



IMPROVING CONSUMER CHARACTERISTICS OF FERMENTED SAUSAGES WITH PROBIOTICS USING

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Abstract

Consuming a food product that contains beneficial microorganisms provides a wide range of health benefits, starting from improved digestion and support of intestinal microflora and to an impact on metabolism, improving immune system and normalizing psychological and emotional status. The purpose of this article is to systematize and evaluate the results of experimental studies conducted over the past ten years on using of probiotic cultures in the dry fermented sausage manufacturing technology, including the casting, selection and adaptation of probiotic strains to meat systems. The influence of probiotics on recipes and process parameters was studied. An analysis of research results conducted in various countries shows that the strategy of combining the advantages of controlled fermentation with potential health benefits for consumers is of the greatest interest. Meat technologies make it possible to produce and reliably preserve fermented sausages with a probiotic content of 10^6 – 10^8 CFU/g, even during long-cycle production and subsequent storage. Moreover, the probiotic effect of consuming 30 g of fermented meat products per day is evident even with a probiotic content of less than 10^6 CFU/g. The observed trends in the recipe composition of fermented sausages, aimed at reducing fat and sodium content, underline the striving of the scientists from various countries to make this type of food product more adapted to the modern dietary requirements. Another promising area of research is the casting and selection of probiotic strains for their using in the specialized starter cultures, as well as the development of technological standards for fermentation processes where those cultures are involved. The results of this study prove the feasibility of implementing a technology for the production of probiotic fermented sausages, which will enable the market to offer a fully functional food product.

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Introduction

The origins of using health-beneficial microorganisms in human nutrition to support human health date back to the works of the great Russian biologist and one of immunology founders Mechnikov Ilya Ilyich. Later this approach was widely developed by the Russian and foreign scientists. The concept proposed by I. I. Mechnikov was extended to various food groups, including meat products.

The scientific researches aimed to the development of fermented sausages with health-beneficial probiotic properties have been actively conducted since the end of the last century. However, despite their potential, those food products still have to become mass-produced. The meat industry was not fast to introduce them, primarily for two reasons: persistently high consumer demand for traditional dry-smoked and dry-cured sausages (even those without probiotics added), and the lack/shortage of commercially available probiotic microorganisms suitable for fermented meat products.

In the last decade, the idea of the health benefits of processed meat, including dry fermented sausages, has been subjected to a radical revision along with the suggestion of a global golden-reference diet based primarily on plant foods, with a complete rejection of meat or its consumption in very small quantities (no more than 14 g per day) [1]. This study, published in 2015 in the journal “The Lancet Oncology”, although was heavily criticized by the scientists [2], nevertheless influenced the consumers’ behavior and caused a decrease in the consumption of fermented sausages [3–5]. In its turn, against the background of changing consumer perceptions about the benefits of processed meat products, the interest of researchers in finding practical ways to integrate the new properties beneficial to human health into traditional fermented meat products has increased. Using of probiotics for these purposes allows for a comprehensive solution to this problem, since the consumption of a product containing health-beneficial microorganisms provides a wide range of positive health effects — from improved food digestion and support of

healthy intestinal microflora to beneficial effect on metabolism, improving the immune system and normalizing the psychological and emotional status [6,7].

In this regard, this review aims to systematization and critical assessment of the results of experimental studies (within the period 2016–2026) on using of probiotic cultures in dry fermented sausage manufacturing technology, including the casting, selection and adaptation of probiotic strains to meat systems; the influence of probiotics on recipes compositions and technological parameters; the evidences base that proves the beneficial effect on consumer health.

Objects and methods

ScienceDirect database was used to search for reference sources. The search was performed with the keywords “probiotic dry fermented sausages” based on the studies published over the past 10years (2016–2026). The initial selection was conducted by two authors independently; discrepancies were resolved through the discussion with the other authors.

Initially ScienceDirect database have found 362 sources using the above-specified keywords. Meanwhile, the dynamics of studies publication activity devoted to this topic indicated growing research interest in the use of probiotics in fermented meat products (Figure 1).

The review articles and articles that were not published in open access were excluded from the primary search and casting, because the purpose of this study was to analyze primary experimental data obtained directly during the development and analysis of quality of the fermented sausages fortified with probiotic cultures.

After excluding the review articles, 171 research articles remained. To ensure a high level of evidence representation and reproducibility of the results, the following additional criteria were applied to the content of the study:

- selection of the suitable probiotic strains, doses and methods of their adding to improve the consumer qualities of the fermented food products;
- sausage recipe composition and technology for the production of the fermented food products with probiotics added;

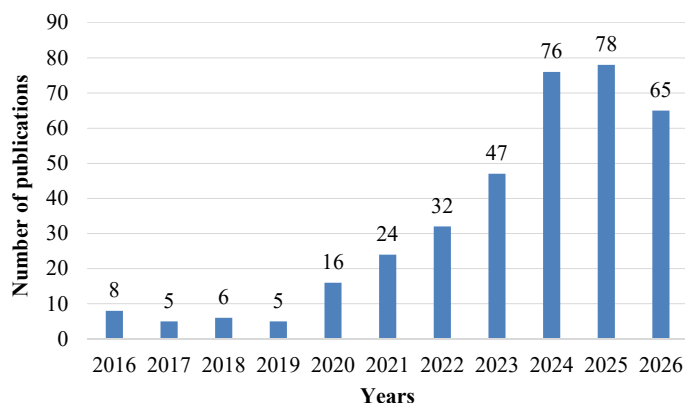


Figure 1. Number of publications with the keywords “probiotic dry fermented sausages”, presented by years in the ScienceDirect database

- survival of probiotic cultures in the fermented food products and their impact on consumer health;
- achieved effects in improving the consumer characteristics of the fermented food products.

The application of the listed criteria allowed us to select 17 articles [8–24] for the final analysis, which at their most extent fully correspond to the purposes of this review and ensure representativeness of the data for formulating justified conclusions.

