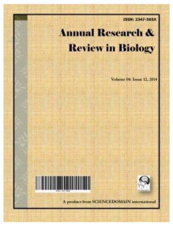

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Effects of Starter Cultures on the Properties of Meat Products: A Review

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Authors' contributions

This work was carried out in collaboration between both authors. Authors ZP and HH designed the study, wrote the protocol, and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Given that competitive microorganisms such as lactic acid bacteria (LAB) are considered as "generally recognized as safe" (GRAS) they must not adversely affect the organoleptic properties of food products. Lactic acid bacteria have been extensively studied and are usually used commercially as biopreservatives to decrease the population of pathogenic and spoilage bacteria in meat and meat products throughout different mechanisms including depletion of nutrients, creation of acidic environment or production of antimicrobial metabolites such as bacteriocins, reuterin and hydrogen peroxide. Additionally, several types of starter cultures are added to traditional meat products that can have interactions with pathogens and probiotics. These bacteria may develop texture, color and flavoring properties, produce buffering components, prevent lipid oxidation, restrict formation of biogenic amines and cause protein changes or even accelerate polychlorinated biphenyls degradation. In this review article after a brief introduction to the subject different types of starter culture and their modes of action are explained. Moreover, application of starter cultures with

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other hurdles in meat industry as well as their safety and toxicity aspects from different microbial and chemical points of view is comprehensively evaluated.

Keywords: Starter cultures; mycotoxins; nitrosamines; polychlorinated biphenyls; biogenic amines; sensory evaluation.

1. INTRODUCTION

Over the past decade global meat production has been increased by 20% [1] or by nearly 70 million tons [2] with an amount of 4.1 kg/ha of agricultural land [3]. Promotion of safe products has led to the manufacturing of fermented meat products. European countries including Germany with more than 350 different types of fermented sausages as the leading country are considered as the major producers and consumers of fermented meat products [4,5]. In European Union (EU) approximately 750 000 tons of fermented sausage was estimated to be produced every year [6] which is equal to 20-40% of total processed meat products [5].

These products are considered natural as the inoculated microorganisms as starter cultures are the normal inhabitants of gastrointestinal tract [7]. Amongst production processes for safe products, fermentation is one of the oldest processing methods. This method was in use before bacterial discovery and has been improved by using specific microbial competition [8]. Microbial competition has been formally explained by ecologists as the process by which one organism reduces the survival or growth of the others. The phenomenon is divided into two main types: interference or exploitative competitions. The first occurs when organisms directly injure each other by secretion of products including bacteriocins and secondary metabolites [9]. The second type is indirect and is accompanied by nutrition limitation, particularly when the microorganisms lie on surface and form biofilms [10].

For production of dry fermented sausages, high populations of desired microorganisms, known as starter cultures, are used. Fermented products can be obtained by bacteria that already exist in the product or by using the selected starter cultures under controlled conditions that ensures a predictable process [11, 12]. Spontaneous fermentation possesses a distinctive quality compared to controlled fermentation, due to specific batter and bacteria. As [13] have indicated, natural starter cultures and selected starter cultures are preferred to be used in comparison to adventitious microbiota. The superiority of natural starters might be due to better flavor and overall acceptability, for further control and better ripening of final product. It should be stated that selected types of lactic acid bacteria and yeasts which produce bacteriocin and ethanol, such as antimicrobial agents, are more common in use than the natural starter cultures. This might be due to variation of natural starter cultures in bacterial strain and population load, which results in quality instability, higher risk of spoilage and also resistance to bacteriophages attacks [14].

For production of meat products different types of starter cultures including (1) lactic acid bacteria producing lactic acid, (2) bio protective bacteria producing bacteriocins, (3) staphylococci and micrococci bacteria as curing agent cultures and (4) mold and yeast for surface treatments can be employed for autochthonous and commercial starter cultures in meat industry [15].

In aging and fermentation process bacterial starter cultures are of great importance in deviation of new and distinct meat products through acidification and effects on sensory acceptability and physical properties [13].

Though the effectiveness of some of starter cultures in safety and quality of meat products have been well established, few reviews have been managed to study the potential of fermentation by starter cultures in order to preserve meat products with explanation of advantages and disadvantages. Hence, the aim of this article is to review starter cultures and discuss their mechanism of action, benefits, limitations and different aspects of application in meat industry.

