Chapter 5

Probiotic Meat Products

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1. Introduction

The growing concern of consumers regarding the food health and safety issues has led to the development of products that promote health and well-being beyond its nutritional effect [1]. Functional foods are those which promote beneficial effects to human´s health beyond nutrition. Their effects are due to the addition of active ingredients, the removal or the replacement of undesirable compounds in its composition [2].

The marketing of food for health benefits began in 1960s. In 1970s the trend was to eliminate or reduce the harmful constituents like sugars and fats from food. In 1980s, the trend continued with the reduction or elimination of food additives, which led to the induction and addition of useful components like vitamins, minerals and probiotics in 1990s [1, 3].

Among the different types of functional food, probiotics represent a large share of the functional food market, being used mainly in dairy beverages, cereal products, infant feeding formulas, fruit juices and ice cream [4-7].

In meat industry, the demand for new products has greatly influenced its development, especially for sausage type products. However, lately, those meat products are considered unhealthy by a part of population because of their fat content and the use of additives and spices in their formulation. Therefore, the addition of probiotics to the fermented sausages could promote the health benefits associated with lactic acid bacteria and contribute to the increase in the consumption of such products [7, 8].

The use of probiotics seems more promising in raw fermented meat products like salami as they are made with raw meat and consumed without prior heating, which would kill the probiotic bacteria [9, 10]. However, the incorporation of probiotic bacteria to these products also represents a technological challenge because of the known sensitivity of probiotic to curing salts, spices and other ingredients used in the formulation of the fermented sausages [11]. Furthermore, this addition requires the use of microorganisms that are resistant to the fermentation process and that remain in a minimal viable number of cells to survive the stomach pH and exert beneficial effects in the intestines [8].

Additionally, the processing of probiotic meat products implies taking into account the appropriateness of the probiotic culture to the target consumer, the intestinal functionality expected for the probiotic species, the rate of survival of probiotic during food processing and the need of maintenance in the probiotic product of the same sensory attributes that characterize the regular product [8, 10, 12].

This chapter presents the potential applications of probiotics in fermented meat products, focusing on the technological challenges, the functional effects of probiotics and on the researches that address the addition of probiotics in fermented meat products.

2. Fermented meat as a probiotic product

2.1. Fermented sausages

Fermented sausages are defined as a mixture of ground lean meat and minced fat, curing salts, sugar and spices, which are embedded into a casing and subjected to fermentation and drying [6, 13, 14].

The quality of fermented sausages is closely related to the ripening process that gives color, flavor, aroma, and firmness to the product which are developed by a complex interaction of chemical and physical reactions associated with the fermentative action of the microbiological flora present in the sausage. In handmade production processes of fermented sausages, fermentation occurs spontaneously by the action of *in nature* bacteria present on meat. In industrial processes the microbiological flora, responsible for the fermentation process, is known as starter culture [6]. Starter cultures are defined as preparations containing live microorganisms capable of developing desirable metabolic activity in meat. They are used to increase the microbiological safety, to maintain stability by inhibiting the growth of undesirable microorganisms and to improve the sensory characteristics of fermented sausages [1].

Starter cultures are formed by mixing of different types of microorganisms, where each one has a specific function. Lactic bacteria are used in order to generate controlled and intense acidification which inhibits the development of undesirable microorganisms, and provides increased safety and stability to the product. On the other hand, coccus catalase positive type bacteria, as *Staphylococccus* and *Kocuria*, yeasts as *Debaryomyces,* and molds as *Penicillium* usually provide desirable sensory characteristics to the product [1, 2, 8].

Table 1 shows the microorganism species most commonly used as starter cultures to fermented meat products.

Microorganism	Genus and Species
Lactic acid bacteria	*Lactobacillus acidophilus* [a], *L. alimentarius* [b], *L. brevis, L. casei* [a], *L. curvatus, L. fermentum, L. plantarum, L. pentosus, L. sakei* *Lactococcus lactis* *Pediococcus acidilactici, P. pentosaceus*
Actinobacteria	*Kocuria varians* [c] *Streptomyces griséus* *Bifidobacterium sp.* [a]
Staphylococcus	*S. xylosus, S. carnosus subsp. carnosus, S. carnosus subsp. utilis, S. equorum* [b]
Halomanadaceae	*Halomonas elongata* [b] (tested in dry cured ham)
Enterobacter	*Aeromonas sp.*
Mold	*Penicillium nalgiovense, P. chrysogeum, P. camemberti*
Yeast	*Debaryomyces hansenii, Candida famata*

Table 1. Microorganism species most commonly used as starter cultures in fermented meat products SOURCE: [15-17].
[a] Used as probiotic cultures.
[b] Used in commercial tests in industrial scale (Laboratorium Wiesby, Niebüll and Rudolf Müller and Co)
[c] formerly known as *Micrococcus varians.*

The selection of starter cultures for use in fermented meat products must be carried out according to the product formulation and the technological processing employed, since environmental factors can select a limited number of strains with the ability to compete and overcome on product. Typically, the species used as the starter culture are selected from strains naturally predominant in meat products and hence, well adapted to this environment. Therefore, these species present a tendency to have greater metabolic capacity which is reflected on the development of the proper sensory and physical-chemical characteristics on the product [6].

Given the adverse conditions of the meat matrix for a number of microorganisms, including those considered probiotics, several studies suggest the selection of probiotic properties in lactic bacteria from commercial starter culture traditionally used in fermented meat products and therefore, already adapted to grow in these conditions. These cultures will provide to the product the same sensory and technological characteristics than the traditional starter cultures, and exert beneficial effects to health [8, 15, 18]. Among the starter lactic acid bacteria, *Lactobacillus brevis*, *L. plantarum*, *L. fermentum* and *Pedioccus pentosaceus* have been characterized as probiotics [19-21]. Strains of *L. sakei* and *P. acidilactici* have also been proposed as potential probiotic in meat products, due to its survival under acid conditions and high concentrations of bile [22]. Probiotic cultures can also be selected from the lactic acid bacteria (LAB) naturally presented in fermented meat products [7, 21, 23-25].