Selection of probiotic strains, doses and methods of administration

The first stage of article analysis revealed that the casted and selected research papers were devoted to using 11 species of probiotic microorganisms, including *bifidobacteria*, *enterococci*, *lactococci*, and *lactobacilli*, with the latter species being the most diversely represented (Table 1¹). The selected articles did not include studies on using propionic acid bacteria as probiotics for the fermented sausages.

As it can be seen from the Table 1, the absolute leaders in the researchers’ choice were strains of *L. rhamnosus* and *L. plantarum*. Each of these microorganism species was used and assessed as a probiotic culture in 29 % of the selected studies. The second place was occupied by the strains of *L. casei*. (24 %). They were followed by *L. acidophilus* (18 %) and *E. faecium* (12 %). Strains of *B. animalis*, *B. longum*, *E. durans*, *L. c. lactis*, *L. paracasei* were used less frequently than the above — in no more than one of the 17 studies.

When selecting strains, the majority (35 %) of studies preferred to use commercially available probiotics, whose health-beneficial properties have been well-studied and proven over long-term use. In 24 % of studies, the authors selected the probiotics from available government and public collections, which properties had also been previously studied.

In 18 % of the studies, the meat origin of the probiotic cultures was indicated: strains were extracted and isolated from traditional fermented sausage, raw meat (pork) and beef (cattle).

Traditional fermented meat products are rich in diverse lactic acid microflora, well adapted to the conditions of survival in meat systems. Among these microflora, strains with probiotic properties have been repeatedly isolated and characterized. Scientists around the world are actively searching for new promising strains isolated from the meat products cooked in traditional, artisan way. For example,

¹ Further on in this review the traditional names are used for all microorganisms (*Lactobacillus* spp.) regardless of the names nomenclature used in the cited sources. This decision was made in order to standardize terminology throughout the work, because the analyzed studies cover the period both before and after the 2020 taxonomic reclassification, and using different names for the same species could complicate the understanding of the material. The modern names are given in this footnote: *Lacticaseibacillus casei* (formerly *Lactobacillus casei*), *Lacticaseibacillus paracasei* (formerly *Lactobacillus paracasei*), *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus*), *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*), *Levilactobacillus brevis* (formerly *Lactobacillus brevis*)

Table 1. Probiotic strains, doses and methods of administration

Probiotic	Origin of the strain	Method of preparation for application	Dosage and method of administration	Ist.
<i>Bifidobacterium longum</i> KACC 9156	Stool of healthy Korean newborns	Suspension in saline solution; microencapsulation of freeze-dried culture with glycerol	0.02 % of the minced meat weight in microencapsulated form; or 0.02 % in microencapsulated form with the addition of 0.02 % freeze-dried culture in saline. Used as a probiotic and starter culture	[8]
<i>Enterococcus durans</i> ED0207, <i>Lactococcus lactis</i> LL8307	Croatian artisanal cheese, selected after assessment of rate of survival in minced meat for 40 days	Suspension in skim milk solution	Addition of cultures at a rate of 10^7 CFU/g of minced meat. Used as a probiotic and starter culture	[9]
<i>Bifidobacterium animalis</i> , <i>Lactobacillus acidophilus</i>	Commercial probiotic cultures from the company Chr. Hansen (Denmark)	Suspension in saline solution containing 10^{10} CFU/ml	Addition of cultures at a rate of 10^7 CFU/g of minced meat. Used as a probiotic and starter culture	[10]
<i>Lactobacillus casei</i> CRL-431	Commercial probiotic culture from the company Chr. Hansen (Denmark)	-	1 g/kg of minced meat. Combined with the commercial starter culture <i>L. plantarum</i> + <i>St. carnosus</i> (Germany)	[11]
<i>Lactobacillus rhamnosus</i>	Commercial probiotic culture from Danisco (USA)	Suspension in saline solution; microencapsulation (coating composition: sodium alginate, gellan gum, gelatin, fructooligosaccharides, etc.)	Addition of culture at a rate of 10^7 CFU/g of minced meat in combination with the same amount of commercial starter culture <i>L. plantarum</i> from the company Blessing-Biotech (Germany)	[12]
<i>Lactobacillus rhamnosus</i> FERM P-15120	The human gastrointestinal tract	Warming and incubation before use of a frozen culture suspended in saline with the addition of 20 % glycerol	Addition of culture in the amount of 10^8 CFU/g. Used as a probiotic and starter culture	[13]
<i>Lactobacillus plantarum</i> L125	Traditional Greek fermented sausage	Warming and incubation before use of a frozen culture suspended in saline with the addition of 20 % glycerol	Addition of the culture in the amount of 10^8 CFU/g together with the commercial starter culture BACTOFERM BFL-F02 from the company Chr. Hansen, containing <i>Pediacoccus pentosaceus</i> and <i>Staphylococcus carnosus</i>	[14]
1) <i>Lactobacillus plantarum</i> 178; 2) <i>Lactobacillus casei</i> LC01	1) pork; 2) commercial probiotic culture from the company Chr. Hansen	Freeze-drying of the cultures	Addition of culture in the amount of 10^8 CFU/g. Used as a probiotic and starter culture	[15]
<i>Lactobacillus plantarum</i> IIA-2C12 and <i>Lactobacillus acidophilus</i> IIA-2B4	Beef cattle	Suspension in 10 % skim milk solution containing over 10^8 CFU/ml	Addition of culture in the amount of 10^9 CFU/g. Used as a probiotic and starter culture	[16]
<i>Lactobacillus plantarum</i>	No data	Suspension in saline in the form of free cells (10^9 CFU/ml); encapsulated form (alginate, dry milk, dextrin); simple emulsions; double emulsions	Addition of culture in the amount of 10^7 CFU/g (suspension — 0.63 %; encapsulated form — 3.75 %; simple emulsion W/O 2.30 %; double emulsion W/O/W — 5.70 %). Used as a probiotic and starter culture	[17]
<i>Lactobacillus plantarum</i>	Commercial probiotic product Bioflora™ from BIOSIDUS SA (Argentina)	Suspension in saline solution	Addition of culture in the amount of 3.16×10^8 CFU/g. Used as a probiotic and starter culture	[18]
<i>Lactobacillus rhamnosus</i> LOCK900	Collection of the Lodz University of Technology (Poland)	Activation of the frozen culture and its suspension in food broth. The bacterial content in the inoculum is approximately 10^9 CFU/ml.	Addition of a culture in the form of a preparation containing 10^6 CFU/ml, at a rate of 2 ml per 1 kg of meat. Used as a probiotic and starter culture	[19]
<i>Lactobacillus rhamnosus</i> HN001	no data	-	Together with the starter cultures, the application rate is not specified	[20]
<i>Enterococcus faecium</i> CRL183 and <i>Lactobacillus acidophilus</i> CRL 1014	Reference center for lactobacilli — CERELA (Argentina)	no data	Used as a probiotic and starter culture. Replacement for starter culture (<i>P. pentosaceus</i> , <i>S. xylosus</i>)	[21]
<i>Lactobacillus paracasei</i> , <i>Lactobacillus rhamnosus</i> GG	Commercial probiotic cultures from the companies SaccoSystem (Italy) and Chr. Hansen (Denmark)	-	0.01 g/kg. Together with the commercial starter culture from Chr. Hansen (Denmark) Bactoferm T-SPX (<i>P. pentosaceus</i> , <i>St. xylosus</i> ; 0.025 g/100 g)	[22]
<i>Lactobacillus casei</i> SJRP66, <i>Lactobacillus casei</i> SJRP169	mozzarella. Lactic acid bacteria culture collection, University of São Paulo (Brazil)	Thawing and activation of the culture in MSR broth; resuspension in saline (10^{10} CFU/ml)	At the level of 10^6 CFU/g. Together with the commercial starter culture Chr-Hansen (Denmark) Bactoferm T-SPX (<i>P. pentosaceus</i> , <i>St. xylosus</i> ; 0.025 g/100 g), (Chr. Hansen, Denmark)	[23]
<i>Enterococcus faecium</i> CECT 410	Collection of Spanish-type cultures CECT (Spain)	Suspension in saline solution containing 10^9 CFU/ml	The application rate is 10^7 CFU/g. Inoculation into minced meat in the form of free cells in the amount of 0.63 %, in encapsulated form (alginate) — 3.75 %. Used as a probiotic and starter culture	[24]