2. STARTER MICROBIAL CULTURE IN MEAT INDUSTRY

Yeast starter cultures such as *Candida famata* and its teleomorph (*Debaryomyces hansenii*), fungi starter cultures including *Penicillium chryosogenum and Penicillium nalgiovense* and bacterial starter cultures including LAB (*Lactobacillus sakei*, *Lactobacillus pentosus, Lactobacillus buchneri*, *Lactobacillus curvatus,* *Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus pentosum, Lactobacillus Brevis, Lactobacillus alimentarius, Carnobacterium maltaromaticum)*, *Staphylococcus* species (*Staphylococcus carnosus, Staphylococcus xylosus, Staphylococcus saprophyticus)*, *Pediococcus* (*Pediococcus pentosaceus, Pediococcus acidilactici*), *Micrococci* (*Micrococcus varians)* and Actinomycetes are usually used for fermentation of meat and meat products in mixture consisting 10^5 to 10^6 cfu/g of miroorganisms [5,16-21].

3. ANTIMICROBIAL EFFECTS IN MEAT PRODUCTS

Meat and meat products are considered as one of the main sources of European food borne outbreaks that are almost associated with *Salmonella* [22]. Given that *Salmonella* and other pathogens related to consumption of meat products including Enterobacteria, *Staphylococcus aureus, E. coli* O157, *Clostridium perfringens* and *Listeria monocytogenes* can be suppressed by application of starter cultures [23,24]. Fermented meat products have been reported to contain food borne pathogens such as verotoxigenic *E. coli* (VTEC) exhibiting microbial hazards [25].

In Sweden (2002) an outbreak (39 cases) associated with consumption of fermented sausage containing enterohaemorrhagic *Escherichia coli* (EHEC) was reported, postponed fermentation had caused unheated beef to be the main source of infection [26]. In consistent to this survey an EHEC outbreak in Canada was linked to unheated product which necessitates further prevention treatments to restrict infection [27]. Similar outbreaks of *E. coli* (shigatoxigenic) have been reported in meat products such as undercooked hamburgers (1993, 700 sick cases and 4 dead in USA) [28] and cured sausage (2006, 17 cases in Norway) [29].

The preservation by LAB is primarily due to the production of antimicrobial metabolites such as organic acids, hydrogen peroxide through strong oxidizing effect on membrane lipids and cell proteins, bacteriocins and reuterin as well as competition for growth with pathogenic or spoilage bacteria [30, 31]. LAB may possess attacks against phages through ineffective infection, restricting or modifying systems and interference in bacteriophage adsorption [8].

Bacteriocins as antimicrobial metabolites of fermentation have been well studied [32]. At least one bacteriocin is produced by almost all the bacteria, but varieties of bacteriocins are made by food grade LAB that are easily introduced into fermented meat products [33]. Another metabolite of reuterin or hydroxyl-propionaldehyde is a broad-spectrum antimicrobial substance produced by *Lactobacillus reuteri* during fermentation of glycerol. Growth of some harmful Gram-negative and Gram-positive bacteria, yeasts, molds, and protozoa can be inhibited by reuterin through inhibitory effect on ribonucleotide reductase [34].

Amongst Lactobacillus strains, *L. plantarum* isolated from Kimchi has shown to convert sugar into lactic acid in fermented sausages by homolactic fermentation process [35]. Nontraditional meat starter cultures (NTMS) contain *L. paracasei* LAFTIkL26, *Lactobacillus acidophilus* LAFTIkL10, *Lactobacillus* sp. L24, *L. paracasei* 5119, and *Bifidobacterium lactis* LAFTIkB94 that are added to extend the shelf life of Hungarian salami, as these cultures show strong inhibitory effect against both *L. monocytogenes* and *E. coli* O111 [36].

Many investigations have been performed on the application of LAB as the dominant bacteria of fermented meat products to prohibit pathogens and spoilage microorganisms during pre and post processing, and handling through a safe metabolic activity and rapid production of organic acids [5]. Homofermentative *L. sakei* is the dominant bacteria of fermented meat sausages which possess different mechanisms to cope with low temperature, pH and water activity and produces the bacteriocin of sakacins exhibiting antimicrobial effect against both spoilage and pathogen bacteria [37]. Dry fermented sausages are mostly produced by a combination of homofermentative LAB and Stapylococci bacteria [37]. In raw sausages a mixed culture consisting of *L. sakei* and *S. carnosus* providing a pleasant mild taste is usually used which shows synergetic properties within the culture, whereas it exhibits antagonistic effect against undesirable spoilage and pathogen microorganisms [38].

Interestingly, there has not been a significant sanitary risk via developing indigenous starters which is promising higher sensory acceptability and better sensory properties [39]. However, to ensure safety in meat products manufacturers usually add lactic acid bacteria to starter cultures for higher acidification. In addition, appropriate contents of nitrate, carbohydrate, salt and other factors associated with acidity should also be considered in formulation of fermented meat products as well as using specific strains of starter cultures producing bacteriocins and reuterin/reutericyclin [40].

