Chapter 11

Probiotic Fermented Sausage

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Abstract

Many changes and innovations in fermented meat products have occurred over time, especially when it comes to health-promoting products. Some studies have been conducted with the aim of developing probiotic meat products that can improve the functionality of the gut microbiota. However, the technological challenges faced during the production of fermented meat sausages make it difficult to apply probiotics in these food matrices. For probiotics to deliver the expected health outcomes for consumers, they need to grow in the products and at the end the viable cell count must be sufficient for the microorganisms to reach the consumer's gut. Therefore, in this chapter we describe a protocol for probiotic Friolano-type sausage. Furthermore, the possible sources of defects in the production of probiotic salami and the best alternatives to overcome them are presented.

Key words Functional foods, Health-promoting compounds, Probiotic salami, Probiotic *Friolano salami*, Probiotic meat product

1 Introduction

Meat sausages are products elaborated with meat or edible organs, seasoned and smoked, and can be cured, cooked or dried, wrapped in tripe, bladder or other animal membrane properly cleaned [1]. Fermented sausages are products that undergo a rapid initial fermentation followed by partial dehydration and may or may not be smoked. They are meat products consisting mainly of pork or beef, but can be produced with other meat types, in addition to pork fat, salt, sugar, curing agent, spices and starter cultures. They do not require refrigeration and have great stability compared to other meat products [2].

Friolano Salami is a kind of fermented sausage made exclusively from pork and lard, ground to a medium particle size of 6–9 mm and with the addition of the other ingredients required. It has an irregular cylindrical shape (defined by the shape of the natural wrap) with length ranging from 15 to 130 cm. Its weight ranges from 0.2 to 4.5 kg and presents non-elastic consistency, compact mass,

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delicate aroma, sweet and delicate flavor, and ruby red color without spots. It is a cured product, which can go through the smoking process, being fermented, matured, and dried [2].

In the world of fermented meat sausages many changes and innovations have taken place over time, especially when it comes to products that are beneficial to health, since the demand for these foods has become a priority for many consumers. With this in mind, studies have been conducted with the addition of probiotics in meat sausages [3].

Probiotics are live microorganisms that when properly added to products present benefits to the consumer's health, with specific effects and functional properties [4]. In addition, they contribute to the balance of intestinal microflora, helping the intestinal transit and facilitating digestion, relieve the symptoms of lactose intolerance, prevent colon cancer, reduce cholesterol and blood pressure, stimulate the immune system, produce B-complex vitamins, digestive and protective enzymes, protect against pathogenic microorganisms and control inflammatory vessel diseases [4–6].

However, for them to present the expected results they need to grow in the products, and at the end of the shelf life the viable cell count should be enough for the microorganisms to reach the consumer's intestine, which makes their application in fermented sausages difficult, due to their high acidity and salt content, and lower water activity (a_w) [4]. Therefore, in some studies probiotics are added microencapsulated in foods, which ensures the viability of the probiotic microorganisms during the process and in the final product [6]. Therefore, this chapter is directed towards the design of a probiotic *Friolano Salami* protocol. Furthermore, the possible sources of defects in meat sausages and the best alternatives to overcome them are presented.

2 Materials

1. Pork shank meat.
2. Back fat (lard).
3. Sodium chloride.
4. Curing salts (sodium nitrate).
5. Sugar.
6. Garlic powder.
7. Chili powder.
8. Probiotic culture (Table 1).
9. Starter culture (Table 2).
10. Collagen wrap with 50 a 60 mm.

Table 1
Probiotic or potentially probiotic cultures in meat products and the main results found in the studies