Chinese scientists have studied the probiotic properties of *Pedioroccus* lactic acid bacteria strains *Pedioroccus pentosaceus* R1, *Lactobacillus brevis* R4, *Lactobacillus curvatus* R5 и *Lactobacillus fermentum* R6 isolated from Harbin dry-cured sausages compared with probiotics from fermented milk products — *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *L. curvatus*, *Lactobacillus sake*, *Lactobacillus pentosaceus*, and *L. fermentum*. The results of the study confirmed that the strains isolated from sausages could be used as potential probiotics for the food production industry [25].

In 12 % of the studies, the authors indicated the human origin of the strains. The selection of strains of human origin is not a random accident. Those strains should initially be characterized by high adaptability to living in the human gastrointestinal tract [26]. However, they may not be as adaptable to the environment conditions typical of the meat system — long exposure to high NaCl concentrations, availability of sodium nitrite, and reduced water activity.

In addition, 12 % of the studies cited traditional dairy products (cheeses) as the source of the strains. This source of probiotic strains is of interest because the duration of the production cycle and the ripening conditions of cheese are comparable to the fermentation process of meat products. It is noteworthy that not a single study was found in the selection of articles that discussed the creation of fermented sausages using recombinant probiotic strains. Scientists have been studying this area for over 30 years, demonstrating the potential of genetic engineering in producing new probiotic preparations with the desired characteristics [27]. This indicates that consumers are still not ready to accept the benefits of such strains in food products and, consequently, prefer not to choose GMO strains in scientific research. Nevertheless, given the active work in the field of DNA research and its genetic engineering, the development of new types of sausages with genetically modified strains that combine the best characteristics of probiotics of various origins can be expected in the future.

Table 1 also shows that scientists suggest at least 2 ways of probiotics using:

- as a functional starter culture, that features both probiotic and technological properties, and which is able to replace this way the starter cultures previously used that have no probiotic potential;
- as an exclusively probiotic culture, which main function is its ability to be preserved in the finished food product until its consumption and then in the human gastrointestinal tract.

Moreover, the first method was chosen as the researches direction in 65 % of the works.

Recipe composition and production technology of fermented food products with probiotics

The idea of creating a fermented sausage with health-beneficial properties may also involve certain amendments to the composition of traditional food products, caused by

the selection of base ingredients and additional ingredients in the recipe, as well as their ratios, thus providing greater health benefits to the consumers.

In this regard, the analysis of the recipes of fermented sausages with probiotics added, which were selected by the scientists during the research (Table 2), was of the special interest.

As Table 2 shows, the great majority (65 %) of recipes used pork only or pork in combination with other kinds of meat (beef, wild boar) as lean meat. The remaining recipes used beef, lamb, and goat meat, which was obviously caused by the religious traditions of the countries where the studies were conducted — it was based on necessity of compliance with Halal product requirements.

One of the trends in the new types of probiotic sausages development that is clearly observed from the data analysis of Table 2 is the reduction of the habitual using of fatty raw materials in the recipe. Typically, a 70:30 ratio of lean to fatty raw meat materials is used for dry-smoked and dry-cured sausages. This high level of fatty raw material content was used in recipes in 12 % of studies only. In one study [11], a 70:30 ratio of lean to fatty raw meat materials was chosen as a comparison with other recipes that contained less fatty raw materials. A ratio of 80:20 or an even greater change in favor of lean raw materials was used in 59 % of studies and has become the “gold” standard when selecting recipes for sausages with probiotic properties. In addition, two more research trends should be highlighted: improving the fatty acid profile of sausages through, for example, using olive oil emulsion [21] and complete refusal from fatty raw materials (i.e. lard) [9,10,11,13,16].

