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ABOUT THE JOURNAL

The journal "Theory and practice of meat processing" is an international peer-reviewed scientific journal covering a wide range of meat science issues.

We publish original results of the fundamental and applied research in the field of:

- regulation of feeding rations and keeping conditions of animals (including poultry);
- targeted modification (selection, hybridization, operative manipulation);
- processing of meat raw materials;
- improvement of technologies for meat product manufacture;
- study of effects of meat and meat product consumption on human health;
- rational use of secondary resources of the meat industry and the ecological problems of the industry

The journal "Theory and practice of meat processing" publishes scientific and review articles, reports, communications, critical reviews, short research communications (letters to the editorial office), informative publications covering themes from the raw material base of the meat industry to innovative technologies of meat processing and food production, including the use of food biotechnology, from quality and safety management and risk analysis to information technologies, modeling in the meat industry as well as trophological chain management, marketing, economics, consumer demand analysis.

The primary objectives of the journal "Theory and practice of meat processing" are to present, preserve and distribute results of the new scientific studies in all directions of the research activities in meat science; create a favorable information environment to support the innovative development of the fundamental and applied research in the meat and poultry processing industries; attract attention to promising and relevant directions of the meat science development.

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THE POTENTIAL OF ARTIFICIAL INTELLIGENCE IN THE MEAT INDUSTRY

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Keywords: *artificial intelligence, new technologies, machine learning, neural networks, computer (machine) vision, food product, authentication, identification*

Abstract
This review considers the potential of artificial intelligence (AI) technologies in meat science and the meat processing industry, including its application in livestock and poultry farming, meat production, sensory evaluation, and personalized nutrition. The review presents approaches to classification of AI technologies used in the food industry and provides their characteristics, description of their constituent components, technical concepts and practical applications. AI is an important tool of support in the food industry and animal husbandry. The review thoroughly examines the application of AI in processing plants: 1) for quality control and sorting (computer vision); 2) for food safety improving (machine learning); 3) for optimizing the production lines (forecasting analytics), as well as in animal husbandry: 1) real-time health monitoring; 2) supervision over the animals' living conditions; 3) feeding optimization. In addition, the review pays special attention to AI using for authentication, identification, classification, and forecasting of the meat products. The development of technologies and the expansion of AI application scenarios in the meat industry will keep expanding. However, despite the significant benefits of AI applications, the article highlights several issues, challenges and limitations that AI encounters, such as privacy and security issues, technical complexity, and integration with the traditional methods of food processing. Nevertheless, technology of artificial intelligence possesses great potential in livestock farming and meat processing for increasing productivity, ensuring product quality and safety, and streamlining management. AI's potential will enable more efficient, safe, and sustainable development to provide consumers with high-quality food products.

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Introduction

Nutrition science is a field of knowledge that encompasses multiple scientific fields, including chemistry, physics, biochemistry, microbiology, technology, and food engineering, among others fields, it also offers potential for interdisciplinary research in agriculture, pharmaceuticals, medicine, and economics. In recent decades, a complementary combination of research in food science and technology, as well as the food industry with artificial intelligence, has been rapidly developing. Artificial intelligence plays a significant role in the modern food processing cycle, from the primary processing of agricultural raw materials to the analysis of quality and safety indicators of food raw materials, products, and packaging [1].

Artificial intelligence (AI) is nowadays permeating every aspect of our lives, and meat production is no exception. AI is revolutionizing the food industry by optimizing technological processes, improving food quality and safety, encouraging introducing of innovation into food production [2,3]. In terms of rapid growth of global food consumption, the use of AI algorithms in food processing from farm to mouth promises to ensure both sustainable production and greater efficiency.

AI has become a transformative force in many industries, with its efficiency particularly noticeable in the food industry [4–6]. The study [4] reviewed the ability of AI to address the negative impacts of population aging on economic growth and industrial structure. The rapid development of AI offers new solutions to cope with labor shortages caused by population aging. Using data from 30 provinces in China, the authors analyzed the influence of population aging on food security and the role of AI, thus coming to conclusion that the application of AI is beneficial for food security.

The studies [5,6] summarize the most up-to-date technologies of Food Industry 4.0 and discuss the understanding of new trends in the food sector that have appeared as a result of the technological revolution. It is noted that technologies of Industry 4.0 have significantly changed the food industry and led to significant consequences for the environment, economy, and human health. Despite the importance of each and every of the above-mentioned

technologies, the true revolutionary sustainable solutions can only emerge through the simultaneous integration of many technologies. The era of Food Industry 4.0 features new challenges, opportunities, and trends that have changed current strategies and prospects for food production and consumption models, thus having paved the way to the Industry 5.0.

The historical development of AI in the food industry is shown in the Figure 1.

Initially focused on automating simple tasks, artificial intelligence has evolved into the advanced machine learning, forecasting analytics tool and integrated systems for real-time decision-making. Integrating AI technologies into food production, safety, supply chain management, and quality control can significantly improve efficiency, accuracy, and sustainability. As the global food industry faces increasing pressure caused by population growth, resource deficit and demand for higher food safety and quality standards, the implementation of AI-based solutions offers promising opportunities to cope with these challenges [7].

According to the analytical report of the Federal Center for Applied Development of Artificial Intelligence that functions under aegis of the Ministry of Industry and Trade of the Russian Federation [8], the experience of implementing AI solutions as of 2024 distributes in the following way: 35 % of enterprises have experience using AI solutions; 40 % do not use AI solutions; 25 % are at different stages of implementation. The Center's analysts note that the main advantages of implementing AI solutions are related to: automation and optimization of processes (20 %); improved quality of the products and services (18 %); increased efficiency and productivity (17 %); expenses lowering and cost reduction (13 %); improved decision-making process (10 %); reducing human factor influence (7 %); acceleration of business processes (7 %); control over the staff's tasks (4 %); reduction of injuries (4 %). Despite significant advantages, there are also disadvantages to the implementation of AI solutions. This is mainly explained by the high costs of AI implementation and operation (23 %) and technical difficulties (18 %).

According to the definition of S. J. Russell and P. Norvig in the book Artificial Intelligence: A Modern Approach "The field of artificial intelligence, or AI, is concerned not only with understanding but also with creating intelligent objects — machines that can figure out how to *act effectively and safely in a wide variety of new situations*" [9].

Artificial intelligence includes: 1) machine learning, which focuses on developing algorithms that enable computers learning from data and make forecasts or decisions without explicit programming. By applying machine learning models, patterns in food safety can be established to detect risks of contamination and optimize storage conditions [10]; 2) deep learning, a subdivision of machine learning based on neural networks with many layers included; 3) natural language processing (NLP) deals with the interaction between computers and people through natural language. By analyzing customers' feedback on food safety, it helps producers (retailers, marketers) improve quality and solve safety problems [11]; 4) computer vision (or technical vision). The creation of machines that identify and classify objects visible in images and videos. With the use of computer vision, automated checking for

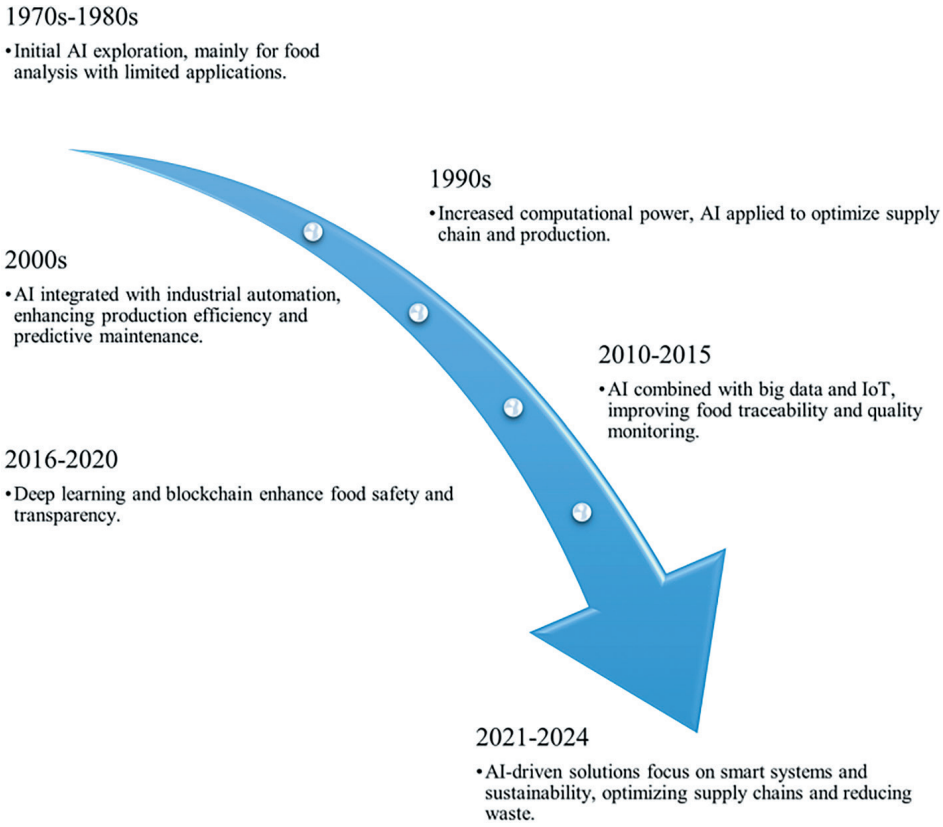

Figure 1. Historical development of AI in the food industry [7]

presence of contamination, checking food quality and labeling accuracy occurs, thus reducing the number of errors related to the human factor [12]; 5) robotics. Integrating AI into physical devices to perform tasks in the real world; 6) expert systems — programs that simulate solving of a problem that requires human expertise in a given field. They use knowledge bases and inference rules to make decisions; 7) agent-based systems — models where agents (software entities) can act autonomously, on their own, in an environment, making decisions based on their observations and goals.

The concept of AI is inseparably linked with the following technologies:

1) Big data is the vast quantity of structured and unstructured data required to train many AI models, especially to machine learning and deep learning. By analyzing big data, food companies can better understand market demands, optimize supply chains, reduce waste, and improve production efficiency. By combining big data technology with blockchain, it is possible to ensure traceability of the food products origin and quality, ensure transparent tracking from field to table, and provide the consumers with reliable, versatile information on the product [13,14];
2) Cloud computing provide the computing power and infrastructure required to train, test, launch and run complex AI models;
3) The Internet of Things (IoT) — sensors and devices that generate data that is analyzed by AI. They enable real-time monitoring of temperature, humidity, and freshness to ensure food quality during its storage and transportation [15].

Artificial neural networks (ANN) are a well-known mathematical tool that has recently been widely used and now being tested to solve problems related to meat production and meat processing technologies. Their advantages include their capability to handle nonlinear data, highly correlated variables, and the potential for problem detection or classification. Specifically, promising applications of ANNs in the meat sector include carcasses classification, quality control and safety control of raw materials and finished products, meat processing, spoilage prevention, ensuring freshness and shelf life assessment, off-flavor detection, authenticity assessment, and many more other functions.

Artificial intelligence and machine learning technologies have now moved from the experimental development stage to integral components for optimizing agricultural processing and food production. Initially, AI technologies were developed to analyze complex data sets, predict consumer behavior, and improve production efficiency. The integration of AI and machine learning in the food industry continues to expand, offering significant opportunities to address quality assessment and control issues, improve food safety, and enable future innovation. However, it is important to consider the potential drawbacks of excessive reliance on AI and machine learning in food production, such as the risk of human jobs losses and the possibility of errors or biases in algorithmic decision-making process. Furthermore, the cost of implementing and maintaining AI technologies may be prohibitive for some small-scale producers of food [16].

With the constant advancement of technology and the expansion of its application scripts, the prospects for AI application in the food industry will keep expanding. It is becoming an important tool for digital transformation and modernization of the food industry, thereby enhancing its competitiveness.

Effective use of AI requires solving of long-term issues, including data protection, complex integration, ethical questions, and staff development. Artificial intelligence offers the potential for supply chain integration, production of personalized nutrition, and implementation of sustainability initiatives, even with initial capital expenses. To scale AI for food industry optimization in the future, balancing ethical obligations with technological advances, effective collaboration of various concerned parties is crucial.

A summary of the literature references shows that the primary goal of AI is to develop systems capable of understanding natural language, identifying patterns, and autonomous adapting to new knowledge. While significant progress has been achieved, ongoing research consistently refines algorithms and strategies to widen the capabilities of artificial intelligence in any fields [16].

The purpose of this review is to systematize the various applications of artificial intelligence in the food industry, with a primary focus on the meat industry, highlighting the potential of artificial intelligence in the field of cattle carcasses classification, automation of various technological operations in meat production, detection of food adulteration, ensuring traceability and determination of meat quality and safety with higher accuracy, the use of machine learning in cultured meat technology, and other areas, while simultaneously encouraging the formation of technological innovations and sustainable development of the meat industry.

Objects and methods

The bibliographic analysis included two stages: (1) collecting research publications relevant to the topic and to the purpose of the study; and (2) detailed review and analysis of the scientific information presented in these publications.

In the first stage, from April to November 2025, a keyword-based search was run for the articles published in English and Russian between 2011 and 2025. The scientific databases Scopus, IEEE Xplore, and ScienceDirect were the sources, as well as the scientific indexing web services RINTS and Google Scholar. These databases were selected to provide a sufficiently large initial tray of samples of the relevant articles. The publication selection process began with an assessment of the titles and abstracts content.

Duplicate references obtained from different databases were ignored. The following keywords were used in the search query: "artificial intelligence", "convolutional neural networks", "machine learning", "deep learning", "computer vision and image processing", "livestock and poultry farming", "meat production", and "food quality and safety." The general term "food safety" was included into the search strings, despite the fact that the study focused on the use of AI technologies in livestock farming and meat production. This was caused by the difficulty of clear definition of the search strings related to these specific aspects of meat technology without excluding too many relevant articles. Using more general terms allowed us obtaining a larger set of references, which in the next step were screened for relevance to the research topic.