Lactic acid bacteria as the dominant bacteria of traditional meat products such as Bosnian [41], Croatian [42] and Botillo [43] extend the shelf life of final product through acidification.

Although dry cured ham and fermented sausages are not exposed to high temperatures (>50°C) but have a longer shelf life than cooked ham and luncheon meat. The longer shelf life might be associated with lower pH value (more significant in Northern Europe products) and lower water activity value particularly in Mediterranean types of fermented meat products via drying and salting processes [44]. In Mediterranean types lactic acid is produced in a slower rate and at the same time it is consumed by surface molds [17].

4. APPLICATION IN MEAT INDUSTRY

The preservation of meat products through fermentation by native organisms has been applied for millennium [45]. As reported, bacterial attachment to meat tissues differs regarded to ionic strength and pH value [46]. Adhesion to animal tissue is necessary for microorganisms' survival. Attachment differences have an effect on safety and spoilage of meat products [47]. *Escherichia coli* mostly adhere to beef muscle with higher ionic strength and it's penetration can be increased by a rise in injection pressure as the integrity of tissue disrupts [46,48]. The quality of fermented meat products can be improved by exploiting non thermal technologies and other novel techniques with starter cultures. High hydrostatic pressure can be applied to the product before inoculation of starter cultures. 200 MPa (10 min in 17 degree Celsius) of pressure could prevent Enterobacteria growth in terms of degradation of proteins, lower moisture content and acidification of inoculated bacteria. By this technique, formation of some biogenic amines such as putrecine and cadavarine was significantly inhibited [49].

Starter cultures are usually added to three types of basic fields: (1) raw cured hams, (2) pasteurized/cold cuts and (3) fermented sausages [13]. Fermented meat products can be grouped according to their dimensions, type of meat, fermentation conditions, materials and ingredients [50]. There are many other classification for meat products, for example as another classification for fermented meat products on the basis of applied starter cultures they are classified into two groups of European products by *Lactobacillus sakei* and *Lactobacillus curvatus* and USA type by *Pediococcus acidilactici* and *Pediococcus pentosaceus* [14].

Novel meat products can be manufactured by using specific starter cultures of lactic acid bacteria such as *Carnobacterium maltaromaticum* or Staphylococci that have shown to produce high concentration of aroma compounds through converting enzymes of amino acids beside inhibitory effects of lactic acid bacteria on pathogenic and spoilage organisms [21]. For production of new fermented meat products types of starter cultures should be chosen according to their health effects and related quality control parameters. For example, *Enterococcus faecium* and *Enterococcus faecalis* are applied for Spanish type of sausage and *Pediococci* is used for Iberian, Italian and American types of fermented sausages [5]. Probiotic meat products are of novel products which are not yet common in meat industry [51]. Bioactive peptides produced by probiotic bacteria are a promising way to develop a new functional food [52].

Some of starter cultures as probiotic bacteria can survive during process and storage conditions increasing functionality of final product and making diversity in the end product through production of different metabolites as well as acidifying the product [53]. Probiotic meat products can be used for bio protectors purposes including improvement in immune system and lactose digestion, strengthening mucosal barriers and preventing gastrointestinal infections by interaction with other microorganisms via site of action or producing antimicrobial agents [54].

These kinds of products should carry a least count of probiotic microorganisms to be called a probiotic product which may possess further health benefits [38]. Processing and storage conditions as well as food passage through the digestive tract can affect survival of probiotic bacteria [55]. Moreover, unlike starter cultures counts of probiotic microorganisms are not expected to increase after inoculation. Therefore, to ensure viability of probiotic bacteria in final product and through passage of digestive system higher dosages of freeze dried probiotic bacteria

(10^8 cfu/g) are required than for starter cultures [56].

Fermented sausages as a carrier of probiotic bacteria have a low pH and water activity as well as containing salt and other microorganisms which exacerbating the conditions for survival of probiotics. In this case *Pediococcus acidilactici* and *Lactobacillus sakei* have shown a good survival whereas *Bifidobacterium* culture have shown a weak survival [5]. Probiotic strains of *Lactobacillus* and *Pediococcus* exhibit acceptable technological properties and flavor profile in production of dry sausages [57]. Probiotic starter culture of *Bifidobacterium animalis* and *Lactobacillus acidophilus* are of good alternatives to common commercial starter cultures exhibiting better sensory and textural properties without any negative effect on technological properties in fermented sausages [58].