With regard to the selection of food ingredients and additives as additional formula components (Table 2), a clear trend toward limiting table salt (sodium chloride) content is evident. While just a decade ago, 2.7–3.5 kg of table salt or nitrite salt was prescribed to be added per 100 kg of primary meat raw material, today scientists prefer more moderate dosages—from 1.8 % to 3.0 %. This is explained not only by nutritional trends but also by the desire to create more favorable conditions for probiotic microorganisms to grow and survive in the meat system [9,11,13,17,24].

On the contrary, regarding the using of sodium nitrite (sodium nitrite and nitrate), the majority (88 %) of studies adhered to the introducing the food additives E251 and E252 at rate of 10–15 g per 100 kg of the base raw material, which is logical from the standpoint of ensuring the safety of fermented sausages produced with a reduced content of table salt.

At the same time, an interesting trend has been observed: the production of probiotic fermented sausages without preservatives, including nitrite salt [9,13] has emerged. This is explained by the fact that some regional dry-cured products, for example, those made from wild animals’ game meat, traditionally did not use nitrites and nitrates. Today, a number of scientists see this as an opportunity to create recipes without E-indices (“clean

Table 2. Recipes compositions of fermented food products samples with probiotics added

Main raw material, % of the main raw material weight		Food additives and ingredients, % of the main raw material weight							Ist.
Lean raw materials	Fatty raw materials	Table salt	Nitrite	Nitrate	Nitrite salt	Carbohydrates	Antioxidant	Spices and other ingredients	
Pork — 80	Lard — 20	2.0	0.010	0.005	—	Sugar — 1.5	E301–0.2	Black pepper — 0.2; Phosphates — 0.2	[8]
Pork — 50; wild boar meat — 50	—	1.9	No nitrite or nitrate	—	—	Sugar — 0.2	—	Red pepper, chili pepper, garlic, red bell pepper, black pepper — 1.1	[9]
Goat meat — 50, beef — 50	—	—	—	—	2.0 (0.6% NaNO ₂)	Lactulose — 1.0	—	Commercial mixture — 5	[10]
Beef — 80, 75, 70	Beef fat — 20, 25, 30	1.8	0.015	—	—	—	E301–0.05	Garlic, red bell pepper, red pepper, black pepper, cumin, allspice — 3.75	[11]
Beef — 80	Beef fat — 20	2.5	0.015	—	—	Sugar — 0.4	—	Garlic, red pepper, black pepper, cumin, allspice — 3.35	[12]
Beef — 100	—	NaCl — 3.0; NaCl — 1.5; NaCl + KCl — 1.5 + 1.5	No nitrite or nitrate	—	—	Glucose — 1.0	—	—	[13]
Pork — 80	Lard — 20	2.0	0.015	—	—	Glucose — 0.5	—	Garlic and spices — 0.7; collagen emulsion — 0.25	[14]
Pork — 69.5	Lard — 17.5	2.2	0.015	—	—	Glucose — 0.4	E301–0.2	Fennel seeds — 0.2; sweet pepper extract — 8.6, commercial mixture — 0.5	[15]*
Beef — 100	—	2.0	+	+	—	Sugar — 2.0	—	Dried ginger, nutmeg, white pepper, black pepper, lime leaves — 2.5	[16]
Pork — 75	Lard — 18	1.5	—	—	0.3	—	—	Commercial mixture — 5.2	[17]*
Pork — 60	Lard — 40	2.0	0.01	—	—	Glucose — 0.02; Chestnut flour — 3.0	E300–0.05	Black pepper and anise — 0.21; Water — 5	[18]
Pork — 70	Lard — 30	—	—	—	2.8 (99.5% sea salt, 0.5% NaNO ₂)	Glucose — 0.5	E301–0.05	Garlic, black pepper, marjoram — 1.3; Water — 5	[19]
Pork — +* *	Lard — +	+	+	+	—	Starch, citrus fiber, inulin, arabinogalactan — 5	—	Black pepper — +	[20]
Pork — 61.5; Beef — 28.5	Lard — 8 Olive oil emulsion with sodium caseinate — 2	2.5	0.07	0.03	—	Sucrose — 0.5; Lactose 0.7	E301–0.5	Garlic powder, white pepper — 0.18	[21]
Pork — 80	Lard — 20; 15	—	—	In the composition of the comp. mixture	—	Fructooligosaccharides (instead of part of the bacon) — 2	—	Water (instead of part of the lard) — 3; commercial mixture — 5 (sugar, salt, dextrins, spices, milk protein, E621, E450i, E451ii, E316, E252, E120)	[22]
Pork — 80	Lard — 20; 15	2.5	0.015	0.015	—	Sucrose — 2 Fructooligosaccharides (instead of part of the bacon) — 2	E316–0.05	Water (instead of part of the bacon) — 3; garlic, white pepper, nutmeg — 0.7;	[23]
Pork — 75	Lard — 18	1.5	—	—	0.3	—	—	Commercial mixture — 5.2	[24]*

* in the asterisk-marked sources, the recipes are given in a form in which all components (in a number of works, probiotics) are included in 100 %;

** „+“ — quantitative values are not specified.

label”), this way meeting the consumers’ desires for eating healthier meat food products. However, while this technology simplifies the task of creating better growth conditions for probiotic cultures in meat systems, it also burdens the researchers with new challenges in selecting strains with well-pronounced antagonistic properties which are able to withstand against pathogenic microorganisms [9,13,28].

The trend of using sea salt in sausage recipes, including in the composition of nitrite salt, is also obviously worth noting as a factor in the possible marketing of developed food products as healthier ones from the point of the consumers’ view [19].

The choice of carbohydrates for fermentation in the majority (71 %) of studies was traditional: the monosaccharide glucose and/or disaccharides (sucrose, lactose). However, in the remaining 29 % of studies, the researchers selected interesting solutions for the carbohydrate component — lactulose, chestnut flour, starch, inulin, arabinogalactan, fructooligosaccharides, and dietary fiber (citrus fiber) were used [10,18,20,22,23]. These components can contribute to a positive perception of fermented sausages composition by the consumers and improve the consumer characteristics, including organoleptic one, for this products; however, the introducing of more complex carbohydrates requires more time for the fermentation process, which manufacturers cannot always afford.

