Biological hurdles as modern strategy for extending shelf life of cooked sausages

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Abstract

Introduction. This review analyses current studies on biological preservation strategies for meat products, with particular emphasis on cooked sausages, and examines the key factors influencing the safety and shelf life of sausage products.

Material and methods. A comprehensive literature search was conducted using international scientific databases, including Scopus, Web of Science, and PubMed, as well as open-access sources such as Google Scholar and ResearchGate.

Results and discussion. Extending the shelf life of cooked sausages through the implementation of biological protective hurdles reduces the proportion of spoiled products, thereby lowering economic losses for producers and supporting the principles of sustainable development. The incorporation of lactic acid bacteria (LAB) into cooked sausage production represents an effective preservation strategy, as these microorganisms exhibit antagonistic activity against pathogenic, opportunistic, and spoilage microflora, improving microbiological safety and prolonging shelf life. Through their natural mechanisms of action, LAB establish a protective barrier by competing for nutrients, producing organic acids, antimicrobial peptides, and other bioactive metabolites, and by regulating microbial interactions via quorum sensing.

Food culture manufacturers face the challenge of selecting LAB strains characterized by low acidification capacity, strong antagonistic activity against undesirable microflora, and the ability to preserve acceptable sensory properties of the final product. For cooked sausage producers, difficulties extend beyond strain selection to include the development of effective methods for LAB application, particularly during post-heat-treatment stages such as vacuum packaging or modified-atmosphere packaging. The use of LAB therefore requires thorough investigation and experimental validation to confirm their efficacy in inhibiting pathogenic and spoilage microorganisms under real industrial conditions and within specific product formulations.

The implementation of effective biological hurdles is consistent with current trends in the food industry and growing consumer demand for healthier products, as it enables the partial or complete replacement and significant reduction of additives, particularly sodium nitrite and certain preservatives.

Conclusions. Biological protection using lactic acid bacteria is an effective strategy to enhance the safety and shelf life of cooked sausages. Future research should focus on the development of biological protection as an additional "barrier" for the preservation of meat products.

Introduction

Sausage products are among the most widely consumed categories of processed meat, constituting a significant part of the human diet. Cooked sausages represent the predominant type globally, owing to their relatively high nutritional value, which is associated with the use of high-quality raw materials and advanced processing technologies (Serikkyzy et al., 2022; Stabnikova et al., 2022).

The growing demand for "clean label" products and the refusal to use chemical preservatives pose significant challenges for the food industry, particularly in the production of meat products (Kim et at., 2025). Traditional preservation methods, such as heat sterilization and use of additives, although effective, often deteriorate sensory properties, reduce nutritional value, and can have negative environmental and economic impacts (Nawaz et al., 2025). This trend stimulates the search for safe and environmentally friendly solutions capable of ensuring microbiological stability without the use of synthetic additives (European Commission, 2020).

One promising approach is hurdle technology, which is based on the combined action of several factors that inhibit the growth of pathogenic and spoilage microflora (Leistner and Gould, 2002). Biological hurdles are especially noteworthy, since they are based on natural defence mechanisms that utilise antagonistic microorganisms, bacteriocins, enzymes, and biopolymers with antimicrobial properties (Bertrand et al., 2024; Rahman, 2015). Their application not only enhances product safety but also helps to preserve product quality, aligns with modern simplified label requirements, and contributes to reducing environmental impact.

Recent studies in collaboration with food manufacturers have explored replacing synthetic ingredients with biologically safe alternatives (Paredes-López et al., 2022). In this context, the antimicrobial properties of natural compounds capable of inhibiting pathogenic and opportunistic microorganisms have been investigated (Bertrand et al., 2024). Research has also focused on the use of selected lactic acid bacteria (LAB) strains and their consortia in various food products to ensure microbiological safety, preserve quality, extend shelf life, and optimize raw material utilization (Kumar et al., 2025).

Considering the substantial global losses of meat and meat products attributable to microbiological spoilage (reaching up to 21% in Europe and North America) (Höll et al., 2016), the implementation of biological solutions could represent a crucial measure for reducing food waste and enhancing the economic efficiency of production. Consequently, the use of biological hurdles is justified and highly relevant in the production of cooked sausages.

The aim of this review is to describe the fundamental principles of biological hurdles, summarise current scientific findings on the use of biological protection in meat product manufacture, and evaluate the potential LAB in the production of ready-to-eat products, particularly cooked sausages.

Material and methods

This review is based on an analysis of scientific articles and publications concerning the effects of microorganisms and their metabolic products on the suppression of undesirable microflora adversely affecting food quality and safety, identified through searches in the Web of Science, Scopus, Google Scholar, and PubMed databases.

Results and discussion

Theory of hurdle technologies

The theory of hurdle technologies is based on the use of several technological factors to preserve product quality, which inhibit the growth of undesirable microorganisms (Food Safety Authority of Ireland, 2022).

The concept of "hurdles" determining the safety and quality of food products was first introduced by Leistner (2017). In his work, he repeatedly emphasized that the broad practical implementation of hurdle theory is possible only when research is carried out to define the quantitative parameters of these hurdles within the technological processes used for specific product types.

Initially, hurdle technologies were based on the application of a limited number of hurdles that can inhibit the development of undesirable microorganisms in meat products.

These hurdles primarily include:

- high temperature (sterilisation and pasteurisation),
- low temperature (processing and storage under chilled and frozen conditions)
- decreasing pH value;
- decreasing water activity a_w;
- decreasing redox potential (Eh);
- presence of preservatives;
- presence of competing microflora.

According to Leistner (2017), when the intensity of a particular hurdle is insufficient, it should be strengthened, whereas if an excessively strong hurdle exerts a detrimental effect on the overall quality of the product, its intensity should be reduced. Following such adjustments, all hurdles should be combined in an optimal value that guarantees effective inhibition of microorganisms while preserving the remaining quality parameters of the product (Abdullahi et al., 2021).

Modern hurdle technologies are designed to ensure high product quality and enhanced food safety, while also supporting environmental sustainability and providing economic benefits by reducing the extent of physical and chemical interventions in industrial processing (Rahman, 2015). In recent years, the range of known and potential physical, chemical, and biological hurdles used to inhibit the development of undesirable microorganisms has increased substantially. Among these approaches, notable examples include irradiation (Jia et al., 2025), ultrasound (Guo et al., 2024), ozone treatment (Ayranci et al., 2020), high-pressure processing (Nguyen et al., 2024), and cold plasma (Zhang et al., 2025). Hurdle technologies in meat production are applied sequentially, beginning at the slaughter stage and continuing through processing, packaging, and storage. The implementation of multiple hurdles at different stages of production is a critical factor in ensuring microbiological safety and preserving the quality of meat and meat products (Gragg et al., 2024). The combined effect of multiple hurdles on the inhibition of undesirable microflora in meat products is shown in Figure 1 (Abdullahi, 2021).

The combination of different barriers, such as reduction of water activity, low- and high-temperature treatments, pH adjustment, and others, provides a synergistic effect that forms the basis of the so-called hurdle technology for food preservation (Rahman et al., 2016). A well-structured combination of several preservation methods creates conditions that inhibit the growth of spoilage microorganisms, thereby enhancing the overall effectiveness of food preservation (Edo et al., 2025). Hurdle technology is an example of a synergistic effect achieved by integrating different preservation methods, which provides an increased level of safety and extends the shelf life of food products (Putnik et al., 2020).

