Chapter 1

Probiotic Fermented Meat Products

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Abstract

The fermentation of meat is an ancient culinary tradition worldwide used mainly with the intention of extending meat shelf life and diversifying. Plenty of products with their respective recipes have been developed throughout the history of civilization. Spain is a country with historical tradition in the production of fermented meat products, highlighting chorizo and salchichón. Specifically, the latter can be divided into different varieties according to aspects, such as size. Thus, products, such as longaniza, fuet, secallona, or didalets, can be classified and named according to the length and width of the piece. The ingredients used for elaboration are practically the same between these salchichónes. In the present chapter, the production of fuet is described in depth since it represents one of the most traditional and consumed fermented meat products in Spain and it is also being internationalized to other countries. On the other hand, the addition of probiotic cultures to meat dough is increasingly practiced, which has potential health benefits. Therefore, the production of fuet with probiotic microorganisms might help to develop novel and healthy alternatives to the traditional recipe. Ingredients including pork lean and belly, spices and other additives incorporated in the form of commercial mixes, and starter and probiotic cultures are used in the elaboration of the fuet proposed in this chapter, throughout different steps, which can be classified as mincing, mixing, stuffing, fermentation, curing, and conservation.

Key words Fermented meat product, Fermented sausage, Probiotic, Fuet, Elaboration process

1 Introduction

The elaboration of fermented meat products is a culinary tradition perpetuated over time by generations in different parts of the world, such as Europe, where a wide and varied offer of these products can be found [1]. Although the technology of these products has undergone significant modifications throughout the history of humankind [2], the purpose of fermenting meat has always been the same, extending shelf life and diversifying [3]. The development of fermented meat products involves dynamic and complex chemical processes, in which lactic acid

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bacteria (LAB) stand as the main muscle-transforming microorganisms, causing the acidification of the medium. This drop in pH helps to stabilize the product, delaying deterioration processes and preventing the development of pathogenic bacteria. In addition, acidification positively impacts on sensory attributes, increasing the final product acceptance [1].

The fermentation of meat can be done in two ways, allowing the indigenous muscle microflora to act alone or using predefined microorganisms (known as starter cultures) to initiate and carry out the transformation processes. These starter cultures mainly consist of one or several LAB species, micrococci, and staphylococci [4], but also yeast and molds can be used [5]. They are specifically designed to meet the food safety criteria specified by the regulatory entity and the technological and organoleptic specifications of the company. In this way, the fermentation can be controlled, and the process standardized, yielding safe and high-quality meat products [6]. The current market trend towards healthier products has led to the research of other microbial cultures capable of exerting health benefits. In this context, special attention has been paid to probiotics, living organisms capable of modifying the gut microbiome and improving health when consumed in adequate amounts [7]. Immunomodulatory effects and anticancer, antimicrobial, antidiabetic, and anti-inflammatory properties have been associated with the consumption of these microorganisms [8–12].

Different studies have been searching for good probiotic candidates to be used in the preparation of fermented meat products (Fig. 1). Strains such as *Bifidobacterium longum* KACC 91563 [13], *Enterococcus faecium* CECT 410 [14], *Lacticaseibacillus casei* ATCC 393 [15], *Lactobacillus paracasei* DTA83 [16], *Lactobacillus rhamnosus* LOCK900 [17], *Lactobacillus acidophilus* CRL1014 [18], and *Lactobacillus sakei* 23 K [19] have been recently assessed in this regard, showing a good ability to produce quality fermented meat products since they are able to satisfactorily colonize the meat dough, reach a reasonably high number of counts, and barely affect sensory attributes, pH, and oxidative status [7]. As can be seen, there are many potential probiotic cultures to be used in the development of fermented meat products, which opens the door to multiple industrial and commercial possibilities [20].