Thus, the studies referencing to the use of AI technologies but not related to the food industry, specifically meat processing, were filtered off. This activity initially found 417 articles. By limiting the search to the appropriate application of AI technologies in the food industry, with specific reference to the meat industry, and substantive conclusions, the initial number of articles was reduced down to 142.

To ensure a thorough and transparent review of the existing literature on the application of AI in food systems, a systematic approach was adopted, based on clearly defined criteria of inclusion and exclusion. These criteria ensured that only high-quality and relevant studies were considered.

The following inclusion criteria were used to confirm the required application:

1. Using AI-based methods and/or approaches as a method for solving the problem under study.
2. Solution of the problem and/or task most related to the agro-industrial complex the following areas: the use of artificial intelligence in livestock and poultry farming, meat product tracking, authentication and detection of meat product adulteration, meat product production, including cultured meat, assessment of its quality and safety, establishment and control of expiration dates and packaging of food products.
3. The studies considered applied artificial intelligence technologies such as machine and deep learning, computer vision, convolutional neural network, artificial neural networks, random forest (RF) method [17] to optimize food systems.
4. Systematization of practical results using some clearly defined performance indicators that indicate the success (efficiency) of the AI method used in the agro-industrial complex.

Exclusion criteria:

- Studies that lack a clear methodology or sufficient data to replicate the results.
- Articles not published in peer-reviewed journals or that exhibit a high degree of bias, thereby compromising the validity of their findings.

In the second stage, the 142 papers selected in the first stage were sequentially analyzed, taking into account the following research questions: (a) the problem they reviewed, (b) the approach used, (c) the data sources used, and (d) general accuracy. Information was also systematized according to the following areas: (e) whether the authors compared their AI- based approach with other methods, and (e) whether the efficiency of the developed method was assessed in comparison with others. It is also taken into account that the use of various artificial intelligence technologies (methods) in the food industry allows for the implementation of specific tasks of a specific research area to ensure its maximum effectiveness.

Technologies of artificial intelligence using in the food industry

Classification of AI technologies used in the food industry

Currently, advanced technologies, artificial intelligence (AI), and especially machine learning (ML), are being actively implemented in various industries and services. Food science and technology and related issues are no exception. Improving agriculture, maximizing the utilization of agricultural raw materials, developing new food products and nutraceuticals, more accurate sensory evaluation, industrial processing, improving food quality, ensuring food safety, supply chain management, waste recycling, and, finally, calorie and nutrient assessment are some of the achievements of the combination of food science and artificial intelligence (AI) [1].

To assess the potential of AI in food industry technologies, AI was classified taking into account the main approaches, methods and components (Figure 2).

I. by capabilities level:

1. specialized (narrow) AI (Artificial Narrow Intelligence, ANI, or Weak AI). The specialized AI is designed to solve one specific problem or some narrow range of problems. It currently dominates. Examples of specialized AI use cases include facial recognition, voice assistants, recommender systems, chess / Go game, spam filters, autopilots. This type of AI does not possess general consciousness, self-awareness, or the ability to apply knowledge outside its specialization;
2. general AI (Artificial General Intelligence, AGI or Strong AI). It is hypothetical AI that features intellectual abilities comparable to those of humans. It is capable to understand, learn, and apply knowledge to any intellectual task;
3. superintelligence (Artificial Superintelligence (ASI). A hypothetical AI that significantly exceeds the intellectual abilities of the best human minds in virtually all areas, including scientific creativity, general wisdom, and social skills.

II. by functionality and approach:

1. Reactive Machines. Basic systems: they have no memory and do not use past experience; they respond only to current input data. An example is the IBM chess

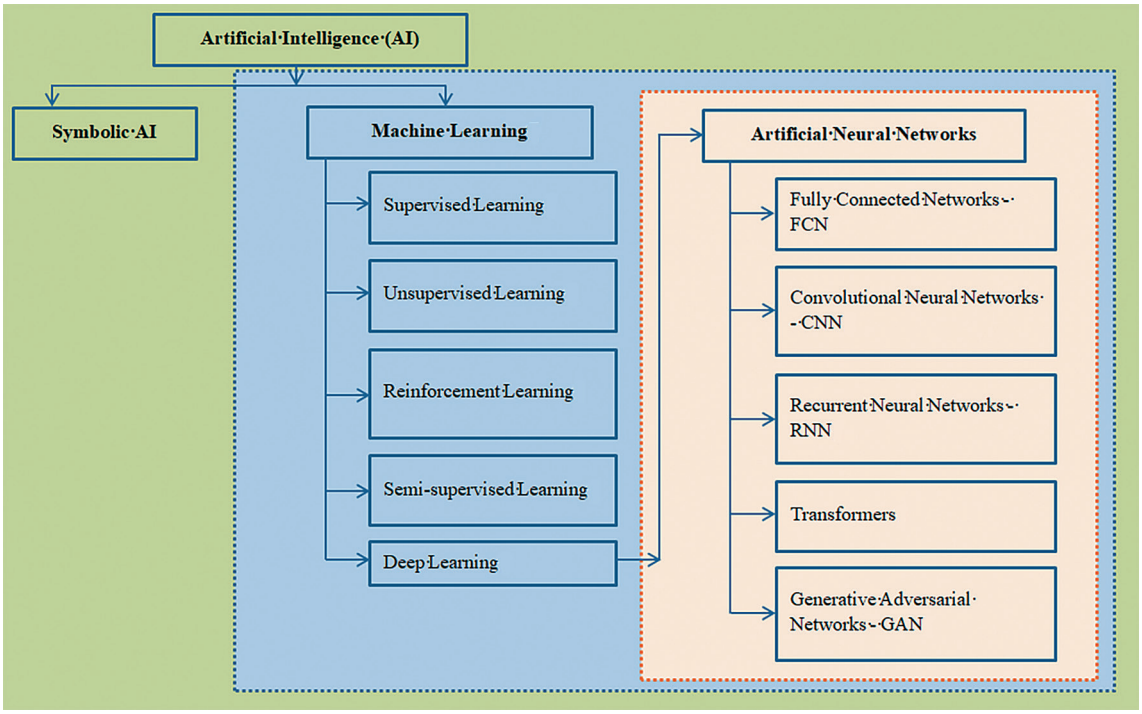

Figure 2. Artificial intelligence (AI) classification split into main approaches, methods, and components

computer — Deep Blue — who won over Kasparov. It analyzed the current position on the board;

2. Limited Memory systems use recent experience (data) to make decisions, it possesses short-term memory. Most modern systems are based on Machine Learning (ML), including self-driving cars (which are capable to analyze recent data obtained from sensors), chatbots (taking into account the context of the dialogue);
3. Theory of Mind. The hypothetical AI capable of understanding the emotions, intentions, beliefs, and thoughts of other beings (people, other AI), and which features forecasting behavior.
4. Self-Awareness. The hypothetical AI that is conscious, self-aware, understands its own state, and is able to forecast the feelings of others.

III. by techniques and methods (technical classification):

1. Symbolic AI or Rule-Based AI. In early approach — it uses written logical rules and symbolic (fact) manipulation to represent knowledge and make decisions. It is a component of expert systems, programming logic (Prolog), and simple decision trees.
2. Machine Learning (ML). A key component of modern AI. The algorithms are used that allow computers to learn from data without being explicitly programmed for each task. They identify patterns and build models for forecast or for making decisions. The following types of machine learning exist: a) Supervised Learning — the algorithm learns from labeled data (there are "correct answers"); b) Unsupervised Learning — an algorithm that searches for hidden structures (patterns) in unlabeled data; c) Reinforcement Learning (RL) — the algorithm (agent) learns by interacting with the environment, receiving "rewards" or "penalties" for actions; d) Semi-Supervised Learning is a combination of labeled and unlabeled data.

   Machine learning includes algorithms such as linear regression, support vector machine (SVM), Random Forest, k-nearest neighbors (KNN), k-means, DBSCAN (Density-Based Spatial Clustering of Applications with Noise), etc.
3. Deep Learning (DL). A subset of ML that uses artificial neural networks (Artificial Neural Networks (ANNs) with many layers ("deep" networks) for learning data representations at increasingly higher levels of abstraction.

   There are the following types of ANN: a) Fully Connected Networks (FCN). The basic type, each neuron in a layer is connected to all neurons in the next layer; b) Convolutional Neural Networks (CNNs) are optimized for processing grid data (images, videos). They use convolutions to detect the local patterns; c) Recurrent Neural Networks (RNNs) are designed for sequential data (text, speech, time series). They have a "memory" of previous inputs; d) Transformers. The modern architecture that dominates in Natural Language Processing (NLP) uses the attention mechanism to analyze the dependencies between all sequence elements, regardless of distance; d) Generative Adversarial Networks (GAN). Two networks (generator and discriminator) compete, which allows generating new, realistic data (images, music, text); e) Autoencoders. Networks for learning the efficient data representations (compression, noise reduction) or generation.

   Evolutionary Algorithms. Optimization methods inspired by biological evolution (selection, mutation, crossbreeding). They are used to solve problems where traditional methods are ineffective.

5. Natural Language Processing (NLP) is a field of AI that deals with the interaction between computers and human language. It utilizes methods from machine learning (especially deep learning) and linguistics.
6. Computer Vision (CV). The field of AI that enables computers to "see" and understand visual information (images, videos) relies on CNNs and other deep learning methods.
7. Robotics. The application of AI to control physical robots. It combines CV, NLP, ML (especially RL), motion planning, and reactions to sensors' signals (sensorics).
8. Expert Systems. An early successful form of AI that uses a knowledge base (rules and facts) and a logical inference engine to solve problems like a human expert in a narrow subject area. It is considered a symbolic AI.

One of the dynamically developing areas for the researchers, scientists and experts in recent decades has been the mutually complementary combination of researches in food science and technology, as well as food industry with artificial intelligence [1,18].

The following AI methods are most actively used in the food industry.

Machine learning

Artificial intelligence is a broad field that models human intelligence processes using machines and computers and develops systems to perform tasks that typically require human intelligence. Machine learning (ML) is a subset of artificial intelligence that enables machines to automatically learn from given data by implementing statistical algorithms and models [1]. Machine learning and automation have become widespread in the field of food safety and quality assurance. Automation combined with artificial intelligence is used for forecasting of crop yield, customers' demands analysis, sorting, quality control of food samples, and many more tasks [19].

Machine learning is divided into four categories: supervised learning, unsupervised learning, reinforcement learning, and representative learning. Supervised learning: Forecast for unobserved points is performed using supervised learning [20,21]. Machine learning has demonstrated its effectiveness in forecasting sales and the amount of food waste generated [21]. Risk assessment of various natures is a decision-making tool in many fields, including the food industry. Developers are trying to incorporate genetic information into the modeling system using machine learning to reduce uncertainty in modeling food safety risks. This supervised learning technology enables forecasting the severity of diseases caused by pathogens such as *Salmonella* and *Listeria* based on genome sequencing data. The author was able to extrapolate clinical cases of foodborne *listeriosis* contamination using multilocus typing of the whole genome sequence [20]. In particular, machine learning is a powerful tool for assessment of food safety risk, assisting in the automatic recognition and classification of food defects, spoilage and shelf life forecast, risk pattern recognition, and rapid detection of food ingredients and hazardous materials [22,23]. Due to the interdisciplinary nature of food-related research, machine learning for food covers various research areas, such as image recognition, ingredient analysis, supply chain optimization, and taste forecast [24].

In [25], a non-invasive and non-destructive automated quality control system based on computer vision and ensemble machine learning methods is proposed for the meat industry. The system determines all physicochemical, textural, and sensory quality characteristics of pork and beef tenderloin in four meat states (fresh, defrosted, cooked, and cured) with high accuracy (0.74 for pork loin, 0.76 for beef loin), thus representing a real alternative to the traditional methods for the food industry processes.

Deep learning

Deep learning is a branch of machine learning. It uses ANN (Artificial Neural A neural network (also known as a deep learning network) is designed to simulate the human brain and is built on a deep hierarchy of layers that solve complex problems. This enhances the capabilities of supervised and unsupervised learning algorithms to solve complex real-world problems by adding various processing methods [20]. Deep learning is used for determination of a range of food characteristics, such as determining the quality of fruits and vegetables, estimating calories content, and so on.

Sensory evaluation plays an important role in determining the quality of food products. Therefore, researchers are interested and willing to invest more effort in this area to forecast sensory characteristics and eating conditions using traditional data processing methods such as PLSR, SVM, and neural networks. Machine learning methods are used to establish the relationship between texture, sensory characteristics, and physicochemical parameters of food products [20]. Research on sensory characteristics is based on traditional data analysis methods, including extraction of features from images, statistical analysis, etc. Deep learning is rarely used for features analysis [26].

The study [27] presents a comparative analysis of the efficiency of various machine learning algorithms based on raw data obtained from the analysis of organoleptic, sensory and nutritional properties of meat, for differentiating categories of commercial lamb from the local Spanish breed (*Mallorquina breed)* obtained in the following production systems: milk-fed lambs; pasture-raised lambs; light breed lambs; and grain-fed pasture lambs. Six machine learning algorithms were evaluated: artificial neural network (ANN), decision tree, k-nearest neighbors (KNN), naive Bayes algorithms, multinomial logistic regression, and support vector machine (SVM). The results of this study demonstrated that machine learning is a useful tool for classifying commercial lamb carcasses. Accuracy in the assessment of the organoleptic and sensory datasets was 88 %, in the nutritional dataset it was 83 %, and in the combined datasets it reached 88 %.

