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Probiotic Microorganisms in Dry Fermented Meat Products

Katarzyna Neffe-Skocińska, Karolina Wójciak and
Dorota Zielińska

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Abstract

In the modern lifestyle, food is supposed not only to feed the hunger but also to provide an appropriate amount and quality of nutrients necessary for proper functioning of the body. The interest of consumers in functional food, including fermented products with probiotic properties, has been growing for several years. Meat and meat products represent one of the most important components of contemporary human diet. Meat fermentation is one of the oldest methods of preserving food. This is a low-energy, biological acidulation which results in unique flavour and palatability, colour, microbiological safety and tenderness. Changes of muscle form into fermented meat product are caused by homo- or heterofermentative starter cultures or “wild” microorganisms which lower the pH. Fermented meat products are one of the most cherished and valuable food products. Fermentation and ageing process would deliver most aromatic and rich in flavour products, which is incomparable with other processes. A new solution is dry-aged meats with the use of new probiotic starter cultures with a high degree of health safety and long shelf life due to the inhibition of growth of the pathogenic microorganisms and therefore reduction of the formation of harmful compounds from protein transformation or lipid oxidation.

Keywords: dry-fermented meat, probiotic starter cultures, lipid oxidation, biogenic amine, bioactive peptides

1. Introduction

Meat and meat products are one of the most important components of human nutrition. Manufacturing raw cured sausages has a very long tradition and its origin is to be sought in the

Roman times in the Mediterranean area. In the past, raw cured sausages were manufactured only in the cool and cold time of the year and, therefore, it was possible to avoid the risk of spoilage. Nowadays, the preservation role of meat fermentation has become largely obsolete due to the introduction of the cold chain. Nevertheless, fermented meat products remain very popular and are still produced in large amounts, especially in Europe, probably because of their unique and specific sensory properties, their convenience and their alleged rootedness in culinary and cultural heritage [1].

One of the most promising areas of development in the human nutritional field over the last two decades has been the use of probiotics and recognition of their role in human health and disease. Lactic acid-producing bacteria are the most commonly used probiotics in foods and supplements. The means by which probiotic bacteria elicit their health effects are not understood fully, but may include competitive exclusion of enteric pathogens, neutralization of dietary carcinogens, production of antimicrobial metabolites and modulation of mucosal and systemic immune function [2]. According to the currently adopted definition by the Food and Agriculture Organization/World Health Organization [3], probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”.

Although dairy products are the most commonly used food vehicles for the delivery of probiotics, several investigations dealing with the use of probiotics in cereal products [4, 5] vegetables and fruit juices [6, 7] and fermented meat products [8–13] to improve their nutritional value as functional foods have been reported.

The commercial application of probiotics in meat products is not yet popular, mainly because of technological issues. As fermented meat products are processed without heating, they could be suitable products for assessing probiotic LAB as starter cultures [14]. However, probiotics may be inactivated due to low pH or water activity value, as well as presence of native microorganisms or curing salt. The most important problem is to find compromise between technology, safety, quality and health-beneficial value of food [15].

2. Lactic acid bacteria in meat fermentation

2.1. Traditional starter cultures

Starter cultures are live, defined and specially selected microorganisms with the GRAS (generally recognized as safe) safety status, responsible for the proper course of meat ageing. Starter cultures may consist of selected bacteria, moulds or yeasts. Their use in the production of dry-aged cold meats is always intentional and aims at obtaining the specified sensory and microbiological characteristics in the end product [16–18].

The fermentation of food is known from centuries. First fermentation processes were driven by adventitious microbiota, represented by unknown microorganisms naturally present in the raw food ingredients and in the environment. A number of fermented foods, such as traditional cheeses, dry-fermented sausages and fermented beverages, are still produced without the addition of microbial inoculation [19]. Over time, with the aim to improve and standard-

ize the foods, the subsequent evolutionary step has been constantly associated with the back-slopping approaches, where higher counts of microorganisms are added to activate the fermentation. However, these natural starter cultures were often variable in load and composition, and if, on one hand, they can confer to the product peculiar characteristics of uniqueness and quality, on the other hand, they are continuously evolving according to seasonal and environmental variations and may result in variable qualities of the final product. For this reason, since the beginning of the past century, strains isolated from the best natural fermentations have been cultivated and studied under defined conditions by industrial companies and research institutions and used as selected starter cultures in food productions [20].