For the elaboration of a fermented meat product, different and varied recipes can be followed since there are innumerable products of this type with very different characteristics, linked to geographical areas of the world [21, 22]. In Spain, there is a long tradition of making these food products and varieties, such as chorizo and salchichón, which can be tasted throughout the different territories of this country. Several classes of salchichón can be found according to parameters, such as size. Thus, products, such as longaniza, fuet, secallona, or didalets, with different lengths and widths are

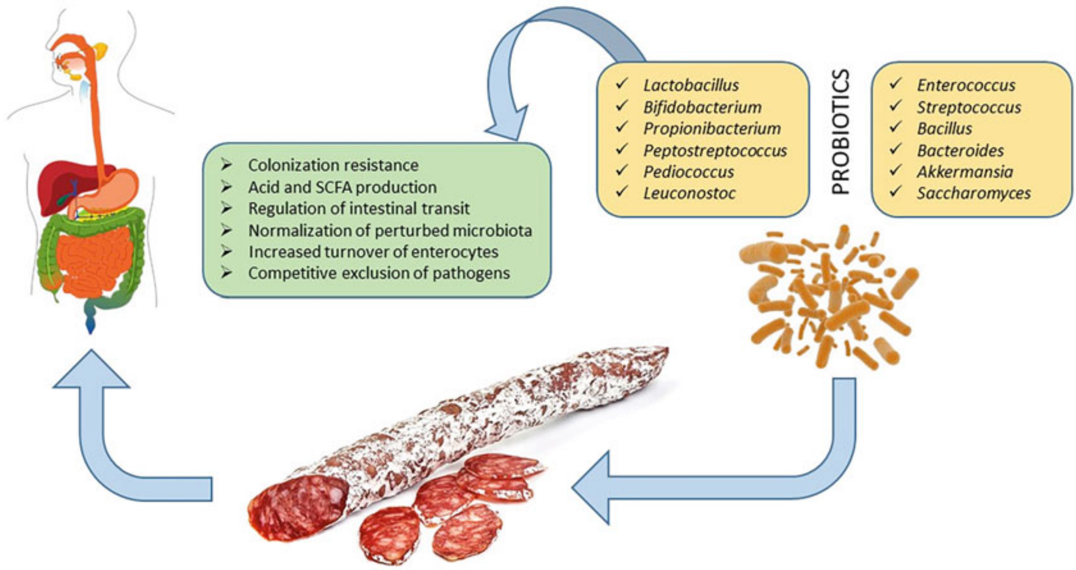

Fig. 1 Potential health benefits of probiotic fuet consumption and possible bacteria involved [24, 25]. *SCFA* short-chain fatty acid

commercialized. In this chapter, we selected the "fuet" as the base product to show its production process in depth. Fuet is a traditional product from the region of Catalonia widely consumed in Spain. Only in 2019 that consumers of this country spent more than 250 million dollars on this meat product and longaniza. In addition, fuet product is becoming international, and its consumption has spread to other neighboring countries such as France and also to the entire European continent and the United States, where it is prized for its presumed high-quality ingredients, exceptional flavor, and superior wholesomeness compared to similar Mediterranean-type sausages [23].

2 Materials

2.1 Ingredients

Fuet is made mainly with pork and fat. In addition, salt, spices, and sugar are also used. Different preparations of this fermented sausage are possible, so in order to avoid conflicts between the multiple existing formulations, we have decided to compile some of the most relevant recipes recently published in the scientific literature to develop our own concept of fuet. Pork lean is a fundamental part of the traditional recipe, but other meats are currently replacing pork in some novel manufacturing protocols. After chopping and blending the lean and fat, salt and ground pepper are added. Other species such as garlic can also be incorporated. These seasonings work as flavor enhancers and can help in the stabilization process by

Table 1
Ingredients and proportions in the elaboration of the probiotic fuet

Ingredients	Proportion (%)
Pork lean	60
Pork fat	30
Water	4
Commercial mix	4
Commercial starter culture	1.98
Commercial probiotic strain	0.02

Commercial mix: salt, dextrin, dextrose, stabilizer (sodium phosphate (E-451)), spices and spice extract, flavor, antioxidants (sodium ascorbate (E-301) and sodium citrate (E-331)), and preservatives (potassium nitrate (E-252) and sodium nitrite (E-250)). Starter culture: *Pediococcus* (50%), *Staphylococcus xylosus* (25%), and *Staphylococcus carnosus* (25%). Probiotic strain: LGG® (*Lacticaseibacillus rhamnosus* GG). Data are based on the studies carried out by Bis-Souza et al. [29], Zamora et al. [30], and Peñaranda et al. [31]

exhibiting antioxidant and antimicrobial properties [26–28]. Differences between fuets, both commercial and homemade, can be found at this point of preparation. The type of meat and the fermentation process also have a significant influence on the final product.