With regard to antioxidants, the researchers’ choice of them in 29 % of studies was classical, favoring sodium ascorbate (E301). Interestingly, it was used in quantities ranging from the standard 50 g per 100 kg of the base raw material to an increased dosage of 200 g per 100 kg [8,15]. Both recipes with an increased dosage of E301 provided for the using of pork only and contained lard in significant quantities (17.5–20.0 %). Antioxidants were not used in the recipes that did not contain fatty raw materials, as well as nitrites and nitrates. In some cases, ascorbic acid (E300) or sodium erythorbate (E316) were used as antioxidants [18,22,23].

Using the spices in sausage production is largely predetermined by the region and local cuisine traditions. However, an analysis of probiotic sausage recipes revealed the following trends. Black pepper was the most commonly used spice, used in at least 47 % of studies (no data on the composition of all complex mixtures is available). It was followed by garlic (35 %), red pepper (18 %), white pepper (18 %), red bell pepper (18 %), allspice (12 %), and nutmeg (12 %). Some recipes also included chili pepper, cumin, marjoram, fennel seeds, dried ginger, anise, and lime leaves. It should be noted that in most studies the researchers did not focus on spices selection and, for the most part, just followed the established cooking practices for their using. However, their selection should be carefully considered when creating a product with probiotic properties. Spices and spice extracts, with appropriate

selection of their combinations, compositions and doses, can serve as an additional “barrier” for control of food pathogens, including *L. monocytogenes*, *Salmonella* spp., *St. aureus* [29–31]. On the one hand, spices can influence the organoleptic characteristics of the food product [29]. Perhaps, when creating probiotic food products, in some cases it is worth abandoning the traditional hot spices, especially those containing capsaicin and other substances, that can have a negative impact on the intestinal microbiome [32]. In this regard, in the future the development of commercial compositions of mixtures for probiotic fermented sausages seems to be a timely and relevant direction of activities.

It is worth to note that using phosphates and water in certain fermented food product recipes as well as the reasons for their using were not explained in the articles [8,18,19]. In the case of phosphate use [8], the production cycle lasted 22 days until the product’s water activity reached 0.81–0.83. It is possible that acidic or pH-neutral phosphates were used, as alkaline phosphates can create poorer conditions for de-moisturizing of the food during its fermentation and drying. The addition of water was apparently related to the use of complex carbohydrates and the need for their better distribution throughout the minced meat.

Thus, an analysis of the chosen articles showed two key trends in the selection of probiotic fermented sausage recipes: reduction or complete elimination of fatty ingredients (lard) and a reduction of table salt mass fraction (sodium chloride). Both trends are fully consistent with modern approaches to improving the composition of meat products and creating functional foods for healthy eating.

Rate of probiotic cultures survival in fermented foods and their impact on the consumers’ health

As Table 1 shows, many researchers point out that without special methods of preparation and introduction into meat systems, probiotic microorganisms may not survive and may not reach their main target for providing a health-promoting effect — the human intestine. In this regard, various methods have been proposed, ranging from probiotic microorganisms’ encapsulation [8,17,24] to emulsification [17]. Some studies have proposed original approaches this problem solution, like encapsulating the purposely grown biofilms of probiotic microorganisms [33].

At the same time the majority of the researchers set the goal to find the strains that can adapt well to fermentation conditions, long-term storage of fermented food products, and which are able to tolerate low acidity of the gastric environment, thus enabling them to reach the human intestine. This approach involves considering target strains both as probiotics and starter cultures, combining health benefits with the advantages of controlled fermentation. This approach in its turn makes unnecessary the use of starter cultures without probiotic properties in the production of

functional fermented food products, as well as declines the additional preparation processes such as encapsulation in the production of probiotics themselves. In general, this approach appears to be the most promising and appealing from an economic and technological process point of view. However, its implementation requires strains with high rate of survivability during the production and storage of fermented food products.

In this regard, the results were analyzed of studies on production and storage of fermented sausages where probiotic strains were used both together with commercial starter cultures and independently, on their own only (Table 3 and Table 4).

The probiotic strain growth during production of the fermented sausage was studied in the articles [8,9,10,11,15,23], and the analysis of the study is presented in Table 3. It should be noted that the authors selected various parameters and process durations. For example, in the article [9], sausages were produced without any climate control equipment (home-made production), when the temperature and humidity of the environment were determined only by weather conditions. The remaining studies primarily used production parameters previously developed for the food products with commercial starter cultures added. Given the complexity and multifactorial nature of the fermentation process, none of the studies considered the possibility of optimizing the production process parameters taking into account the specific properties of probiotic strains.

In general, the culture strains of *B. animalis*, *L. acidophilus*, *L. plantarum*, *L. casei* demonstrated not only survival in meat systems, but also increasing the quantity of viable microbial cells to 2 or more lg CFU/g. In contrast, the strains of culture *B. longum*, *E. durans* and *Lc. lactis* demonstrated decreasing of quantity and even death of microbial cells, which proved the impossibility of using them for manufacturing of probiotic food products or the advisability of selecting optimal conditions for their growth in meat systems. Thus, in the case of sausage production with added strain of *E. durans* ED0207, the manufacturing process was the longest, it lasted 40 days under uncontrolled conditions. The authors focus attention on the fact that nevertheless the strain survived for 15 days [9]. Under less extreme experimental conditions, this strain could possibly have demonstrated better results.

All sausages tested after their production, both without starter cultures and with the addition of starter cultures, were acknowledged to be successfully produced, even despite the ambiguous assessment of the survival of probiotic strains in the final food product, and they were characterized by pH values (with some exceptions [10]) and water activity typical for fermented sausages.

Analysis of the data presented in Table 3 also suggested that probiotic strains (*B. animalis*, *L. acidophilus*, *L. casei*) featured better rate of survival and increasing number of cells viable (NCV) during the fermentation process, due to

the combined use with each other or with starter cultures of lactic acid microorganisms (LAM).