Group of microorganisms	Technological function and changes in meat>
Lactic acid bacteria	Natural preservative: lactic acid production, acidifying bacteria; inhibiting
<i>Lactobacillus sakei</i>	
<i>Lactobacillus curvatus</i>	the development of putrefactive and pathogenic microorganisms;
<i>Lactobacillus plantarum</i>	storage stabilization Good sensory quality, flavour and aroma
<i>Lactobacillus pentosus</i>	development
<i>Lactobacillus casei</i>	Proteolysis and lipolysis stabilization
<i>Pediococcus acidilactici</i>	
<i>Pediococcus pentosaceus</i>	
Gram-positive cocci	Bacteria redox flavouring: nitrates and nitrites reduction; using up
<i>Staphylococcus carnosus</i>	the oxygen; decomposition of peroxides; lipolysis stabilization (delaying
<i>Staphylococcus xylosus</i>	rancidity); colour stabilization; good sensory quality, flavour and aroma
<i>Micrococcus varians</i>	development
Yeasts	The surface microflora: using up the oxygen; decomposition of
<i>Debaryomyces hansenii</i>	peroxides;
<i>Candida famata</i>	delaying rancidity colour stabilization; good sensory quality, flavour and aroma development
Moulds	The surface microflora: using up the oxygen; decomposition of
<i>Penicillium nalgiovense</i>	peroxides;
<i>Penicillium camemberti</i>	proteolysis and lipolysis stabilization; good sensory quality, flavour and
<i>Penicillium chrysogenum</i>	aroma development
Mixed composition of yeasts and moulds	
<i>Penicillium nalgiovense</i> + <i>Debaryomyces hansenii</i>	
<i>Penicillium candidum</i> + <i>Debaryomyces hansenii</i>	

Table 1. The starter cultures composition for dry-fermented meat production [15].

Currently, the production of commercial starter preparations uses primarily lactic acid bacteria which show favourable technological effect. Typical starter cultures (**Table 1**) which are used to all types of fermented cold meats are composed of mildly acidifying bacteria cultures of the following species: *Lactobacillus sakei*, *L. curvatus*, *L. plantarum*, *Pediococcus acidilactici* and *Pediococcus pentosaceus*. In Europe, mainly cultures of *L. sakei* and *L. curvatus* are used to

manufacture dry-aged cold meats [14, 16]. Strains of bacteria belonging to one species often differ in various physiological and metabolic properties among each other. Due to the fact that most strains of *L. curvatus* demonstrate the ability to produce biogenic amines, *L. sakei* bacteria are more often used in practice. These microorganisms are not capable of catalysing the decarboxylation of amino acids, thereby reducing or even inhibiting the formation of biogenic amines in dry-fermented meat products [14, 15, 21–23].

In spite of LAB, starter cultures may also contain micrococci, most frequently those of *Micrococcus varians* species and of *Staphylococcus xylosum*, *Staphylococcus carnosus* and *Streptomyces griseus* [15].

Apart from providing consistent quality and typical sensory features, the primary function of LAB bacteria included in such starter cultures is mainly to preserve the product through the production of lactic acid during metabolic changes and competition with microbiota naturally occurring in the meat product and pathogenic microorganisms. The composition of starter cultures impacts the duration of the ageing process and storage stability of such products but also on their flavour, odour and texture [16, 24].

2.2. Development of novel starter cultures

One of the major focuses of the current innovation in development of novel starter cultures to meat industry seems to be on improved food safety and health properties. The isolation and selection of lactic acid bacteria which can be used as starter cultures in meat fermentation present a considerable challenge to standardization and management of quality of dry-fermented sausage. The basic starter cultures used in meat industry are selected strains of homofermentative *Lactobacillus* (lactic acid bacteria (LAB)) and/or *Pediococcus* and Gram-positive catalase-positive cocci (GCC), nonpathogenic, coagulase-negative staphylococci and/or *Kocuria*. Lactic acid bacteria originating from fermented meats are specially adapted to the ecology of meat fermentation. The rapid production of lactic acid in those products is primarily responsible for the quality and safety of the product [16, 25].

First of all the addition of selected starter cultures usually induces a higher acidification, compared to the standard product, which was reported by several authors [13, 26–28]. Moreover, in Spanish raw-fermented sausage with addition of probiotic starter cultures, the reduction of fat and salt has been achieved [27]. Also flavour, texture and taste are very important components of the final quality of dry-fermented meat products, and most of these traits are related to the metabolic activities of microorganisms [20].

Selected LAB starter cultures could have positive influence on sensorial acceptance of dry-fermented meat products. For example, in [29], they have found that *Lb. sakei* and *Staphylococcus equorum* added to the Dacia sausage resulted in better smell intensity, overall quality and mastication attributes, as well as lower biogenic amines content in comparison to control samples. In Ref. [30], they studied the effect of selected LAB starter cultures in Italian dry-fermented sausage and found that the obtained products were saltier, juicier and more tasty as compared to the control.