For development of new meat products by using starter cultures the safety can be provided through new technologies such as cold pasterurisation (high hydrostatic pressures), introducing bio protective bacteria producing bacteriocins, direct addition of bacteriocins/phytochemicals and application of specific phages of starter cultures [59]. Bio protective type of starter cultures can be applied into sliced prepackaged meat in order to improve sensory properties through elimination of pasteurization after packaging and excess chemical preservatives [38].

Although sensory acceptability from consumer's point of view should be considered in combination of preservation techniques with fermentation process but food safety parameters often determine how much preservatives such as NaCl or nitrate should be added to meat products [44].

Some technologies (hurdles) are prohibited with fermentation of meat products such as smoking of fermented meat products that worsen polycyclic aromatic hydrocarbons (PAH) formation encountering legislative constraints [60]. Some complex innovations are restricted due to unfamiliar and unknown effects on product safety and consumer's health [60].

Although high temperature/long process time, hygienic measures, fat level of product, drying process, salting (increasing pH value) and quality of ingredients may affect viability of starter cultures and probiotic bacteria there are number of probiotic types of starter cultures including spore-forming *Bacillus* species (*B. coagulans* and *B. subtilis*) that are resistant to processing conditions [55]. Even differences in dimension of fermented sausages can lead to microbial changes through significant differences in pH value and lactate/acetate concentrations of the end product [21].

Processing meat by using different methods affects viability of starter cultures. For example ripening in room temperature increases the population of micrococcus and staphylococcus bacteria and *Pediococcus acidilacici* (>40°C) grows at higher temperature compared to *Lactobacillus plantarum* (30-35°C) [5].

Starter culture of dry fermented sausage can degrade glucosinolates of mustard into isothiocyanates showing higher antimicrobial activity against *E. coli* O157:H7 in combination with heat treatments (15 min in 115°C) [61].

Microencapsulation of starter cultures in sausages has not shown any significant increase in antimicrobial effect against *E. coli* [53]. However, counts of viable cells can be increased by micro capsulation with walls used to protect the bacteria from harmful environmental conditions. To have a strong wall structure for capsules different materials have been used. A mixture of starch and alginate improves the stability of structure. The increased stability and cell viability is associated with high viscosity of alginate when it is used alone [62]. Alginate contains calcium and carboxyl groups which are affected by pH changes due to fermentation [63]. Starter cultures distribute in voids of alginate network [64]. Sphered shape of micro capsules are influenced by viscosity of solution.

Fat as a carrier of lipid-soluble compounds is responsible for desirable texture and mouthfeel of fermented meat products [44]. In line with previous works related to fat and moisture contents of fermented products, capsuled bacteria inoculated in fermented meat have shown to be responsible for higher contents of protein and fat [16]. Encapsulated *L. reuteri* ATCC 55730 has been used for inhibition of *E. coli* O157:H7 in fermented sausage. Combined encapsulated and free cells exhibit highest inhibitory activity against *Escherichia coli* O157:H7. The free cells viability is less than the encapsulated cells and shows an immediate effect at the beginning of the process because of environment conditions [65].

5. TEXTURE AND SENSORY PROPERTIES

Organic acids produced by starters' exhibit sensory and texture traits which results in a better mouthful, juiciness, less brittleness and intensive flavor of final product compared to samples without starter cultures [66]. Starter cultures show distinct contribution in acidification. For example, *Pediococcus* exhibits a weak contribution in acidification compared to *L. sakei* and other dominant bacteria of commercial starter cultures [17]. For a desirable sour flavor of dried meat product lactate (main flavoring agent) to acetate (with stronger antimicrobial activity) ratio should be from 7:1 to 20:1 [5].

Staphylococcus strains as well as LAB are responsible for sensory properties of fermented sausages by degradation of amino acids and inhibition of lipid oxidation [39]. Contrary to pediococci and lactobacilli, staphylococci cultures do not contribute in acidification of fermented meat products. Among *Staphylococcus* strains, *S. xylosus* produces lower contents of volatile compounds compared to *S. carnosus* due to amino acids degradation [67]. [68] have analyzed the peptides released by a mixture of *Lactobacillus* and *Staphylococcus* starter cultures from specific proteins of beaker sausages. Besides contribution in flavor, these peptides can be considered as biomarkers. Unlike *S. vitulines, L. carvatus* showed to produce acid and ensures safety [68]. However, LAB bacteria used to inhibit the growth of pathogens in food must not exhibit undesired sensory effects. Some examples for texture and sensory changes due to addition of starter cultures with other hurdles are listed in Table 1.