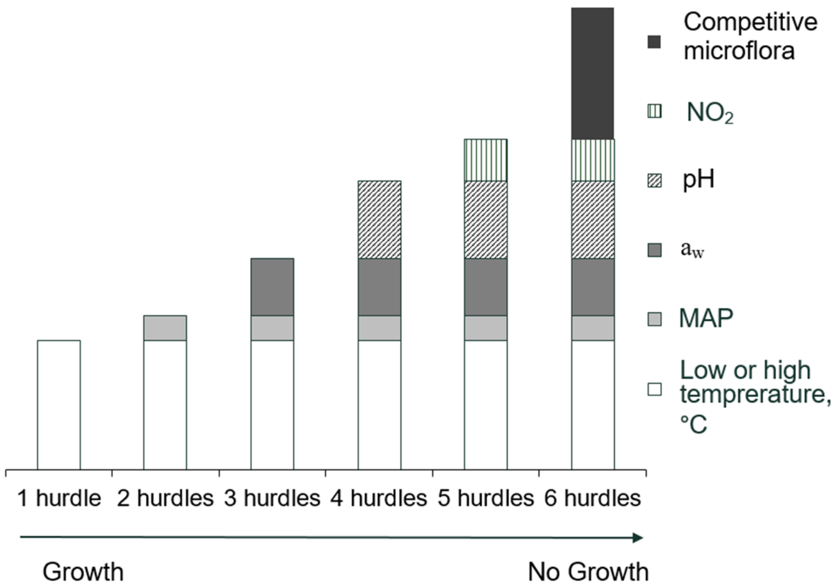

Figure 1. The combined effect of multiple hurdles on the inhibition of undesirable microflora in meat products (adapted from Abdullahi et al., 2021)

Although the total number of potential hurdles may be considerably higher, only a limited subset receives the most attention. Ensuring food safety and microbiological stability in both traditional and novel products requires a synergistic combination of intrinsic and extrinsic factors that function as hurdles (Mafe et al., 2024). An example of how hurdles can be applied to slow down or inhibit the growth of the pathogenic microorganism *Listeria monocytogenes* in packaged cooked ham (Figure 2).

Therefore, to ensure freshness and extend the shelf life of food products, it is essential to create specific conditions that slow down or completely inhibit the growth of pathogenic microorganisms (Khama, 2024). Regulatory criteria aimed at inhibiting the growth of *Listeria monocytogenes* have been incorporated into the legislative frameworks of EU Member States (Begins et al., 2018), the United States, and several other countries (Zhang et al., 2021). Similar requirements are also reflected in Ukrainian food legislation (Ministry of Health, Order No. 548 of 19 July 2012):

– pH $\leq$4.4;
– water activity (a_w) $\leq$0.92;
– pH $\leq$5.0 and a_w $\leq$0.94;
– freezing temperature.

The Food Safety Authority of Ireland (2022) notes that not all hurdles are equally effective in influencing the ability of pathogens to survive and multiply, or in determining the shelf life of food. However, because each hurdle contributes to risk reduction, they may be regarded as critical control points and should be monitored accordingly.

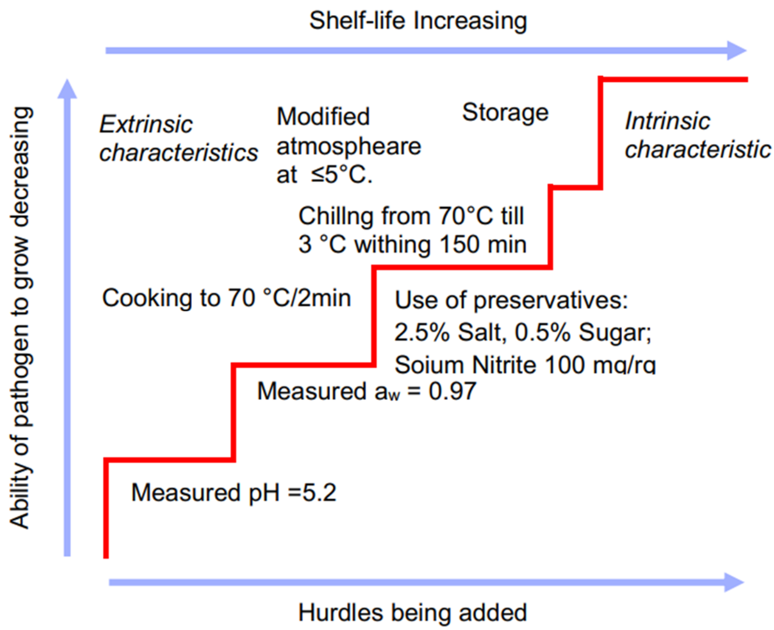

Figure 2. Hurdles applied to inhibit pathogenic microorganisms in cooked sausages (adapted from Food Safety Authority of Ireland, 2022)

Pathogenic microorganisms present in food products are characterized by minimum, maximum, and optimal values that determine their survival and growth (US Food and Drug Administration, 2021). Analysing the intrinsic properties of food products, as well as the extrinsic factors associated with their production and storage, allows for the identification of microorganisms capable of surviving and multiplying in these products, particularly those that pose a risk to human health (Shetty and Singh, 2025).

Factors affecting the microbiological safety and shelf life of foods

All food products possess characteristics that can be classified as intrinsic or extrinsic factors (Table 1). Intrinsic factors are properties inherent to the composition of the food product. Extrinsic factors are properties related to the external production and storage environment that influence the product, such as storage temperature and packaging (Food Safety Authority of Ireland, 2022).

pH value is an important parameter for assessing the suitability of raw materials for further processing. However, the shelf life of meat products cannot be extended solely by lowering pH, as many pathogenic microorganisms exhibit high acid resistance. The optimal pH range for yeast growth is 4.00–6.00 (Riesute et al., 2021), but some yeasts, such as *Saccharomyces cerevisiae*, can grow within a pH range of 2.5–8.5, representatives of the genus *Candida* at pH values below 2.0 (Rane et al., 2019), and mould such as *Aspergillus niger* at pH values from 1.5 to 9.8 (Li et al., 2020).

Lowering the pH of a product can significantly limit the growth of spoilage bacteria. Nevertheless, for meat and meat products, pH can be modified only within approximately from 4.5 to7.5. Products with lower pH values are rarely produced, mainly due to technological constraints and predominant sensory and taste preferences among consumers (Toupchi et al., 2025).

Water activity (a_w) is a key characteristic of a product that indicates the free water that can be utilized by microorganisms for their metabolic activities. A reduction in water activity inhibits microbial proliferation, disrupts intracellular metabolic processes, and adversely affects microbial survival. In meat products, lower water activity values are associated with extended shelf life (Figura et al., 2023). Intrinsic and extrinsic characteristics affecting the microbiological quality and shelf life of food products are shown in Table 1.

Table 1

Intrinsic and extrinsic characteristics affecting the microbiological quality and shelf life of food products*

Extrinsic	Intrinsic
– Temperature (during production, storage, distribution, and display) – Packaging – Gas atmosphere – Relative humidity – Food processing – Historical factors (my also relate to intrinsic characteristics depending on the nature of the data) – Good manufacturing and hygiene practices – Storage and distribution – Consumer practices	– pH and type of acid present – Water activity (a_w) – Redox potential (Eh) – Natural barriers – Nutritional content of food and availability – Antimicrobial substances – Microflora – Microbiological quality of ingredients – Food formulation and composition – Food assembly and structure

*Adapted from Food Safety Authority of Ireland, 2022

Table 2 provides a summary of the effects of water activity on microorganisms.