It has been also found that probiotic starter cultures may have been successfully used in fermentation process of meat products. In Ref. [31], they found that probiotic bacteria did not change the characteristic flavour and aroma of raw sausages in comparison to product obtained from commercial starter cultures. Also in studies [12, 27], they have obtained the sausages with probiotic cultures addition and recorded a satisfactory overall sensory quality without any noticeable off-flavour.

The recent literature is also well consistent in indicating advantages of selected starter cultures in the control of pathogenic bacteria and other spoilage microflora. Fermented meat products are commonly considered safe for consumption, and the acidification by lactic acid starter bacteria is one of the main preserving factors. The most frequently isolated lactic acid bacteria from dry sausages processed with different technologies are *L. sakei*, *L. curvatus* and *L. plantarum* [32, 33].

The lactic acid bacteria (LAB) produce an array of antimicrobial substances (such as organic acids, diacetyl, acetoin, hydrogen peroxide, reuterin, reutericyclin, antifungal peptides and bacteriocins) [34, 35]. Therefore, there is an increasing interest in lactic acid bacteria (LAB) derived from meat that can be used as starter or adjunct cultures in dry sausage fermentation. Their ability to produce bacteriocins and nonproteinaceous low-molecular-mass antimicrobial compounds (mainly lactic acid and hydrogen peroxide) is of importance [36]. As there is no sufficient glucose in meat to reduce the pH, the addition of glucose is essential to develop the desired metabolic activity to produce lactic acid via glycolysis. Hydrogen peroxide is produced after glucose is consumed by cells [37].

Bacteriocins are the peptides produced by lactic acid bacteria with antibacterial properties. These peptides can reduce or inhibit the growth of other Gram-positive [38–40], and thus they can be used to control the growth of food-borne pathogens such as *L. monocytogenes* in food products [41]. In Ref. [38], they isolated *P. acidilactici* from Spanish dry-fermented sausages and found that they had a strong inhibitory effect against members of Gram-positive genera. It has been observed that starter cultures containing *L. sakei* reduced the growth of *Listeria* in fermented sausages [42, 43]. Also *L. curvatus* and *L. plantarum* in sausage starter cultures have shown antilisterial effect [44, 45]. In the other study [46], they reported antilisterial effect of a lactic acid bacterium isolated from Italian salami. In Ref. [47], they found that nine strains of *Lactobacillus casei* and three strains of *L. plantarum* isolated from dry-fermented sausages had an antagonistic activity against the indicator species tested. The bacteriocin produced by *L. casei* was named as Lactocin 705 and showed antibacterial effects against *L. plantarum*, *L. monocytogenes*, *S. aureus* and a wide range of Gram-negative bacteria. Bacteriocinogenic starter cultures are recommended as an additional hurdle to reduce the risk of *L. monocytogenes* in dry sausage [48]. In contrast sakacin P, synthesized by *L. sakei* subsp. *sakei* 2a isolated from pork sausage, inhibits the growth of *Listeria monocytogenes* [49]. The addition of the bacteriocinogenic *L. sakei* CTC494 in combination with some ingredients (i.e. pepper, salt and nitrite) in sausage batter has a dramatic effect on *L. monocytogenes* survival in fermented sausages [50]. Sakacins produced by *L. sakei* are mainly active against other LAB and *L. monocytogenes* as well as against the Gram-negative psychrotroph *Aeromonas hydrophila* [51, 52].

Besides prevention of microbiological hazards, also probiotic starter cultures have been developed. Many advantages and disadvantages are connected with application of probiotic bacteria to dry-fermented meat products.

2.3. Probiotic starter cultures: benefits and problems

Dynamic development of the functional food market has contributed to the attempt to use starter cultures consisting of probiotic LAB in meat processing. Two trends may be observed during development of new probiotic starter cultures. One of them is an attempt to apply already known probiotic cultures (from the gastrointestinal tract of healthy humans) used, e.g. in the production of fermented milk beverages. The second one consists in isolating of the strains of lactic acid bacteria from naturally fermented meat products and examining them in terms of probiotic qualities as well as of safety of use in an industrial scale [25, 53–55].

The number of benefits arising from the use of probiotic starter cultures is worth noticing (Table 2). The important aspect of using cultures of probiotic bacteria in the production of dry-fermented meat, in addition to the possibilities of growth and survival in meat environment and exercising favourable effect of these microorganisms on human body, is the ability to inhibit the growth of pathogenic microflora, which usually is capable of producing biogenic amines. Model studies with the use of probiotic bacteria as a starter culture to manufacture ripening meat products revealed that strains of *Lactobacillus acidophilus*, *L. lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *lactis*, *L. plantarum*, *L. reuteri* and *L. fermentum* reduce or even inhibit the production of biogenic amines in the products discussed [56–58].