2.2. Probiotic fermented sausages

Although the concept of including probiotics in meat products is not entirely new, only a few manufacturers consider the use of fermented sausages as vehicles for probiotics [7, 17].

Several meat products containing probiotics with claims for health benefits have been commercialized. A salami containing three intestinal LAB (*Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium* spp.) was produced by a German company in 1998. In the same year, a meat spread containing an intestinal LAB (*Lactobacillus rhamnosus* FERM P-15120) was produced by a Japanese company [26-28].

Fermented sausages are suitable for the incorporation of probiotic bacteria since mild or no heat treatment is usually required by dry fermented meat products, thus providing the suitable conditions required for the survival of probiotics [3, 14, 26]. The sausage has to be designed in such a way as to keep the number and viability of probiotic strain in the optimum range. Thus, reduction in pH (e.g. < 5.0), extended ripening (e.g. >1 month), dry or excessive heating has to be avoided if the beneficial effects of probiotic are to be harvested [3, 7].

In meat sector, meat cultures are generally added to fermented meat products with the function of inhibiting pathogens and increasing shelf-life, rather than introducing functional or physiological qualities. Those cultures are called protective starter cultures and do not promote significant changes in physical and sensory characteristics of the product. On the other hand, probiotic cultures are, by definition, those that after ingestion in sufficient number employ health benefits in addition to their nutritional effects [6, 8, 15]. However, often, the probiotic cultures have also been used in meat products as protective cultures, since both of these cultures have the ability to survive in adverse environments and to produce organic acids and bacteriocins [18]. Likewise, probiotics added to meat products are also known as functional starter cultures since they contribute to safety, can provide sensory and nutritional benefits and promote health [6].

The success of probiotics in other types of foods, especially dairy products, is based on scientific evidence of beneficial effects provided by some microorganisms. In meat products, the beneficial effects must be proven with the consumption of these products. From the good results obtained with dairy products it is not possible to conclude that a probiotic species will have the same effect on another type of product. This is due to the fact that the performance and properties of microorganisms are environment-dependent. Furthermore, there are few studies about the proper number of probiotic bacteria that should be ingested in meat products to achieve the desired effect [1, 15].

The estimated number of viable cells of probiotic bacteria to be ingested to obtain beneficial effects and temporary colonization of the intestine is around 10^9 to 10^{10} CFU/ g of product, in accordance with the counts of 10^6 to 10^8 viable cells found in 1 g of feces. Therefore, in a fermented meat product containing 10^8 CFU/ g, the minimum daily consumption might be 10-100 g of product [1, 29]. Rivera-Espinoza and Gallardo-Navarro [17] recommended the concentration of probiotic viable cells of at least 10^8 to 10^9 CFU/ g of the product to obtain the physiological effects associated with the use of probiotic food.

Despite the known health benefits provided by the use of probiotics such as the improvement of intestinal transit and digestion, improvement of symptoms of lactose intolerance, increase in immune response, reduction of diarrhea episodes, prevention or suppression of colon cancer and reduction of blood cholesterol [30, 31], much attention has paid to the use of probiotics in meat products in order to increase product safety and few studies evaluated the health benefits associated with the consumption of these products [7, 8, 15].

2.3. Most used probiotic cultures in meat products

Probiotics are mainly the strains from species of *Bifidobacterium* and *Lactobacillus*. Other than these, some species of *Lactococcus, Enterococcus, Saccharomyces* and *Propionibacterium* are considered as probiotics due to their ability to promote health in the host [32].

In fermented meat products several studies have demonstrated the feasibility of using probiotic *Lactobacillus*.

Arihara et al. [33] studied the use of *Lactobacillus gasseri* to improve the microbiological safety of fermented meat product. The use of *Lactobacillus rhamnosus* and *L. paracasei* subsp. *paracasei* for the fermentation of meat products has been studied by Sameshima et al. [9], while Pennacchia et al. [20] report the use of *Lactobacillus plantarum* and *Lactobacillus paracasei* as probiotics in meat products.

Erkkilä et al. [22] conducted experiments using probiotic strains of *L. rhamnosus* GG and potentially probiotic strains of *L. rhamnosus* LC-705, *L. rhamnosus* VTT-97800 and *L. rhamnosus* VTT for the manufacture of dry sausage.

Andersen [10] demonstrated the ability of mix of a traditional starter culture, Bactoferm T-SPX (Chr Hansen), and the potential probiotic cultures of *L. casei* LC-01 and *Bifidobacterium lactis* Bb-12 to ferment meat product.

Also Erkkilä et al. [11] used strains of *Lactobacillus gasseri*, *L. rhamnosus*, *L. paracasei* subsp. *paracasei*, *L. casei* and *Bifidobacterium lactis* for the manufacture of salami.

Pediococcus acidilactici PA-2 and *Lactobacillus sakei* Lb3 showed good survival characteristics in fermented sausages, being considered as probiotic candidates for meat products [7], as well as *Lactobacillus casei* and *Lactobacillus paracasei* isolated from fermented sausages which showed *in vitro* functional abilities [25].

Macedo et al. [34] investigated the viability of the use of probiotic *Lactobacillus paracasei, L. casei* e *L. rhamnosus* in fermented dry sausage with the maintenance of the technological and sensory characteristics of the product.

Vuyst et al. [7] and Khan et al. [3] stated that *Lactobacillus* species currently used as meat starter cultures, as *L. plantarum* and *L. casei*, can have a significant scope for being utilized in probiotic sausage manufacture.

2.3.1. Criteria for the selection of probiotic cultures for meat products

The criteria for a microbial culture to be considered probiotic are the stomach acidity resistance, lysozyme and bile resistance and the ability to colonize the human intestinal tract using mechanisms of adhesion or binding to intestinal cells [7, 8, 23, 35]. Other authors have also included the ability to tolerate pancreatic enzymes as a required characteristic of probiotic cultures [16].