Other ingredients, including stabilizer (e.g., phosphate), antioxidants (e.g., ascorbate and citrate), preservatives (e.g., nitrate and nitrite), dextrin, dextrose, and flavorings, are also added to the meat matrix. These compounds, together with the spices, are usually incorporated in the form of commercial mixes. A starter culture consisting different type of species, including LAB, and a probiotic culture are then inoculated. Finally, the formed dough needs to be completed with water up to a certain percentage of humidity. Artificial pig casings are used to stuff the dough obtained, but natural pork casings are also commonly used, and a food-grade *Penicillium candidum* mold is applied to the surface of sausage to protect it from the invasion of spoilage molds during storage. Moreover, it adds a touch of flavor and extra aroma to the final product. Finally, fermentation and drying processes complete the production protocol. Ingredients and proportions in the preparation of the probiotic fuet are detailed in Table 1.

There are many commercial mixes available on the market, but we suggest using the one sold by Catalina Food Solutions S.L. (El Palmar, Murcia, Spain) [30]. This mix is made up of salt, dextrin, dextrose, stabilizer (sodium phosphate (E-451)), spices and spice extract, flavor, antioxidants (sodium ascorbate (E-301) and sodium citrate (E-331)), and preservatives (potassium nitrate (E-252) and sodium nitrite (E-250)).

Regarding the starter culture, the commercial mix used by Zamora et al. [30] (Microsan-R), also from Catalina Food Solutions S.L. (El Palmar, Murcia, Spain), was chosen for the elaboration of the probiotic fuet. The genus *Pediococcus* at a concentration of 50% and the species *Staphylococcus xylosus* and *Staphylococcus carnosus* at concentrations of 25% each compose the starter culture. On the other hand, the probiotic strain LGG® (*Lacticaseibacillus rhamnosus* GG), marketed by the company Chr. Hansen (Hørsholm, Denmark) (*see* **Note 1**) and successfully tested in the preparation of a salchichón [29], was the one chosen to colonize the fuet. Finally, as previously mentioned, a layer of *Penicillium candidum* spores is applied to the product after stuffing. There are different commercial brands on the market that provide this mold. In this case, we propose the one marketed by the company Danisco S.A. (Barcelona, Spain), according to the elaboration carried out by Marcos et al. [32].

2.2 Equipment

The equipment for making fuet is an essential part of the production protocol since inadequate material can lead to undesirable results. Thus, we have suggested a series of elements and brands that can adequately satisfy the needs of manufacturers during the different stages of production (Table 2). Photos of this machinery are shown in Fig. 2.

A mincing machine is the first piece of equipment necessary for the fuet production process as it allows the chopping of meat and fat, which will form the base of the sausage. For this, a refrigerated mincer from La Minerva di Chiodini Mario (Bologna, Italy) with a 6 mm mincing plate can be used. For fine grinding and mixing of both raw materials, along with the commercial mixes of additives and microorganisms (starter culture and probiotic strain), an Industrial Fuerpla (Benetusser, Valencia, Spain) vacuum grinder is

Table 2
List of suitable industrial equipment for the elaboration of the probiotic fuet and the corresponding brands (*see* Notes 2 and 3)

Equipment	Brand
Mincing machine	La Minerva di Chiodini Mario (Bologna, Italy)
Vacuum grinder	Industrial Fuerpla (Benetusser, Valencia, Spain)
Semiautomatic stuffer	Sia Suministros Industriales (las Torres de Cotillas, Murcia, Spain)
Sausage tying machine	Andher-*Comercial Eliseo Andújar S.L.* (Alcázar de San Juan, Ciudad Real, Spain)
Air-drying chamber	–
Conservation chamber	–