Table 2

Dependence of microbial growth on water activity (a_w)*

Value of a_w	Impact on microbial growth
< 0.90	Most bacteria cease to multiply
< 0.88	Most yeasts cease to multiply
< 0.80	Most mold cease to multiply
0.60–0.75	Halophilic bacteria, xerophilic mold, osmophilic yeasts can be active

* Adapted from Figura et al., 2023

Redox potential (E_h) which depends primarily on the amount of oxygen incorporated during the preparation of minced meat and on conditions that promote its reduction, has an inhibitory effect on aerobic microorganisms that tolerate the presence of sodium nitrite and favours the selection of lactic acid bacteria. Microorganisms differ in their requirements and tolerance for oxygen and redox potential, as follows (Food Safety Authority of Ireland, 2022):

– aerobes require oxygen for growth and can grow at an oxidation–reduction potential of approximately +300 to +500 mV
– facultative anaerobes can grow in the presence or absence of oxygen and are able to grow at an oxidation–reduction potential of approximately +300 to −100 mV
– anaerobes do not require oxygen for growth and can grow at an oxidation–reduction potential of approximately +100 to ≤−250 mV.

The redox potential values for selected products are presented in Table 3.

Table 3

Redox potential of selected food matrices*

Product	Eh, mV	pH
Raw meat (post-rigor)	–200	5.7
Chilled minced meat	+225	5.9
Cooked sausages and canned ham	–20 to –150	≈6.5
Wheat (whole grain)	–320 to –360	6.0
Barley (ground grain)	+225	7.0
Potatoes	≈ –150	≈ 6.0

*Adapter from Adams et al., 2024

Sodium chloride and curing salt. Salt plays a key role in flavour development, modifies the microstructure of minced meat, and affects its texture (Shevchenko et al., 2021). When dissolved in the water present in meat raw materials or mince, salt lowers water activity (a_w), thereby inhibiting or slowing microbial growth and promoting the selective development of particular microbial groups (Food Safety Authority of Ireland, 2022). Furthermore, regulatory authorities recommend the addition of nitrate and nitrite curing salts to impart the characteristic reddish colour to cured meat products and to inhibit the growth of vegetative cells of *Clostridium botulinum* (Lemos et al., 2024).

At the same time, certain microorganisms (e.g., *Staphylococcus* spp.) (Table 4) are able to tolerate high concentrations of sodium chloride in aqueous systems. Therefore, only the combined action of multiple hurdles can ensure prolonged preservation of finished products (Gragg et al., 2024). According to Commission Regulation (EU) No. 1129/2011 (European Commission, 2011), the use of nitrates and nitrites as food additives is permitted in the European Union, specifically sodium nitrate (E251), potassium nitrate (E252), sodium nitrite (E250), and potassium nitrite (E249). This regulation, which amends Annex II to Regulation (EC) No. 1333/2008, establishes maximum levels of nitrates and nitrites in meat products that vary according to product type. Non-heat-treated meat products may contain up to 150 ppm of nitrate or sodium or potassium nitrite, whereas heat-treated meat products (with the exception of sterilized products) may contain up to 150 ppm of sodium or potassium nitrite. Certain traditional smoked meat products are exempt from these general limits and may be permitted higher maximum levels (Melios et al., 2024). Growth characteristics of common foodborne bacterial pathogens are shown in Table 4.

Table 4

Growth characteristics of common foodborne bacterial pathogens

Pathogens	Temperature, °C	pH	Water activity (a_w)	Salt, %
	Min (Optimum) Max *Allowing Growth*		**Min** *Allowing Growth*	**Max** *Allowing Growth*
Salmonella spp.[1]	5 (35–43) 47	3.8 (7–7.5) 9.5	0.94	4
Clostridium botulinum[2] (proteolytic)	10 (35–40) 42	<4.6 (7) 8	>0.94	10
Clostridium botulinum[2] (non-proteolytic)	3 (28–30) 35	<5.0 (7) 8	>0.97	5
Staphylococcus aureus[3]	10 (40–45) 48	4 (7–8) 9.6	0.83	10
Campylobacter spp.[4]	32 (42–43) 45	4.9 (6.5–7.5) 9	>0.98	1.5
Yersinia enterocolitica[5]	–1.3 (25–37) 42	4.2 (7.2) 9.6	0.94	7
Listeria monocytogenes[6]	–1.5 (30–37) 45	4.2 (7) 9.5	0.92	12
Clostridium perfringens[7]	10 (43–47) 50	5.5 (7.2) 9	0.93	6
Shiga toxin (*STEC*) or Verocytotoxin (*VTEC*) producing *Escherichia coli*[8]	6.5 (30–40) 45	3.6 (6–7) 9	0.95	>6.5

Source: Food Safety Authority of Ireland (2022). [1]Egg, meat; [2]Foods which are canned, vacuum packed, modified atmosphere packed, jarred (i.e. low oxygen environments); [3]Eggs, poultry, meats, salads, sandwiches; [4]Poultry meat, unpasteurised/raw drinking milk and dairy products (e.g. cheese, butter); [5]Fresh meats (pork in particular) and unpasteurised/raw drinking milk and dairy products (e.g. cheese, butter); [6]Chilled, ready-to-eat foods (e.g. smoked salmon, sliced cooked meats, coleslaw); [7] Cooked meats; [8]Meat, poultry, unpasteurised/raw dairy products and apple juice, sprouted seeds, salad vegetables, untreated drinking water (e.g. from a well) etc.

Commission Regulation (EU) 2023/2108 reduced the maximum permitted levels of sodium and potassium nitrite to 55-80 ppm (expressed as NO_2 ion) and sodium and potassium nitrate to 90 ppm (expressed as NO_3 ion), with certain exceptions maintained for traditionally cured products (European Commission, 2023). In this most recent legislation, the input quantities are expressed in terms of ions rather than salts; consequently, the actual reduction may not be as substantial as it initially appears.

A major drawback associated with the use of nitrites and nitrates in meat products is the formation of nitrosamines during processing, compounds that may adversely affect human health (Habermeyer et al., 2019). In response to these concerns, the European Parliament's Committee on the Environment, Public Health and Food Safety has recently submitted a motion for a resolution proposing a complete ban on the addition of nitrates and nitrites to smoked meat products (Melios et al., 2024).

Table 4 presents key characteristics of the growth conditions of common foodborne bacterial pathogens that pose a significant risk to consumer health. Pathogenic microorganisms exhibit different requirements for the gaseous composition of their environment, which determines their growth potential (Table 5).

Table 5

Oxygen-related growth requirements of pathogenic microorganisms

Pathogens	Relationship to oxygen
Salmonella spp.	Facultative
Clostridium Botulinum (proteolytic)	Anaerobic
Clostridium botulinum (non-proteolytic)	Anaerobic
Staphylococcus aureus	Facultative
Campylobacter spp.	Microaerophilic
Yersinia enterocolitica	Facultative
Listeria monocytogenes	Facultative
Clostridium perfringens	Anaerobic
Shiga toxin (*STEC*) or Verocytotoxin (*VTEC*) producing *Escherichia coli*	Facultative

Source: Food Safety Authority of Ireland (2022)

The values presented in Table 4 and Table 5 are approximate and apply under optimal conditions; therefore, they should be used for guidance only. Pathogenic microorganisms may be capable of growth outside these ranges (Food Safety Authority of Ireland, 2022).

Cooking process is a sequence of complex physicochemical transformations in the meat matrix aimed at fixing the product's shape and structure, inactivating vegetative microflora, developing desirable sensory properties, and enhancing resistance to mould growth and microbiological spoilage during storage (Andrade et al., 2025).

Non-optimized or uncontrolled heat treatment regimes may result in souring of minced meat and the reduction of nitrite to molecular nitrogen. Consequently, grey discolorations can appear on the cut surface of sausage batons, porosity may develop due to gas production by heterofermentative microflora, and microbiological spoilage of the finished products can occur during storage (Andrade et al., 2025).

Packaging process. Cooked sausages are typically packaged and marketed under vacuum. During storage, it is necessary to consider process-related factors such as synergistic microbial interactions and the proliferation of rod-shaped, slime-producing bacteria (e.g., *Bacillus* spp.), which are among the main causes of defects in vacuum-packed meat products. Deterioration in the appearance and colour of products can adversely affect consumer perception and may lead to rejection of the product before the end of its shelf life (de Lima et al., 2022).