Product	Probiotic or potential probiotic used	Main results	References
Fresh pork sausage	*L. sakei* BAS0117 isolated from Brazilian fermented meat products (Italian salami, Calabrian sausage, ham, and mortadella)	The strains added allowed desirable characteristics to the product during storage	[7]
Italian salami sausage	*L. acidophilus*, *Bifidobacterium lactis* (potentially probiotic)	The addition of probiotic cultures produced sausages with good physical-chemical, microbiological, and sensory properties	[8]
Fermented pork sausage and loin	*L. rhamnosus* LOCK900 (probiotic strain)	Many lactic acid-producing bacteria, including 90% *L. rhamnosus,* were found during all stages of the meat process The added probiotics inhibited lipid oxidation in loins and pork sausage	[9]
Sausage and pork neck fermented and dry-cured	Pure cultures of probiotic strains: *Bifidobacterium animalis* subsp. *lactis* BB-12 (strain deposit number: DSM15954), *L. rhamnosus* LOCK900 (strain deposit number: CP005484), *L. acidophilus* Bauer (potentially probiotic)	The three starter strains could be applied to smoked meat products. However, the culture *L. acidophilus* Bauer did not allow lipid oxidation and discoloration of the products	[10]
Fermented sausage	Isolated from human intestinal tracts: *L. acidophilus* FERM P-15119, *L. rhamnosus* FERM P-15120, *L. paracasei subsp. paracasei* FERM P-15121 Commercial starter culture: *L. sake* (Chr. Hansen's)	The three strains examined could inhibit the growth of *S. aureus* and enterotoxins during the sausage fermentation process at different temperature variations	[11]
Fermented sausage	Probiotic lactobacilli strain isolated from infants' feces: *L. casei/paracasei CTC1677*, *L. casei/paracasei CTC1678*, *L. rhamnosus CTC1679* (confirmed probiotic lactobacilli) Commercial probiotic strains: *L. plantarum 299v*, *L. rhamnosus GG*, *L. casei Shirota*	It was observed that the *L. rhamnosus* remained viable at high levels (10^8 CFU/g) and survived the passage in TGI during sausage consumption	[12]

(continued)

Table 1 (continued)

Product	Probiotic or potential probiotic used	Main results	References
Fermented sausage	LAB isolated from infants qualified as potential probiotics: *L. gasseri CTC1700*, *L. gasseri CTC1704*, *L. fermentum CTC1693* Potential probiotic strains with proved ability, isolated from infant feces: *L. casei/paracasei CTC1677*, *L. casei/paracasei CTC1678*, *L. rhamnosus CTC1679*	*L. rhamnosus CTC1679* was the only strain capable of mastering both repetitions A putative probiotic effect can be achieved by eating 10 g/day of fuet with CTC1679	[13]
Fermented sausage	Probiotic strain: *E. faecium ATCC 8459*	*E. faecium* was efficient as a starter for producing fermented sausage with resistance to curing salts and sodium chloride and maintained its viability during the ripening process	[14]
Fermented sausage	*E. faecium CRL183* (potential probiotic)	It demonstrated a positive influence of sausage fermented with *E. faecium* CRL183 on microbial diversity	[15]
Fermented sausage	*L. rhamnosus* CTC1679 (potential probiotic)	*L. rhamnosus CTC1679* used as a probiotic starter culture produced safe, nutritionally enhanced fermented sausages The strain showed the ability to act as probiotic starter cultures remaining viable at high levels (108 CFU/g) in ripened fuets and surviving the passage through the human GIT during the consumption of the sausages	[16]
Fermented sausage	*Commercial probiotic strain: L. sakei* (potential probiotic)	The strains presented technological characteristics expected for application in sausage maturation processes as a starter culture	[17]

Raw fermented sausage	*L. casei* LOCK 0900 isolated from feces of healthy infants (potential probiotic)	Raw fermented sausages with probiotic strain *L. casei* LOCK 0900 showed good microbiological quality. The environment of raw fermented sausages is suitable for the growth and survival of the probiotic strain *L. casei* LOCK 0900	[18]
Fermented lamb sausage	*Commercial probiotic strain: L. acidophilus* CCDM 476, *Bifidobacterium animalis* 241a (potential probiotic)	The number of *Lactobacillus* (10^7 CFU/g) and *Bifidobacterium* (10^3 CFU/g) in the final product did not alter its technological properties. Despite this, there were problems in using *Bifidobacterium* as a starter because of its low concentration after fermentation and absence after 60 days of storage	[19]
Norwegian fermented sausage, Swedish fermented sausage, and Norwegian cured ham	Potential probiotic cultures isolated from fermented meat: *L. sakei* MF1295, *L. sakei* MF1296, *L. farciminis* MF1288, *L. plantarum/pentosus* MF1290, *L. plantarum/pentosus* MF1299, *L. plantarum* MF1291, *L. plantarum* MF1297, *L. pentosus* MF1300, *L. alimentarius* MF1297	The strains met all probiotic criteria and proved to be rapidly producing lactic acid, demonstrating the successful application of the selected strains as starter cultures for Scandinavian-type fermented sausages	[20]
Dry fermented sausage	*L. rhamnosus* GG (probiotic strain), *L. rhamnosus* E-97800 (potential probiotic), *L. rhamnosus* LC-705 (potential probiotic) Commercial strains: *Pediococcus pentosaceus*	*L. rhamnosus* E-97800 showed the fastest growth and acidification rate. Therefore, *L. rhamnosus* GG and *L. rhamnosus* E-97800 were considered tasty as the sausages fermented by the control	[21]
Dry fermented sausage	Commercial probiotic strains documented: *L. rhamnosus* R0011, *L. helveticus* R0052, *L. rhamnosus* Lr- 32, *L. paracasei* Lpc-37, *L. casei* Shirota, *L. reuteri* DSM17938, *L. reuteri* DSM17918, *Enterococcus faecium* MXVK29	The evaluated strains demonstrated different technological capacities in the other conditions in which the tests were performed *L. rhamnosus* Lr-32, *L. rhamnosus* R0011, *L. paracasei* Lpc-37, *E. faecium* MXVK29, and *L. casei* Shirota strains are the primary candidates to be used as sausages starters culture	[22]