Table 4 presents the results of the production of probiotic fermented sausages and the studies of their storage. In general, all probiotic strains, except *L. plantarum* L125 [14] showed high results in preserving viable cells during storage of sausages, even in the case of a 6-month storage period, and in storage for 60 days, an increase in the probiotic content was even observed. It should be noted that in two studies [17,24] encapsulation and emulsification did not give any pronounced advantages in probiotic strains preserving.

In general, most of the analyzed studies achieved probiotic content levels of 10^7 – 10^8 CFU/g in fermented products. This number of viable probiotic cells was maintained during sausage storage. However, in some cases, probiotic content was lower — at 10^6 CFU/g. This depended on both the application rate and other factors. A decrease in the content of beneficial (starter) microflora in finished products is typical of fermented sausages. This occurs as a result of drying and achieving low water activity. It should be noted that even for commercial starter cultures that were specially selected for their survival in the meat system, a decrease in number of cells viable down to 2–3 lg CFU/g is observed by the end of sausage production.

In the technological standard TR CU 021/2011² the requirements are set out for the content of probiotic microorganisms in the fortified (functional) food products — there should be not less than 10^9 CFU/g (ml) or 9 lg CFU/g (ml). This level was not achieved in any of the studies devoted to the analysis of probiotic fermented sausages. At the same time, the authors of the study [19] state that the minimum amount of probiotic in the food product which is able to achieve a probiotic effect should be at least 6 lg CFU/g of product. However, the majority of scientific data indicate that the minimum doses of probiotic consumption, at which a positive effect for human health is achieved and visibly observed, range from 10^6 to 10^9 CFU/g per day [34]. Taking into account the daily dose of the product consumption, the minimum probiotic content in the product, at which a positive effect is observed, may also be 10^5 CFU/g.

Clinical data obtained from the analysis of industrially produced salami dry sausage with added probiotic content of less than 10^6 CFU/g (4 – 5^8 CFU/g) confirm that even these concentrations are capable of providing a probiotic effect with a daily consumption of 30 g of product [20]. This reduces the generality of the regulatory threshold of 10^6 CFU/g, established for the fortified foods without taking into account the specifics of the meat matrix and actual volumes of consumption. In case of fermented sausages, the development of separate efficacy criteria is required, which criteria should be based on evidence-proven clinical trials.

² TR CU 021/2011 Technical Regulations of the Customs Union “TR CU 021/2011 “On Food Safety” (as amended as of April 22, 2024)” Retrieved from <https://docs.cntd.ru/document/902320560> Accessed July 12, 2026 (In Russian)

Table 3. Technological parameters, production time and survival rate of probiotics in the production of fermented sausages

Probiotic	Ambient temperature range / ambient relative humidity range / manufacturing time	pH / a _w finished product	The amount of probiotic, lg CFU/g, in the original minced meat / in the final product	Probiotic survival assessment	Ist.
When using a probiotic as a starter culture					
<i>B. longum</i> KACC 9156, encapsulated form	25–14 °C / 90–75 % / 22 days	5.24 / 0.810	5.88 / 2.83	Decrease by 3 lg CFU/g	[8]
<i>B. longum</i> KACC 9156, encapsulated. form+ cuspensia	– “ –	4.88 / 0.810	7.05 / 3.17	Decrease by 4 lg CFU/g	[8]
<i>E. Durans</i> ED0207	5.5–22–12–1 °C / 74–90 % / 40 days *	5.25 / 0.860	6.92 / 6.22	Decrease by 0.7 lg CFU/g	[9]
<i>L. lactis</i> LL8307	– “ –	5.16 / 0.860	6.98 / not detected	Death	[9]
1) <i>B. animalis</i> + 2) <i>L. acidophilus</i> , 1 % lactulose	22–18 °C / 95–75 % / 6 days	6.16 / 0.870	1) 6.87 / 7.92 2) 7.30 / 7.88	Increase by: 1) 1 lg CFU/g 2) 0.58 lg CFU/g	[10]
1) <i>B. animalis</i> + 2) <i>L. acidophilus</i> , 1 % lactulose	– “ –	6.16 / 0.830	1) 7.07 / 8.07 2) 7.05 / 7.41	Increase by: 1) 1 lg CFU/g 2) 0.36 lg CFU/g	[10]
	14–16 °C / 73–77 % / 5 days	5.71 / 0.900	8.50 / 8.60	Slight increase	[18]
	– “ –	5.63 / 0.920	8.50 / 8.67	– “ –	[18]
	22–12 °C / 99–65–75 % / 21 days	5.00 / 0.870	ICD: 7.00 / 8.00	Increase by 1 lg CFU/g	[18]
When using a probiotic together with a starter culture (SC)					
<i>L. casei</i> CRL-431 (SC: <i>L. plantarum</i> + <i>St. carnosus</i>)	24–17 °C / 94–76 % / 9 days	4.94–5.06 / — **	5.89–6.58 / 6.62–7.24	Increase by 0.66–0.73 lg CFU/g	[11]
<i>L. casei</i> SJRP66 (SC: <i>P. pentosaceus</i> + <i>St. xylosum</i>)	25–15 °C / 90–75 % / 18 days	4.76 / — **	6.00 / 8.15	Increase by 2 lg CFU/g	[23]
<i>L. casei</i> SJRP169 (SC: <i>P. pentosaceus</i> + <i>St. xylosum</i>)	– “ –	4.79 / — **	6.00 / 8.10	Increase by 2 lg CFU/g	[23]

* — artisanal production conditions, smoking was used; ** — a_w was not determined, the mass fraction of moisture in the product was for sausages with: *L. casei* CRL-431–35.28–39.37 %; *L. casei* SJRP 66–37.12 %, *L. casei* SJRP 169–38.19 %.