As stated in Table 1 fermentation by starter cultures can be improved in combination with other bacteria or components including sugar, enzymes and unsaturated fatty acids. Sensory and texture properties of fermented meat products may not change in encapsulation form (alginate) or may change by using vacuum packing or high hydrostatic pressure.

Application of commercial starter cultures in horse sausages have revealed that counts of LAB and total viable counts can be increased, whereas Enterobacteriaceae count decreases. Use of commercial starter cultures results in lower intense flavor and higher acid taste [76]. However, at the final stages of fermentation process production of acetic acid and acetoin in high concentration might possess sensory issues [17]. Commercial starter culture of *Staphylococcus* and *Lactobacillus* was studied for further changes of proteins during process of a type of Turkish meat product called pastirma. Myofibrillar proteins of actin and myosin were both affected by starter culture, but myosin was more stable to heat treatments than actin. Among genus *Staphylococcus*, *S. carnosus* species denatured the proteins hardly when exploited without *L. pentosus*. This investigation represents that by use of starter cultures lower temperature and energy input are needed to degrade myofibrillar proteins [77].

A study showed the relation between type of starter cultures used in fermented meat and profile of volatile organic compound (VOCs). The aroma profile differed significantly between sausages with *P. pentosaceus* and *L. sakei* cultures. Metabolism of pyruvate and the pathway starter cultures use lactic fermentation products exhibit the main role in differences between volatile profiles of fermented products at variance starter cultures [78].

Moreover, lipid oxidation was controlled by *L. sakei* in long time. Unlike lactic acid bacteria, *L. sakei* has shown to decrease peroxidation and malondialdehyde (MDA) in vitro [79], in production of refrigerated vacuum packed sliced beef [80] and fermented sausages. The Nitrite content was decreased significantly and the color values, texture and overall acceptability of fermented sausage were all developed by using *L. sakei* culture. Free fatty acid content was not significantly increased [81]. 75 ppm of nitrite shows inhibitory effect against formation of biogenic amines in Sucuk [82].

In contrary, there were not any significant changes in sensory evaluation of manufactured sausage samples containing *L. sakei* with other starter cultures. All the examined batches showed to have a pH fall related to breakdown of carbohydrates to organic acids including lactic acid. As mentioned before, this acidification suppresses growth of undesired bacteria; which results in flavor changes, formation of red color and a reduction in water binding capacity of proteins that ensures drying process in fermented sausages [83].

Pork meat inoculated with a commercial starter culture has emerged out to have higher contents of moisture and lipid oxidation which might be associated with amounts of total free acids produced by starter cultures. Excluding the hardness, none of the textural parameters were affected by inoculation of starter cultures, but color and flavor scores showed to be higher in comparison to inoculated samples [84]. It should be considered that the products of bacterial proteolysis such as amino acids and amines show a buffering effect [85]. Chemical acidulation by glucono delata lactone (GDL) has shown to control microbial contamination [86] and decrease formation of biogenic amines [87]. However, GDL may change the traditional flavor of dry sausages [88].

Table 1. Sensory and texture properties of fermented meat products under different conditions

Combined factor with starter culture	Type of meat product	Type of starter culture	Sensory/texture changes	References
Probiotic (protective strains of Lactobacillus strains and Pediococcus)	Experimental dry sausage	Without commercial starter cultures	Similar profile analysis to commercial dry sausages	[57]
Protease EPg222	Salchichon	*Staphylococcus* strains and *L. sakei*	Better sensory evaluation (intensive flavor and lower hardness)	[69]
Fish oil (high content of n-3)	Functional dry fermented sausage	Typical starter culture	Without unpleasant and rancidity flavor	[70]
Heat treatment	Fermented dry and semi-dry sausages	*Pediococcus pentosaceus*	Lower pH (tangy flavor and chewiness)	[20]
Sugar (>0.5%)	Chorizo	*Lactobacillus* and *Pediococcus* strains	Higher hardness and chewiness	[71]
Microencapsulation (alginate)	Dry fermented sausages	Pediococcus and *Staphylococcus*	Similar sensory properties to control not microcapsulated	[65]
High hydrostatic pressure (400 MPa, 10 min) after ripening	Fuet and chorizo	*Lactobacillus sakei* and *Staphylococcus xylosus*	Higher chewiness, springiness and cohesiveness	[72]
Lactobacillus acidifying strain	Valla di Diano	*Staphylococcus* strains	lower greasy and chewiness properties	[73]
Vacuum packaging at resting (drying room)	Dry cured RIB ham	Yeasts, LAB, catalase positive cocci (Gram-positive)	Crustiness and lower oil drip	[74]
Vacuum packaging (chill temperatures)	Fresh meat	*Lactobacillus curvatus*	Similar sensory properties to control not vacuumed	[19]
Cherry powder	Cured cooked sausages	Bacterial starte culture	Similar sensory properties to control containing nitrite	[75]
Probiotic (protective strains of Lactobacillus strains and Bifidobacterium)	Fermented mutton sausages	Without commercial starter cultures	Better texture properties and reduction in typical mutton smell	[58]
Encapsulation (alginate-starch)	Sucuk like product	*Lactobacillus plantarum* and *Staphylococcus xylosus*	Sour taste intensity compared to free form and heat treated controls	[16]