(continued)

Table 1 (continued)

Product	Probiotic or potential probiotic used	Main results	References
Iberian dry fermented sausage	Probiotic culture: *L. fermentum* HL57, *Pediococcus acidilactici* SP979	Inoculation with *L. fermentum* HL57 increased the amount of acetic acid and lipid degradation products, such as malonaldehyde in Iberian dry fermented sausages, resulting in a negative impact on relevant sensory parameters related to color and flavor. On the contrary, *P. acidilactici* SP979 did not remarkably modify the physical–chemical parameters or sensory quality of Iberian dry-fermented sausages	[23]
Tunisian dry fermented sausage	Autochthonous strains isolated from a Tunisian traditional salted meat "kadid": *L. plantarum*, *S. xylosus* (not confirmed probiotic)	The use of bacterial strains can inhibit the growth of Gram-negative bacteria and may improve the sensory properties of sausage due to nitrate reductase and protease activity of *the S. xylosus* strain and the acidifying activity of the *L. plantarum* strain	[24]
Harbin dry sausage	Potential probiotics: *P. pentosaceus* R1, *L. brevis* R4, *L. curvatus* R5, *L. fermentum* R6 Confirmed probiotics from fermented dairy products (used for comparison with the isolated LAB): *L. acidophilus* AD1, *L. plantarum* KLDS1.0391, *L. curvatus* KLDS1.0505, *L. sake* AS1, *L. pentosaceus* KLDS1.0412, *L. fermentum* KLDS1.0709	Except for *L. curvatus* R5, all strains isolated from Harbin dry sausages supported passage in the gastrointestinal tract (GIT). Different components of the strains have different modes of antioxidant action. LAB isolated from Harbin dry sausages has strong probiotic properties and can be used as potential probiotics for food processing	[25]
Sucuk-type dry sausage	Twenty-three probiotic *L. plantarum* strains producing the conjugated linoleic acid (CLA) were screened: *L. plantarum* LMG 11405 and *L. plantarum* LMG 23521 were selected from the catalog of	*L. plantarum* AA1-2 and *L. plantarum* AB20-961 were identified as potential strains for CLA production. The strain of *L. plantarum* AB20-961 can be used to produce sucuk by an increase in the amount of linoleic acid of the	[26]

	BCCM/LMG (Belgian Coordinated Collections of Microorganisms/Laboratory of Microbiology) at the University of Ghent Twenty one *L. plantarum* strains (AA1-2, AA13-59, AA17-73, AB6-25, AB7-35, AB16-65, AB20-961, AC3-27, AC10-40, AC18-82, AC21-101, AC21-1031, AC18-88, AC3-10, AC3-14, AK4-11, AK6-27, AK6-28, AK8-31B, BC18-81, BK10-48) isolated from human sources in Suleyman Demirel University	product, without alteration on the product's final characteristics Production conditions such as temperature and pH were probably the most limiting factors for linoleic acid production	
Salami	Commercial probiotics: *L. plantarum* 299v, *L. plantarum* DSM 9843, *L. rhamnosus* LbGG or ATCC 53103, *L. casei* Shirota YIT 9029, *L. reuteri* DSM 17938, *L. casei* ATCC 393	The microbiological counts were different according to the type of starter strain used. *L. plantarum* 299v kept a concentration higher than 106 CFU g^{-1}, level of probiotic bacteria recommended at the time of consumption to exert a beneficial effect in humans The experimental salami proved to be safe since coagulase-positive coliforms and *Staphylococci* were not detected in the salami at the moment of consumption and after more than 1 month of storage at a cooling temperature	[27]
Nostrano salami	Bacterial strains: *L. lactis ssp. lactis* strain 340, *L. lactis ssp. lactis* strain 16, *L. casei ssp. casei* strain 208, *E. faecium* UBEF-41	These strains were selected for its ability to grow under low temperatures and modulate the aroma by converting amino acids and fatty acids, making it possible to produce fermented matured at low temperature without adding nitrates and nitrites, resulting in a potentially safer product with no adverse effect on the quality of Italian salami	[28]