Table 4. Technological parameters, production time and survival rate of probiotics in fermented sausages

Probiotic	Ambient temperature range / ambient relative humidity range / manufacturing time	pH / a _w finished product	Storage conditions / duration	The amount of probiotic, lg CFU/g, in the finished product before/after storage	Probiotic survival after storage	Ist.
When using a probiotic as a starter culture						
<i>L. plantarum</i> , suspension	23–13 °C / 90–75 % / 22 days	4.43 / 0.858	4 ± 1 °C / 60 days	7.06 / 7.77	Increase by 0.7 lg CFU/g	[17]
<i>L. plantarum</i> , encapsulir. form	– “ –	4.41 / 0.879	– “ –	7.28 / 8.34	Increase by 1 lg CFU/g	[17]
<i>L. plantarum</i> , O / W emulsion	– “ –	4.93 / 0.888	– “ –	6.28 / 7.13	Increase by 0.85 lg CFU/g	[17]
<i>L. plantarum</i> , W/O/W emulsion	– “ –	4.84 / 0.896	– “ –	5.95 / 7.05	Increase by 1 lg CFU/g	[17]
<i>L. rhamnosus</i> LOCK900	18–16 °C / 92–75 % / 21 days *	4.90 / — **	4 ± 1 °C / 42 days***	ICD: 8.08, of which 84 % <i>L. rhamnosus</i> / 8.21, of which 94 % <i>L. rhamnosus</i>	Slight increase	[19]
<i>E. faecium</i> CECT 410, suspension	23–13 °C / 90–75 % / 22 days	4.66 / 0.899	4 ± 1 °C, 83 ± 2 % / 42 days***	8.02 / 8.02	Preservation of housing cooperatives	[24]
<i>E. faecium</i> CECT 410, encapsulated. form	– “ –	4.68 / 0.870	– “ –	8.63 / 8.25	Minor decrease	[24]
When using a probiotic together with a starter culture (SC)						
<i>L. rhamnosus</i> susp. (SC: <i>L. plantarum</i>)	4–26–24 °C / 95–75 % / 21 days	4.51 / 0.870	10–15 °C / 6 months*	7.35 / 6.59***	Minor decrease	[12]
<i>L. rhamnosus</i> , encapsulated form, (SC: <i>L. plantarum</i>)	– “ –	4.54 / 0.870	– “ –	8.19 / 7.99***	Minor decrease	[12]
<i>L. plantarum</i> L125 (SC: <i>P. pentosaceus</i> + <i>St. carnosus</i>)	Industrial parameters / 22 days	No data	4 °C, 12 °C / 160 days	8.21 / 6.00	Reduction by more than 2 lg CFU / g	[14]

* — smoking was used; ** — a_w was not determined; *** — vacuum packaging; **** — at the same time the number of SC decreased by more than 3 lg CFU / g.

Achieved effects on improving consumer characteristics of fermented products

Even the healthiest, the most beneficial and nutritious functional food product can be of no consumers' demand, if its characteristics do not align with the consumers' eating habits and expectations of delicious food sensations. So, creating fermented sausages with probiotics requires not only preserving the beneficial microflora but also ensuring certain specific organoleptic characteristics that will be appreciated by the consumers. On the other hand, it's important for company's specialists to ensure that the required technological characteristics (pH, a_w , moisture content, color, density, etc.) are achieved during the production of these sausages with probiotic strains added, thus ensuring the quality of the final food product during its storage.

In this regard, the comprehensive approach of many researchers to analyzing the quality and consumption safety of fermented sausages was noted, including an assessment of the various effects achieved through the use of probiotic strains. Overall, the positive impact of probiotics on the organoleptic and technological characteristics of sausage products was demonstrated, highlighting the possibility of producing high-quality functional meat products under industrial conditions. The most frequently detected positive effects of probiotic addition are listed below in the Table 5.

In addition to the rapid lowering of pH, to the effective suppression of undesirable microflora and improvement of the organoleptic characteristics profile of the fermented sausages, the authors also pointed out a row of measures that can provide an additional contribution to the development of functional food products for healthy nutrition. Among them there is control over the formation of biogenic amines, which, when consumed in excessive amount together with food, can cause gastrointestinal issues, pseudo-allergic reactions, vegetative-vascular and cardiovascular disorders, and even have a carcinogenic effect [21,35]. Probiotics can make an equally important contribution to the preservation of essential polyunsaturated fatty acids of Omega-3 and Omega-6 groups, which are most susceptible to oxidative spoilage, especially during the long process

of production cycle and subsequent storage of the fermented meat products. Probiotic cultures create an antioxidant system during the fermentation process, which includes the synthesis of enzymes that neutralize active oxygen species, decompose hydrogen peroxide (H_2O_2) and participate in detoxification peroxide radicals and compounds. Probiotic cultures are capable of binding atmospheric oxygen and reducing the oxidation-reduction potential of the environment, as well as forming complexes with metals of variable valence, creating less favorable conditions for oxidative processes. Antioxidant properties are exhibited by some bactericide agents, exopolysaccharides, and other metabolites of probiotic cultures [36]. The complex action of the antioxidant system created by probiotic strains not only provides for better preservation of unsaturated fatty acids and reduces accumulation of oxidative spoilage substances in the food products, but also provides a positive impact on the organoleptic properties of fermented sausages and their stability during storage.

In the recent years the experimental studies have emerged confirming that fermented sausages with added probiotic cultures can serve as an effective matrix for delivering of the health-beneficial microorganisms into the gastrointestinal tract and thus providing a wide range of the physiological effects. The study that involved five healthy volunteers who daily consumed 25 g of fermented sausage with reduced fat and sodium content made with a *Lactobacillus strain, rhamnosus* CTC1679 for 21 days in a row, showed that this strain was able to pass through the gastrointestinal tract and colonize the intestine temporarily [37].

In a clinical study that involved 20 healthy volunteers (10 women and 10 men) who daily consumed 50 g of probiotic sausage with added *Lactobacillus strain paracasei* LTH 2579 for five weeks in a row, a positive correlation was detected between the number of *L. paracasei* in feces and the percentage of CD4⁺T- helper lymphocytes [38].