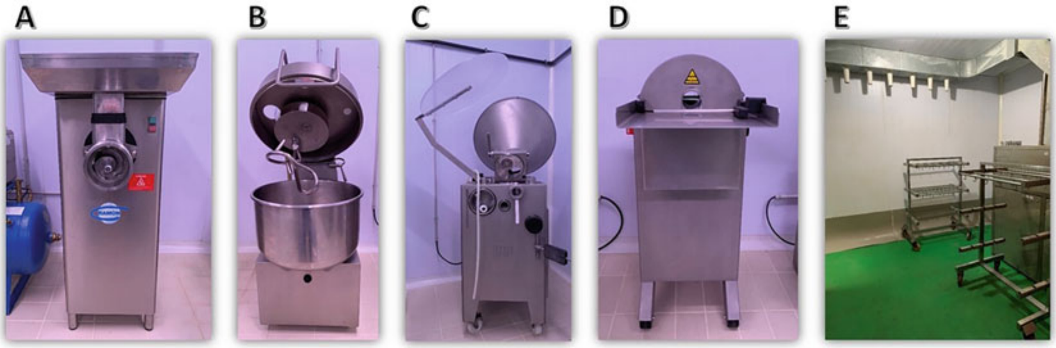

Fig. 2 Suggested equipment for the elaboration of a probiotic fuet. (**a**) mincing machine; (**b**) vacuum grinder; (**c**) semiautomatic stuffer; (**d**) sausage tying machine; (**e**) air-drying chamber

recommended. A conventional or industrial refrigerator will be necessary, depending on the amount of dough produced, to store it and allow the compaction to occur. Stuffing can be performed using a Sia Suministros Industriales (Las Torres de Cotillas, Murcia, Spain) semiautomatic stuffer and a 45 mm diameter artificial casings (Edicas, Salamanca, Spain). The sausages formed can be divided and tied using an Andher sausage tying machine (Alcázar de San Juan, Ciudad Real, Spain). Finally, the fermentation and drying processes can be carried out in an air-drying chamber. After elaboration, the product can be moved to another automated chamber to preserve it until consumption.

3 Methods (*See* Note 4)

Fuet requires a strict production protocol and good raw materials (lean meat and belly) to obtain a quality product. Once these elements are minced and mixed with the commercial mix and both the starter and probiotic cultures, the dough formed is stuffed, fermented and dried, and conserved (Fig. 3). This highly summarized production procedure consists of a series of detailed stages that will be described in depth in the following paragraphs.

3.1 Chopping of Raw Materials

The first step when making fuet is to obtain good-quality raw materials. A product made with meat and fat of little aptitude for the preparation of sausages will affect the quality of the product. The classical recipe for fuet uses pork lean and pork belly. However, recently modifications of this model recipe have been appearing, which attempt to diversify this product by incorporating proteins from different animals. Meat from animals, such as goat, sheep, beef, and other less common, such as duck, foal, or game, can be used in the production of salchichón [33–35]. In our case, both the meat and fat for making fuet will be from pork.