In the study [28], the integrated systems for real-time food freshness measurement were developed to improve the reliability, safety, and sustainability of food supplies. The system combined a metal-organic mixed-matrix framework membrane with deep learning technology. Four state-of-the-art deep convolutional neural networks were used to recognize color changes, thus enabling highly accurate freshness assessment. In a simulation test for assessing chicken freshness, an accuracy of up to 98.95 % was achieved via using the WISeR-50 algorithm.

In the article [29] an open database of Chinese food images with nutrient data was represented. The database got the name ChinaFood-100, it contains 10,074 images systematized into 100 categories of popular Chinese dishes, including staples, meat, fish, and vegetable dishes. The database structure includes two main groups: staples and dishes. For each image of a single dish, detailed information on the content of 23 food nutrients is provided. The data is based on the official document "Dietary Guidelines for the Chinese Population", which ensures compliance with national standards and the scientific validity of the annotation to the dish image. The ChinaFood-100 database is a structured, annotated, and scientifically valid resource designed to support research at the intersection of computer vision, dietetics, and food habit analysis, with an emphasis on Chinese cooking specifics. Based on this database, four state-of-the-art deep learning neural network architectures were studied. The results showed that Inception V3 achieved the highest image recognition accuracy, reaching 78.26 % and 96.62 % for top-1 and top-5 accuracy, respectively, while the lowest score was achieved using VGG. The results of ROC and mAP showed that the diversity of food images in each category significantly impacted the model's performance. The authors suggested that increasing the image sample size in some categories with low recognition accuracy (e.g., braised pork leg (#66), fried minced celery (#87)) are able to improve image recognition accuracy in the future.

Computer vision and image processing

Computer vision systems (CVS) is an important branch of artificial intelligence that studies how computers receive, process, analyze, and understand digital images, videos, and other visual data to enable computers to mimic the human visual system.

Computer vision is the acquisition and processing of images. The past decade has witnessed a significant growth in machine vision-based researches in the food industry. Machine vision-based food image analysis has become an important research area aimed at solving complex problems in the food industry, providing broad development prospects for scientific research to solve food safety and quality issues, food system development, and food processing. Machine vision-based image recognition refers to the classification and recognition of images with the help of machine vision technology, in which various objects in some image are distinguished into certain types and get category label to identify the object, function, or content in the image. The workflow of image recognition can be divided into the following steps: image receiving, image preprocessing, image segmentation, features extraction, image recognition classification, models training and optimization, and models testing and evaluation [30].

Machine vision (MV) is a technology that automatically extracts information from images via a computer. Machine vision, a synonym for computer vision, refers to the use of a device for contactless optical sensing and computational processing for automatically receiving and interpreting the image obtained. The goal of the technology is to match the functions of human vision through electronic perception and evaluation with the help of images. MV systems offer extensive capabilities for automating manual assessing, standardizing methods and eliminating the tedious task of data verification by human. These systems work by capturing an object image, like a piece of meat, processing the image to measure desired parameters, and comparing these parameters with predetermined inspection criteria, helping to make decisions about corrective actions for the object itself or production process. The most significant advantage of meat inspection with MV systems is its non-destructive nature when examining a meat sample. Recently, there has been significant growth of MV application in various fields, such as: poultry carcasses inspection, weight forecast, beef color determination, meat tenderness forecast, and chemical component forecast of meat and meat products. However, certain disadvantages of this method should be noted. MV technology requires uniform illumination and calibration. It is often challenging to separate overlapping objects from the background when it is necessary to assess both sides of a meat product [31].

The study [32] explored the feasibility of using computer vision technology to improve traceability and quality control in the red meat industry. Extensive experiments with beef steaks (602 pcs) demonstrated that internal meat characteristics can serve as reliable identifiers for traceability while simultaneously providing automated quality assessment. The developed quality forecast module, based on the EfficientNet model, achieves high accuracy in forecasting marbling scores (96.24 % top-1 ± 1, 99.57 % top-1 ± 2), breed identification (91.23 %), and cattle diet determination (90.90 %).

Consumers often struggle to assess the organoleptic qualities of meat, which are influenced by tenderness and intramuscular fat. In study [33], the computer vision system (CVS) was developed which uses smartphone images to determine the tenderness of beef and pork steaks (1), predicts shear force and intramuscular fat content (2), and runs a comparative analysis between consumers' ratings and the results of the method (3). When classifying beef as tender, the model achieved an accuracy score (F1) of 68.1 %. After changing the dataset category to "tender" and "tough", the F1 score for tenderness increased to 76.6 %.

For pork loin tenderness, the model achieved an F1 score of 81.4 %. This score improved slightly to 81.5 % after splitting into two classes.

Fuzzy Logic Technique (FLT)

FLT is widely used in industry due to its ease of use and rapid and accurate problem-solving capabilities. By controlling human thinking in linguistic terms, FLT is used in the food business to model, control, and classify food products, as well as to solve food-related problems [34]. FLT can analyze factors like temperature fluctuations during transportation, humidity level, and ethylene level to forecast the remaining shelf life of fruits and vegetables more accurately than traditional methods. FLT can be used for sensory evaluation of bread or cakes to assess texture, taste, and appearance, integrating subjective feedback from panels into quantitative data for product development. Fuzzy analysis of the organoleptic properties of food products at various stages of machine operation can be used to optimize processing steps based on desired product characteristics [2]. Fuzzy modeling can be used to optimize process parameters like soaking time, cooking time, frying temperature, and raw material properties (particularly — the slice thickness) in the production of taro chips [35].

AI technologies in livestock and poultry farming

Careful management of farm cattle rearing has become an important means of improving production efficiency in the livestock industry. Monitoring and recognizing livestock behavior are of great importance for the development of precise farming of livestock. For example, individual animal behavior is related to the amount of water and feed consumed and is important for improving animal productivity. The social behavior of animals can provide important information about their well-being; for example, aggression between pigs can cause skin lesions, infections, and even lead to fatal injuries. Feeding behavior, as one of the behaviors of sows during lactation, is crucial for the early survival and growth of their piglets before weaning, which has a significant impact on the economic benefits of pig farms. Furthermore, the movement of animal body parts can be used to detect diseases; for example, clinical lameness provides a significant impact on milk yield and reproductive function. Assessing animal's posture is a key step in analyzing its behavior and assessing health, and therefore is of great importance for rational livestock breeding [36].

However, despite the growing demand for smart farm management solutions, the implementation of advanced technologies like artificial intelligence and big data to improve livestock productivity is relatively slow compared to other sectors such as healthcare, infectious diseases surveillance, and production. The importance of such AI-related research and development is underestimated, and additional efforts are required. The combination of artificial intelligence with the end-to-end Internet of Things (IoT), fog computing, and cloud computing will undoubtedly accelerate the development of smart farm management [37].

Traditional manual observation of pig and cattle behavior is labor-intensive, human subjective, and difficult to achieve in continuous and large-scale operations. It is not surprising that computer vision technology, with its advantages of objectivity, non-invasiveness, and continuous operation, has been widely explored for its use in livestock behavior recognition in recent years. However, computer vision technology faces certain challenges in livestock behavior recognition research, such as complex animal interaction scenes, variable lighting, occlusion, touch, and overlap between livestock, thus limiting the rapid transfer of the technology to industry [36,38].

Image segmentation and identification are the foundation of livestock behavior recognition. Therefore, the development of reliable algorithms for livestock identification, behavior recognition at different growth stages, and subsequent quantitative evaluation of behavior recognition results are the foundation for constructing a system for assessing the growth, health, and well-being of animals. The role of image segmentation is to extract target foreground objects from the background, and the effect of image segmentation directly affects the accuracy of features extraction and behavior recognition among the livestock [39]. The role of identification is to determine the identity of each individual in a group, and this identity can localize the recognizable behavior down to a specific animal, thereby implementing the transition from group behavior recognition to recognition of the individual behavior [40].

Accurate forecast of cattle body weight is critical for herd monitoring, assessing biological efficiency, and optimizing feed management. Researchers evaluated a cattle body weight forecast model using morphological data from 465 lactating cows of Holstein breed, including dorsum length (DL), thoracic width (TW), abdominal width (AW), rump width (RW), hip height (HH), body depth (BD), thoracic perimeter (TP), and abdominal perimeter (AP). Spearman's correlation analysis identified the measurements TP (r = 0.89), AP (r = 0.88), and RW (r = 0.80) as the strongest predictors. Simple and multiple linear regression models, artificial neural networks (ANN), and support vector regression (SVR) were tested to forecast dairy cattle body weight. The dataset was divided into 90 % for training (419 samples), 5 % for validation (23 samples), and 5 % for testing (23 samples). The results achieved in this study demonstrated that the use of morphological measurements, even manually collected, allows for the creation of highly accurate forecasting models for estimating cattle body weight based on machine learning methods. In the best simple model using only the TP indicator, R^2 (the coefficient of determination) was 0.7763, and RMSE (root mean square error) was 43.69 kg. The multiple regression model with TP, AP, and RW improved the performance (R^2 = 0.9067, RMSE = 28.00 kg). As a result of the evaluation of the obtained results, it was

noted that although regression models are quite effective, the use of artificial neural network (ANN) (R^2 = 0.9125, RMSE = 25.86 kg) and support vector regression (SVR) (R^2 = 0.9046, RMSE = 27.41 kg) ensures greater accuracy, which enhances their potential for herd management. However, simpler models still remain viable alternatives for their practical application in farms [41].

Measuring animals with a tape measure to determine their weight is quite labor-intensive and causes animals' stress, which leads to weight loss and productivity of 5–10 %. Therefore, computer vision technologies are increasingly being used to assess the live weight of cattle through contactless morphometric measurements, which eliminates the need for linear measurements or weighing cattle on scales. In developing an effective model for forecasting the live weight of cattle, methods were proposed for preprocessing RGB images and a depth map (side view of the animal) and creating a color RGB projection and 2.5D depth map for forecasting live weight based on image regression using deep learning methods. Contactless measurement of live weight of cattle can be used: for an objective assessment of breeding animals during grading; livestock valuation; justification of the further use of young animals, and the development of a technology for assessing the health and productivity of animals in industrial fattening complexes [42].

The well-being and productivity of dairy cows are closely linked to their behavior, with factors such as time of lying and standing, feeding, and movement, which serve as indicators of health and well-being [43]. The main objective of this study was to evaluate and optimize deep learning-based object detection models for automated classification of cattle activity. The authors compared different variants of the YOLOv5 network (v5x, v5l, v5m, v5s, and v5n), learning rates (0.1, 0.01, and 0.001), data pack sizes (4, 8, 16, and 32), and the performance of the optimizer used (SGD and Adam) to identify the most suitable model in terms of accuracy and computational efficiency. The acquired images from a barn with 100 cows were annotated using polygonal masks, and all labeled objects were divided into two classes, cow_stay and cow_lay (Figure 3).

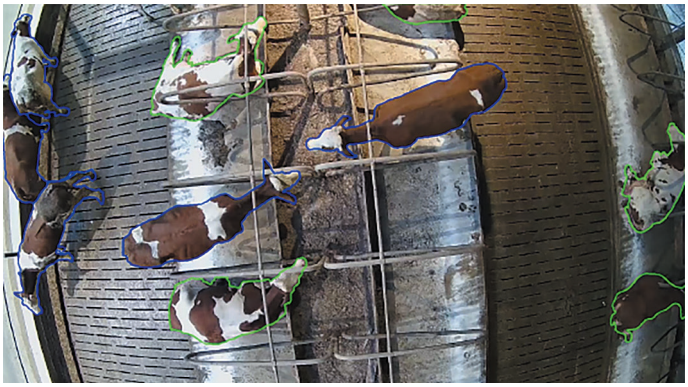

Figure 3. Annotation using polygonal masks with assignment of a specific class (cow laying is contoured with green, and a cow standing is contoured with dark blue) [43]

YOLOv5m showed the best results in terms of time and accuracy optimization, with a ratio of 0.5:0.95 of 0.8969 (compared to 0.9070 for YOLOv5x). The training time for YOLOv5m was 7:48:19, while YOLOv5x required 16:53:27. The highest accuracy (0.9028) was achieved with a learning rate of 0.001, and the lowest (0.8897) with a learning rate of 0.1 [43].

Accurate and timely information on the livestock quantity and location in the farms is difficult to obtain, especially in case of large agricultural enterprises. Livestock counting is primarily done manually, which is labor-intensive and prone to error. Currently, many farmers count their animals approximately once a month, while others only count their livestock when loading or unloading them from trucks, arriving at or leaving the farm. Therefore, many farmers are interested in monitoring their livestock using a reliable automated system. Monitoring the distribution and population of animal species over time is also a key element of successful saving the herd. In this regard, the development of deep learning algorithms and unmanned aerial vehicles (UAVs) is creating a new area of research for remote monitoring and counting of various animal species in various geographic and climatic conditions. In this paper, the authors focused on the detection and counting of sheep in a pen via UAV video. Sheep counting is performed using a model based on regional convolutional neural networks, and the results are then compared with other methods to assess their performance [44].