Blowing of the packaging and the presence of a milky-coloured exudate in vacuum-packed cooked sausages, irrespective of the formulation, are associated in approximately 50% of cases with contamination by bacteria of the genus *Leuconostoc*. Substantial batch-to-batch variation in bacterial counts and initial contamination levels is likely related to cross-contamination under industrial production conditions. Experimental findings indicate that the reduction in the shelf life of cooked hams is specifically linked to cross-contamination occurring during slicing (Blanco–Lizarazo, 2022).

If no additional processing is applied at the packaging stage, the only hurdle capable of significantly reducing initial microbial contamination is the heat treatment of the product. External hurdles, typically applied in the form of preservatives, exert primarily a bacteriostatic rather than a bactericidal effect (Rogovski et al., 2021).

Sources of contamination in cooked sausages

Raw meat is an excellent medium for bacterial proliferation because of its rich nutrient composition, high a_w, and slightly acidic pH (Cauchie et al., 2020). In combination with the action of endogenous autolytic enzymes, these conditions accelerate the progression of meat spoilage (Shao et al., 2021). Meat spoilage is primarily caused by bacterial activity and is influenced by multiple factors, including product composition and the microbiological status of the carcass at slaughter. Additional parameters include the nature of surface treatments, subsequent processing steps, hygienic conditions during production, storage temperature, and the type of packaging use (Mafe et al., 2024). Moreover, the processing environment itself may act as a source of contamination with pathogenic and spoilage microorganisms, thereby compromising the quality and safety of meat and meat products, including cooked sausages (Barcenilla et al., 2024). Source of contamination of cooked sausages are shown in Table 6.

Table 6

Source of contamination of cooked sausages

Process	Source of contamination	Reference
Slaughter and primary processing	Equipment, knifes, surfaces	Salama et al., 2024
Production environment	Air, water, dust	Barcenilla et al., 2024
Cutting and mixing	Surface of equipment	Salama et al., 2024
Packaging (MAP/vacuum)	Packaging material, packaging equipment	Pellissery et al., 2020
Storage and logistics	Temperature, duration of storage	Park et al., 2024

The level of contamination in meat raw materials is influenced by their temperature prior to processing (fresh, chilled, or frozen) and by the sanitary conditions within the production environment. The use of thawed meat, combined with non-compliance with prescribed temperature requirements in processing and cutting areas, leads to an elevated microbiological load (Park et al., 2024; Shevchenko and Tunik, 2024).

Contamination originating from slaughter equipment, air, water, soil, and workers' hands can facilitate the growth of spoilage bacteria, thereby degrading the quality of meat components (Holman et al., 2018).

The natural microbiota of meat comprises a diverse range of spoilage microorganisms, including Gram-positive and Gram-negative bacteria, filamentous fungi, and yeasts belonging to the genera *Trichosporon* and *Candida* (Lemos, 2024).

Meat and meat products are recognized as common sources of major foodborne pathogens (Ali et al., 2022), including members of the *Enterobacteriaceae* family (Pellissery et al., 2020). Significant quality deterioration and alterations in the physicochemical and sensory properties of meat can result from putrefactive microorganisms present in raw materials, such as *Pseudomonas* spp. and *Brochothrix thermosphacta* (Pellissery et al., 2020). Proteolytic bacteria produce sulfur compounds, ammonia, and amines, which contribute to putrid and unpleasant odors (Wang et al., 2023). Additionally, bacteria such as *Pseudomonas* spp. synthesize pigments that cause green, grey, or brown discoloration (Wang et al., 2023).

In addition, meat spoilage can be caused by representatives of the genera *Acinetobacter, Bacillus, Shewanella, Aeromonas*, and *Clostridium*, as well as by lactic acid bacteria belonging to the genera *Weissella, Leuconostoc, Carnobacterium, Lactococcus*, and *Lactobacillus* (Poirier, 2018; Shao et al., 2021).

Microorganisms present in meat raw materials can contaminate the internal matrix of the sausage during production (Salama and Chennaoui, 2024). Thermal processing remains a key factor in ensuring the microbiological safety of cooked sausages. During cooking, when the core temperature of the product reaches 72 °C, up to 99% of vegetative bacterial cells are inactivated; however, spore-forming microorganisms, such as clostridia, may survive. Among them, *Clostridium botulinum*, the causative agent of botulism, is considered the most critical pathogen (Devine et al., 2023). Microorganisms contaminating the surface of sausages can also contribute to product spoilage (Tian et al., 2025). Cooked sausages produced from raw meat materials with a high initial microbial load, such as meat trimmings, heavily processed trimmings, and offal, exhibit the lowest storage stability. In addition, cooked sausages generally have a higher moisture content than semi-dry and dry products (e.g., cooked-smoked, semi-smoked, and fermented sausages), which consequently leads to a reduced shelf life (Halagarda and Wójciak, 2022фдцфны.

Sausage casings have an impact on overall quality of cooked sausages. Both natural casings (e.g., hog, sheep, and beef intestines) and artificial casings (e.g., cellulose, collagen, and plastic) are used in production, but artificial casings generally provide better sanitary conditions and protection. Because natural casings are obtained from different regions around the world, their microbiological quality can be highly variable (Liu et al., 2023). They contain protein, water, and often a fat layer, which together create favourable conditions for microbial growth. If natural casings are not properly cleaned, they may develop a musty odour and carry large numbers of spoilage microorganisms, especially bacteria of the genera *Proteus* and *Clostridium*, which can cause sausage spoilage during storage (de Araújo et al., 2022).

When the microbial load reaches 10^7–10^8 CFU/cm², it leads to significant sensory changes in the muscle tissue (Ndoye et al., 2025). Meat exhibiting a high level of microbiological contamination by mesophilic, aerobic, and facultative anaerobic microorganisms, despite retaining an acceptable visual appearance, can result in technological defects and a decline in the overall quality of the final product (Tian et al., 2025). Meat raw materials with an approximate total contamination level of 1×10^7 CFU/g exhibit all characteristic of spoilage, including greening, persistent off-flavor, surface slime, rancidity of fat (Cauchie et al., 2020; Shevchenko et al., 2020).

The total number of viable microorganisms in the finished product depends on the initial microbial load of the raw materials (Cauchie et al., 2020). Consequently, a low initial level of microbial contamination in meat raw materials represents the first critical hurdle and has a significant impact on the shelf life of the final product (Salama and Chennaoui, 2024).

A modern view of bioprotection for cooked sausages

A review of publications in the Scopus database (www.scopus.com) conducted in November 2024 using the keywords “bacteriocins” and “meat” identified 691 publications, including 94 review articles, with more than 60% published within the last decade. This trend reflects the strong and growing interest of both the scientific community and the food industry in developing strategies to partially or entirely replace chemical preservatives in food products. Bioprotection was initially defined as an approach to control specific pathogens in food systems. However, in recent years, it has acquired broader significance and is now recognized as a key strategy for inhibiting spoilage-associated microflora, extending shelf life, and improving the overall stability of food systems. Within this framework, bacteriocin-producing cultures and their metabolites are increasingly being explored as promising bioprotective agents for application in meat products (Souza et al., 2022).

During extended storage of food products, spoilage is a complex process often driven by consortia of microorganisms from various species. Consequently, starter cultures must exhibit a broad spectrum of inhibitory activity (Alessandria et al., 2023). The use of bacteriocinogenic strains (mainly lactic acid bacteria) is described in the works (Azevedo et al., 2024; Urso et al., 2006). To identify suitable strains capable of producing bacteriocins, it is necessary to adapt and reproduce the conditions under which the suppression of consortia of microorganisms that spoil food products will occur. Some studies show that it is more promising to search for suitable strains in fresh products while the microbiological characteristics are still satisfactory and autochthonous populations can be used to protect them (Cocolin, 2025). Since microorganisms responsible for food spoilage are not necessarily pathogenic, recent research has focused on developing novel preservation strategies, while the underlying principle remains the same: employing natural or microbially derived agents to inhibit undesirable microflora and maintain product quality. By inhibiting microorganisms or bacterial groups responsible for spoilage, it is possible to extend the shelf life of food products and improve the stability of food systems. Methods for identifying specific spoilage organisms (SSO) were described by Gram et al. (1987).