Advantages	Disadvantages
Meat, as a rich source of nutrition components, is a suitable medium for probiotic bacteria growth	Technological issues: • Presence of native microflora can inhibit growth of probiotic bacteria • Presence of other inhibitory factors: low water activity, low sugar contents, nitrates and salt additives • Difficulties with inoculation probiotic in appropriate number of bacteria • Stability of probiotic during storage
Beneficial effect on human health corresponding with applied probiotic strain	Difficulties with identification of probiotic strains in meat matrix
Inhibition of pathogen growth, production of bacteriocin and antimicrobial compounds	Non-sterile environment can affect the spontaneous fermentation or spoilage
Unique sensorial quality	No detailed procedure for production of probiotic meat products
Reduction of fat oxidation, proteolytic reactions	Possibilities of biogenic amine production

Table 2. Benefits and issues related to application of probiotic starter cultures.

As a result of their own metabolic changes, the probiotic starter cultures produce a number of bacteriostatic and bactericidal substances (e.g. lactic acid, acetic acid, formic acid, ethanol and bacteriocins), thereby inhibiting the growth of undesired and pathogenic microbiota, including Gram-negative strains from family *Enterobacteriaceae* (e.g. *Escherichia coli*) and *Pseudomonaceae* as well as species such as *Listeria monocytogenes* or *Staphylococcus aureus*, thus naturally preserving the products discussed [14, 48, 59, 60].

Another benefit arising from the use of probiotic bacteria to manufacture dry-fermented meat products is reduction of fat oxidation and proteolytic reactions. Primary and secondary products resulting from degradation of lipids and proteins have significant impact on deterioration of sensory quality (i.e. negative aftertastes: bitter, of old fat) but also of features such as colour and texture. Additionally, they affect the health safety and shorten the use by date of the meat products discussed [18, 61–63]. Research on autooxidation of the fat in ripening products clearly proves that probiotic strains protect lipids from oxidation during storage which is indicated by substantially lower values of TBARS parameter in comparison to the control sample [64, 65].

Proteolysis is one of the most important biochemical processes in dry-aged meat products where proteins are metabolized and broken down to polypeptides, peptides and free amino acids [62, 66]. Protein breakdown takes place with the participation of microbiological enzymes, which in turn leads to deamination and decarboxylation. These reactions occur faster at low pH values. The basic products of protein decarboxylation in meat are cadaverine, putrescine, tyramine and histamine. Large concentration of biogenic amines in meat products may result in adverse symptoms in consumers, such as increase in blood pressure, increase in rate and strength of heart contraction and problems with the central nervous system, including migraines. Additionally, it may cause stomachaches, vomiting or severe sweating [56]. It has been observed that the proper choice of probiotic strains limits proteolytic changes in dry-aged meat products [15, 26, 67]. This relation has been observed in studies [66] and [23] which examined dry-aged pork tenderloin with addition of *L. casei* LOCK 0900 strain with documented probiotic and very good technological properties. Controlling and proper conduct of the process of fermentation and ageing ensure low concentration of biogenic amines in raw cold-meat products, thereby preventing poisoning [15].

It is currently believed that dry-fermented meat products are an appropriate medium for probiotic bacteria. Many studies have shown that growth and survival rate of probiotic LAB in dry-fermented sausages are possible [18, 60]. However, the previous attempts to manufacture meat product in controlled process of fermentation and ageing conducted by proven and selected probiotic strains mainly pertain to sausages [1, 10, 23, 33, 54, 57, 68, 69]. Moreover, there are few research works published which will unequivocally confirm the technological suitability of probiotic cultures and their healthy impact on human body caused by regular consumption of such meat products [70–72].

Probiotic strains used in the production of dry-aged meat products must demonstrate suitable technological properties without departing from the traditional starter cultures used in meat processing. Therefore, the idea to use probiotic starter cultures in meat processing industry

raises many issues from the technological, microbiological and analytical perspective (Table 2).

The task consisting in introducing the probiotic starter cultures to meat is not easy to perform, as these bacteria which are in fact the intestinal bacteria do not demonstrate very good technological properties. It was determined that from 50 to 500 million of lactic acid bacteria, including mainly *L. sakei* and *L. curvatus*, may be placed into one gram of the product discussed. Therefore, the raw meat material itself constitutes a problem, because spontaneous growth of LAB may occur there.

Secondly, inoculation of the probiotic bacteria is performed to the raw material which is not sterile as is the case of fermented milk products or fermented juices [73, 74]. Thirdly, certain analytic difficulties also emerge here. Identification of selected probiotic strains in meat with the use of traditional microbiological methods is not complete, because only the general number of LAB is assayed. Only advanced identification methods based on genetic analysis of nucleotide sequences typical for given bacterial strain may ensure that all of them will be assayed [54].