To improve sheep counting accuracy and avoid mutual occlusion interference caused by different moving speeds among sheep, Chinese researchers proposed a fusion concept between an improved YOLOv5x model based on an attention mechanism and the DeepSort algorithm. The improved, high-precision YOLOv5x model for sheep detection reduces the false detection rate and missed detections. At the same time, the DeepSort algorithm is used to address the problem of sheep that were not counted due to mutual occlusion. Then, in the sorting algorithm, during the target (sheep number) forecast and appearance recognition stages, newly appearing objects after occlusion are distinguished and compared to complete target tracking. In this study, dynamic sheep counting is primarily applied to a single-family sheep farm, where the number of sheep is relatively small, averaging approximately to 50 heads, which is insufficient for generalization to the dataset. Therefore, a dataset-based optimization technique is proposed to address the sample insufficiency problem. That is, low-resolution images collected by a binocular vision sensor are used as input, the SRGAN network model is used for super-resolution reconstruction to generate 500 high-resolution datasets, and then 800 high-resolution images are obtained through autonomous data enhancement and expansion in the form of sheep datasets. The high-resolution images are used as the training dataset of the YOLOv5x-ECA model, which integrates the structure of the ECA channel attention mechanism to adaptively

enhance the channel weight information. In this case, the sparrow search algorithm (Bird Swarm the YOLOv5x-ECA (a bioinspired evolutionary algorithm using swarm intelligence inspired by social interactions and bird flock behavior) algorithm, based on the elite opposition learning strategy, is used to optimize the training speed of the detection model to obtain optimal group weight information for further improvement of performance in sheep recognition. In the experiment, 800 high-resolution sheep images augmented with the SRGAN network and data augmentation are used as the model datasets, and weight information obtained by the YOLOv5x-ECA-SSA* model is used to accurately recognize sheep. According to the DeepSort algorithm, recognized sheep are tracked, forecast, and matched as efficiently as possible. The experimental results show that the testing accuracy of YOLOv5x*, YOLOv5x-ECA*, and YOLOv5x-ECA-SSA* based on SRGAN and training data improvement are 95.74 %, 96.50 %, and 97.10 %, respectively. The error rate of each model when combined with the DeepSort algorithm for completing the dynamic sheep counting is 13 %, 12 %, and 5 %, respectively. Among them, the YOLOv5x-ECA-SSA* model has the highest mAP and the best sheep counting effect. The result can provide a new theoretical method for realizing intelligent dynamic counting and tracking in the grazing process and provide a new technical application for intelligent livestock farming [45].

Tiana et al. [46] proposed a method for automated pig counting using deep learning. The reported convolutional neural network (CNN) model learns the reconciliation of an image object to a density map and obtains the total number of pigs in the entire image by integrating the density map. Accurate pig counting can improve the management of pig feeding, barn construction, etc., which can help farmers reduce costs and unnecessary losses, and make farms more competitive. However, accurately counting pigs is difficult due to pig image occlusion, differences in group density, camera perspective, and lighting variations. Manual counting can easily miss some pigs or add several extra pigs, is time-consuming and expensive, and also leads to the introduction of false data in reports. These problems are common in large breeding enterprises. Deep learning has proven to be the most promising solution to ensure accurate object counting in various environments. The authors of the automated model proposed to use a modified version of Counting Convolutional Neural Network in an end-to-end mode as a homogeneous multi-branch architecture for pig counting. The deep learning architecture combined Counting Convolutional Neural Network (CNN) and ResNeXt, so the proposed model should not depend on the results of image foreground segmentation, as the model only takes into account the appearance information. The results of the study showed that on real-world data, the proposed counting method yields an average absolute error of 1.67, regardless of the pig image, such as those covered with shadows, occlusions, or from different perspectives. The model's object detection and identification speed is 42 ms, which complies with the requirements of agricultural applications [46].

Automatic identification of individual animals is an important step towards achieving personalized care in terms of disease detection and management, as well as improved feed quality. Marsot et al. [37] propose a new structure consisting of computer vision, machine learning, and deep learning algorithms to offer a relatively low-cost and scalable solution for pig recognition. In the implementation of this project: first, pig faces and eyes are automatically detected by two Haar function-based cascade classifiers and one shallow convolutional neural network to produce higher quality images. The Haar function-based cascade classifier [47] was proposed by Viola-Jones company, and it is trained to recognize the pig face. This solution is preferable to color segmentation because it avoids detecting not only the pig's ears/body but also piglets that may appear in the background. Second, pig face recognition is accomplished using a deep convolutional neural network. Additionally, class activation maps generated by grad-CAM and noticeability maps are used to visually understand how the neural network learns discriminative features. Applying the proposed approach to 10 randomly selected pigs photographed in a farm setting, the proposed method showed its high performance with an accuracy of 83 % on 320 test images. However, by examining the noticeability and activation maps, it was determined that the neural network benefits from interesting features, such as the eyes and specific markings on the pig face, but may be sensitive to parasitic patterns caused by dirt stains or food remains. The results of this study will contribute to the practical application of AI-based animal identification in pig farming [37].

Pen soiling is an undesirable behavior of slaughter pigs that can take place in facilities where pigs are housed in pens with a designated resting area (solid floor) and a designated washing area (slatted floor). It increases farmer labor costs, reduces pig hygiene and well-being, and provides negative environmental consequences. Previous researches suggest that monitoring the positional behavior of weaner / finisher pigs in their pen can be used in early warning systems that can alert farmers about coming pen soiling event 1–3 days in advance. For example, Jensen et al. [48] showed that manual counts of the number of pigs located in different areas of a pen can be used as input to machine learning methods to forecast pen soiling events a day before they occur. In order to make this alert system feasible, monitoring of pig positioning must be automated. One method that can be used for such automation is a convolutional neural network (CNN) with a linear regression output, where the output value is the estimated number of pigs in a given area of the pen. The proposed CNN takes partial images of the pen corresponding to different areas of the pen and outputs the estimated number of pigs in the partial image. In such a model, convolutional layers automatically extract relevant features from the image, and subsequent fully connected

layers then perform further processing before the final numerical output is obtained [49]. Similar CNN concepts and architectures have previously been used to estimate the live weight of slaughter pigs [50].

Sheep producers need to identify different sheep breeds to assess the commercial value of their herd and the cost of sheep raising. DNA testing is an alternative method for breed identification; however, this method is not effective for evaluating large numbers of sheep in real time in a production environment. Therefore, methods are needed that can efficiently and accurately replicate the ability of an expert to identify sheep by breed in a farm environment. To differentiate sheep breeds on a farm for automatic sheep breed identification, the following work steps were completed: setting up a prototype computer vision system on a sheep farm, creating a database containing 1,642 images of sheep of four breeds, labeled by an expert with breed information, and creating a sheep breed classifier using machine learning and computer vision to achieve an average accuracy of 95.8 % with a standard deviation of 1.7. The processing time for one image takes on average 0.7 c. To train a sheep breed classifier on a farm, minimal image preprocessing (image resizing and upscaling) was required, as the convolutional neural network (CNN) model was found to be invariant to the sheep's viewpoint, pose, size, or illumination after being trained using the full set of images. Furthermore, the CNN model was able to process images of the sheep's entire body, as well as enlarged images of its face, which contains key information that allows it to distinguish between different breeds. The experiments examined the effect of fine-tuning different numbers of VGG-16 layers (the VGG-16 model is a 16-layer convolutional neural network developed by Visual Geometry Group at the University of Oxford) to analyze its impact on the performance of a sheep breed classifier. A model with six fine-tuned layers showed more stable behavior and higher accuracy of classification than a model with three fine-tuned layers. To determine the best training method for the sheep breeds classifier, two transfer learning approaches were assessed: one by fine-tuning of six VGG-16 layers, and the other using a pre-trained VGG-16 model with an SVM support vectors over it. Training the SVM using features from the pre-trained VGG-16 took twenty minutes, while fine-tuning VGG-16 on the same computer took twelve hours. The fine-tuned VGG-16 model achieved a high average accuracy of 94 %, 15 % higher than the pre-trained VGG-16. It also showed a lower standard deviation than the second model (1.9 versus 3.9), reflecting the consistency of its results. This makes sheep breed classification highly reliable and practical for use on farms. The developed classifier can help sheep farmers accurately and efficiently differentiate breeds without the need for an expert, ensuring more accurate meat yield assessment and cost management [51].

Growing demand for livestock and poultry products requires new approaches to animal breeding and raising. Breeding programs are currently seeking the ways to integrate animal behavioral phenotypes, as they are linked to rearing technologies, feeding, health, and animal well-being, and can thus impact animal productivity and the economic benefits of the industry.

Artificial neural networks (ANNs) have been used in poultry farming to solve complex nonlinear problems: problems of management and forecast of economically important traits, such as estimation of egg production curve parameters [52], growth curve of broiler chickens, reproductive performance of poultry and their demand for nutrients [53].

Forecasting the slaughter weight of quails in the early stages of the growing period is cost-effective for producers, given that quail production for meat production is growing worldwide [54]. Therefore, the aim of the study conducted by Jahan et al, was the forecast and optimization of slaughter weight of meat Japanese quails (*Coturnix coturnix*) at the age of 45 days based on their early growth performance, sex, and egg weight as predictors using artificial neural network modeling. The multilayer feedforward perceptron neural network structure was used to build the artificial neural network model [53]. The ANN structure defines the arrangement of neurons in 3 separate layers (input, hidden, and output). Therefore, the input layer outputs the data to the network, the hidden layer processes the data, and the results are extracted in the output layer. From the research results, it was determined that the most suitable network on the input data for forecasting slaughter weight in Japanese quail was identified with 7 neurons in the input layer, 11 neurons in the hidden layer, and one neuron in the output layer. The coefficient of determination (R2) was 0.9404, 0.9359, and 0.9223 for the training, validation, and testing phases of the system, respectively. According to the sensitivity analysis, the most important input variable for forecasting slaughter weight was body weight at 20 days of age, while less important input variables were bird weight at hatching and body weight at 5 days of age. Among the 7 input variables for forecasting slaughter weight (body weight at 5, 10; 15, 20, 45 days; egg weight; body weight at hatching; body weight at 45 days), bird sex as a discontinuous variable was the second important predictor. Egg weight was the fifth important predictor and even more important than body weight at hatching and weight at 5 days. Moreover, egg weight can be a marker of the quail chicks' health and rate of survival, at least in the early stages of life. To forecast the slaughter weight of Japanese quail, a neural network was trained using the backpropagation algorithm [53].

The objectives of the study, aimed at developing and validating machine learning models for monitoring individual behavior of group-housed broiler chickens, were: 1) developing and optimizing machine learning models for detecting, tracking, and classifying individual behavior of group-housed broiler chickens using continuous video recordings; and to use a set of independent data to evaluate the performance of the developed machine learning model

for differentiating the behavior of individual poultry [55]. Forty-two video recordings (total video duration = 1620 minutes) from 4 different poultry pens were analyzed in developing the machine learning models for monitoring individual behavior of broiler chickens. Four behavior types were classified: feeding, drinking, active (any movement other than feeding and drinking, but including feather fluffing and/or ruffling, pecking at the walls of another pen, scratching, stretching, and floor clawing), and inactive (the bird sits with its abdomen resting on the floor covering or stands with its feet touching any floor covering. The bird's head may be hidden under its wing or be positioned at body level or even lower. The bird may stand without showing any other behavior).

The optimal model was used to continuously analyze an external dataset of 11 videos (duration = 326 minutes), capturing the second-by-second behavior of each individual broiler (16 chickens were used). After comparing model performance, YOLOv5l was selected from five detection models for detecting individual broilers in a pen, as it is the most widely used and reliable object detection model. Pre-trained tracking algorithms were then compared to determine the optimal model. Using the pre-trained codes, 5 different models (osnet_x0_25_msmt17, osnet_x0_25_market1501, osnet_x0_75_imagenet, osnet_ibn_x1_0_imagenet, osnet_ain_x1_0_imagenet) were randomly selected with different data fidelity thresholds of 0.5, 0.6, 0.75, 0.8, 0.9 and 0.95. Based on the study results, osnet_x0_25_msmt17 was selected to track each detected bird in continuous frames; and the Gradient Boosting Classifier, out of 12 machine learning classifiers. Most models were able to retain previously assigned individual chick identities for a limited period of time but lost them over the study period (≥ 4 min). The final system was able to accurately predict the time of feeding (accuracy = 0.895) and drinking (accuracy = 0.9), but underperformed for the time of active (accuracy = 0.545) and inactive (accuracy = 0.505) behavior. The algorithms used by the machine learning models were able to accurately predict feeding and drinking behavior but require improvement in maintaining individual chicks' identities and determining active and inactive behavior [55].

The role of AI in meat tracking at the stages of the technological process (identification/classification/forecasting) of meat products

Meat traceability is important for analyzing the quality of individual meat cuts, including the farmer who produced the animal and the parameters of the technological process from which they were processed to the finished product. RFID tags are currently the dominant technology for food tracking [56,57]. However, RFID tagging of meat at the slaughterhouse has its drawbacks: tags can disappear within the meat product and end up on the consumer's plate, leading slaughterhouses to avoid directly tagging meat cuts, attaching the tag to the device transporting the meat.

Other methods have been proposed for tracking meat cuts. For example, a conveyor belt system capable of tracking meat cuts in a deboning facility has been developed. A disadvantage of this method is that it is prone to cases where identification tags are lost or interchanged between the carrying devices. To simplify cut tracking, it has been proposed to embed RFID chips in the carrier hooks for meat cuts [58]. The possibility of using barcodes printed on the beak and legs of chickens has been studied [59]. This approach is not well suited for meat tracking, as it requires the meat product to have inedible parts suitable for barcode printing. A number of researchers are conducting cattle breed identification when identifying beef based on DNA analysis or are investigating the possibility of using DNA analysis to trace individual cuts of meat back to the original carcass [56]. However, DNA identification is a rather labor-intensive method for slaughterhouse conditions.

The approach proposed by Larsen et al. [56], based on modern computer vision and image processing techniques, resembles DNA identification in the sense that identification comes from the object rather than from a tag attached to the object.

In industrial meat processing plants, individual cuts of meat are hard to track after carcass cutting. A technology for ensuring traceability of individual cuts of meat has been proposed in Denmark, using computer vision and image processing methods for pork loin (211 samples) as an example. For image analysis, the pork loin is photographed in two sessions, separated by a day. The loin samples are subjected to various disturbance scenarios to simulate real-world working conditions at the plant [56]. All disturbances took place after the first and before the second photo session. These disturbances include:

- Rough processing of 19 loin samples, which are heavily dropped and tightly squeezed on the table before the second photo session.
- Incorrect cutting. Additional pieces of meat and bones are cut from 18 loin samples before the second photo shoot.
- Incorrectly hung 19 samples where loins are hung on their sides when stored overnight, thus causing their bowing.
- Illumination and orientation change: Before photographing, 37 meat samples are rotated from 45° to 180° around the optical axis. This creates illumination variations, as the light falls differently on the rotated object. This evaluates whether the proposed image analysis algorithm is invariant to different object orientations.