Additionally to the criteria described above, the probiotic bacteria need to have *GRAS* (*Generally Recognized as Safe*) status [36]. Currently, this concept also includes the antibiotic resistance evaluated by Qualified Prediction Security Program suggested by EFSA (*European Food Safety Authority*). The ability of probiotic bacteria used in meat products to resist to some antibiotics can be genetically transmitted to other bacteria. Scientific studies report genetic determinants for bacterial resistance to chloramphenicol, erythromycin and tetracycline [14]. Normally, the lactic acid bacteria are sensitive to penicillin G, ampicillin, tetracycline, erythromycin, chloramphenicol and aminoglycosides, quinolones and glycopeptides [18]. Thus, the selection of probiotic cultures for meat products implies confirmation of the absence of antibiotic resistance transferable gens in selected strains [14].

However, among the criteria for the selection of probiotic cultures, the main condition to be evaluated is the ability of strains to promote beneficial effects in the host through interactions probiotic/ host and to prevent diseases [37]. These effects on human health may occur in three different ways according to the specificity of the strain: the antagonist action against other microorganisms in the same environment (by nutrient competition, bacteriocin production or competitive exclusion), the barrier effect on the intestinal mucosa and the boosting of immune system [7, 36].

2.3.2. Technological characteristics of probiotic cultures for meat products

For addition in fermented meat products, the probiotic bacteria need to maintain their viability towards the adverse conditions generated during the fermented sausages manufacture: low pH (<5.0), high salt content (2-3%), high nitrite content (around 120 ppm) and low water activity (<0.85). The probiotic cultures should also be capable of growing fast during the fermentation, be easily cultivated on an industrial scale, resist to freezing and lyophilization processes, provide longer shelf life to the product as well as contribute to the sensory quality of the final product [7, 11].

Probiotic cultures can be added in fermented sausage as part of the starter culture or as an additional culture incorporated during the mass mixing (Figure 1).

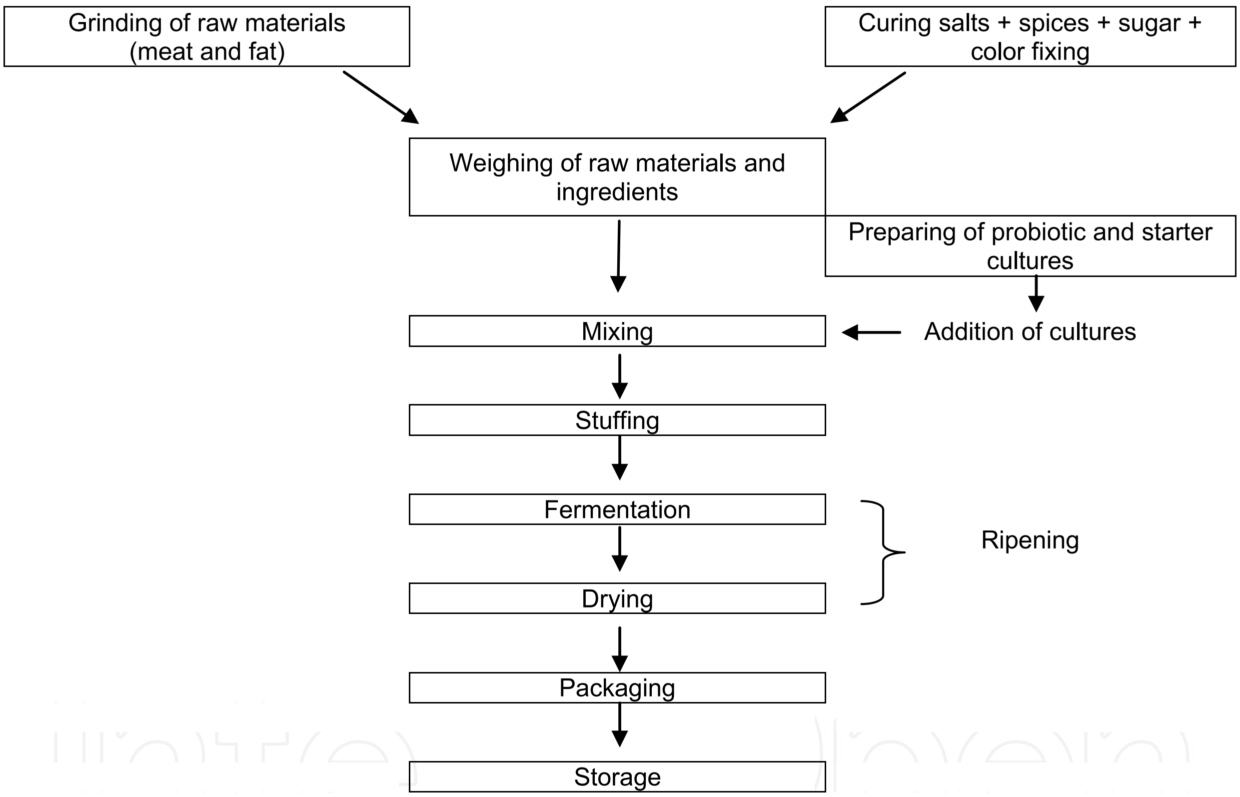

Figure 1. Basic flowchart of the processing of fermented dry sausage with the addition of probiotic cultures

Probiotic cultures may be added to the sausage batter as liquid inoculum, in high concentrations, or lyophilized. However, the addition of lyophilized culture can delay the fermentation time and reduce the culture viability in the final product. These effects can be reduced with the culture microencapsulation prior to lyophilization. This procedure is also indicated when probiotic strains are inhibited by ingredients of the sausage composition [6, 38].