While that, all changes in immunological parameters still remained within physiological norms, thus indicating a mild modulating rather than hyperstimulating effect of the probiotics. A study of 2023 demonstrated that the

Table 5. Positive organoleptic and technological effects of using the probiotic strains in the technology of fermented sausages manufacturing

Effect\Probiotic	<i>B. longum</i>	<i>B. animalis</i>	<i>L. acidophilus</i>	<i>L. casei</i>	<i>L. plantarum</i>	<i>L. rhamnosus</i>	<i>L. paracasei</i>	<i>E. durans</i>	<i>E. faecium</i>	<i>Lc. lactis</i>
Positive changes in product quality		[10]	[10,16]	[11,23]	[14,16,18]	[22]			[24]	
Positive effect on smell and taste	[8]							[9]		[9]
Improving the aromatic profile			[16]		[16]	[12]				
Absence of color and consistency defects	[8]			[23]				[9]		[9]
Rapid pH reduction and pathogen suppression			[16]		[15,16,18]	[19,22]	[22]	[9]		[9]
Antioxidant effect/preservation of PUFAs	[8]		[16]	[23]	[16,17]	[19,20,22]	[22]		[24]	
Reduction of biogenic amines	[8]		[21]						[21]	
Accumulation of bioactive peptides						[12]				

Note: green color — the effect is present; gray color — this effect was not studied in [8–24].

probiotic strain *Lactiplantibacillus plantarum* 1K isolated from a traditional fermented meat product featured high antioxidant activity both as viable cells and as a metabolite fraction [39].

Viable cells maintained compliant antioxidant activity after being exposed to simulation of gastrointestinal transit, and extracted probiotic metabolites significantly inhibited TNF- α production in LPS-stimulated human peripheral blood mononuclear cells, thus proving a pronounced anti-inflammatory effect.

Studies using a dynamic colon model have shown that consumption of a potentially probiotic sausage that contained *Enterococcus faecium* CRL 183 may result in altered microbial diversity and metabolite profiles, which in general represents a healthier alternative to fermented meat products.

Adding of food fiber (citrus fiber, arabinogalactan, and inulin) to a probiotic-fortified salami composition not only increased the product's antioxidant capacity but also boosted the production of short-chain fatty acids during salami fermentation by the intestinal microbiota. The most trustworthy evidence of a systemic effect on human health was obtained in an intervention study that involved 24 healthy volunteers who consumed fermented *Lactobacillus rhamnosus* HN001 probiotic-fortified salami with added citrus fiber [20]. The authors recorded a decrease in inflammatory markers (CRP and TNF α), an improvement in plasma antioxidant parameters, an increase in butyrate production, and presence of *L. rhamnosus* in the feces of the intervention group participants, which facts confirmed the probiotic effect.

The results of clinical and experimental studies accumulated to this date demonstrate that fermented sausages with probiotic cultures added can provide significant health benefits, including modulation of the immune response, mitigation of systemic inflammation, increasing antioxidant activity status, improving intestinal microbiota composition, and increasing production of physiologically active metabolites. These data significantly support the position of probiotic meat products as complete functional foods and justify their further development and implementation in the industrial production scale.

Conclusion

The systematic review of the experimental studies which have been conducted over the past decade allows making conclusion that using the probiotic cultures in dry fermented sausage technology goes beyond academic interest only, and represents a truly achievable way to improve the consumer properties of meat products. The modern technological solutions ensure the content of viable probiotic cells in the ready-to-eat food products at a level of 10^6 – 10^8 CFU/g, and this value is maintained for at least six months of storage, even in case of long fermentation and long drying cycles. Although this concentration does not reach the regulatory requirement of

TR CU 021/2011² on the content threshold, which prescribes it to be at least 10^9 CFU/g for the fortified food products, the clinical survey data convincingly prove that the daily consumption of 30 g of sausage with a probiotic content of approximately 10^6 CFU/g provides a pronounced physiological effect — decreasing systemic inflammation markers levels, increasing rate of production of short-chain fatty acids, and temporary colonization of the intestine with the target strain.

Among the studied probiotic microorganisms, *Lactobacillus plantarum* and *Lactocaseibacillus rhamnosus* are the one most mentioned ones in terms of frequency of their use and the totality of positive effects. Their advantage lies in their ability to simultaneously act as a starter culture, thus providing rapid acidification of the medium and suppression of pathogenic microflora, and the ability to maintain high viability under conditions typical of meat systems. Moreover, strains isolated directly from fermented meat products demonstrate better adaptation to these stress factors compared to isolates from dairy products or the human gastrointestinal tract, indicating the feasibility of targeted screening of probiotic cultures specifically among traditional meat microflora. Analysis of recipes revealed a consistent trend toward a reduction in the fat content among the raw materials from the classic ratio of 70:30 of lean meat: fat to 80:20 in favor of lean meat, as well as a decrease of table salt mass fraction to 1.8–2.5 % in comparison with the traditional 2.7–3.5 % ratio. Moreover, these changes not only comply with modern dietary recommendations but also create more favorable conditions for the growth and survival of probiotic cultures. In a third of the studies, the prebiotic components (fructooligosaccharides, inulin, lactulose or dietary fiber) are additionally introduced into the food composition, which this way enhance the functional potential of the food product and provide the positive effect on its organoleptic characteristics.

In addition to their probiotic activity, the introduced strains provide a number of additional technological and quality benefits, including effective control of biogenic amine accumulation, antioxidant protection of polyunsaturated fatty acids from oxidative degradation, and the formation of a more intense aromatic profile, which collectively increases the food product's shelf stability and improve its consumer appeal.

The current level of scientific and technological development allows affirming that the fermented sausages fortified with probiotics are moving from the sector of experimental food models to the sector of practicably scalable innovations capable of not only rehabilitating the processed meat products in the consumers' eyes, but also offering the market a fully functional product that combines traditional taste qualities with clinically proven health benefits.

In spite of the accumulated scope of knowledge, still the issues exist such as the lack of proven technological standards for the selection of temperature and humidity

parameters suitable for fermentation; drying, packaging solutions and storage conditions that take into account the specifics of probiotic cultures; still there is an insufficient number of randomized clinical trials involving people

of various ages and nosological groups; and there are no studies on using the genetically modified probiotics strains which in the future perspective could ensure the combination of the best technological and functional properties.

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