Numerous authors of available literature focus on the survival rate of probiotic starter bacteria added to dry-fermented meat products. The factors limiting and even inhibiting the growth of the discussed microbes in meat environment include mainly the native microflora but also low water activity and the content of sugars naturally occurring in meat, as well as technological additives: sodium chloride, nitrates and other curing agents [15].

Low content of simple sugars in fresh meat, necessary for lactic acid bacteria, including probiotic ones, to conduct metabolic change, also poses a significant problem (4.5–7 mM/g of raw material). Therefore, microorganisms discussed start to use amino acids as an alternative source of carbon which starts the spoilage process of meat and results in intensive bitter taste. For this reason, saccharides are added in amount of 0.4–0.8% during the production of aged meat products [13, 48].

There is also a technological difficulty in inoculating bacterial strain (form, number, application method) to cured element to an unground meat in particular. Starter cultures are most often manufactured in lyophilized or frozen form. In the case of dry-fermented sausages, they are inoculated to the sausage meat, usually after they are mixed with cold water or curing brine. Adding starter cultures to tenderloin or ham is significantly more difficult, as they are also posed by varied consistency of different muscles. They result, i.e. from the presence of fat at the meat's surface which may uneven drying up and thereby excessive or inhibited growth of microorganisms [15].

One of the major technological problems involves no detailed procedure for production of probiotic meat products developed. This primarily requires determination of optimal temperature for fermentation and ageing, which will allow probiotic strains to grow and dominate natural microbiota in the meat. Fermentation and ageing of meat products are mainly conducted in the temperature within the range of 15–26°C. Lower temperature allows to obtain high-quality product with a long ageing period and use by date. In contrast, the use of higher

temperature decreases the duration of fermentation; however there is a risk that microorganisms responsible for product's spoilage will grow in the meat [13, 15].

Additionally, one of the important technological criteria to be met by a probiotic strain includes stability during storage, i.e. capability to retain the number of 6.00–7.00 log cfu/g in the product in the last use by date [70, 71].

Another aspect of the selection of bacterial cultures pertains mainly to their beneficial impact on sensory quality and also moderate acidifying activity, low thermal activity within range of 0–15°C, antagonism with respect to undesired microbes and food pathogens as well as resistance to bacteriophages [18, 56, 75].

Probiotic microorganisms selected to the production of aged meat products must meet not only the necessary requirements of safety and functionality but also the technological criteria discussed above.

3. Technological and health aspects of probiotic meat products

3.1. Influence of probiotic bacteria on lipid oxidation

The shelf life of fermented meat products is generally not limited by bacterial deterioration but by chemical spoilage [76]. It is oxidation stability that is the main restriction on the shelf life of probiotic meat products [77]. Processed products, which are minced, mixed with salt and heated, expose muscle tissue to oxidative stress responsible for loss of quality and lead to oxidative flavours and loss of haem iron and vitamins and finally cause discoloration [77]. Oxidation of lipids can also have a negative effect on nutritional value and may be responsible for the production of toxic compounds. In Ref. [76], they have reported that enzymatic hydrolysis during fermentation accelerates lipid peroxidation. In addition, a strong correlation between lipid and myoglobin oxidation, especially in fresh meat, has been documented by scientists [78]. In [78], they have reported that secondary lipid oxidation products (2-heptenal, 2-nonenal, 4-hydroxy-2-nonenal) promote pigment oxidation. However, there is also strong evidence that haem pigments may initiate lipid oxidation through the reaction of hydrogen peroxide with metmyoglobin to form ferryl and perferrylmyoglobin, which have powerful prooxidant effects on lipids. In probiotic meat product, the fact that the high 20–30% concentration of fat (dry-fermented ham, neck, sausage) does not limit the adaptive capacity of probiotic bacteria in fermented meat products is interesting. Probiotic bacteria can stabilize the oxidation process taking during the maturing and prolonged storage period. In Ref. [79], they found that neither the presence nor the level of probiotic *Bifidobacterium animalis* ssp. *lactis* BB-12 has a negative influence on colour and oxidative stability of dry-cured neck during 12 months of ageing. The authors observed the significantly ($P < 0.05$) lower TBARS values in neck B1 and B2 (1.54 and 1.69 mg MDA/kg) compared to the values with spontaneously added LAB (2.26 mg MDA/kg). In [80], they pointed out that inoculation with *L. fermentum* HL57 potential probiotic strain increased the amount of malondialdehyde in Iberian dry-fermented sausages resulting in a negative colour and taste. The interaction of myoglo-