Microencapsulation increases the viability of bacteria due to the protective effect of a polymeric membrane formed around the bacterial cells. The methods used for microencapsulation of lactic acid bacteria are extrusion and emulsification. Extrusion produces microcapsules with 2-3 mm in diameter which are 60 times greater than the microcapsule formed by emulsification. The materials most commonly used for the microencapsulation of probiotics include alginate, starch, k-carrageenan, guar gum, xanthan gum, gelatin and milk whey proteins. Muthukumarasamy and Holley [38] tested the microencapsulation of *Lactobacillus reuteri* ATCC 55730 in alginate for use in fermented meat product and found no adverse effect on the sensory quality of the product. Despite the microcapsules were visible to naked eye, they were detected as fat particles by the panelists due to their size and color similarity.

Rivera-Espinoza and Gallardo-Navarro [17] encapsulated *Bifidobacterium longum* and *Lactobacillus reuteri* in alginate to increase the survival of probiotics in fermented meat. Recently, Poulin, Caillard, and Subirade [39] created succinylated β-lactoglobulin tablet to protect *B. longum* strain and proved its protection effect *in-vivo* and *in-vitro*. Heidebach, Först and Kulozik [40] reported higher viability of *Lactobacillus* F19 encapsulated with casein during freeze storage compared to *Bifidobacterium* Bb12. Furthermore, the same authors [41] microencapsulated these two strains with rennet-induced gelation of milk, obtaining higher yields and improved survival rates.

2.3.2.1. Lactic acid production

One of the most important characteristics of *Lactobacillus* in fermented meat products is the production of lactic acid. The acidification has positive effects on safety and on the sensory characteristics of the product. The pH decrease in fermented sausages provides the coagulation of myofibrillar proteins, resulting in the increase of firmness and cohesiveness of the final product, and contributes to the flavor and red color. Inhibition of spoilage and pathogenic microorganisms is also provided by the fast decrease of pH and lactic acid production in appropriate quantities. The fast decrease in pH values during fermentation of sausages can also contribute to the prevention of the accumulation of biogenic amines, which are harmful to health [14].

However, it is important to confirm that the lactic acid bacteria used as probiotic produce the L(+) isomer lactic acid and do not produce the D(-) isomer lactic acid, due to the higher inhibitory effect on undesirable microorganisms of the L(+) lactic acid. Moreover, the D(-) lactic acid form is not metabolized by the human body and may cause health problems in consumers [7, 14, 42].

2.3.2.2. Resistance to salt (NaCl) and nitrite (NO_2)

According to Arihara and Itoh [43] and Sameshima et al. [9], the addition of 3% sodium chloride (NaCl) and 200 ppm sodium nitrite ($NaNO_2$) to fermented sausage is mandatory in Japan in order to maintain the microbiological safety of the product. Thus, the use of cultures resistant to curing salts is the first condition for the production of sausage with probiotic properties [23].

Sameshima et al. [9] tested the resistance of 202 *Lactobacillus* species of intestinal origin to sodium nitrite and sodium chloride in liquid medium and found that strains of *L. paracasei* ssp. *paracasei*, *L. rhamnosus* and *L. acidophilus* were tolerant to these salts. Similar results were obtained by Macedo et al. [44] who found resistance of *Lactobacillus rhamnosus*, *Lactobacillus paracasei* and *Lactobacillus casei* to the simultaneous use of sodium chloride and sodium nitrite at the concentrations of 3% and 200 ppm, respectively.

2.3.2.3. Bacteriocin production in meat products

Bacteriocins are peptides or proteins produced by microorganisms which destroy or inhibit the growth of gram positive bacteria, in particular *Listeria monocytogenes*. The use of bacteriocin-producing cultures in meat products may represent a considerable benefit to the consumers health and safety of the product, since bacteriocins do not pose toxicological hazards arising from their consumption and act as a natural form of preservation in the products. The production of bacteriocins has been detected in several lactic acid bacteria isolated from meat products such as *L. sakei*, *L. curvatus*, *L. plantarum*, *L. brevis* and *L. casei* [6].

2.3.3. Physiological characteristics of probiotic cultures for meat products

2.3.3.1. Resistance to low pH

The tolerance to acidity and bile salts are two fundamental properties that indicate the ability of a probiotic microorganism to survive through the gastrointestinal tract, resisting the acidic conditions of the stomach and the bile salts in the initial portion of the small intestine [22, 45].

The acidity is considered the most important deleterious factor that affects the viability and growth of lactic acid bacteria, since its growth is greatly inhibited at pH lower than 4.5. Such inhibition is related to a reduction in intracellular pH of the bacteria caused by non-dissociated lactic acid form, which due to its lipophilic nature, it diffuses through the cell membrane and causes collapse of the electrochemical gradient, promoting bacteriostatic or bactericidal effects [14, 36].

The survival of the probiotic to the gastric juice depends on its ability to tolerate low pH. At the time of hydrochloric acid excretion, the stomach pH is 0.9, however, during the digestive process the pH increases to around 3 due to the presence of food, remaining under this condition for a period of 2-4 hours [1, 22].

Due to the sensitivity of most bacteria to the low pH of the stomach, probiotic bacteria have to be ingested with food, because it acts as a buffer on the high acidity of the stomach, allowing the survival of the bacteria during gastric transit [46]. Meat, as well as milk, has buffers characteristics in acid environment and can thereby protect the probiotic from the adverse environment of the stomach [1].

Erkkilä and Petäjä [22] reported the resistance of species of *Lactobacillus pentosus*, *L. sakei*, *Pediococcus pentosaceus* e *P. acidilactici* to low pH and observed that at pH 4 and pH 5, the number of viable cells of these species remained unchanged compared to its initial value, indicating that the growth of the cultures was not affected by low pH.

Taking into account the pH conditions of stomach and the digestion time, probiotic bacteria ingested with food must be capable of resisting pH value 3 for a period of 2-4 hours to allow their survival during gastric transit. Macedo et al. [44] found that *Lactobacillus paracasei* used in probiotic salami was able to resist and grow in a medium at pH 3, showing a 20% increase in the initial number of cells during the 4 hours of exposure to this acidic condition.