The objective of the visual meat pattern recognition method is to correctly match images of pork loin between two photo sessions. The image recognition method is divided into four steps: 1. Segmentation. First, the pork loin is segmented. That is, the pork loin is cut out from the pixels of the background image. 2. Canonization. Then, the segmented pork loin images are brought to a canonical

form, which minimizes variability from external sources, such as lighting. 3. Description. From the canonical images we generate a description of the image structure. 4. Matching. The pork loin samples are matched by comparing the descriptors from the previous step. In total, the recognition method takes less than 2 s per one image. The proposed image analysis-based meat pattern tracking method is a promising alternative to more invasive methods currently available [56].

In the meat processing industry, the decision to implement automated or robotic processes is typically driven by return on investment, which in turn typically depends on improved product quality, reduced labor costs, or a reduction in safety incidents [60]. However, beef deboning on modern deboning lines is still primarily a manual operation. Operators at the end of the line identify products, check their quality characteristics, and then manually redirect them to the relevant packing stations.

Meat identification for labeling and quality control on production lines is largely performed manually, which can lead not only to errors in meat labeling but also to bacterial cross-contamination. Artificial intelligence is used in many disciplines to identify objects in images, but these approaches typically require a significant volume of images for training and validation. A study was conducted in a commercial Irish meat processing plant to identify meat by image and weight for its subsequent labeling. Images of 7,987 individual cuts of beef, trimmed of fat and tendons, isolated from *M. semimembranosus,* and their weight data were available for analysis to systematize the experimental data. Various classical neural networks and an integrated machine learning approach were then tasked with identifying each cut of meat; The performance of these approaches was measured by accuracy (the percentage of correct forecasts), precision (the ratio of correctly forecast objects to the number of objects identified as positive), and sensitivity (also known as the true positive rate or sensitivity). Preprocessing and application of deep learning algorithms were implemented using the Python programming language. The new comprehensive approach to meat identification outperformed a number of classical neural networks, including the convolutional neural network (CNN) convolutional neural network) and residual network ResNet. Accuracy, precision, and sensitivity for the new method were 99.13 %, 99.00 %, and 98.00 %, respectively, while the next-best method achieved 98.00 %, 98.00 %, and 95.00 %, respectively. The new integrated approach demonstrated positive results for identifying each piece of meat, with the use of color images improving categorization accuracy compared to grayscale images while also taking into account the product's weight. By collecting and systematizing image and weight data for individual chunks of meat, it is possible to develop a methodology for accurate classification and increased automation of the meat-piece identification process. Improved object detection algorithms and the incorporation of weighting factors appear to have eliminated the need for infrared spectroscopy and can potentially be used in many other areas of the food industry. The accuracy of the Ensemble machine learning model demonstrates the capability of artificial intelligence to replicate the behavior of a human operator. The resulting algorithm eliminates the need for a human operator, thus reducing the risk of cross-contamination of samples and potentially increasing the shelf life of the product [61].

In South Korea a mobile app has been developed that classifies beef quality using artificial intelligence, which has undergone rapid technological advancement in recent years. The app allows users accessing information, including cut labeling, freshness, and marbling of the beef they are about to purchase. Deep learning technology was used to classify beef cuts, and OpenCV (OpenCV) technology was used to determine quality. Source Computer Vision). The application was developed in a client-server system for real-time operation. The server part of the program is hosted on a website, which contains a module for classifying beef parts, assessing freshness and marbling. The user's (client's) mobile phone takes a photo of the beef and sends it to the server, and the server analyzes the resulting image to identify and determine the quality, freshness, and marbling of the beef. The results are then sent back from the server to the client. The developed system has proven itself effective for tenderloin, sirloin, and ribs. For these types of meat, it provided a high accuracy rate of over 75 %. However, for other types of beef cuts, it performed poorly, which is due to the problem of training for image classifiers. The proposed application is designed to help the average consumer make the right decision when purchasing beef by providing data on this beef [62].

To develop a beef cut classification system based on the existing TensorFlow deep learning neural network, we evaluated its capabilities for quickly classifying seven different types of beef cuts (bone-in ribeye steak, boneless ribeye steak, chuck roll steak, flank steak, striploin steak, short ribs, and tenderloin). Color images of the beef samples were obtained using a laboratory computer vision system and collected from an internet platform Google Images. In total of 1,113 images of beef cuts were used as training, validation, and test datasets. A model developed using a deep learning neural network algorithm was able to classify specific cuts of beef (flank steak and tenderloin) with 100 % accuracy. Two pre-trained neutral convolutional network (CNN) models, Visual Geometry Group (VGG16) and Inception ResNet V2 was used to train, validate, and test these models for classifying beef images. Image augmentation was incorporated into the neutral convolutional network models to avoid re-learning issues, resulting in improved image classifier performance. The VGG16 model outperformed the model Inception ResNet V2. The VGG16 model, combined with data augmentation technology (data augmentation method), was able to achieve a high accuracy of 98.6 % on 116 test images, while Inception ResNet

V2 achieved a maximum accuracy of 95.7 % on the same test images. Based on the performance of both models, the deep learning technology clearly showed promising prospects for identifying beef cuts in the meat industry [63].

García-Infante et al. [27] have tested artificial intelligence algorithms for classifying commercial lamb meat categories. The dataset used in this study was obtained from 78 indigenous *Mallorquina* lambs. The samples were classified according to production model and commercial categories as follows: milk-suckler lambs (SL; $n = 30$); light lambs (Ternasco) (*TP*, $n = 26$), which were reared with their mothers on the natural pastures of the Balearic Islands, including natural pastures and pastures of oats, ryegrass and barley, and on cultivated pastures as green forage during the spring months; and Ternasco TC ($n = 22$) lambs, that were reared with their mothers on cereal crops and natural pastures on the Balearic Islands, including oats, ryegrass and barley pastures as the source of green forage for two months. The performance of six machine learning algorithms of artificial neural network (ANN), decision tree, k-nearest neighbors (KNN), naive Bayes (NB) function, multinomial logistic regression (MLR), and support vector machine (SVM) were evaluated for their ability to perform descriptive data analysis and classification tasks using various types of raw data. Each algorithm was tested on three datasets, namely, sensory and sensory attributes (CIELAB color, water holding capacity, Warner-Bratzler shear force, volatile content) and nutritional attributes (raw composition and fatty acid profile). The evaluated algorithms demonstrated overall accuracies ranging from 0.88 (ANN) to 0.54 (KNN) in classifying those three lamb categories. Among the six tested algorithms, ANN demonstrated the highest performance in processing the organoleptic and sensory meat dataset, achieving an overall accuracy of 0.88. In fact, the overall accuracy of the ANN was 24–39 % higher than the results obtained with the other tested algorithms. Furthermore, the ANN algorithm was able to automatically adjust internal parameters to improve forecast accuracy. Based on these results, it can be concluded that the use of machine learning algorithms is an effective alternative for differentiating the meat of native Spanish lambs. Further research aimed at improving classification methods based on machine learning will be useful for lamb meat authentication and quality certification [27].

Machine learning algorithms are widely used to forecast carcass yield. Deep Learning (DL) has proven its being successful in solving many image classification problems, particularly for forecasting bovine carcass yield and comparing these forecasts with methods of standard machine learning (ML). The following approaches were selected as DL methods: (1) animal phenotypic data used as features for a number of machine learning algorithms, (2) carcass images used to train convolutional neural networks, and (3) analysis of carcass dimensions measured directly from carcass images, combined with corresponding phenotypic data and used as feature data for the machine learning algorithms. The final approach to forecasting grouped carcass cuts included 346 carcass dimension measurements at predetermined image points and recorded across all digital images. One of the key findings of the study is that the difference in performance between DL and ML models is insignificant. From a practical standpoint, trained DL models are easier to use than ML models. This is because DL models only require carcass images captured at the slaughterhouse within a few minutes after slaughter. In contrast, machine learning models require significantly more data collection [64].

Traditional approaches to forecasting pork quality parameters have relied primarily on statistical methods such as regression analysis [65]. However, these methods exhibit significant limitations in both forecasting performance and practical applicability. The main limitations of existing studies on pork quality forecast can be summarized by the following criteria: (1) focusing on specific time points rather than the dynamic changes; (2) reliance on single algorithms rather than ensemble approaches; (3) limited incorporation of multivariate data, including genetic and feed information; (4) insufficient sample sizes and genetic diversity; and (5) inadequate integration of the chemical mechanisms underlying quality development with forecasting modeling approaches [66]. Artificial intelligence-based ensemble quality forecast models achieve high accuracy in forecasting dynamic changes in pork quality [67]. Based on experimental data obtained from 1,284 pigs of 24 breeds, Chinese scientists developed and optimized ensemble machine learning models, including LightGBM, XGBoost, and Random. Forest, which takes into account the biochemical mechanisms governing pH changes and color development in pork meat within 48 hours after slaughter. The proposed collective learning method achieved high forecast accuracy: R^2 values exceeded 0.7 for both pH and color parameters. Although meat quality parameters generally stabilize for 24 hours after slaughter, forecasting quality within 48 hours possesses significant practical value for commercial meat processing and distribution. For example, early forecast of quality parameters within 48 hours allows processors to determine which products are suitable for premium processing, which are optimal for fresh market sales, and which may require promotional pricing. This forecasting decision-making capability is critical for optimizing supply chain management and maximizing product value. Therefore, the development of accurate 48-hour quality forecast models possesses significant commercial value. Trait importance analysis identified early pH values and muscle structural characteristics as critical predictors consistent with key biochemical processes such as anaerobic glycolysis, protein denaturation kinetics, and myoglobin redox reactions, confirming the complex interplay between chemical mechanisms in meat and sensory traits, providing an accurate alternative to traditional assessment methods. The established chemical basis for forecasting pork quality offers a scalable framework

for integrating genetic, environmental, and technological factors, ultimately improving the accuracy of quality control and sustainability in meat production through the application of food chemistry principles [67].

In [68], an analysis of a multi-component meat system (pork and "Doktorskaya" cooked sausage) was performed using a convolutional neural network (CNN) with two convolution layers (convolutional, C-layer) and pooling (subsampling, S-layer). The gradient backpropagation algorithm was used to train the CNN. The authors determined the main microstructural characteristics (classification parameters) for identifying plant components in raw meat and finished products using CNN. The concentration of plant components and their nature (carrageenan, starch, soy isolate, vegetable gum) did not matter; the CNN identified them regardless of concentration. The best result was obtained using the ReLu activation function (0.9843), followed by the SoftPlus (0.9765) and eLu (0.9687) activation functions.

The study [69] demonstrates the capabilities of AI in recognizing the undeclared components in histological sections of raw meat. A study of digital images of histological sections of raw meat using five optimization algorithms (Adam, NAdam, FTRL, Adamax, AMSGrad) included in the TensorFlow pack revealed that the NAdam algorithm demonstrated the best results in recognizing/identifying the undeclared herbal preparations among protein substance. Recognition accuracy on the test sample was 80 %. Classification metrics: AUC = 94 %, Recall = 78 %, Precision = 79 %.

A comparative analysis of the capabilities of the YOLO and ResNet convolutional neural networks in recognizing undeclared components in histological sections of raw meat using the NAdam optimization algorithm was conducted in the study [70]. The study found that both ResNet and YOLO convolutional neural networks both possessed equally high potential for identifying undeclared components in raw meat. Both demonstrated virtually identical results in the accuracy of recognizing/identifying undeclared components in images of histological section.

AI in meat products authentication

Confirming the authenticity of meat and meat products is becoming increasingly important due to the spread of food adulteration. Meat adulteration, such as deliberate adulteration or mislabeling of meat varieties, undermines the meat market by causing consumers' mistrust. These fraudulent actions affect both consumers and producers of authentic food products. This phenomenon represents an information asymmetry that the market is not able to correct spontaneously and on its own. Meat authentication methods are crucial for market legitimacy and, thus, ensuring fair trade.

Meat authenticity and traceability are issues of the highest importance in modern society, as evidenced, for example, by widely publicized events involving the adulteration of meat products with undeclared meats, such as horse meat [71]. This underscores the demand of consumers worldwide for clear and reliable information about the food they consume. This is particularly true for processed meat products, where simple visual inspection cannot readily distinguish the various components as is the case with intact fresh meat. More than ever, consumers are focused on the link between food and health, seeking reliable information about the safety, quality, composition, and origin of food products. Food authenticity has become a key issue in the production chain [72].

The aim of the research work by Brazilian scientists was to obtain classification models for the authentication of beef cuts from animals raised under fully controlled conditions of genetics, nutrition, and geographic origin, using multi-element profiling and supervised learning algorithms. The feasibility of combining classification algorithms was assessed based on the content of the main microelements: Br, Co, Cs, Fe, K, Na, Rb, Se, and Zn, selected as a tool for the authentication of beef cuts [73].

To build classification models based on the multielement beef profile determined using neutron activation analysis, the following were used: classification and regression tree (CART); multilayer perceptron (MLP), widely used in deep learning technologies; naive Bayes classifier as a probabilistic machine learning algorithm based on Bayes' theorem and used in different classification problems (NB); random forest (RF) method — machine learning algorithm for solving classification and regression problems; and sequential minimal optimization (SMO) — an algorithm for solving the quadratic programming problem. The classification accuracy values obtained for beef cuts were 96 % (MLP), 95 % (SMO), 91 % (RF), 86 % (NB) and 70 % (CART) [73]. The multilayer perceptron (MLP) algorithm provided the best classification for the authentication of beef cuts based on the content of major and trace elements, as it provides the best accuracy, sensitivity and specificity of the classification.