bin with H₂O₂ activates metmyoglobin, which may be a ferrylmyoglobin radical that is very unstable and can transform rapidly into the peroxy radical form. On the other hand, in research [81], they proposed that *L. casei*, *L. plantarum*, *L. curvatus* and *L. sakei* strains actively contribute to the hydrolysis of sarcoplasmic proteins such as myoglobin. Also in Ref. [82], they found that some strains of lactic acid bacteria demonstrated antioxidative activity with inhibition rates of ascorbate autoxidation in the range of 7-12%. In another paper the same authors presented that six strains of *L. acidophilus* and two strains of *B. longum* demonstrated an inhibitory effect on linoleic acid peroxidation [83]. The inhibitory rates of linoleic acid peroxidation ranged from 33 to 46% when 1 mL of intracellular cell-free extract was tested [83]. Authors of the Ref. [18] found out that the addition of probiotic strain *L. casei* LOCK 0900 changed physicochemical profile of dry-fermented sausages. Sausages with lower probiotic bacteria inoculation (6.0 log cfu/g) had better quality, inclusive colour and lipid oxidative stability (lower peroxide value, conjugated dienes and TBARS value) than those with 6.3 log cfu/g of probiotic strain. Based on research [18], it can be concluded that dry-fermented sausages produced with probiotic *L. casei* 0900 LOCK are oxidatively stable and the stability of the fat does not limit the shelf life of probiotic-treated dry-fermented sausages. The study suggests that the probiotic strain can be used in the production of edible sausage. The study conducted by authors of [84] proved that the use of potential probiotic *L. acidophilus* Bauer in dry-fermented pork neck production process decreases the hydroperoxide concentration at a level comparable to synthetic antioxidant.

Studies of the effects of the probiotic *L. rhamnosus* LOCK 0900 strain with green tea extract on the oxidative stability of ageing dry-cured pork loin showed that adding the probiotic strain with natural antioxidant increased the antioxidant potential of meat product by lowering the oxidation-reduction potential and TBARS values and improving the part of red in the general tone of colour [13].

The results presented by authors of Ref. [85] clearly demonstrated that the use of probiotic strains mixture (*L. casei* LOCK 0900, *L. casei* LOCK 0908 and *Lactobacillus paracasei* LOCK 0919) is possible in manufacturing process of organic dry-fermented sausages without nitrate and/or nitrite. The uncured fermented sausages with probiotic strains have appropriate oxidative stability and are shelf-stable during 180 days of storage period [85].

Examples of starter cultures that represent significant influences on fermented meat products regarding lipid oxidation, proteolysis, biogenic amine formation and sensorial quality were collected in **Table 3**.

Starter culture	Product	Influence	Literature
<i>L. rhamnosus</i> LOCK900 (Formerly <i>Lactobacillus casei</i> LOCK 0900)	Dry-fermented sausage	The probiotic strain can be used in the production of edible sausage Dry-fermented sausage produced with probiotic is oxidatively stable during storage	[18]
	Dry-fermented pork loin	Pork loin inoculation with a probiotic strain has different free amino acid concentrations, the taste	[92]

Starter culture	Product	Influence	Literature
		and flavour attributes	
	Dry-fermented pork loin	Inoculation with a probiotic strain proved to be a protective measure against the formation and accumulation of biogenic amine	[91]
<i>L. acidophilus</i> Bauer	Dry-fermented pork neck	Probiotic strain decreases the hydroperoxide concentration at a level comparable to synthetic antioxidant <i>L. acidophilus</i> Bauer has antioxidant properties	[84]
<i>L. rhamnosus</i> LOCK900 (Formerly <i>Lactobacillus casei</i> LOCK 0900)	Dry-cured pork loin	Addition of probiotic strain with green tea extract increased the antioxidant potential of meat product and improved the part of red in the general tone of colour	[13]
<i>L. rhamnosus</i> LOCK900, <i>L. rhamnosus</i> LOCK908 and <i>L. casei</i> LOCK919 (Formerly <i>L. casei</i> LOCK 0900, <i>L. casei</i> LOCK 0908 and <i>L. paracasei</i> LOCK 0919)	Uncured fermented pork sausage (organic sausage)	The uncured fermented sausages with probiotic strain mixture have appropriate oxidative stability and are shelf-stable during 180 days of storage period	[85]

Table 3. Influence of probiotic starter cultures on lipid oxidation, proteolysis, biogenic amine formation and sensorial quality in meat processing.

3.2. Influence of probiotic bacteria on proteolysis and biogenic amine formation

Proteolysis results in generation of peptides, oligopeptides and finally free amino acids (FAAs). Proteolysis is one of the most important biochemical changes, which take place during ageing of fermented meat products. FAAs contribute to the basic taste and aroma of fermented meat products [86]. However, an excessive amount of FAAs seems to be responsible for the biogenic amine formation.