6. SAFETY AND TOXICITY IMPROVEMENT IN MEAT AND MEAT PRODUCTS

6.1 Microbial Aspects

6.1.1 Food infectious bacteria

For safety purposes and increasing responsibility of producers in meat industry EU regulation defines food safety criteria on six hazards including *E coli* O157H7 and *L. monocytogenes* found in meat products [89].

Given that starter cultures including lactic acid bacteria provide a safe condition for fermented sausages it can't compensate poor hygienic conditions. Some pathogens of *Yersinia, enterocolitica*, *E. coli* and *L. monocytogenes* might survive and grow particularly when they are stored for a long period at refrigeration temperatures without any maturation process [38]. *Listeria monocytogenes* with a high mortality rate and ubiquitous nature often present in most of meat products thus many countries have banned meat products containing any pathogen in 25 gram of samples [17].

E. coli and *L. monocytogenes* have been responsible for reported outbreaks in short ripened semi dry sausage and end product originated from environment, respectively [17].

To help this issue functional starter cultures producing bacteriocins as well as providing health benefits and acidifying the product have been proposed. Probiotic bacteria can tolerate harsh conditions and exhibit two types of inhibition in sausage and also in digestive system [58]. For example enterococci have an antilisterial effect through producing bacteriocins in fermented sausages [31,17]. *L. sakei* suppresses *Escherichia coli* O157:H7 and *Listeria monocytogenes* growth in fermented sausages [90]. Starter cultures of *Bifidobacterium longum* ATCC 15708 and *Lactobacillus reuteri* ATCC 55730 have shown to inactivate *Escherichia coli* O157:H7 during production of fermented sausage [65]. Low molecular mass molecules produced by *L. plantarum* and *L. pentosus* exhibit anti-*E. coli* and anti-listerial activities [91].

6.1.2 Food intoxicant bacteria

Spore forming *Clostridium botolinum* and *S. aureus* can produce toxins causing numerous outbreaks of food poisoning [89]. In fermentation process *Staphylococcus aureus* can cause poising in raw meat through producing enterotoxin at initial stages [17].

Although many of LAB bacteriocins are effective against listeria there are a few of bacteriocins exhibiting antimicrobial effect against *Clostridium botolinum* and *S. aureus* [6].

However, protective cultures may inhibit the growth of main food intoxication bacterial pathogens including *Clostridium botolinum* and *S. aureus* via acidification the product [38]. Probiotic bacteria may exhibit synergetic effect with nitrite as an antimicrobial agent for *C. botulinum and L. monocytogenes* [51].

As reported, Enterobacteria and *Staphylococcus aureus* counts decreases about 6 log CFU/g in Argentinan dry-fermented sausages by adding *Enterococcus faecalis* CECT7121 [24]. *Enterococcus faecalis* as a bio protective culture proves to be effective against *E. coli* O157, *Clostridium perfringens* and *Listeria monocytogenes* when added to ground beef (4 log CFU/g) [23]. Other bio protective agent such as fresh immobilized LAB including *L. casei* ATCC 393 is added to dry-fermented sausages increases the shelf-life even with reduced levels or zero levels of preservatives (sodium chloride, sodium nitrite and sodium nitrate) [92]. Resistance to spoilage (white spots by yeast and mold on sausage surface) is significant in products with immobilized *L. casei*. There is an extreme reduction in *pseudomonas*, *enterobacteria*, and staphylococci bacteria in sausages containing immobilized *L. casei* and are significantly resistant to spoilage caused by yeast and mold [92].

[81] manufactured a type of fermented pork sausage using *L. sakei* isolates (7 log CFU/g) isolated from Chinese cabbage. This strain established itself and more than 87.5 percentage of inoculated population existed at the end of process in pork sausages.