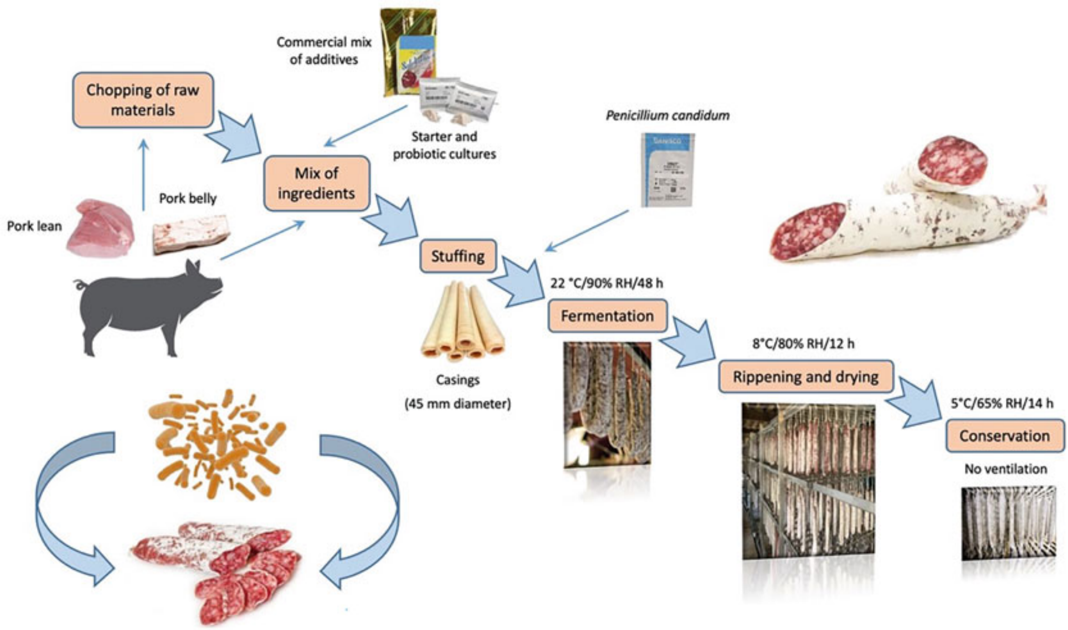

Fig. 3 Production scheme of probiotic fuet

The previously refrigerated pork lean and pork belly are cut into chunks of between approximately 10 × 10 and 20 × 20 cm to allow their easy passage through the mincer tube. The meat and fat are placed separately in the mincer's loading hopper and are pushed by an auger towards the hole that ends in a perforated disc that grinds the raw material. The chopped lean and fat are then collected in a clean stainless steel container. In this operation, the sharpness of the blades must be adequate; otherwise, the mincing may be poor, causing tears in the meat and overheating. This leads to problems in the ripening and drying stage, giving rise to fuets with poorly defined short surfaces.

3.2 Mix of Raw Materials with Additives and Starter and Probiotic Cultures

After the mincing process, the lean and fat are properly mixed under refrigerated (< 4 °C) vacuum. The absence of air is essential to prevent subsequent problems, such as discoloration and a higher development of microorganisms. At this point in processing, the commercial mix of additives in powdered form, incorporating salt, spices and spice extract, dextrin, dextrose, and flavoring, along with a stabilizer, antioxidants, and preservatives, is poured over the meat mixture and fat. This operation should last the time enough to allow the formation of the most uniform paste as possible. Around 5 min would be needed to process 20 kg of dough. During the mixing process, both the starter and probiotic cultures are added. The moment and the order of addition are indifferent. Specifically, the commercial starter culture will be incorporated in the form of lyophilized powder (commercial presentation), being previously rehydrated for 8 h (approximately 7 g per 100 mL of water) (adaptation of the preparation carried out by Zamora et al. [30]). On the other hand, the probiotic culture will be prepared and added according to the instructions for use provided by the trader. Along with all the mentioned ingredients, water will be added according to the proportion indicated in Table 1.

Once the different ingredients are mixed, the dough formed is stored under refrigeration for 24–48 h. This storage favors the obtaining of a higher-quality dough by allowing better integration of the aromas in the meat and fat. Similarly, this period helps salt penetration. In this way, protein coagulation can be increased, and dough with superior rheological characteristics can be formed.

3.3 Stuffing of Meat Dough

The meat dough adequately prepared is introduced into artificial casings by means of a stuffer, trying to avoid the presence of air as much as possible. The mixing of ingredients under vacuum conditions makes it possible to considerably reduce the formation of air cavities in the dough, but this condition could be reversed if the stuffing process is not carried out properly. Insufficient fill pressure could add air into the casing and form small air-filled spaces that may cause discoloration, abnormal coloring, moldiness, and other abnormalities. In addition, the dough must be kept away from any source of moisture as it could also cause abnormal coloring. At the end of the stuffing process, casings are divided into portions about 30–35 cm long that are tied with a string specially indicated for this use. Finally, all the formed pieces must be washed with clean water to remove any kind of leftover material from the surface.