The biogenic amines (BAs) are the compounds in which one, two or three hydrogens of ammonia are replaced by alkyl or aryl groups. Tyramine and phenylethylamine have aromatic structure, while putrescine, cadaverine, spermine and spermidine have the aliphatic one. Heterocyclic structures were proved for histamine and tryptamine. Based on number of amine group, we can divide the BA into the monoamines (phenylethylamine, tyramine), diamines (cadaverine, putrescine) and polyamines (spermidine, spermine) [87]. Biogenic amines have been reported in variety of foods, such as fish, meat, cheese, vegetable and wine [88]. They can be formed but also degraded as a result of normal metabolism of living cells in plant, animal and microorganism. BA can be produced by two different pathways: firstly by the decarboxylation of free amino acids and secondly by the amination and transamination of aldehydes and ketones [15]. The control of biogenic amines formation mainly focused on the controlling the growth of biogenic amine-forming bacteria. Microorganisms have a different ability

to synthesize decarboxylases. *Pseudomonas*, *Enterobacteriaceae*, enterococci and lactobacilli were found to have a high decarboxylase activity [88]. Within the same species, the presence, the activity and the specificity of decarboxylases are strain dependent. Bacterial amino acid decarboxylases usually have an acidic pH optimum 4.9–5.3, because the BA productions have been recognized as defence microbial mechanisms against an acidic environment [88, 89]. On the other hand, rapid and intense acidifications of environment reduce the growth of *Enterobacteriaceae* and enterococci. The factors that could significantly influence the BA formations in fermented meat are pH value, redox potential, environment microorganisms, starter culture, temperature of maturing, salt concentration, additives, water activity and hygienic quality of meat [89]. Authors of paper [90] proved that when salt concentration increased from 0 to 6%, the rate of BA production of *Lactobacillus delbrueckii* subsp. *bulgaricus* decreased. Other authors proved that concentration of sodium chloride at the level from 3.5 to 5.5% inhibited the histamine formation. Several studies showed that temperature of fermentation, maturing and storing have influence on BA accumulation in meat product [88]. Higher maturing temperature (20–25°C) could stimulate the growth of LAB which inhibits the amine-positive bacteria. The storing temperature below 4°C inhibits most of amine-positive bacteria except from psychrotrophic *Pseudomonas*, so the BA concentration in this kind of meat product is relatively low compared with product stored at 14–16°C. The addition of sugar (glucose, lactose) to fermented meat products has some influence on bacterial population dynamics and BA production. The absence of sugar stimulated the proteolysis and tyramine, cadaverine, putrescine and tryptamine formation. The preservative sodium nitrite could be added to fermented meat products because of their ability to reduce the putrescine and cadaverine accumulation. The addition of pure or mixture of various probiotic strains can decrease BA formation in fermented meat products. It is extremely important that probiotic starter culture should not form BA and have to be competitive in suppressing growth of amine-positive microbiota. In Ref. [91], they pointed out that pork loin inoculation with a probiotic strain *L. casei* LOCK 0900 has different free amino acid concentrations, which could influence the taste and flavour attributes. Also in [92], they presented evidence that some sorts of probiotic starter cultures (*L. casei* LOCK 0900, *Bifidobacterium bifidum*, *L. acidophilus* Bauer) have different abilities to create the high- or low-molecular-weight peptides and free amino acids. Another authors [93] found out that LAB reduced the pH values during ageing and thereby activated the endogenous acid protease (cathepsin B, L). Inoculation with a probiotic strain *L. casei* LOCK 0900 proved to be a protective measure against the formation and accumulation of cadaverine, putrescine, spermine and tryptamine. In Ref. [91] the author has not observed the correlation between the higher content of free amino acids (potential precursor of BA) and the level of biogenic amines. A 50% BA decrease was observed in comminuted fermented meat products with mixture of *L. curvatus* CTC371 and *S. xylosus* despite the increase of free amino acid availability. In Ref. [23] BA changes during maturation were presented. Potential probiotic pork loins were analysed in 4-, 8- and 16-month-old samples. The authors have not detected histamine and spermidine. Spermine was present at very low levels (4.0–5.8 mg/kg), while cadaverine (10.8–39.6 mg/kg) and tryptamine (17.8–49.2 mg/kg) were the most abundant BA. The level of all BA did not exceed the suggested toxic limits [23].