Recently, spectroscopic methods of hyperspectral imaging (HSI) systems have been used to identify meat and meat product types. These methods allow for the simultaneous receiving of both spatial (textural) and spectral information from meat product samples. The main advantage of the line-scanning HSI system is that it provides a wide spectral range. However, image acquisition with line-scanning is slow, and the image size requires large computational resources. To mitigate these drawbacks, a new deep 3D convolutional neural network (3D-CNN) model for extracting combined features and subsequent identification of red meat (lamb, beef, pork) in the HSI image and the innovative graph-based post-processing method to improve the forecast of the 3D-CNN approach were proposed [74].

During the study, meat samples were randomly divided based on meat species and product type into training set and test set: a set of 105 meat samples was used for training purposes, including the processes of training and selecting

(or validating) the best model. An independent set of 79 meat samples was used to obtain the final quantitative and qualitative results (i.e., accuracy and classification maps). This experimental design was used to ensure that the analysis was independent of the influence of the training and experimental processes. The results show that the deep learning 3D-CNN approach for red meat classification by combining spectral and spatial features of HSI data of red meat products significantly improved the overall accuracy of state-of-the-art models. Despite the limitations in spectral information when processing HSI imagery, the 3D-CNN model demonstrates its reliability in red meat classification with an overall accuracy of 96.9 % and 97.1 % for HSI images in the near-infrared range (NIR) and visible (VIS) range, respectively. The conducted study allows for further development of researches in the field of real-time red meat authenticity verification and mobile HSI systems using HSI snapshots and deep learning models [74].

A study was conducted in China to identify a specific counterfeit product — adulterated lamb or beef slices used in hot pot, a dish cooked from duck and fat. The goal is to develop a quick and convenient authentication method that customers could use directly in the restaurants [75]. A large number of adulterated lamb or beef slices exist, which are made from duck meat and lamb or beef fat [76] due to their low price. Compared to other cheap meats, such as chicken, the color of duck meat is closer to red meat. This product is sold legally on the market under labels such as "Meatloaf" or "Stuffed Meatloaf." Manufacturers of these products have their own secret methods to make these meatloaves visually resemble lamb rolls. Due to the lamb fat content, these meatloaves taste somewhat like lamb. Dishonest hot-food restaurants buy those meat products and serve them as "lamb cuts". The same applies to adulterated beef cuts. Therefore, it would be advisable to provide restaurant customers with a convenient authentication tool so that such meat fraud can be reported right at the spot [75].

The task of authenticating meat slices in developing a detection method for the aforementioned counterfeit product was formalized as a classification problem of RGB images as input using the textural features of meat slices. Authentication is performed quickly and non-destructively. The only device required is a mobile phone with a camera. For this purpose, a lightweight (and therefore highly efficient) convolutional neural network architecture called MTx-Net was created. Fourteen convolutional layers in four blocks were used to extract reliable visual features. The neural network utilized techniques such as residual values, depthwise convolution, dropout, and batch normalization. A total of 77,956 meat images were collected using 225 kg of meat for training and testing the neural network. This method provides identification accuracy of 99.38 % and 98.20 % for lamb and beef slices, respectively [75].

Kozan et al. [77] developed and tested a mobile application for meat freshness assessment using deep learning technology. The main focus was on the system methodology and validation of the obtained results. A "black-box" testing method was used to evaluate the functionality. This approach allows for checking the system's functionality from the end user's perspective, without taking into account its internal implementation, which increases the relevance of the assessment for practical use. Testing included processing images of fresh and spoiled meat in the environment Google Teachable Machine. The classification results demonstrate the system's ability to identify the product state correctly (Figures 4, 5).

The mobile app interface is presented as a user-friendly tool that visualizes the analysis results directly on the device screen (Figure 6). To verify the results obtained in Google Teachable Machine, the deep convolutional neural network ResNet-50 was used.

Song et al. [78] used a mobile phone front camera and screen to form a "hyperspectral imaging" system to detect adulteration of ground beef with pork based on smartphone videos recorded in a sequence of different colors. For the study, ground beef samples were mixed with ground pork in the range of 10–100 % (weight) with 10 % weight increments. Light of different colors was generated on the smartphone screen and used to illuminate the sample surface. Short videos were recorded and converted into spectral-like data through image processing. Data samples were collected under different conditions in terms of smartphone type, recording, distance, and lighting conditions, resulting in seven datasets. A partial least squares regression model was used to forecast the adulteration level, yielding coefficients of determination of 0.73–0.98 and root mean square errors of 0.04–0.16. Furthermore, smartphone videos were used to map the prevalence of adulteration levels. The results demonstrate the potential of a simple and inexpensive approach to detecting adulteration in ground meat. The video-based approach to detecting adulteration is more user-friendly in terms of cost and data collection. Therefore, it can be used as an effective tool for processors and regulators for the preliminary detection of adulteration in ground meat [78].

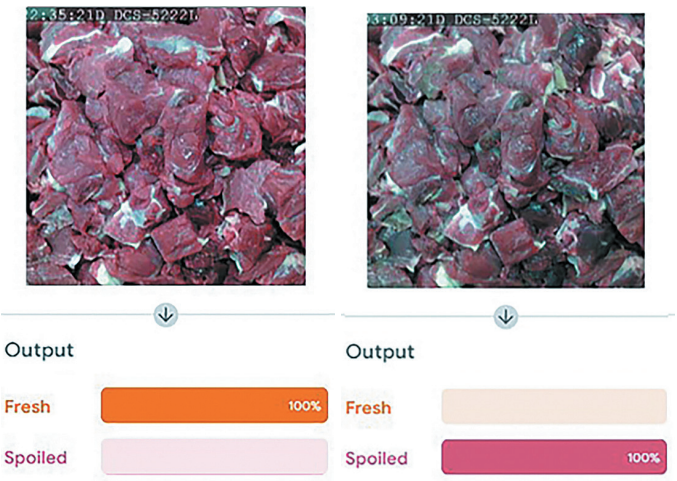

Figure 4. The result of the test classification on an image of fresh meat [77]

Figure 5. The results of test classifications on images of spoiled meat [77]

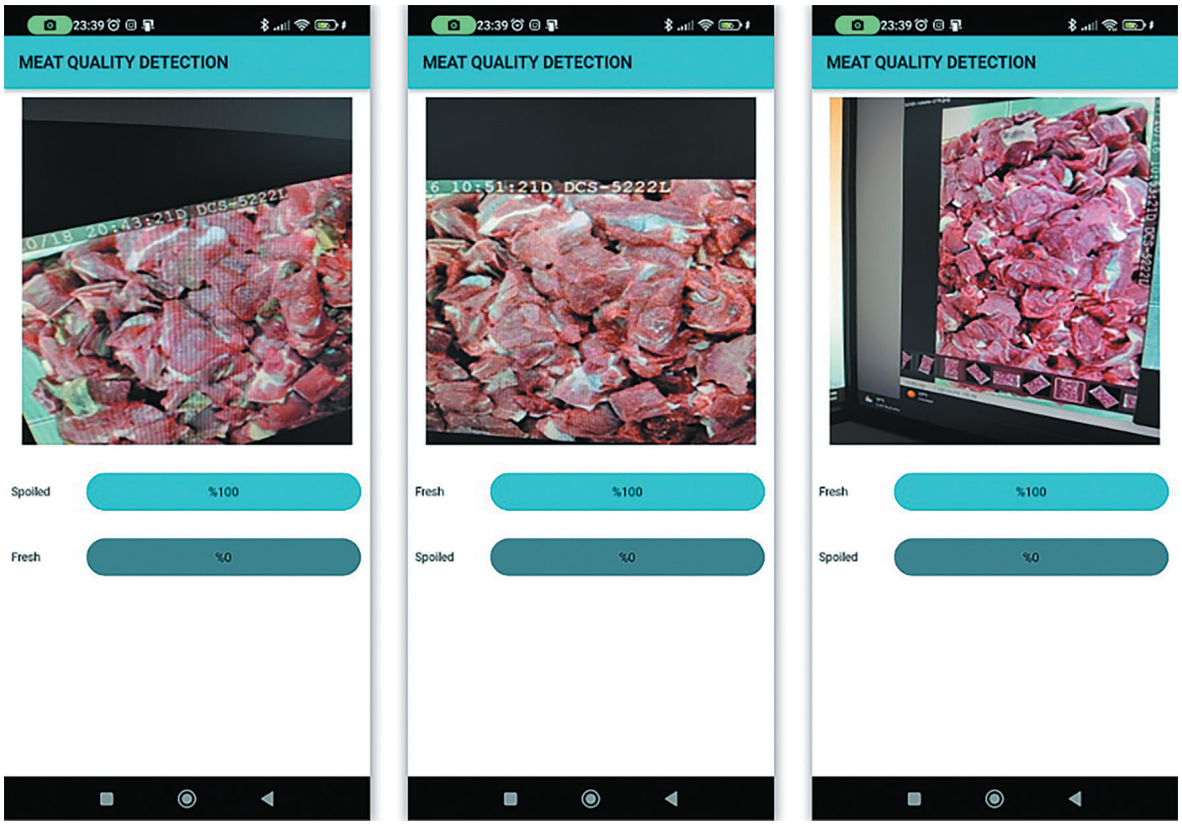

Figure 6. Screenshots from the mobile application developed for this testing [77].

It should be noted that research into detecting meat adulteration has made significant progress by combining the use of electronic noses and artificial intelligence methods. Han et al. [79] developed a low-cost electronic nose using colorimetric sensors to detect pork adulteration in beef, with the extreme learning machine model outperforming traditional methods (91.3 % and 87.5 % on training and test sets). Furthermore, Huang and Gu [80] presented a combined one-dimensional convolutional neural network (1 DC NN) and random forest framework (RFR) for quantitative detection of pork-adulterated beef with the help of 10 different MOS sensors as an electronic nose. The determination coefficient (R^2) for this model was estimated to be 99.7 %.

Assessing the quality and safety of meat and meat products using artificial intelligence technologies

Ensuring food safety is the most important and critical requirement for any food industry, as spoiled food gives the rise of foodborne illnesses. Meat and meat products become contaminated due to various factors, plus frequent human handling on the production line increases the risk of contamination. Growing demands for meat products to meet high quality and safety standards have led to the development of technologies that enable accurate, rapid, and more objective quality assessment. Machine vision technology matches the functions of human vision through electronic image perception and evaluation and can be used for quality assessment and classification of meat and meat products [31].

With the rapid development of computer technology, non-destructive testing systems based on image processing and machine vision have been widely used to obtain product characteristics based on image analysis and recognition of characteristics related to determining the quality and safety of meat products.

The review article [80] is devoted to the possibilities of using ANN. A special role in the work is given to convolutional neural networks, structured like the visual cortex of the brain. In recent decades, CNNs have achieved great success in image recognition, since they are able to concentrate on a small area and highlight important features in it. The prospects for using ANNs in the food industry for incoming quality control of raw materials are noted. In world practice, various methods of remote control of raw materials are used, for this purpose mainly devices based on ultrasound scanning are used. Such devices and analysis systems run control of raw materials based on the ratio of meat tissues (muscle, connective, fat) in a carcass or half-carcass, without affecting the structure of tissues, and do not conduct quality assessment at the cellular (microstructural) level. It has been established that the structure of muscle tissue (diameter of muscle fibers, preservation of cellular elements, tissue porosity, integrity of muscle fibers) reflects the quality of the raw material, its thermal state [81].

Machine vision systems operate by capturing an image of an object, such as a piece of meat, processing the image to measure desired parameters, and comparing these parameters. Parameters with predetermined inspection criteria help inform decisions about corrective actions for the object or production process. The most important advantage of meat inspection using a machine vision system is its

non-destructive nature when examining a meat sample. Recently, there has been significant growth in the use of machine vision in various fields, such as poultry carcass inspection, weight forecast, beef color determination, tenderness forecast, and chemical component forecast of meat and meat products. However, certain disadvantages of this method should be noted. MV technology requires uniform illumination and calibration. It is often difficult to separate overlapping objects from the background when it is necessary to assess both sides of a meat product [31].

Color characteristics are considered one of the most important indicators for assessing the quality of meat and meat products. The first and most important quality assessment any consumer makes before purchasing a food product is color. Rahman et al. studied the ability of computer vision technology to forecast the quality characteristics of beef and concluded that the highest forecast accuracy was obtained for lightness (L*), average for redness (a*), pH, moisture loss, protein and ash content in a beef sample [82]. Chmiel et al. [83] investigated the color parameters using image analysis technology to identify PSE in pork. The color parameters were assessed using computer vision systems (CVS) and the CIE — L*a*b* scale. The obtained correlation and determination coefficients between the color parameters characterized by lightness reached low values of 0.44 and 19.4 %, respectively [83].

The efficiency of using a computer vision system (CVS) to calculate the color characteristics of beef and pork meat compared to experimental color data obtained with a Minolta colorimeter revealed significant differences in color parameters (L*a*b*, hue change angle, and chromaticity) between the two different methods. The CVS methodology produced colors very similar to visual tests, unlike the Minolta colorimeter. The match rate of colors obtained by CVS with actual meat colors, as judged by trained experts, was 100 %. These results indicate that CVS may be an excellent alternative to the traditional Minolta colorimeter, as it provides higher sensitivity and accuracy. In addition to objective color measurement, computer vision offers other capabilities that may be useful for further quality control or research in the meat industry [84]. Thus, the advantage of CVS is that it determines the L*, a*, b* values for each pixel of the meat sample image, providing speed, accuracy, objectivity, efficiency and allowing the identification of meat quality defects PSE or DFD [83,85], or for forecasting the color and marbling of pork and beef [86].