The bioprotection strategy is comparable to that employed for controlling foodborne pathogens but is applied to any microorganism responsible for spoilage (Cocolin, 2025). It involves conducting microbiological analyses of ready-to-eat products, such as cooked sausages, both at the beginning and at the end of their shelf life to monitor microbial dynamics and ensure product safety and quality.

At the beginning of shelf life, the food ecosystem is characterized by a balanced microflora that reflects both the intrinsic properties of the product and the conditions under which it was produced. However, during storage, shifts in the microbial population may occur, adversely affecting quality attributes such as color, taste, and aroma and ultimately leading to product spoilage (Cocolin, 2025). Microbial colonies isolated at the beginning of shelf life and representing different microbial groups, cultivated on selective and non-selective media (de Man, Rogosa and Sharpe (MRS) agar for lactic acid bacteria and Brain Heart Infusion (BHI) agar for total microflora), can be evaluated for their inhibitory activity against spoilage consortia that develop at the end of shelf life. This approach enables the identification of spoilage-associated microorganisms and their isolation in pure culture, allowing their subsequent use as indicator strains for screening bacteriocin-producing bacteria by means of agar well diffusion assays (Cocolin, 2025).

A comparable strategy has been successfully employed to evaluate the effectiveness of starter cultures in sliced cooked ham. In that study, nine lactic acid bacteria strains were selected from 140 isolates based on their capacity to inhibit a broad spectrum of spoilage microorganisms through bacteriocin production at 4 °C (Alessandria et al., 2023).

Biological protection mode of action

Bacteriocins are antimicrobial peptides synthesized by bacteria that exhibit activity against other microorganisms. Their production confers a competitive advantage on the producer strains in shared ecosystems by inhibiting the growth of undesirable or pathogenic microflora. Notably, bacteriocins are generally considered as non-toxic, can display either narrow- or broad-spectrum antimicrobial activity, and are effective at low concentrations. These properties make them promising candidates for use as natural biopreservatives in meat products and other perishable foods (Arbulu et al., 2022) and stimulated considerable scientific interest in bacteriocins as potential natural alternatives to conventional preservatives for use in food products.

Particular attention is given to bacteriocins produced by lactic acid bacteria, as these microorganisms are already widely employed in the food industry (Stabnikov et al., 2025). Such cultures can enhance the safety and quality of products through active biological interactions with both the food matrix and the product microbiota (Nascimento et al., 2025). The mode of action of biological hurdles is shown in Figure 3.

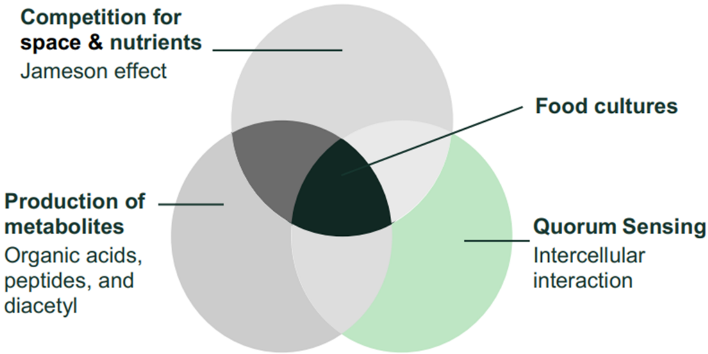

Figure 3. Mode of action of biological hurdles
Adapted from Mgomi et al., 2023

Currently, the concept of the microbiome refers to the microbial community of a defined environment, occupying a specific niche with characteristic physicochemical conditions, and encompasses not only the microorganisms present but also their interactions, forming an integrated ecosystem (Berg et al., 2020).

Scientific research on the role and application of the microbiome in the food industry has intensified in recent years. Beyond the field of human health, these studies have increasingly focused on biotechnological processes based on microbial fermentation of food systems, aiming to improve technological solutions (Zhao et al., 2025).

In the context of microbiome functioning, particularly the mechanisms of bacterial interaction, increasing attention is being paid to the concept of microbial competition as a potential tool for improving food safety and quality. It has been demonstrated that microorganisms can establish complex relationships based on positive and negative interactions, thereby shaping population equilibrium and suppressing other microbial groups (Raimondi et al., 2019).

It has been established that LAB can employ various competitive strategies, ultimately enabling them to dominate specific ecosystems. The most common mechanisms include the production of organic acids, hydrogen peroxide, diacetyl, antimicrobial compounds (often referred to as “killer factors”), as well as competition for space and nutrient resources (Raimondi et al., 2019).

Natural antimicrobial metabolites also include bacteriocins – peptides whose production provides the producing strains with a competitive advantage over other microorganisms occupying the same ecological niche (Smaoui et al., 2023). Lactic acid bacteria have considerable potential for application in bioprotection technologies, and are naturally capable of dominating the microbiota of many products during storage (Zapaśnik et al., 2022).

In raw meat products stored at low temperatures under vacuum or in an atmosphere with elevated CO_2 levels, lactic acid bacteria become the dominant microbial group and contribute to preservation of the raw material through their "hidden" metabolism. A similar phenomenon is observed in ready-to-eat meat products when these bacteria partially survive heat treatment or are reintroduced after processing (Cocolin, 2025).

In recent years, this strategy has been increasingly adopted in industrial production as an additional hurdle for biological protection during food storage. Notably, Novonesis A/S (Denmark) has reported substantial progress in utilizing starter cultures as a source of natural microbial biopreservation. These commercial starter cultures are now widely incorporated into meat and other perishable products to enhance safety and extend shelf life without exclusive reliance on preservatives.

Antimicrobial activity and classification of bacteriocins

According to the classification proposed by Smaoui et al. (2023), bacteriocins are categorized into three classes: Class I (lantibiotics), they are low-molecular-weight peptides (<5 kDa), thermostable, resistant to proteolytic degradation, and post-translationally modified; Class II (non-lantibiotics), they are low-molecular-weight peptides (<10 kDa), thermostable, stable across a wide pH range, and not post-translationally modified; and Class III, comprising high-molecular-weight proteins (>30 kDa) that are thermolabile (Fig. 2). It is well established that bacteriocins can be produced by both Gram-positive and Gram-negative microorganisms (Ageitos et al., 2017), as reflected in the classification proposed by Simons et al. (2020).

Class I lantibiotics comprise small bacteriocins (<5 kDa) that undergo post-translational modifications. A distinctive feature of this group is the presence of unusual amino acids, including dehydrated residues, lanthionine, and 3-methyllanthionine, which form ring structures and confer structural stability and heat resistance.

This class is typically associated with the inhibition of Gram-positive bacteria, including foodborne pathogens (He and Deber, 2024). It also encompasses lantibiotics such as lacticin, cytolysin, and salivaricin, whose antimicrobial activity is linked to the inhibition of specific enzymes that are essential for the survival of target bacteria.

