salami preparation, *L. plantarum* PCS20 could proffer antimicrobial activity against *Clostridium* spp. As well as reduced nitrite concentration in fermented meat products (Di Gioia et al. 2016).

Curing agents are employed in hardening the surfaces of materials like polymers. Sodium nitrite is used as a curing agent plus other additives to meat batter, especially in European-type fermented sausages to inhibit the growth of common spoilage bacteria and *Clostridium* spp. and the development of botulism toxin, thus preventing lipid oxidation and promoting color development and the typical cured flavor and aroma (Papagianni and Sergelidis 2013). In this regard, 0.005 g/L of this $NaNO_2$ introduced into medium consisting *W. paramesenteroides* DX and a complex meat batter confirmed that the presence of nitrite suppressed bacteriocin (weissellin A) production, which increases with increasing nitrite concentrations (Papagianni and Sergelidis 2013).

The use of *E. coli* SafeFerment (EcSF) as a predictive tool was also suggested for the evaluation of the impact of modifications, interventions, or unexpected events during the manufacturing process and/or storage period on verotoxigenic *E. coli* (VTEC) survival. As EcSF integrates growth, probability of growth, and thermal and non-thermal inactivation models to predict the VTEC concentration throughout fermented raw-meat sausages manufacturing and storage under constant or fluctuating environmental conditions (Quinto et al. 2014).

7. Future perspectives of microflora functions in fermented meats

7.1. Development of novel starter cultures

Safety and quality for fermented meat products is one of the future promising areas of development through several variables, such as applying novel and nontraditional meat starter (NTMS) cultures and is always to evolve to keep path with improved fermented meat product with best health attributes. For example, adding NTMS to the Hungarian salami increased the safety due to the inhibition effect pathogenic organisms *E. coli* O111 and *L. Monocytogenes* (Pidcock, Heard, and Henriksson 2002). Multi-functional meat starters should be designed to target not only increased safety but also higher quality and mass production feasibility (Leroy, Verluyten, and De Vuyst 2006).

In some fermented meat products like sausages, selection of culture starters that rapidly initiate acidification simultaneously with desirable level of sensory quality is a crucial step for developing fermented end-product. A number of research documents tackled functional culture starters which add extra functionality in comparison with traditional starters and show a method of increasing and optimizing fermented sausages or meat's qualities of safety, taste and health (Leroy, Verluyten, and De Vuyst 2006). Such qualities are represented in various examples, such as generated aroma from microorganisms, components that promote health status, antimicrobials or bacteriocins as preservatives (Biscola et al., 2013; Hu et al. 2017; Zhang et al. 2013), emerging meat color, probiotic properties and minimal production of toxic components of biogenic amines, nitrite and nitrate, and others (Leroy, Verluyten, and De Vuyst 2006).

7.2. Probiotic starter cultures

Starter cultures that have probiotic characters are promising base for meat fermentation process. They contain organisms in a living state represented in some supplements or food. Upon consumption in appropriate amounts, they positively benefit the host by enhancing the microbiota naturally living in the intestine. Examples of qualified organisms are some *Lactobacillus* strains, and they can be utilized in functional fermented meat production. (Leroy, Verluyten, and De Vuyst 2006). Thus, upon selection of cultures that act as a starter in fermentation, probiotic activities should be one of the criteria to be considered and investigated. Moreover, studies about microbiota and the dynamics of a desired meat population will dramatically enhance not only the process of fermentation but also the standardization and safety of fermented meat. With that, better economic state and market positioning will be achieved (Leroy, Verluyten, and De Vuyst 2006).

7.3. Bioinformatics, omics technologies and data modeling

Bioinformatics also contribute to the advancement of fermented meat products. With the number of bioinformatics means, more data can be gathered to search the genomes for several crucial compounds. For example, components involved in flavor generation, such as peptidases, aminotransferases, enzymes for amino acids production, and those which are produced during fermentation process. Such technologies can support the advancement of this industry in several dimensions mainly in creating molecules that do not naturally exist using genetically engineering methods (Leroy, Verluyten, and De Vuyst 2006), and or identifying novel microorganisms from unexplored resources. Metabolomics as an omic technology for identification of fermented meat composition and record biochemical changes due to bacterial activity during fermentation process and more upon storage that can be readily applied to predict the sensory, nutritional and safety measures of fermented meat products. With the advances in analytics, it is now feasible to monitor several hundreds of metabolites instead of the previous rather chemically targeted approach that shall aids provide a better insight on the fermentation process (Farag, El Hawary, and Elmassry 2020). Most of the studies assessed the influence of a single variable on the fermented meat quality or composition while the interactions between the variables have not been fully examined. Therefore, in order to optimize the fermentation conditions, it would be necessary to simultaneously investigate the influence of different variables on products quality using statistical design such as surface response methodology.

7.4. Production and cleaning

Another aspect that should not be ignored in terms of future directions of fermented meat items production is cross contamination. Several studies should be conducted to include cleaning process validation that can be used before and after mass production on each fermented meat product so as to ensure both safety and high quality of the product. There are several toxic, unwanted components or microorganisms that might be remnant over machine used in the production process or in some cases, one production line is used for manufacture somehow related meat fermented products. In these cases, cleaning validation application is recommended to monitor and control the process with safe and higher quality (Blackstone et al., 2018).

Market requirements for high quality and safe fermented meat products led to increased research into developing alternative preservation and processing methods at no or least amounts of chemical additives. Such improved criteria of safety and quality level for fermented meat products can be achieved, for example, by applying novel decontamination strategies like irradiation, pulsed UV light, high pressure processing (HPP) and hurdle technology, either alone or combined with conventional application (Farag et al. 2020; Ojha et al. 2015).

8. Conclusions

Fermentation is an important tool that can be used to improve meat products for overall healthful impact. Meat fermentation involves starters, bacterial, yeast and mold cultures, which play crucial roles on texture change, flavor formation, and biogenic amines accumulation. These microorganisms and their harbored enzymes affect organoleptic characteristics of fermented meat products. Although "omics"-based biotechnological development of novel probiotic starter cultures have led to engineering newer strains with much better strengths than their progenitors, yet presuming that the new strains are as safe as their progenitors will be preposterous because gene mutation can occur at the molecular level. Therefore, safety and efficacy of novel probiotic organisms for meat fermentation should be carefully ascertained before commercialization. In addition, the production conditions and the major biochemical changes in fermented meat products could be manipulated to present the best factors for enhanced quality of fermented meat in terms of nutritive value and/or health outcome. Moreover, overall safety concerns and prospects for further research of fermented meat should be of special interests to both consumers and the food industry.

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Authors contribution

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Disclosure statement

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