As reported, concentration of acid and bacteriocin increases due to higher counts of *L. curvatus* and *L. sakei* that suppresses growth of harmful microorganisms [19].

Microbial hazards in meat products are related to the presence and interactions of not only pathogens, but also probiotic bacteria (so-called side effects). It has been concluded that

Enterococcus species are the most serious risk for consumers associated with consumption of probiotic meat products [51]. Members of the *Enterococcus* and *Streptococcus* and some other LAB genera that are used in meat industry may contain certain opportunistic pathogens. Therefore, probiotics might be theoretically responsible for five types of side effects: systemic infections, deleterious metabolic activities, risk of adjuvant side effects, excessive immune stimulation in susceptible individuals and risk of gene transfer [93].

Using food grade lactic acid bacteria (LAB) in food products have shown to be safe and generally recognized as safe (GRAS). Some LAB as genera *Lactococcus* and *Lactobacillus* are GRAS due to their occurrence as normally commensally mammalian flora and their worldwide established safe use in variety of food and dietary supplements [94]. Combination of spices with starter cultures in mutton sausages has shown to improve safety and shelf life of product due to lower pH and water activity resulted from changes in microbial population of LAB [95].

The reservoir role of starter cultures which are resistant to antibiotic is of great concern due to their spread ability to human by food consumption [96]. Strains of *S. carnosus* have not been resistant to 17 types of antibiotics including ampicillin, but 13 and 10.5 percentages of strains were resistant to beta-lactam and chloramphenicol, respectively due presence of beta lactamase enzyme. None of examined strains contained toxigenic encoding genes, which is considered as low toxigenic potency [97].

Molds might produce antibiotics or some secondary metabolites and beta lactams that are considered toxic; thus starter cultures should be monitored for further safety of final fermented meat products [98].

6.2 Chemical Aspects

6.2.1 Mycotoxins

Many strains of Penicillium and Aspergillus species available on meat products may produce myctoxins. Mycotoxin can have tetratogenic, nephrotoxic and carcinogenic effects by different mechanisms such as oxidative stresses, damages to DNA and synthesis of proteins [99].

For selection of starter cultures from molds it should be considered that they must not produce mycotoxins which are harmful to health [31]. Although mold growth on the surface of fermented meat products are desirable in terms of visual appearance but many of toxigenic *Penicillium* species are able to produce mycotoxins including ochratoxin, citrinin, patulin and cyclopiazonic acid which leads manufacturers to use specific kinds of molds like *P. nalgiovense* with lesser toxigenic potential [4,5]. Mechanical removal of surface molds including *Penicillium nordicum* and *Aspergillus ochraceus* seems insufficient to control ochratoxin because they may contaminate the product through cross contamination via environment and air [100].

6.2.2 Heavy metals

Histidyl dipeptides such as carnosine and anserine are the most available antioxidant agents in meat and meat products [101]. Low molecular weight peptides can be produced via fermentation of commercial and autochthonous starter cultures including lactic acid bacteria [68]. These bioactive peptides of fermented sausages produced by bacteria such as *L. sakei* or *L. curvatus* can show antioxidant activity and exhibit chelating properties on metal ions including zinc, cobalt and copper [52]. Marine products might be contaminated to heavy metals that can be substituted by marine microorganisms of fermented products for production of omega 3 fatty acids [31].

6.2.3 Nitrosamines

There are many factors which affect formation of nitrosamines including processing conditions, contents of spices, salts and other preservatives and also the type of starter culture used for fermented meat products [102]. One of the selection criteria for LAB is that they are able to reduce nitrate [38]. Lactic acid bacteria can reduce NO_2 to NO by nitrite reductase enzyme and through acidification, which can decrease formation of nitrosamines and its harmful effects [103]. *Staphylococcus xylosus* shows high activity for nitrite reduction [91, 104]. Unlike *Pediococcus pentosaceus*, *Staphylococcus xylosus* converts metmyoglobin to nitrosylmyoglobin in pork batters without adding any nitrite or nitrate [105].

6.2.4 Polychlorinated biphenyl (PCB)

Several studies have investigated microbial degradation of polychlorinated biphenyl (PCB). The microbial degradation of PCBs related to starter cultures has also been studied. In contrary to meat products, in liquid media *Staphylococcus* strains are known for their PCB degradation ability. The Rate of degradation accelerates in exponential phase of starter cultures growth. As [106] reported half of PCB degradation occurs within two days of incubation, which coincides to rapid acidification of product. It should be considered that products of PCB degradation might exhibit higher risk to human health compared to PCBs itself. Effect of a common commercial starter cultures containing *Staphylococcus* on PCB degradation has been studied. The results showed that starter treatment degrades PCB as well as heat treatment in frankfurter meat products [107].