Class II bacteriocins, or non-lantibiotics, do not contain unusual amino acids in their structure (Yang et al., 2024), and post-translational modification is limited to the formation of disulfide bonds in only a few members (e.g., pediocin PA-1, pediocin AcH). Similar to class I bacteriocins, these peptides are thermostable and relatively small (<10 kDa). Their antimicrobial activity is mainly exerted through destabilization and increased permeability of bacterial membranes or via pore formation in the membrane (Marr et al., 2006; Pontes et al., 2022). This group can be further divided into four subclasses. The first subclass comprises bacteriocins with a linear structure stabilized by disulfide bonds and exhibiting broad antilisterial activity; therefore, they are commonly referred to as antilisterial bacteriocins (e.g., leucocin A, acidocin A, pediocin PA-1) (Batoni et al., 2011; Zasloff et al., 2002).

The second subclass consists of two-peptide (α/β) bacteriocins, in which both peptides are produced and are required for full antimicrobial activity (e.g., lactococcin G, lactococcin Q, and plantaricin NC8) (Fontanot et al., 2020). The third subclass includes small bacteriocins associated with a signal sequence and containing one or two cysteine residues in their structure; these are referred to as cystibiotics and thiolbiotics, respectively. This subgroup comprises several molecules, such as lactococcin A, divercin A, and acidocin B (Marr et al., 2006). The fourth subclass encompasses all remaining class II bacteriocins that do not fall into any of the aforementioned categories.

Unlike Class I and Class II bacteriocins, Class III bacteriocins are large peptides (>30 kDa) that are generally thermolabile and may or may not induce cell lysis (Beis et al., 2019; Kapil et al., 2020). This class includes bacteriocins such as zoocin A, lysostaphin, and helveticins J and V (Kapil et al., 2020). Their antibacterial activity is primarily associated with enzymatic functions (e.g., endopeptidase activity) that lead to degradation of the bacterial cell wall.

Class IV bacteriocins are distinguished by a structure incorporating lipid or carbohydrate moieties (He and Deber, 2024), as exemplified by plantaricin S (Kuma et al., 2018) and leuconocin S, which disrupt bacterial cell membranes. This structural characteristic renders these molecules susceptible to degradation by various enzymes, including glycolytic and lipolytic enzymes.

The antimicrobial activity of bacteriocins is primarily associated with their ability to disrupt the cell membrane of Gram-positive bacteria by forming pores, which trigger the passive leakage of essential intracellular components and ultimately lead to cell death. Biological protection in food products is mainly achieved either by adding starter cultures (predominantly lactic acid bacteria) capable of producing bacteriocins in *situ*, or by incorporating purified bacteriocins (Stabnikov et al., 2025). Bacteriocins are effective against foodborne pathogens as well as a wide range of spoilage microorganisms (Chen et al., 2020). The overall classification scheme of bacteriocins produced by lactic acid bacteria and their main characteristics (Smaoui et al., 2024) is presented in Figure 4. In a study by Dal Bello et al. (2010), approximately 1,000 autochthonous LAB strains isolated from artisanal fermented products were examined, among which 98 bacteriocin-producing cultures (around 10%) were identified. The bacteriocins synthesized by these strains exhibited antimicrobial activity against various spoilage microorganisms and inhibited the growth of pathogenic and opportunistic bacteria, including *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium tyrobutyricum*, and *Brochothrix thermosphacta.*

It has been demonstrated that bacteria belonging to the genera *Lactococcus* and *Enterococcus* are effective against several microorganisms responsible for meat product spoilage and are also capable of inhibiting pathogenic bacteria (Smaoui et al., 2024). This is evidence that bacteriocin-producing strains are frequently present in competitive ecosystems, such as fermented foods, where the ability to synthesize antimicrobial compounds confers an ecological advantage and promotes their dominance within the microbiota (Cocolin, 2025). The classification scheme of bacteriocins produced by lactic acid bacteria, along with their main characteristics, is shown in Figure 4.

In accordance with the regulatory status of food ingredients, microorganisms recognized as safe for consumption (GRAS) by the U.S. FDA and granted Qualified Presumption of Safety (QPS) status in Europe (EFSA, 2020) can be used under specified conditions, but their application must still comply with relevant food safety regulations and labelling requirements. However, the incorporation of purified or partially purified bacteriocins is not yet fully supported at the regulatory level, and their use depends on national authorities and intended applications (Stabnikova et al., 2023; Verma et al., 2022). For instance, nisin (E234) is the best-known and most extensively studied bacteriocin and remains the only substance approved as a food additive for some type of products in the European Union (European Commission, 2011).

Barcenilla et al. (2022) investigated the potential application of LAB and bacteriocins in meat products to enhance safety, including their possible use as functional components in active packaging.

However, manufacturers face emerging challenges related to improving technological processes and implementing biological methods for food system protection, which would contribute to increased product safety and extended shelf life.

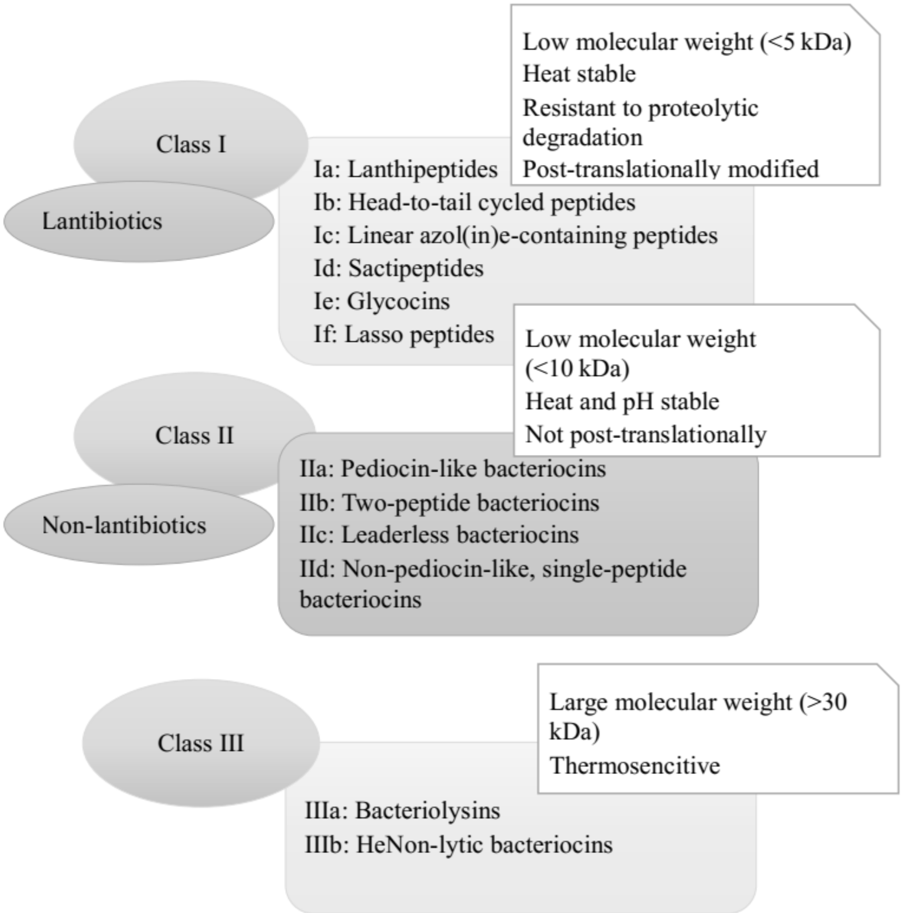

Figure 4. Classification scheme of bacteriocins produced by lactic acid bacteria and their main characteristics
Adapted from Smaoui et al., 2024

Bacteriocin production by bacteriocinogenic strains in cooked sausage manufacturing

Interest in bioprotection and lactic acid bacteria strains capable of producing bacteriocins has increased substantially in recent years, accompanied by numerous studies on their detection and characterization (Cocolin, 2025). However, research specifically addressing the effective release of bacteriocins during food processing remains limited. This limitation is largely due to the difficulty of assessing bacteriocin relising in the specific food matrix in which they reside, although this parameter is critical for selecting a strain as a bioprotective component (Cocolin, 2025).