(continued)

Table 1 (continued)

Product	Probiotic or potential probiotic used	Main results	References
Italian-type salami	Probiotic cultures: *L. casei* LC 01, *L. paracasei* ssp. *paracasei* ATCC 10746/CCT 0566, *L. rhamnosus* ATCC 7469/CCT 6645	The addition of these strains to the sausages caused a reduction in the development of *S. xylosus*. On the other hand, probiotic cultures did not interfere in the growth of *P. pentosaceus*, which, for most evaluation periods, showed better development when together with *Lacticaseibacillus*	[29]

Adapted from ref. [3]

Table 2
Main starter cultures used in meat sausages

Starter culture	Strain
Acid lactic bacteria	*Latilactobacillus sakei* *Latilactobacillus curvatus* *Lactiplantibacillus plantarum* *Lacticaseibacillus rhamnosus*
Pediococus	*Pediococcus acidilactici* *Pediococcus pentosaceus*
Staphylococcus and *Kocuria*	*Staphylococcus xylosus* *Staphylococcus carnosus* *Staphylococcus equorum* *Kocuria varians*
Micrococcaceae	*Micrococcaceae candidus* *Micrococcaceae aquatilis*

2.1 Equipment

1. Meat grinder with 6–9 mm disc.
2. Lard cuber.
3. Mixer.
4. Maturation chamber.
5. Drying chamber.

3 Methods

The protocol for making a probiotic *Friolano Salami* is illustrated in Fig. 1.

3.1 Probiotic Cultures

1. Use direct vat set (DVS) or direct vat inoculation (DVI) cultures that allow the addition of probiotic strains directly into the food matrix. See Chapter 13 for the main suppliers of DVS probiotic strains (Table 1).

3.2 Friolano Salami Manufacture

1. Grind the raw pork meat (85% of the raw material) with a 6–9 mm disc at a temperature of 4–7 °C and then transfer it to the blender.
2. Cut the bacon (15% of the raw material) in an incubator at 0 °C into cubes of no more than 1 cm. Add the lard to the meat in the blender.
3. Add 2.5% sodium chloride, 0.3% sugar, 0.03% chili powder, 0.3% garlic powder, and 0.015% sodium nitrate over the meat in the blender.