6.2.5 Biogenic amines (BA)

High levels of BA cause diarrhea, sweating, nausea and tachycardia [108]. It should be stated that tyramine shows severe symptoms of necrosis compared to apoptosis caused by histamine [109]. Biogenic amines in fermented products such as cadaverine and histamine might be a risk factor which seems to be related to probiotics, since responsible microorganisms for the fermentation process may contribute to accumulation of biogenic amines [110].

There are some restrictions to control formation of biogenic amines in traditional meat products such as: (1) thermal process might kill desired bacteria and enzymes; (2) addition of artificial additi;2ves alters the expected flavor of traditional products, and (3) in irradiated and MAP products aroma changes could occur in final product [83].

As an alternative method to inhibit production of biogenic amines competitive LAB should be chosen based on their growth ability and survival during fermentation or storage conditions. Amongst LAB isolated from Spanish fermented dry sausage, strains of *L. sakei* Al-143 and *L. paracasei* are considered as safe starter cultures due to sensitivity to antibiotics and formation of biogenic amines [111]. Starter cultures with a mixture of *S. carnosus* and *L. sakei* have shown to positively affect quality parameters of meat products and have a lower content of biogenic amines [14]. Some strains of *L. curvatus* which are used as starter cultures form up to four different biogenic amines and should not be used [112,113]. In contrary, strains of *L. sakei* [114] and *T. koreensis* [115] do not have this potential. Phenethylamine as a biogenic amine is produced by *S. carnosus* strains, but other biogenic amines including histamine, cadavarine and putrescine are insignificantly produced [97].

Amino acid content and proteolysis activity of fermented meat products differ by type starter cultures. Decarboxylation of amino acids which produce biogenic amines is related to availability of encoding genes of enzyme and suitable condition for bacterial growth. These encoding genes could be horizontally transmitted between bacteria which add to risk of intoxication in specific conditions of acid concentration and temperature [116]. Higher contents of biogenic amines are produced with slow acidification of fermented products [117]. Most of bacterial decarboxylases have an acidic pH optimum. Therefore, reduced pH increases decarboxylase activity in bacteria as part of their protective mechanism and reduces the growth of amine-positive microorganisms [6]. Post fermentation storage in refrigerator have been suggested for Herkules fermented sausages due to formation of high content of most toxigenic biogenic amine-tyramine by micro flora [118]. Therefore, in screening of desired bacteria presence of encoding gene of decarboxylase, acidification rate and temperature should be considered.

7. CONCLUSION

Using starter culture bacteria is an old energy efficient method to extend the shelf life of highly perishable meat products and improve functionality in order to fulfill consumer demands and maintain diversity in the end product.

Consumption of meat and meat products such as beef burgers is related to many chronic diseases including cancer and cardiovascular diseases that can be relieved by application of starter cultures. In comparable to meat and other meat products, fermented meat products are known as nutritional and healthy safe products with good sensory quality from ancient times and have been always appreciated by various cultures and societies. In this case, application of probiotic bacteria causes more health effects via synthesis of vitamins and some secondary metabolites.

Application of starter cultures and probiotics leads to microbial and physical changes which results in safety and quality of meat products. Beside acidification and production of bacteriocins through fermentation, salting and drying processes can lead to longer shelf life of fermented meat products that makes these products more digestible and also easy to keep and truck. Moreover, acidification may lead to a favorable texture by denaturing protein as well as modifying fat emulsifying properties.

As concluded, these kinds of products show a high overall acceptability and pleasant flavor due to high moisture content (lower water activity) and juiciness, less brittleness and hardness, and intensive taste of product with rarely rancidity flavor.

Besides all these positive effects starter cultures might develop antibiotic resistant bacteria or possess the risk of biogenic amines formation. Hence, starter culture bacteria should be selected according to their metabolic and physiological properties in order to maintain a desirable meat product by a controlled fermentation process with development of desired microorganisms.

As a safety hazard, LAB genera may contain/interact with pathogen/probiotic organisms. Formation of biogenic amines, side effects of probiotics and degraded PCBs are of great concerns in using starter cultures in meat and meat products. Therefore, it is necessary to investigate the side effects and toxicity before using starter cultures in meat and meat products.

Competitive organisms, particularly LAB can be a good choice for meat preservation due to their ability in preventing pathogen growth and their positive effect on sensory properties through fermentation. However, more investigations are required to solve the problem on survival of probiotic type of starter culture bacteria in high concentrations of salt and acid as well as low water activity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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