Several conditions encountered by microorganisms during food production can influence bacteriocin synthesis. Factors such as salt concentration, pH range, heat treatment, microbial competition, and the presence of preservatives significantly affect the protective efficacy of bacteriocin-producing LAB strains (Todorov et al., 2022). One approach to demonstrate bacteriocin synthesis in food products is to monitor the expression of the gene encoding the bacteriocin and correlate this expression with evidence of active bacteriocin production by the protective culture during processing (Cocolin, 2025). Such confirmation typically involves combining molecular techniques, such as quantitative PCR or transcriptomic analysis, with biochemical assays that detect the presence and activity of the bacteriocin in the food matrix.

For example, Urso et al. (2006) demonstrated that during sausage production with *Latilactibacillus sakei* I151, the strain was capable of producing sakacin P. The approach involved manufacturing sausages using *L. sakei* I151 as a starter culture and collecting samples at regular intervals throughout fermentation. A representative sample was subjected to RNA extraction, followed by membrane transfer and hybridization using a *sakP* gene-specific probe. Gene expression *in vitro* peaked after approximately 15 hours and persisted until the end of the experiment, which lasted nearly five days.

This hypothesis was further supported by agar activity tests using the agar diffusion method. Aliquots of fermented sausage homogenate, previously neutralized in physiological solution, were distributed into wells of an agar layer inoculated with *Listeria monocytogenes*. Clear inhibition zones were observed around the wells on days 3, 7, and 14 of fermentation (Cocolin, 2025). These results confirmed antimicrobial activity associated with the secretion of sakacin P, which correlated perfectly with the expression of the *sakP* gene. Further evidence was provided by the sustained expression of the *sakP* gene, which remained active even after 14 days of fermentation. This finding indicates the ability of *Latilactibacillus sakei* I151 to maintain bioprotective activity against *Listeria monocytogenes* throughout the production process by utilizing a biological mechanism (Cocolin, 2025).

García-López et al. (2023) demonstrated the feasibility of incorporating two autochthonous bacteriocin-producing strains during salami production. For comparison, a control batch underwent spontaneous fermentation with the selected strains, while another batch used a commercial starter culture. The results showed that inhibition of *Listeria monocytogenes* was equally effective in salami produced with either the autochthonous strains or the commercial starter. These findings underscore the ability of native LAB to produce bacteriocins within real food matrices, such as cooked sausages.

Competition for nutrients

The synthesis of antimicrobial compounds is among the most extensively studied mechanisms for inhibiting both pathogenic and spoilage microorganisms. However, because the concentrations of these compounds may be insufficient to suppress certain species, additional mechanisms are likely involved (Mgomi et al., 2023). One such mechanism is nutrient competition: spoilage bacteria and LAB compete for available resources, often in combination with the action of bioactive compounds. By efficiently utilizing nutrients and limiting or completely depleting them in the environment, lactic acid bacteria can effectively inhibit the growth of spoilage organisms (Siedler et al., 2019).

However, the competitive exclusion of microorganisms in different food matrices remains insufficiently studied, and its role within the overall bioprotective mechanism is still unclear. It has been shown that three strains of *Lactobacillus paracasei* utilized metabolites to varying degrees, revealing an inverse correlation between glucose and glutamine consumption and the inhibition of spoilage mold growth: more intensive nutrient utilization by the bacteria was associated with reduced mold development (Honoré et al., 2016). In the human body, competitive exclusion is described as "nutritional immunity," in which limited availability of iron and zinc in specific tissues suppresses the growth of pathogenic microorganisms. Although this mechanism may not apply to lactic acid bacteria, competition for other nutrients in nutrient-rich environments cannot be excluded (Gerwien et al., 2018).

The presence of oxygen in contact with food products can significantly deteriorate quality by stimulating mold growth and promoting lipid oxidation, which reduces shelf life (Marin et al., 2021). Molds and yeasts require water, minerals, and carbon and nitrogen sources for growth and reproduction. They exhibit high resistance to environmental stress, such as elevated salt or sugar concentrations, due to the structural properties of their cell walls, which contain chitin and cellulose. For example, yeasts belonging to the genera *Candida*, *Saccharomyces*, *Zygosaccharomyces*, *Pichia*, *Meyerozyma*, *Metschnikowia*, and *Wickerhamomyces* are capable of growing in products with high sugar concentrations and low moisture content (15–50%) (Hu, 2016; Tarlak, 2023).

Molds responsible for food spoilage, including those affecting cooked sausages, are generally strict aerobes (Walker et al., 2017). Therefore, limiting oxygen availability is an effective strategy to inhibit their growth. However, certain molds, such as *Penicillium roqueforti*, *Mucor plumbeus*, and some *Fusarium* species, can grow at oxygen concentrations as low as 0.5–2%. In addition, molds are capable of utilizing oxygen present within the food matrix itself, not solely from the surrounding atmosphere. Consequently, reducing atmospheric oxygen in packaged foods should be combined with additional inhibitory measures, such as the application of carbon dioxide or inert gases (Dangas et al., 2013).

The presence of 5% O_2 in a modified atmosphere delayed the growth of *A. niger*, *Eurotium amstelodami*, *P. chrysogenum*, and *Fusarium oxysporum*. Alternatively, anaerobic conditions combined with CO_2 resulted in complete inhibition of mold growth (Dagnas and Membré, 2013).

Traditional methods for preserving meat and meat products include air-permeable packaging, vacuum packaging, and modified atmosphere packaging (Gómez et al., 2020; Pasichnyi et al., 2022). Reducing residual oxygen in a modified atmosphere helps minimize lipid oxidation, inhibit undesirable microorganisms, stabilize color, and maintain nutritional quality (Niazmand and Yeganehzad, 2020). However, complete removal of oxygen during packaging is not feasible due to the limited efficiency of inert gas flushing or vacuuming. Furthermore, oxygen can permeate packaging materials during storage, leading to deterioration of product quality (Röcker et al., 2021). The residual oxygen level in vacuum-packaged cooked pork products typically ranges from 0.11–0.15%, while in modified atmosphere packaging it is approximately 0.9–1.1% (Smiddy et al., 2002). Since many foods release oxygen after packaging, residual oxygen levels of 0.5–5% may remain in the headspace of modified atmosphere packages. This can result from leakage through the packaging material, improper sealing, or oxygen released from the product itself (Sängerlaub et al., 2021).

Studies conducted over several decades have demonstrated that cooked-smoked ham undergoes color changes under the combined influence of oxygen and light (Andersen et al., 1988). Even residual oxygen levels as low as 0.1% in the packaging can induce photooxidation of nitrosomyoglobin and nitrohemoglobin, resulting in the formation of grey-brown metmyoglobin (Juncher et al., 2003; Møller et al., 2000, 2002).

Chemical oxygen scavengers can be applied to reduce oxygen levels in MAP. Modern packaging technologies employ one or more of the following approaches: oxidation of divalent iron powder, oxidation of ascorbic acid, oxidation of photosensitive dyes, enzymatic oxidation (e.g., glucose oxidase and alcohol oxidase), unsaturated fatty acids (e.g., oleic or linolenic acid), or immobilized yeast on solid substrates (Kordjazi and Ajji, 2022). Most commercially available oxygen absorbers rely on iron powder oxidation (Jain et al., 2025), and the use of oxygen-absorbing materials is typically indicated on product labels (Gupta et al., 2024). The main disadvantages of these solutions are their relatively low efficiency and the prolonged activation time required the system to become effective. Additionally, food legislation in certain regions restricts or prohibits the use of chemical oxygen absorbers. Consequently, alternative or complementary strategies are needed to reduce residual oxygen in packaged products. Such methods must comply with regulatory requirements for safety and quality while meeting the expectations of increasingly demanding consumers (Jain et al., 2025).