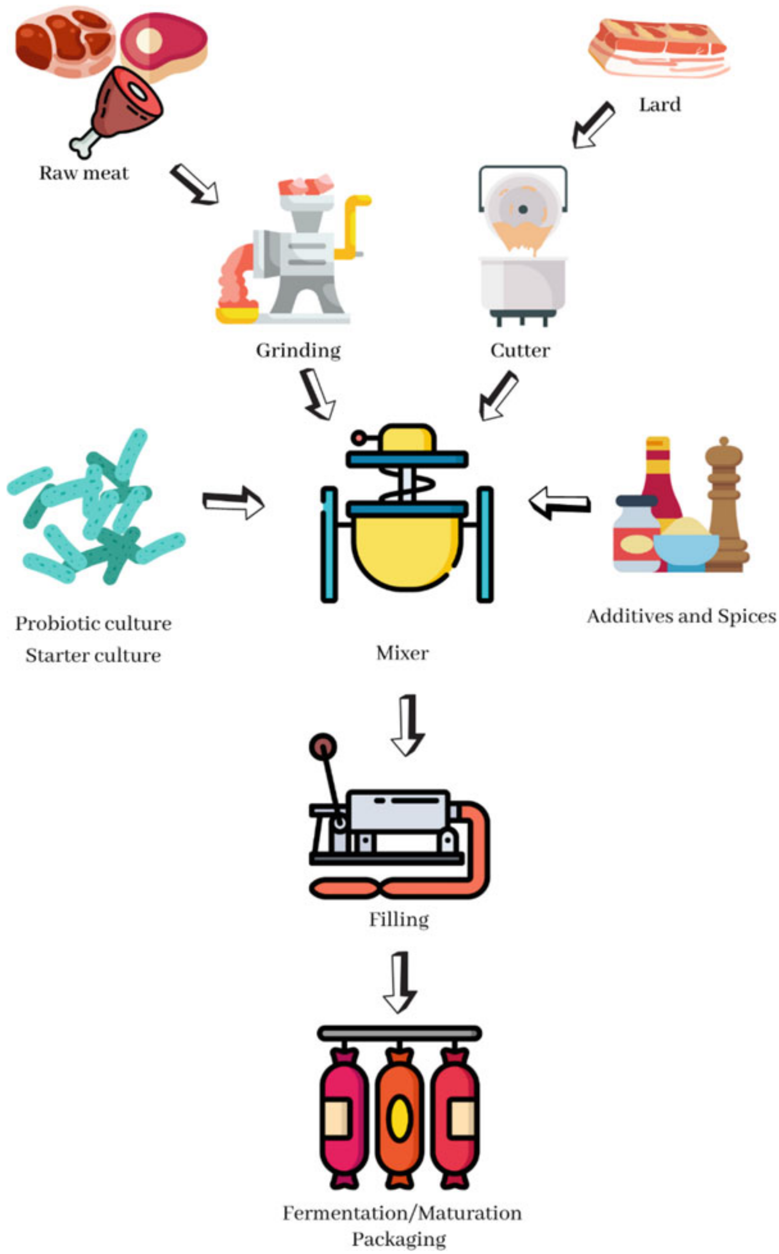

Fig. 1 Steps for the elaboration of a probiotic *Friolano Salami*

4. Mix the ground meat, minced lard, and the other ingredients and additives for 2 min in the mixer at 4 °C.
5. Add the starter culture and the probiotic culture at 10^8 CFU/g. Mix for about 2 min (*See* **Notes 1–3**).
6. After a resting phase of 24 h at 0–2 °C, the mixture must be filled using a vacuum filler (*See* **Note 4**).

7. Clip the salami and spray an aqueous mold solution on the surface wrap (*See* **Note 5**).
8. Hang the salami and transfer it to the fermentation chamber (*See* **Note 6**).
9. In the fermentation chamber, the dripping phase occurs at 20 °C for 14–20 h.
10. Keep the salami in the drying room at 20 °C, relative humidity 60 to 80% for 96 to 144 h (*See* **Note 7**).
11. Finally, after drying, transfer the salami to the ripening room at 12–18 °C for 23 days (*See* **Note 8**).
12. The salami can be washed and, thus, packaged in packs without light and oxygen permeability (*See* **Note 9**).

4 Notes

1. The starter culture commonly used in salami is *Staphylococcus xylosus*, which produces lipolytic and proteolytic activity enzymes that are fundamental in the formation and color stability of the final product, and is involved in aroma formation; *Latilactobacillus sakei* with fermenting action, producer of lactic acid and antibacterial metabolites, has also protective action; and *Staphylococcus carnosus*, which adds flavor, has protective and fermenting action.
2. At the end of this stage, the temperature of the mixture rises to about 6 °C.
3. The lactic acid bacteria lower the pH and produce aromatic compounds in the sausage, also masking the bitterness of the curing salts. In addition, they produce reducing conditions, helping to not develop oxidized flavors, and improving color, since they favor the development of meat pigments by stabilizing Fe^{2+}. The also protect the pigments from oxidation by blocking the formation of undesirable compounds in the product.
4. To avoid air residue in the meat paste, it is very important that no air be trapped in the salami.
5. Optionally smoking can be done, but knowing the bacteriostatic effect it can have on some probiotics, this step is not recommended for probiotic Salami.
6. Until pH 4.6–5.4 is reached and for color development.
7. The time of the drying step is given by the weight loss function chosen as the target, which in turn depends on the quality of the lean meat fraction used. If an initial step aimed at losing water from fresh meat is performed in ventilation systems

before grinding, with the objective of losing moisture, the next drying step can be shorter. At the end of the drying stage, the temperature of the drying environment is usually a third lower than it was at the beginning.

8. The length of the ripening chamber will depend on the targeted weight loss. A time of 23 days is sufficient to lower the water activity (a_w < 0.9) and achieve the physical-chemical characteristics of *Friolano Salami*. The weight loss at the end of ripening (intended as a complete cycle) is about 38%. This weight loss value can vary according to the lean to fat ratio, diameter, salt concentration, etc. If the previous drying phase has been carried out correctly in terms of weight loss, during the first days of maturation some mold colonies appear on the surface of the wrap. However, this step must be carefully considered since the lower a_w can lead to the loss of probiotic viability.
9. The viability of probiotics over storage depends on the individual strain. Some examples of viability time in storage can be seen in Table 1.

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