The artificial casings used in this protocol should be soaked for about 2 h before stuffing to avoid breakage during the filling process. A small handful of salt can be added to provide an extra touch of saltiness to the sausage.

3.4 Addition of Protective Mold on the Surface of Sausage

Just after stuffing and before fermentation, a layer of specific mold is added to the surface of sausages to prevent possible contamination with spoilage molds. A spore solution of the mold *Penicillium candidum* is prepared according to the manufacturer's instructions, and the sausages are immersed in the liquid [32].

During the drying stage, the internal water of the sausage slowly escapes to the outside, leaving the surface of the product wet. Under these conditions, molds can proliferate and spoil the fuet, causing possible health problems. For this reason, spores of certain types of mold should be added in order to protect the sausage during storage. Furthermore, the white layer formed by these microorganisms helps control water loss, promoting more uniform dehydration, and adds aroma and taste to the final product, being actually considered an important quality attribute.

3.5 Fermentation Process

The stage after stuffing and adding mold is fermentation. During this brief period of time, fundamental biochemical processes for the proper sensory development of fuet will take place. The sausages are transferred in a drying chamber under special humidity and temperature conditions. According to the parameters used by Zamora et al. [30], temperature and relative humidity (RH) will be set at 22 °C and 90%, respectively, for 48 h. In this stage, the starter culture will colonize the meat substrate and metabolize the sugar, transforming it into lactic acid. This will cause a drop in pH, which will lead to a series of phenomena, such as protein and fat hydrolysis, color changes, and texture modification, among others.

3.6 Ripening and Drying Period

The post-fermentation period, commonly known as the curing stage, encompasses the most prominent changes (chemical, physical, physicochemical, microbiological, and sensory) in the sausage matrix. In fact, one of the most important events that occur in this stage is the redness of sausages. This phenomenon is produced by the formation of nitropigments, favored by the conversion of nitrates into nitrites due to the action of reducing microorganisms. After 48 hours of fermentation, the RH and temperature values will be reduced to 80% and 8 °C, respectively, and the sausages will be maintained for the next 12 days in these new environmental conditions. At the end of this time, adequate microbiological stability and unique organoleptic properties will be achieved.

On the other hand, the ripening and drying period is characterized by the loss of moisture in the dough, known as "merma." Thus, a salchichón can suffer a reduction of at least 30% of the initial content [35]. During post-fermentation, the sausages must remain hung in the darkness to avoid the appearance of rancidity in the crust due to the action of light. However, they should not be hung too close together because proper ventilation is prevented. This promotes the accumulation of humidity between pieces, which may lead to mold on the product.

3.7 Conservation

At the end of the production protocol, the freshly finished fuets must be kept under specific storage conditions that maintain their unique sensory quality and also prevent microbiological spoilage. Therefore, they are transferred from the curing chamber to the conservation chamber, using a lower refrigeration temperature and RH than those used during the previous stage. Specifically, fuets should be stored at 5 °C and 65% HR in the absence of ventilation for 14 days.

4 Notes

1. The probiotic culture proposed in the fuet elaboration was highly researched, and the most notable results discovered can be consulted on the website of the trading company.
2. The models of the machines suggested for the elaboration of the probiotic fuet were not provided on purpose because the model may vary depending on the amount of product. Only the proportions of the ingredients were given, leaving the decision about the production size in the hands of the reader.
3. The trademarks of the ingredients and machines provided in this chapter are only indicative. They were suggested due to previous satisfactory experience by the authors.
4. All the processes before fermentation have to be done under refrigeration temperature. This implies that the work rooms employed need to be prepared to produce cold. Otherwise, microorganisms can grow over the recommendable limits, spoiling the fuet prematurely.

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