Pennacchia et al. [20] tested the resistance of *Lactobacillus* isolated from 10 different types of salami to low pH. The authors found that from a total of 14 lactic acid bacteria that survived at pH 2.5 during 3 hours, 5 belonged to the *Lactobacillus casei* group. These authors also mention studies on the resistance of 20 strains of *Lactobacillus* isolated from infant faeces to acidic conditions and report the high viability rate of 3 strains of *L. paracasei* and one of *L. rhamnosus* at low pH.

2.3.3.2. Resistance to bile salts

Bile plays an important role in intestinal defense mechanism. The intensity of its inhibitory effect on microorganisms is determined by the concentration of salts in the bile composition [47]. Bile salts act by destroying the lipid layer and the fatty acids of the cell membrane of microorganisms. However, some *Lactobacillus* strains are able to hydrolyze bile salts by excreting bile salt hydrolase enzyme that weakens the detergent power of the bile [23]. *Lactobacillus* bile resistance has also been associated with other factors such as the stress response system as well as with the elements that involve the maintenance of cellular wall integrity, the energetic metabolism, the amino acid transport and the fatty acid biosynthesis [48].

According to Erkkilä and Petaja [22] and Pennacchia et al. [20], the average concentration of bile salts in the human intestinal tract is 0.3%, thus this is the critical concentration used for the selection of probiotic bacteria. Papamanoli et al. [23] consider as bile salts tolerance when a bacterial population reduces the number of viable cells from 10^6 - 10^7 CFU/ mL to 10^5 CFU/ mL in a 4 hour period.

Erkkilä and Petaja [22] observed a reduction of 1 log cycle in the initial number of viable cells of *Lactobacillus curvatus* and *Pediococcus acidilactici* when grown in a medium containing 0.3% bile salts and pH 6 after 4 hours of exposure.

From a total of 63 bacterial strains isolated from fermented sausages, canned fish, bakery dough and jellies, 9 strains of *Lactobacillus* sp. were able to survive at pH 2.5, while only strains of *Lactobacillus casei* e *Lactobacillus plantarum* showed survival at pH 2 and in the presence of bile salt [49].

Macedo et al. [44] found resistance of *Lactobacillus paracasei* to 0.3% bile salt.

Meat has also been reported to protect microbes against bile [50]. During meat sausage processing, *Lactobacillus* added to the batter are encapsulated by the matrix consisting of meat and fat. Due to the protection exerted by the food, the survival of *Lactobacillus in vivo* during transit through the stomach and intestine appears to be higher than that observed by the *in vitro* exposure of the microorganisms to low pH and bile salts [1, 22].

2.3.3.3. Detoxification capacity of biogenic amines produced in meat products

The biogenic amines, organic bases with aliphatic, aromatic or heterocyclic structures, are produced by the microbial decarboxylation of amino acids present in meat products, either by naturally occurring microorganisms or from the starter culture. The biogenic amines such as histamine, tryptamine, tyramine, cadaverine, putrescine and spermidine can cause toxic effects, especially in consumers with amino oxidase deficiency. In fermented meat products, biogenic amines producing microorganisms have a favorable environment due to the high protein content and the intense proteolytic activity that occurs during the long ripening time of these products. However, some strains of *Lactobacillus* are able to produce amino acid descarboxylase that prevents the accumulation of biogenic amines in the product. Thus, the selection of probiotic bacteria for use in fermented meat products must also be based on its ability to oxidate biogenic amines formed in the product and to prevent the formation of new amine by the rapid drop of pH that inhibits the growth of amine producing microorganisms. In fermented meat products, amine oxidase activity was detected in strains of *Lactobacillus casei* and *L. plantarum* [6, 14].

Ergönül and Kundakçi [51] found low biogenic amine contents in a Turkish fermented sausage manufactured by using three different probiotic starter culture combinations (*Lactobacillus casei*, *L. acidophilus* or their combination). Putrescine contents of the samples were ranging between 1.98 and 35.48 ppm during manufacturing and refrigerated storage (8 months), respectively, whereas the values were 0.96–18.50 ppm for cadaverine, 1.41– 10.84 ppm for histamine and 1.75–9.36 ppm for tyramine.

2.4. Beneficial effects associated with the consumption of probiotic meat products

As described earlier, most research involving probiotics in meat products focuses on the survival of probiotic species in the meat matrix and its influence on the technological and sensory characteristics of the final product. Few studies report the effects of consumption of these products on host health [7]. This condition is mainly due to the fact that *in vivo* tests are expensive, require more time for experimentation and the approval by ethics committees [36].

One of the few studies reporting the effects of the consumption of probiotic meat product on the human health was carried out by Jahreis et al. [52]. These authors evaluated the effect of daily consumption of 50g of probiotic salami containing *L. paracasei* LTH 2579 on the immunity system and blood triglycerides and cholesterol levels of healthy volunteers for a few week period, and obtained moderately satisfactory results. Although it has been observed effect on immunity of the host, small effect was observed on the plasmatic lipid levels.

In laboratory animals probiotic administration has shown to decrease the blood cholesterol level and increase the feed-conversion rate [53]. *L. plantarum* administration was reported to increase CD-8 and CD-4 lymphocytes in lab rats [54].

Other important physiological properties to be considered for the potential probiotics are the adhesive capacity toward Caco-2 cells and the antagonism toward pathogenic organisms [3].

Klingberg et al. [21] evaluated the ability of probiotic cultures to colonize the human intestinal tract by *in vitro* study using Caco-2 cells isolated from human colon adenocarcinoma. The starter strains *Pediococcus pentosaceus*, *Lactobacillus pentosus* and *L. plantarum* showed higher ability to adhere to cells in comparison to *Lactobacillus rhamnosus* used as control strain in the experiment.