Researches were conducted to evaluate the quality of pork loin using online computer vision. Eighteen color images of pork loin, images of intramuscular fat (IMF) content, and 88 texture images were used as input indicators for pork quality forecast models. To evaluate loin color, measured color scores were divided into six groups according to a specific range of lightness (L*) values. For marbling, objective scores were divided into ten groups according to the range of fat content (CF%) values. Based on the research results, an artificial intelligence-based forecasting model (support vector machine (SVM)) was developed for determining pork quality based on color and marbling, with the highest forecast accuracy of 92.5 % and 75.0 %, respectively. This machine learning method has great potential for forecasting pork loin product quality. However, further research is still needed before a computer vision system (CVS) with artificial intelligence and SVM modeling can be used in pork processing plants. First, it is necessary to optimize the model's performance to achieve higher forecast accuracy rates, and also to select the most effective type of learning algorithm to use. In this study, the kernel radial basis function (RBF) was used, as it is the most common function used for the SVM forecast method. However, there are other learning algorithms that are worth testing and comparing to validate the model's forecast results [86].

Mortensen et al. used a 3D computer vision camera to calculate the weight of broiler chickens with an accuracy of 92.2 % [87]. Moral et al. [88] investigated the potential of image analysis for automated quantification of intramuscular connective tissue in meat and found that its application allows for automatic, accurate, objective, and reliable determination of the amount of intramuscular connective tissue in meat and fiber contraction in muscle. The software they developed allows for the analysis of 20 images per minute and provides more accurate measurements than traditional morphometric methods [88].

Research has identified the potential of computer vision technology for assessing the color of fresh pork loin. Software was developed to segment pork loin images into background, muscle, and fat. Color features were then extracted from the segmented images. This study utilized characteristics such as the mean and standard deviation of red, green, and blue bands within the segmented muscle region. To assess the color characteristics of lean meat, sensory ratings of pork loin were collected from a trained panel using a 5-point color scale and compared with data of computer images analysis. Both statistical and neural network models were used to forecast color gamut using image characteristics as input. The statistical model used partial least squares to derive latent variables. The latent variables were then used in multiple linear regression. The neural network utilized a backpropagation learning algorithm. The correlation coefficients between the forecast and original sensory scores were 0.75 and 0.52 for the neural network and statistical model, respectively. The forecast error was the difference between the mean sensory score and the forecast color score. An error of 0.6 or less was considered insignificant from a practical point of view. For 93.2 % of the 44 pork loin samples, the forecast error in the neural network modeling was less than 0.6. In addition, 84.1 % of the samples yielded a statistical forecast error of less than 0.6. The results of this study showed that the image processing system combined with the neural network is an effective tool for assessing the color of fresh pork [89].

Liu et al. investigated the feasibility of using a computer vision system to forecast the percentage of intramuscular fat in pork and developed a stepwise regression model and a support vector machine (SVM) model. The accuracy rates of the regression models were 0.63 for stepwise regression and 0.75 for the support vector machine [90].

Pork color is crucial for assessing its safety and freshness, and traditional human-eye observation methods are ineffective and subjective. In recent years, several methods based on computer vision and deep learning have been proposed that can provide objective and stable assessments. To improve the performance and accuracy of this method, which was limited by the lack of effective preprocessing of background noise, a standardized pork image acquisition device was developed by processing 1,707 high-quality pork images, and a new deep learning model for color forecast was proposed. The deep learning framework, called Porkolor, consists of two modules: an image preprocessing module and a pork color classification module. The image preprocessing module uses the Segment Anything Model (SAM) was developed to extract pork patterns and remove background noise, thereby improving the accuracy and stability of the model. The pork color classification module uses a ResNet-101 model trained with a patch-based learning strategy as a baseline. The resulting model achieved 91.50 % classification accuracy on a high-quality pork image dataset and 89.00 % on an external validation dataset. A POPO color features dataset was also created to address the current lack of a large-scale, publicly available pork color classification dataset. The pork color classification system operates as follows. First, an image of pork captured by an image collector is sent to SAM, and the trained SAM generates several pork pattern masks and relevant confidence scores. The mask with the highest confidence score is selected for segmentation, resulting in a clear segmented image of the pork piece. The segmented image is then fed into a trained patch-based ResNet-101 network to obtain the final pork color estimate. However, the authors of the study note that their work has some limitations. First, although the POPO dataset is a valuable resource, its size is relatively small compared to other large-scale datasets, which may limit the generalizability of the proposed image analysis model. Second, the annotation process, although thorough, is inherently subjective and may introduce some level of bias. Finally, the performance of our model, like many deep learning models, depends on the quality of the input data. Any deviations in the standardized image collection process may impact the accuracy of the model [91].

In the food industry, risk assessment is a fundamental component of efficient food safety management, which involves a systematic approach to identifying, assessing and controlling potential hazards that may compromise food safety at various stages of the food supply chain.

Traditional food safety risk assessment is primarily performed manually using methods such as HACCP and quantitative microbial risk assessment (QMRA) [24,92]. However, these methods face several challenges: difficulty integrating different data sources and efficiently processing heterogeneous information; reliance on expert knowledge limits their ability to forecast emerging risks; and lacking real-time adaptability, making it difficult to track rapid changes in food production and distribution. These limitations highlight the need for advanced technologies such as machine learning (ML) to improve the accuracy and efficiency of risk assessment. Unlike traditional methods, machine learning can automatically detect and predict foodborne hazards by extracting patterns from data, integrating information from multiple sources, and dynamically adapting to new risks, making assessments more effective and proactive [93].

Rapid and accurate detection of foodborne pathogens in lamb using shortwave infrared hyperspectral imaging (SWIR-HSI) is essential for ensuring the safety of lamb and its products, as well as consumer health. The feasibility of using SWIR-HSI to determine contamination status and *Escherichia coli* (EC), *Staphylococcus aureus* (SA) and *Salmonella typhimurium* (ST) species detected on lamb. The potential of combining SWIR-HSI with traditional machine and deep learning methods for the effective detection of lamb contamination with pathogenic microorganisms was investigated. Hyperspectral images of uncontaminated and contaminated lamb samples with different concentrations (10^8, 10^7, 10^6, 10^5, 10^4, 10^3 and 10^2 CFU/mL) of EC, SA and ST were obtained. A one-dimensional convolutional neural network (1D-CNN) model was created and the influence of structural hyper parameters per model. The best full-range model was the 1D-CNN model with 16.64 convolution kernels and a tanh activation function (hyperbolic tangent), installed using the original spectra, and its accuracy on the training set, test set, and external validation set were 100.00, 92.86, and 97.62 %, respectively. The optimal simplified model was the genetic algorithm optimization using the support vector machine (GA-SVM). For pathogen species recognition, the accuracy of the SVM models fitted using the full-band spectra preprocessed with 2D and all 1D-CNN models with the convolution kernel number (16.32) and the tanh activation function was 100.00 %. The researchers noted that the performance of the deep learning model was higher than that of machine learning. This study highlights the importance of developing fast and reliable methods for detecting foodborne pathogens that are tailored to the specific food characteristics [94].

The potential of a computer vision-based method as an intelligent, non-destructive, and robust online freshness forecast method for chicken meat is discussed [95]. The proposed method includes the following steps: image capture, image pre-processing, image processing, computational pipelines, feature extraction, feature selection using a hybrid of a genetic algorithm (GA) and an artificial neural network (ANN), and forecast using ANN. A total of 3000 images of chicken thigh meat (with bones, skin,

and muscle tissue) from both sides (with skin and skinless) were acquired. Images were received every 6 hours for 300 hours long.

Mean square error *(MSE)* and correlation coefficient (*R)* were used as statistical indices to evaluate the performance and accuracy of the network. An *R* value greater than 0.9 indicates high model performance, an *R* value within the range from 0.8 to 0.9 characterizes adequate performance, and an *R value* below 0.8 demonstrates unacceptable performance of the model [96]. High model performance was confirmed by a correlation coefficient of 0.98734 and an MSE of 0.002045. The MSE of the selected model at the forecast step was 0.002045 h (0.1227 min), which confirms a reliable match between the forecast and actual values for all considered time intervals for determining chicken meat spoilage indicators. Thus, the results of the study indicate a high potential for using GA-ANN computer vision as a reliable, fast, non-destructive and online method for assessing the freshness of chicken meat [95].

Integrating sensory assessment with sophisticated machine learning models is an effective approach to solving various problems [97–100], especially in the field of food quality assessment and safety monitoring [79,101].

An off-flavor, such as boar odor, is primarily associated with the presence of androstenone and skatole and which can indicate poor meat quality. Meat odor profiles vary significantly over time, influenced by natural hormonal processes, gut bacterial metabolism, feed type, breed, age, sexual status, environment, and maturing/decomposition of the meat. Therefore, the development of detection methods that capture dynamic changes in odor profiles is essential for improving the accuracy and reliability of meat quality assessments and provides cost-effective, practical solutions for enhancing safety measures [102].

Swedish scientists have developed a reliable, accurate, and non-invasive method for the rapid detection of chemical contaminants in meat. Initially, they conducted studies to distinguish between the odor of fresh pork and samples with an odor similar to urine, using urine as the chemical contaminant. The study also examined another important aspect of meat quality: assessing freshness during storage of pork for 1 to 31 days at +5 ± 1°C. Using an electronic nose based on a metal oxide gas sensor with Optimizable Using Ensemble ML models, the proposed method achieved a sensitivity of 96.5 % and a specificity of 95.3 % in categorizing fresh and urine-contaminated meat samples. The model demonstrated robust forecasting performance with Kappa value of approximately 0.926, indicating near-to-perfect agreement between forecasts and actual classifications. The high sensitivity of e-nose to detect volatile organic compounds (VOCs), combined with the high forecasting power of the developed ML models and consensus-based decision algorithms, enabled not only accurate classification of urine-contaminated meat with a validation accuracy of 95.9 % and a test accuracy of 96.3 % but can also be used to quickly determine meat shelf life in real time as an indicator of meat freshness. The model demonstrated the capability to distinguish between fresh pork meat and meat aged for 1 to 2 days with an accuracy of up to 93.5 %, as well as to identify meat with a storage period of 3–31 days and 17–31 days [102].

Artificial intelligence in meat processing and meat product manufacturing

Primary processing of livestock, including stunning, butchering, evisceration, abdominal dissection, and other operations, is an extremely critical step, requiring high standards of hygiene, quality, and precision to ensure proper subsequent operations [103]. A high degree of automation in the meat industry can be achieved for repetitive technological tasks. For example, the initial stages of meat processing on the slaughter line have undergone significant automation.

With the growing use of machine vision in meat analysis and livestock identification, robots powered by machine vision have been explored. Based on a genetic algorithm, Liu et al. [104] proposed a flexible robotic system for cutting the pig abdomen while hanging it by the hind legs by the hooks, based on an algorithm using trajectory planning: a 2D camera captures the side view of the pig, and a programming platform with the Matlab application package identifies the curve of the pig abdomen from the image, which is fitted with a spline of fifth-order. Then, the trajectory is optimized using a genetic algorithm, minimizing the cutting segments and errors. The optimized trajectory is divided into six segments with a maximum cutting error of 1.6 mm, which ensures accurate cuts of the skin and muscles and avoids damage to the internal organs. The system proved that machine vision is suitable for optimizing the cutting of the pig abdomen [104]. However, the recognized accuracy of this automated cutting system is still low [103]. Methods such as deep learning and 3D modeling can provide higher accuracy than machine vision and offer increasing opportunities for the creation of robotic systems of meat processing [103].

Based on the New Zealand sheep carcass segmentation specification, Mu et al. [105] developed a segmentation robot for cutting sheep carcasses. An image processing system based on Deeplab v3+ networks was used to determine the location of ribs and spine. A 3D camera was used to obtain an image of the sheep carcass depth, and deep image processing algorithms were used to obtain key points of cutting [105].

Another method for cutting sheep carcasses based on a 3D machine vision system with a dual robot system was proposed by Bao et al. [106]. The dual-robot system consists of a 3D scanning system, a sheep carcass fixation device, and a cutting robot. A 3D model of the sheep carcass was constructed using a 3D scanner. The cutting pattern of the cutting robot was planned based on the processed 3D points clouds. The fixed carcass fixation device is designed to prevent the carcass from swinging during the cutting

process, and the dual-robot system performs precise cutting according to the trajectory calculated from the spatial coordinates of the 3D point cloud. The system proposed in this study can improve the efficiency and accuracy of sheep carcass cutting [106]. These methods have shown promising results, which are able to improve the efficiency, accuracy, and consistency of processing while reducing labor costs and improving the workers' safety [105].

An alternative approach to creating automated single-cut deboning systems is to create a human-machine interaction platform — using a robotic arm to assist manual cutting to improve efficiency and reduce the risk of human injury. Research has been conducted on replacing the human hand with a robotic arm for deboning operations by learning the motions from the butcher during meat processing [103]. Wei et al. [107] developed a robotic arm to replace the human hand for ham deboning. The robotic arm consists of a reconfigurable palm and four fingers that perform abduction, flexion, and extension, with the palm configuration adjustable for different tasks and changing conditions. The operator's left hand motion trajectories were recorded using instrumented gloves with associated force/torque and position sensors to map the deboning operation workspace to the robotic hand joint space and perform the deboning operation by both humans and robots. However, some critical issues, such as reducing tendon-driven hand friction and increasing friction at the contact point between the meat and the robotic arm, still require further research [103].

Electrical stimulation technology improves the texture and flavor of meat by using electrical current to stimulate muscle tissue after slaughter. Simmons et al. [108] developed a computer expert system that allows the stimulation parameters (electrical stimulation parameters, intensity and duration) to be adjusted to the characteristics of a specific beef or lamb carcass as it moves along the production line, and to forecast the final pH value and meat temperature.