The potential of microorganisms to reduce residual oxygen in MAP has been demonstrated and described in patent WO 2007/057026 A1. The microbial oxygen "absorber" consists of microorganisms that consume oxygen while producing minimal or no metabolic by-products such as carbon dioxide or organic acids. According to this invention, such microorganisms can be applied in packaged food products.

An example is the *Lactococcus lactis* strain with a defect in the pyruvate dehydrogenase and/or lactate dehydrogenase complex, as detailed in patents EP 0937774 and EP 0928333. This strain exhibits oxygen absorption due to a natural mutation that alters its metabolism, resulting in the loss of lactate dehydrogenase activity and enabling active oxygen uptake without lactic acid formation.

Company Novonesis has developed and commercially implemented a starter culture capable of absorbing residual oxygen in the packaging of meat products and other food items. This innovation enhances the preservation of a wide range of products, particularly ready-to-eat meat products and prepared meals, including those produced using sous-vide technology.

By employing diverse strategies to minimize residual oxygen in packaging, meat product manufacturers can enhance the microbiological safety and shelf life of ready-to-eat products (Gupta, 2024).

Quorum Sensing: recent insights and evolutionary aspects

Quorum sensing (QS) is a bacterial cell-to-cell communication mechanism that relies on the synthesis and detection of small signalling molecules known as autoinducers. This system enables microorganisms to monitor population density and coordinate the expression of genes that confer collective benefits only when a sufficient number of cells are present. Such QS-regulated processes include biofilm development, production of virulence factors, sporulation, and genetic transformation (Miller and Bassler, 2001; Waters and Bassler, 2005).

However, recent studies challenge the universality of this paradigm, emphasizing that autoinducer concentrations are strongly influenced by environmental factors, rendering population density estimates unreliable (West et al., 2012). An alternative perspective proposes quorum sensing as a mechanism of collective environmental sensing, wherein bacteria exchange information about surrounding conditions rather than merely assessing cell numbers. Moreno-Gámez et al. (2003) developed a theoretical model demonstrating that QS can function as a distributed system for environmental perception, enabling microbial communities to integrate and disseminate information at appropriate spatial scales through a form of "collective intelligence". This approach explains why QS regulates not only shared resources (exoenzymes) but also individual functions that are useful to a single cell regardless of the behavior of neighboring cells (Schuster et al., 2017). Evolutionary models suggest that the development of collective sensing is facilitated by local cell interactions and limited signal diffusion. Spatial organization at the micron scale promotes the formation of bacterial clusters, thereby enhancing the efficiency of signal exchange and coordinated responses. Conversely, excessive diffusion or pronounced environmental heterogeneity reduces the adaptive significance of QS, as signalling molecules become less informative and collective coordination is weakened (Moreno-Gámez et al., 2023).

Biological hurdles and color retention of meat products

Studies investigating the microbiota of packaged meat products have revealed a correlation between microbial activity and color changes during storage. Li et al. (2019) reported noticeable discoloration in vacuum-packed bacon after seven days of refrigerated storage and identified *Leuconostoc* and *Lactobacillus* as the dominant spoilage-associated genera.

Peirson et al. (2003) reported visible greening in slices of Bologna sausage, with the degree of discoloration linked to the presence of *Weissella viridescens*, *Aerococcus viridans*, and *Carnobacterium viridans*. Furthermore, recent literature highlights that heat-resistant spore-forming bacteria, particularly *Bacillus subtilis*, adversely affect the color stability of vacuum-packed cooked sausages, posing a significant challenge to product quality during storage (Guerra et al., 2023).

The growing scientific interest in the bacterial ecosystem of meat products (Wu et al., 2024) was stimulated by an unexpected observation: certain cooked hams retained their characteristic color throughout their shelf life, even under conditions of microbiological spoilage (Wu et al., 2023). Remarkably, the color remained stable and unaffected by light exposure, consistently exhibiting a pink hue. Pigment analysis revealed the simultaneous presence of Zn-protoporphyrin IX (ZnPP) and nitrosomyoglobin. Notably, ZnPP – formed by the substitution of iron in the heme group with zinc – demonstrated exceptional resistance to light and heat, distinguishing it from other known myoglobin derivatives (Wakamatsu, 2022).

Numerous studies have examined the mechanism of ZnPP formation in nitrite-free hams, as evidence indicates that nitrite and nitrate inhibit its synthesis (Higuero et al., 2020). Furthermore, Asaduzzaman et al. (2020) identified bacterial strains capable of promoting high levels of ZnPP in minced meat homogenates, resulting in the preservation of a brighter red color for up to 14 days. Even after thermal treatment at 75 °C for 15 min, the color of cooked meat remained relatively stable, with residual ZnPP concentrations significantly higher than those in uninoculated controls. Despite these findings, the biochemical pathway underlying ZnPP formation remains incompletely understood. The potential involvement of endogenous and bacterial ferrochelatases (FECH), along with non-enzymatic reactions influencing iron and zinc incorporation into the porphyrin ring, has yet to be fully elucidated (Wakamatsu, 2022).

Therefore, Wu et al. (2024) proposed three conceptual models for ZnPP formation: (a) non-enzymatic model: ZnPP forms through a progressive reaction between Zn(II) ions and protoporphyrin IX during processing and storage; (b) endogenous enzymatic model: ZnPP formation occurs via an enzymatic pathway prior to complete thermal processing, likely resulting from structural modifications of endogenous ferrochelatase (FECH) that alter its catalytic activity; (c) combined enzymatic–microbial model: Certain spoilage-associated microorganisms exhibit elevated FECH activity, facilitating zinc incorporation into protoporphyrin IX during ham deterioration.

The studies conducted by Wu et al. (2023, 2024), which aimed to investigate storage conditions and quality changes in cooked hams, as well as identify key factors influencing pigment transformation associated with the substitution of iron by zinc in heme, do not fully elucidate the mechanisms linking ZnPP formation to variations in nitrosomyoglobin content. Moreover, aspects related to lipid and protein oxidation, along with shifts in the product's microbiota, remain insufficiently explored. Consequently, future research should focus on elucidating ZnPP formation in cooked meat products containing nitrite, determining factors that promote ZnPP synthesis under predominantly non-enzymatic conditions, and clarifying the dominant role of microorganisms and their contribution to ZnPP formation.

Conclusions

Bioprotection is key modern strategy that contribute to the sustainable production of food. Harnessing microbial competition mechanisms inherent to natural microbial ecosystems offers additional opportunities for the development of safe products without the use of chemical preservatives or with their minimal application. This, in turn, promotes the extension of shelf life for substantial volumes of food products, supports the preservation of raw material resources, and has a positive impact on consumer health. The use of microbial competition is not a new approach in food technology, as clearly demonstrated by fermented products. In this context, bacteriocins, which are natural antimicrobial compounds, may serve as an important additional component of the meat product ecosystem when combined with starter cultures that form the dominant microbiota. Lactic acid bacteria are capable of producing a wide range of compounds with antimicrobial activity; however, it should be noted that the concentration of individual metabolites might sometimes be insufficient for effective inhibition of undesirable microflora. Moreover, there is a need for a deeper understanding of the biological characteristics of microorganisms responsible for spoilage of cooked sausages in order to elucidate the mechanisms of action of bioactive antimicrobial compounds. An important aspect is also to clarify the role of peptides with pronounced antifungal activity that are not produced directly by lactic acid bacteria, but may be generated through proteolysis of plant- or animal-derived proteins present in the matrix.

There are several questions remain that require further investigation to enable the effective use of microorganisms as tools of competitive interaction and bioprotection in cooked meat products. In particular, promising directions include the development of new methods for identifying bacteriocinogenic strains and the experimental validation of their efficacy in specific food matrices and under relevant production conditions.

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