Lactobacillus plantarum isolated from sausages exhibited superior adhesive properties toward Caco-2 cell lines as compared to *L. paracasei* and *L. brevis* [55].

The majority of studies on probiotic meat products focuses on the inhibition of pathogens by probiotics, increasing the safety of meat products. Mahoney and Henriksson [56] tested the inhibition of colonization and virulence of *Listeria monocytogenes* in the intestinal tract of rats by the consumption of fermented meat product with the addition of starter cultures, probiotic cultures and *Listeria monocytogenes*. The results showed that the starter culture consisting of *Pediococcus pentosaceus* and *Staphylococcus xylosus*, and the probiotic culture consisting of *Lactobacillus acidophilus*, *L. paracasei* and *Bifidobacterium* sp. were able to inhibit the growth of *Listeria monocytogenes* during its passage through the gastrointestinal tract. There was also a possible protective effect of the sausage on the intestinal mucosa by involving the pathogenic bacteria in its matrix and thus, not allowing it to adhere and colonize the intestine.

Autoaggregation of probiotic strains appears necessary for their adhesion to intestinal epithelial cells and coaggregation presents a barrier that prevents colonization by pathogenic microorganisms. Yuksekdag and Aslim [57] reported autoaggregation capacity of five *Pediococcus* strains isolated from a Turkish-type fermented sausages (sucuk) ranging from 35% to 84%. The high EPS (exopolysaccharide) producing *P. pentosaceus* Z12P and Z13P strains showed greater autoaggregation (79% and 84%, respectively) than the other strains. The coaggregation scores of those *Pediococcus* species with *L. monocytogenes* ATCC 7644 ranged from good (Z12P and Z13P) to partial (Z9P, Z10P, and Z11P).

Growth inhibition of *Escherichia coli* O157:H7 by the use of *Lactobacillus reuteri* ATCC 55730 and *Bifidobacterium longum* ATCC 15708 in the production of salami was confirmed by Muthukumarasamy and Holley [38]. Sameshima et al. [9] found that *Lactobacillus rhamnosus* FERM P-15120, *L. paracasei* subsp. *paracasei* FERM P-15121 and starter culture *L. sakei* were able to inhibit the growth and the toxin production of *Staphylococcus aureus* in fermented meat product.

Nedelcheva et al. [58] demonstrated the ability of *Lactobacillus plantarum* NBIMCC 2415 to inhibit the growth of pathogenic microorganisms such as *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 8739, *Proteus vulgaris* G, *Salmonella* sp., *Salmonella abony* NTCC 6017, *Staphylococcus aureus* ATCC 25093, *Staphylococcus aureus* ATCC 6538 P and *Listeria monocytogenes* at drying temperature (15-18 ^{o}C) for use in raw-dried meat products.

In addition to the studies related to the improvement of the safety of meat products with the use of probiotics, these bacteria have also been assessed for *in situ* production of nutraceutical compounds in meat products. Ammor and Mayo [14] describe studies related to high production of folate (vitamin B11) by a genetically modified *Lactobacillus plantarum*. Likewise, the production of conjugated linoleic acid (CLA), which has anticancer, antiobesity, antidiabetic, and antiatherogenic properties as well as stimulates the immune response, has been reported in some probiotic bacteria. Thus, the property of some probiotic bacteria to produce micronutrients and nutraceuticals compounds may allow *in situ* fortification of meat products, making them more nutritious and healthy.

The combined effect of the addition of probiotics and other active ingredients such as dietary fiber in meat products has also been studied. Sayas-Barberá et al. [59] reported that the addition of *Lactobacillus cas*ei CECT 475 to a traditional Spanish dry-cured sausage (*Longaniza de Pascua*) accelerates the curing process and that the incorporation of 1% orange fiber promotes the growth and survival of lactobacilli and micrococci, enhancing the microbial quality and safety of the sausages.

3. Conclusion

The fermented sausages fit perfectly in the current consumption trend due to their ease of preparation (ready to eat), ease of conservation, versatility of use (individually or as an garnish in cooking plates), nutritional appeal and variety of forms of presentation [60]. In this regard, probiotic fermented meat products might be the trend setters for development of innovative meat products.

Despite the selling of probiotic meat products occurs since 1998 in countries like Germany and Japan, further human-based studies are needed to establish documented proofs of the beneficial effect of these products, mainly with research on health promotion in humans [7]. Only after these studies will be possible to confirm the intrinsic value of fermented meat products and contribute to the recognition of such products as health foods.

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4. References

[1] Työppönen S, Petäjä E, Mattila-Sandholm T. Bioprotectives and probiotics for dry sausages. International Journal of Food Microbiology 2003;83:233-244.
[2] Erkkilä S, Petäjä E, Eerola S, Lilleberg L, Mattila-Sandholm T, Suihko ML. Flavour profiles of dry sausages fermented by selected novel meat starter cultures. Meat Science 2001b;58:111-116.
[3] Khan MI, Arshad MS, Anjum FM, Sameen A, Aneeq-ur-Rehman, Gill WT. Meat as a functional food with special reference to probiotic sausages. Food Research International 2011;44(10):3125-3133.
[4] Santos FL, Ferreira CLLF, Costa NMB. Modulação da colesterolemia por meio de prebióticos e probióticos. In: Célia Lúcia de Luces Fortes Ferreira, editor. Prébióticos e Probióticos: Atualização e Prospecção. Viçosa, Rubio; 2003.
[5] Bejder HC. Probiotics: today dairy, tomorrow the world. Danish Dairy & Food Industry 2004;14:42-43.
[6] Leroy F, Verluyten J, Vuyst L. Functional meat starter cultures for improved sausage fermentation. International Journal of Food Microbiology 2006;106:270-285.
[7] Vuyst LD, Falony G, Leroy F. Probiotics in fermented sausages. Meat Science 2008;80:75-78.
[8] Lücke FK. Utilization of microbes to process and preserve meat. Meat Science 2000;56:105-115.
[9] Sameshima T, Magome C, Takeshita K, Arihara K, Itoh M, Kondo Y. Effect of intestinal *Lactobacillus* starter cultures on the behaviour of *Staphylococcus aureus* in fermented sausage. International Journal of Food Microbiology 1998;41:1-7.
[10] Andersen L. Fermented dry sausages produced with the admixture of probiotic cultures. In: Proceedings of the 44th International Commitment of Meat Science and Technology;1998; Barcelona, Spain. Barcelona: Institut de Recerca i Tecnologia Agroalimentaries, 1998. P. 826-827.
[11] Erkkilä S, Suihko ML, Eerola S, Petäjä E, Mattila-Sandholm T. Dry sausage fermented by *Lactobacillus rhamnosus* strains. International Journal of Food Microbiology 2001a;64:205-210.