3.3. Bioactive peptides in probiotic fermented meat products

The proteolytic activity during meat processing generates a large amount of peptides and free amino acids because of calpain, cathepsin and peptidase enzymatic activity [86]. The most interesting peptides are those that can be considered as bioactive peptides because of their different health-care abilities like antihypertensive activity, antioxidant activity, antimicrobial activity, etc. The activities of bioactive peptides depend on their chemical structure (amino acids composition, kind of amino acid in N- and C-terminal), the length of molecule chain and their weight, charge character of amino acids and the hydrophobic/hydrophilic property. Meat has been reported to contribute to the generation of bioactive peptides. As such peptides, antihypertensive, opioid, immunostimulating, antimicrobial, antithrombotic, hypocholesterolemic, antioxidative and prebiotic activities have been studied. Angiotensin I-converting enzyme (ACE) plays an important physiological role in the regulation of blood pressure. Fermented food with probiotic bacteria, especially *Lactobacillus*, produce bioactive peptides known to inhibit the activity of angiotensin-converting enzyme (ACE) and thus alleviate high blood pressure (hypertension). Authors of the study [94] used calpis sour milk fermented with *Lactobacillus helveticus* and *Saccharomyces cerevisiae* and identified two peptides Ile-Pro-Pro and Val-Pro-Pro, both of which possessed ACE inhibitory activity in vitro. These two bioactive peptides were released from b-casein and k-casein by lactobacilli enzymes. In Ref. [95], hypertensive subjects were fed with milk fermented with *L. helveticus* LBK-16H containing bioactive peptides. After 21 weeks test subjects showed a significant lowering of their blood pressure. Even in antihypertensive peptides, it has been found that antioxidative (VW, DLYA, SLYA, DLQEKLE) and prebiotic (ELM) peptides are generated from meat protein by enzymatic digestion. In [96], they reported that bioactive peptides from hydrolysis of sarcoplasmic porcine proteins by the activity of *L. sakei* CRL 1862 and *L. curvatus* CRL 705 showed high ACE inhibitory activity. In [97], they pointed out that some peptides from Spanish dry-cured ham have DPPH radical-scavenging activity (39–92%) as well as superoxide ion-extinguishing ability with values ranging from 41.67 to 50.27% of the antioxidant activity, suggesting the presence of peptides with antioxidant activity. Moreover every sample exhibited pooled fractions corresponding to 1700 Da or lower were the most antihypertensive with a decrease of 38.38 mm Hg in systolic blood pressure [97]. The antioxidant activity of low-molecular-weight compounds isolated from Iberian-fermented sausage (chorizo) was tested in Ref. [98]. Authors did not observe many bioactive peptides; however plenty free amino acids, bacterial metabolites and β -alanyl-peptides have been identified. Bioactive peptides from meat and fermented meat products exhibit various biological activities which are favourable for human health.

4. Conclusion

In recent years, the possibility of development of probiotic meat products has been discussed in the field of meat science and industry. Probiotics in meat may exert their benefits by way of several mechanisms; therefore human clinical studies are needed to assess the health-

promoting effect of probiotic dry-fermented meat products. So far, there is a little scientific evidence related to such studies. Therefore, further studies are required to demonstrate the clear benefits of probiotic meat products for human health.

On the other hand, the use of probiotic strain which possesses the ability to create the bioactive compounds is the challenge for research and meat industry and could develop novel functional meat products. Possible generation of bioactive peptides in dry meat products fermented by probiotics as starter cultures seems the most promising way for designing novel functional food. Therefore meat products with probiotics have a great future potential and it is expected that increasing interest will be shown in basic research and potential applications for designing new meat products.

Target products with probiotic bacteria are mainly dry sausages but also hams and loins, which are processed by fermentation without heat treatment. Technically, it has already become possible to produce probiotic meat products; moreover probiotic raw-fermented meat products exist on a German and Japanese market. However, the production of probiotic meat products requires overcoming certain technological limitations, such as the native microflora of meat, a need to use additives such as nitrites and salt and also low water activity and low content or absence of natural sugars. Probiotic bacteria strains that can be used in the manufacturing of dry-fermented meat products should be capable of surviving in conditions found in fermented products. Moreover, the product should maintain its sensory characteristics.

Additionally, since food safety is another critical aspect of food quality, efforts should also be directed to ensure that new functional meat products are safe. The dry-fermented meat products with probiotic starter cultures addition have to possess appropriate biogenic amine profile and should be oxidative and shelf-stable. Along with accumulation of scientific data, there is an urgent need to inform consumers of the exact physiological value of probiotic meat products. Without proof of product safety, most consumers would hesitate to adopt new foods in their diet.

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Author details

Katarzyna Neffe-Skocińska^{1*}, Karolina Wójciak² and Dorota Zielińska¹

*Address all correspondence to: katarzyna_neffe_skocinska@sggw.pl

1 Department of Food Gastronomy and Food Hygiene, Warsaw University of Life Sciences (WULS), Warsaw, Poland

2 Department of Meat Technology and Food Quality, University of Life Sciences (LULS), Lublin, Poland

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