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[12] Ferreira CLLF. Grupo de bactérias lácticas – Caracterização e aplicação tecnológica de bactérias probióticas. In: Célia Lúcia de Luces Fortes Ferreira, editor. Prébióticos e Probióticos: Atualização e Prospecção. Viçosa, Rubio; 2003.

[13] Campbell-Platt G. Fermented meats - a world perspective. In: Campbell-Platt G, Cook PE, editor. Fermented Meats. Glasgow: Chapman & Hall, 1995.

[14] Ammor M, Mayo B. Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production: An update. Meat Science 2007;76(1):138-146.

[15] Hammes WP, Hertel C. New developments in meat starter cultures. Meat Science 1998;49:125-138.

[16] Ruiz-Moyano S, Martín A, Benito MJ, Nevado FP, Córdoba MG. Screening of lactic acid bacteria and bifidobacteria for potential probiotic use in iberian dry fermented sausage. Meat Science 2008;80:715-721.

[17] Rivera-Espinoza Y, Gallardo-Navarro Y. Non-dairy probiotic products. Food Microbiology, doi: 101016/ j.fm.2008.06.008, 2008.

[18] Maragkoudakis PA, Mountzouris KC, Psyrras D, Cremonese S, Fischer J, Cantor MD, Tsakalidou E. Functional properties of novel protective lactic acid bacteria and application in raw chicken meat against *Listeria monocytogenes* and *Salmonella enteritidis*. International Journal of Food Microbiology 2009;130:219-226.

[19] Silvi S, Oripianesi MC, Cresci A. Functional foods, gut microflora and healthy ageing. Isolation and identification of *Lactobacillus* and *Bifidobacterium* strains from faecal samples of elderly subjects for possible probiotic use in functional foods. Journal of Food Engineering 2003;56:195-200.

[20] Pennacchia C, Ercolini D, Blaiotta G, Pepe O, Mauriello G, Villani F. Selection of *Lactobacillus* strains from fermented sausages for their potential use as probiotics. Meat Science 2004;67:309-317.

[21] Klingberg TD, Axelsson L, Naterstad K, Elsser D, Budde BB. Identification of potential probiotic starter cultures for Scandinavian-type fermented sausage. International Journal of Food Microbiology 2005;105:419-431.

[22] Erkkilä S, Petäjä E. Screening of commercial meat starter cultures at low pH and in the presence of bile salts for potential probiotic use. Meat Science 2000;55:297-300.

[23] Papamanoli E, Tzanetakis N, Litopoulou-Tzanetaki E, Kotzekidou P. Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. Meat Science 2003;65:859-867.

[24] Villani F, Mauriello G, Pepe O, Blaiotta G, Ercolini D, Casaburi A. Technological and probiotic characteristics of *Lactobacillus* and coagulase negative *Staphylococcus* strains as starter for fermented sausage manufacture. Italian Journal of Animal Science 2005;4:498.

[25] Rebucci R, Sangalli L, Fava M, Bersani C, Cantoni C, Baldi A. Evaluation of functional aspects in *Lactobacillus* strains isolated from dry fermented sausages. Journal of Food Quality 2007;30:187-201.

[26] Arihara K. Strategies for designing novel functional meat products. Meat Science 2006;74: 219–229.

[27] Arihara K, Ohata M. Functional meat products. In F. Toldra, editor. Handbook of meat processing (pp. Ames, Iowa: Wiley-Blackwell, 2010. P. 423-439.

[28] Toldrá F, Reig M. Innovations for healthier processed meats. Trends in Food Science & Technology 2011;22(9):517-522.

[29] Penna ALB. Probióticos & Saúde. In: Proceedings of the XVIII Congresso Brasileiro de Ciência e Tecnologia de Alimentos; 2002; Porto Alegre, Brazil. Porto Alegre: SBCTA, 2002. P. 4045-4046.

[30] Heenan CN, Adams MC, Hosken RW. Growth medium for culturing Probiotics bacteria for applications in vegetarian food products. Lebensmittel-Wissenschaft und-Technologie 2002;35:171-176.

[31] Tharmaraj N, Shah NP. Selective enumeration of *Lactobacillus delbrueckii ssp. Bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Bifidobacteria, Lactobacillus casei, Lactobacillus rhamnosus, and Propionibacteria*. Journal of Dairy Science 2003;86:2288-2296.

[32] Zhang W, Xiao S, Samaraweera H, Lee EJ, Ahn DU. Improving functional value of meat products. Meat Science 2010; 86(1):15-31.

[33] Arihara K, Ota H, Itoh M, Kondo Y, Sameshim T, Yamanaka H. *Lactobacillus acidophilus* group lactic acid bacteria applied to meat fermentation. Journal of Food Science1998;63:544-547.

[34] Macedo REF, Pflanzer SB, Terra NN, Freitas RJS. Desenvolvimento de embutido fermentado por *Lactobacillus* probióticos: características de qualidade. Ciência e Tecnologia de Alimentos 2008;28:509-519.

[35] Pidcock K, Heard GM, Henriksson A. Application of nontraditional meat starter cultures in production of Hungarian salami. International Journal of Food Microbiology 2002;76:75-81.

[36] Pan X, Chen F, Wu T, Tang H, Zhao Z. The acid, bile tolerance and antimicrobial property of *Lactobacillus acidophilus* NIT. Food Control 2009;20:598-602.

[37] FAO/WHO [Internet]. 2009 [updated 2001; cited 2009 May 25] Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Report of a joint FAO/WHO expert consultation, Córdoba, Argentina. 2001. Available from:
http://www.who.int/foodsafety/publications/fs_management/probiotics/en/index.html

[38] Muthukumarasamy P, Holley RA. Microbiological and sensory quality of dry fermented sausages containing alginate-microencapsulated *Lactobacillus reuteri*. International Journal of Food Microbiology 2006;111:164-169.

[39] Poulin JF, Caillard R, Subirade M. -Lactoglobulin tablets as a suitable vehicle for protection and intestinal delivery of probiotic bacteria. International Journal of Pharmaceutics 2011;405(1-2):47-54.

[40] Heidebach T, Först P, Kulozik U. Microencapsulation of probiotic cells by means of rennet-gelation of milk proteins. Food Hydrocolloids 2009;23(7):1670-1677.

[41] Heidebach T, Först P, Kulozik U. Influence of casein-based microencapsulation on freeze-drying and storage of probiotic cells. Journal of Food Engineering 2010;98(3):309-316.

[42] Caplice E, Fitzgerald GF. Food fermentations: role of microorganisms in food production and preservation. International Journal of Food Microbiology 1999;50:131-149.

[43] Arihara K, Itoh M. UV-induced *Lactobacillus gasseri* mutants resisting sodium choride and sodium nitrite for meat fermentation. International Journal of Food Microbiology 2000;56:227-230.

[44] Macedo REF, Pflanzer SB, Terra NN, Freitas RJS. Características de culturas lácticas probióticas para uso em produtos cárneos fermentados: sensibilidade aos sais de cura e uso de antibióticos para contagem seletiva. Boletim do CEPPA 2005;23(1):123-134.

[45] Annuk H, Shchepetova J, Kullisaar T, Songisepp E, Zilmer M, Mikelsaar M. Characterization of intestinal lactobacilli as putative probiotic candidates. Journal of Applied Microbiology 2003;94:403-412.

[46] Collado MC, Sanz Y. Method for direct selection of potencially probiotic *Bifidobacterium* strains from human feces based on their acid-adaptation ability. Journal of Microbiological Methods 2006;66:560-563.

[47] Charteris WP, Kelly PM, Morelli L, Collins JK. Effect of conjugated bile salts on antibiotic susceptibility of bile salt-tolerant *Lactobacillus* and *Bifidobacterium* isolates. Journal of Food Protection 2000;63(10):1369-1376.

[48] Taranto MP, Perez-Martinez G, Valdez GF. Effect of bile acid on the cell membrane functionality of lactic acid bacteria for oral administration. Research in Microbiology 2006;157:720-725.

[49] Haller D, Scherenbacher P, Bode C, Hammes WP. Selection of potentially probiotic bacteria. Zeitschrift fuer Ernaehrungswissenschaft 1997;36(1):87.

[50] Gänzle MG, Hertel C, van der Vossen JMBM, Hammes WP. Effect of bacteriocin-producing lactobacilli on the survival of *Escherichia coli* and *Listeria* in a dynamic model of the stomach and the small intestine. International Journal of Food Microbiology 1999;48:21-35.

[51] Ergönül B, Kundakçı A. Microbiological attributes and biogenic amine content of probiotic Turkish fermented sausage. Journal für Verbraucherschutz und Lebensmittelsicherheit 2011;6(1):49-56.

[52] Jahreis G, Vogelsang H, Kiessling G, Schubert R, Bunte C, Hammes WP. Influence of probiotic sausage (*Lactobacillus paracasei*) on blood lipids and immunological parameters of health volunteers. Food Research International 2002;35:133-138.

[53] Alkhalf A, Alhaj M, Al-Homidan I. Influence of probiotic supplementation on immune response of broiler chicks. Egypt Poultry Science 2010;30(1):271-280.

[54] Mao Y, Nobaek S, Kasravi B, Adawi D, Stenram U, Molin G, Jeppsson B. The effects of *Lactobacillus* strains and oat fiber on methotrexate-induced enterocolitis in rats. Gastroenterology 1996;111(2):334-44.

[55] Pennacchia C, Vaughan EE, Villani F. Potential probiotic *Lactobacillus* strains from fermented sausages: Further investigations on their probiotic properties. Meat Science 2006;73(1):90-101.

[56] Mahoney M, Henriksson A. The effect of processed meat and meat starter cultures on gastrointestinal colonization and virulence of *Listeria monocytogenes* in mice. International Journal of Food Microbiology 2003;84:255-261.

[57] Yuksekdag ZN, Aslim B. Assessment of potential probiotic- and starter properties of *Pediococcus* spp. Isolated from Turkish-type fermented sausages (sucuk). Journal of Microbiology and Biotechnology 2010;20(1):161-168.

[58] Nedelcheva P, Denkova Z, Denev P, Slavchev A, Krastanov A. Probiotic strain *Lactobacillus plantarum* NBIMCC 2415 with antioxidant activity as a starter culture in the production of dried fermented meat products. Biotechnology and Biotechnological Equipment 2012;24(1):1624-1630.

[59] Sayas-Barberá E, Viuda-Martos M, Fernández-López F, Pérez-Alvarez JA, Sendra-Nadal E. Combined use of a probiotic culture and citrus fiber in a traditional sausage '*Longaniza de pascua*'. Food Control 2012; doi 10.1016/j.foodcont.2012.04.009.

[60] Monfort JM. Los productos carnicos crudos curados. In: Proceedings of the XVIII Congresso Brasileiro de Ciência e Tecnologia de Alimentos; 2002; Porto Alegre, Brazil. Porto Alegre: SBCTA, 2002. P. 3984-3992.