The effectiveness of the sterilization process is determined by the temperature and duration of heating, the pressure in the autoclave, and the properties of the product. Chung et al. [109] developed a fuzzy logic controller that can maintain the sterilization temperature with an accuracy of ±0.5 °C throughout the sterilization duration, adapting to process variations in the autoclave. Precise temperature control coupled with online updating ensures that food products are heated at the set temperature for the expected time and, therefore, successful sterilization. Sterilization is controlled by a programmable logic controller (PLC), which is remotely controlled by SCADA system. The SCADA system monitors the controller's operation and sterilization response and, accordingly, calculates and updates the sterilization duration value (F_0) in real time. The temperature control system must adapt to process variations caused by periodic pressure releases. Compared with sterilization using the traditional F_0 — calculation method, the proposed system allows for batch processing with less time, less steam consumption and less risk of over-sterilization [109].

Drying is one of the most important step in extracting moisture from meat to achieve the desired moisture level. This process prevents spoilage and impacts product quality, safety, and overall production efficiency. Traditional approaches to drying time estimation are often based on rules of thumb or manual observations, which can be labor-intensive, subjective, and prone to human error. Therefore, implementing an automated solution by developing a meat drying time forecast model is essential for optimizing the product lifecycle. Taking into consideration the potential of machine learning algorithms, they have demonstrated promising results in recent years in solving various forecasting problems. Building on this, research was conducted to explore the use of machine learning methods to forecast the drying time of meat-based food products, taking into account multiple factors, including product structure and properties, environmental parameters, drying chamber characteristics, the number of products in the drying chamber, and the volume of food being processed. SCADA (Supervisory Control and Data Acquisition) system software used to monitor and control industrial processes by recording real-time measurements of room parameters such as temperature, humidity, fan speed, and other environmental conditions, was used to control the meat block drying process. The developed forecasting model demonstrated high performance in forecasting the drying time (in hours) of meat products based on features obtained from ERP and SCADA systems. Using the XGBoost collaborative machine learning algorithm, the model achieved a high correlation of 0.96 between actual and forecast values. These results highlight the model's potential for practical application in the food industry, providing a reliable tool for resource optimization and improved production planning [110].

Using an expert system (ES), an information management system for the production of sausages of certain predetermined quality has been adapted. This system takes into account potential nonlinear constraints on the quality characteristics of sausage products, or potential nonlinear criteria. The automated expert system for managing the technological process of meat and sausage production — the software package (SP) MultiMit Expert is based on two components: a database and a knowledge base, which enable the creation of an optimal recipe that meets consumer requirements. The ES has been tested for managing the production process and identifying defects in the recipe for Stolichnye boiled sausages with a high fat content. For example, the fatty pork content in the basic recipe was 32.65 %; taking into account the recommendations of the developed expert system, the fatty pork content was adjusted to 27 %. Thus, the recommendations of the developed expert system make it possible to improve the quality of the ready-to-eat meat product, increase the moisture-

holding capacity of the minced meat, which contributes to an increase in the yield of the finished product and an increase in the profitability of the meat product [111].

Using artificial intelligence and machine learning to improve the efficiency of cultured meat technology

CM) technology and assessed its future potential [112]. Optimizing cell lines for cell-based meat production is often challenging because it requires understanding the "state" of a cell or cell population (i.e., what genes and protein networks do) and selecting or engineering desired cell states. Measuring and forecasting these states involves interpreting complex interactions between genes and proteins, identifying those important for generating specific traits, and forecasting how perturbations will affect the overall process. Machine learning can be used to model these interactions using network analysis. In recent years, graph neural networks (GNNs) have been actively used to analyze biological networks [112].

Graph neural networks (GNNs) are another area of machine learning that works with graph-structured data and biological networks, such as protein interaction networks, gene co-expression networks, and metabolic networks [113]. GNNs have been implemented to forecast protein interactions [114], molecular interactions [115], metabolite-disease associations to understand disease causation [116], and others.

In addition, network analysis can help determine aroma and flavor [117]. Since meat aroma and flavor are largely controlled by metabolic pathways [118], network analysis can be applied in the biosynthesis of cultured meat to enhance aroma and flavor or add flavorings [112].

Technologies of RNA sequencing (RNK-seq) are commonly used to quantify cellular gene expression for cell line validation and optimization. However, analyzing RNA-seq or other omic data across many candidate cells is a complex and challenging analytical task. Machine learning can assist in this analysis in a variety of ways, including by grouping cells related to functionality using an unsupervised approach, gene expression profiling using a supervised approach, and identifying different types of tissue using unsupervised and semi-supervised approaches [112].

Using gene expression data to cluster cells based on cell type or behavior can help explain heterogeneity among cell populations and identify subpopulations with beneficial characteristics. When engineering cells for cultured meat production, scientists may want to isolate only certain cell types with optimal characteristics or remove undesirable cell types. For example, using single-cell RNA sequencing (scRNA-seq), Messmer et al. found that samples isolated from bovine muscle contain 11 different cell types [119]. Unsupervised machine learning can help map cellular heterogeneity by grouping cells related to functionality, identifying cell subpopulations, and performing dimensionality reduction. Typically, the input to an unsupervised machine learning model is gene expression data obtained from RNA sequencing. In traditional machine learning frameworks that are not based on neural networks, the result is typically the assignment of a cluster number to each cell or to each gene [112].

Machine learning models have been successfully applied [112,120] in gene editing technology to identify regulatory areas that determine where, when, and how much a gene is expressed. The most relevant modern machine learning methods that have demonstrated their usefulness for solving these problems are generative adversarial networks, trained directly on genomic and transcriptomic data [121], and convolutional neural networks [112].

Microscopy is a fundamental technique in cell culture, providing information such as cell health (e.g., whether they are mitotic, senescent, or apoptotic); cell behavior (e.g., whether they are invasive, contractile, or secretory); and cell lineage (e.g., whether they are stem cells, progenitor cells, or terminally differentiated cells). The relevance of microscopy during large-scale production will particularly depend on its ability to serve as a low-cost, high-throughput tool. However, microscopy has historically been limited by the complexity of its analysis. Microscopic analysis is typically performed manually by researchers with well-trained vision, and its automated execution requires systems that can incorporate the many nuances of image data. Furthermore, the use of dyes to enhance image quality is undesirable due to cost and time constraints in the production of cultured meat (CM). For CM production, cell segmentation and classification are fundamental and indispensable due to their multifaceted contributions, including quality control, cell culture health monitoring, and production optimization. The use of machine learning approaches for automated cell segmentation and classification can reduce the time, costs, and errors associated with preparing for manual image data analysis [112].

Developing robust algorithms for cell detection and segmentation using computer vision requires access to large, diverse, and well-curated datasets with detailed annotations, and additional CM-specific datasets are needed to develop robust algorithms for accurate cell segmentation in microscopic images [112]. Much of the research interest has been directed toward deep neural network architectures, driven by convolutional networks for cell segmentation [122,123]. These architectures use encoder-decoder blocks to transfer features from multiple scales and levels to efficiently segment cells in histopathological and microscopic images. The U-Net model, a variant of the fully convolutional network architecture, has shown particular promise for this task. Unlike fully convolutional networks, U-Net incorporates skip connections that facilitate accurate semantic segmentation by fusing features from different resolutions, enhancing the model's ability to capture complex details [112].

Transitioning CM cultivation from laboratory to industrial scale requires efficient bioprocess design. This involves using large bioreactors to create a controlled environment

for cell growth and differentiation that maximizes biomass and minimizes byproduct yield. The most straightforward application of machine learning to bioreactors is the use of machine learning-based models to manage bioreactor inputs. For monitoring and controlling bioreactors and industrial processes, wide range of machine learning models are used in bioprocesses, especially supervised learning models such as neural networks [124], random forests [125], and gradient boosting [126]. These learning methods do not necessarily require a prior understanding of the bioreactor biochemistry [112].

The sensory properties of CM, such as flavor and texture, are crucial to its commercial success. They can be obtained during cell culture from the flavor or textural components of cells, media, or scaffolds. Machine learning plays an important role in improving the analysis and identification of flavors and textures in other food products through the analysis of various types of data, and these methods will likely also play a role in the development of CM cultivation technologies. Recent research focuses on using the molecular structure and physicochemical properties of aroma compounds to forecast aromas, including taste or odor [127,128]. These characteristics are quantified as molecular descriptors — numeric representations that encapsulate the properties of the molecules involved. These descriptors then serve as input to machine learning models, which are trained to identify and predict flavor profiles and odor characteristics with greater objectivity. A wide range of traditional machine learning approaches can be used for this purpose, such as support vector machines, random forests, k-nearest neighbor, and AdaBoost trees [112,129]. In addition, deep learning approaches such as CNNs and multilayer perceptrons, and unsupervised learning approaches such as cluster analysis using principal component analysis, have been applied. Collectively, these methodologies have demonstrated that ML can make a significant contribution to improving the sensory properties of cultured meat and a range of food products [112].

Artificial intelligence for shelf life forecast

Accurate shelf life assessment is essential to ensure and maintain food safety, reduce waste and improve supply chain efficiency.

Traditionally, the shelf life of food products has been assessed using microbiological and chemical analysis, and sensory evaluation. Although these methods are effective, they are labor-intensive, time-consuming, and cannot fully account for dynamic environmental conditions, such as fluctuations in temperature, humidity, and microbial load during storage and transportation [130–132]. To address these issues, mathematical models have been introduced to forecast shelf life based on experimental data obtained using destructive or non-destructive analytical methods. The reliability of these models largely depends on the quality and accuracy of the analytical methods used to collect the data [132].

AI can be used to study data obtained from non-destructive testing methods such as hyperspectral imaging, spectroscopy, machine vision and electronic sensors to improve shelf life forecast [132].

The accuracy and reliability of artificial intelligence models for shelf life forecast depend significantly on the quality of the data obtained through analytical methods. Machine learning (ML) is a powerful tool capable of processing massive amounts of data, including food chemical composition, storage temperature, humidity, and non-destructive testing data, to accurately forecast product shelf life. Various traditional ML and new ML models have been developed and applied in the food industry. Traditional ML models typically require structured and manually developed features that are interpretable and struggle with high-dimensional and unstructured data. Deep learning technology, on the other hand, was developed to automatically learn feature representations from raw data using multiple layers of computation, which works exceptionally well with unstructured data such as images. The key difference lies in their complexity and feature extraction. While classical machine learning involves manual feature engineering, deep learning models are trained hierarchically from raw data and are therefore flexible but also computationally expensive. However, some of them have been used in forecasting the shelf life of food products [132].

Artificial intelligence is improving the accuracy of programs used to determine the shelf life of meat in food quality control. Current microbiological kinetic equations predict freshness under specific temperature conditions, but they become ineffective when the temperature fluctuates. To address this issue, deep learning methods were used to identify the internal relationships between temperature fluctuations during storage and a new CNN_LSTM (convolutional neural network with long short-term memory) model was proposed that predicts freshness parameters over a range of temperature fluctuations. Results showed that using the CNN_LSTM model yields more accurate forecast results than classical microbial kinetics methods such as the logistic equation, the Gompertz equation, and the Aarhenius equation, which are effective under fixed temperature conditions. Under temperature fluctuations, the model could still accurately predict the total viable cell count (TVC) under variable temperature conditions, with the coefficient of determination (R^2) exceeding 0.95 and the root mean square error (RMSE) less than 0.2. Furthermore, the model had the potential to forecast freshness under various changes in factors other than temperature fluctuations, which opened up new perspectives for forecasting produce freshness [133].

A number of researchers have developed machine learning algorithms to detect color, texture, and volatile organic compounds, as well as a range of other spoilage indicators, to assess meat quality in real time [132,134].

In a study by Italian scientists, various parts of chicken (breast, leg and thigh) were analyzed in terms of the content of ten biocompounds in the meat: nicotinamide, anserine,

carnosine, malonic acid Dialdehyde and biogenic amines (putrescine, cadaverine, histamine, tyramine, spermidine, and spermine). The analysis was conducted on chilled chicken meat parts using three different packaging solutions: modified atmosphere packaging (MAP), vacuum packaging (SKIN), and packaging in O_2-permeable plastic film (STRETCH). The results showed that nicotinamide was the most discriminant compound, monitored for all chicken parts and reduced the research time. Notably, its concentration decreased over time, regardless of the packaging type. The cumulative contribution of all studied biocompounds to shelf life was identified using multivariate statistical analysis using partial least squares discriminant analysis (PLS-DA) and support vector machines (SVM). Both machine learning models demonstrated high classification accuracy: for different poultry parts, shelf life, and packaging used. The PLS-DA method achieved classification accuracies of 87 %, 85 %, and 70 %, respectively. SVM achieved higher accuracy rates of 97 %, 99 %, and 94 %, respectively. These results highlight the importance of considering the combined effects of all studied biological compounds. Furthermore, the obtained results underscore SVM efficiency as a discriminative multivariate approach to food classification [134].

Some key parameters used as chemical indicators in assessing quality and shelf life using digital technologies combined with machine learning (ML) include pH, water activity, lipid oxidation, and protein degradation. They have been regularly monitored to assess spoilage processes in various food products during storage and transportation [135]. Future research is expected to explore advanced data analysis approaches, such as deep learning, to facilitate the integration of spectral and spatial information in joint modeling.

The results of digital image processing technologies that detect color, texture, and changes in the appearance of products [132,135], combined with data from an electronic nose that detects volatile organic compounds released during spoilage [101,131], are transmitted into ML models that, by comparing these parameters with the rate of spoilage, predict shelf life in real time. The combination of digital technologies and machine learning forms a universal approach to quality management of a perishable product — meat [132].

The development of wireless sensor technologies (WST), which enable interconnection between the internet cloud and the physical world, based on smartphones with sensing and networking capabilities, has the potential to continuously monitor the temperature and humidity of meat storage in real time, inform consumers of the corresponding results, and further control the storage environment. The hardware requirements of a standard WST include: (1) a flexible wireless signal transceiver, (2) an energy-efficient microcontroller, (3) a long-life power supply, and (4) a high-performance sensor [136]. The revolution in information and communication technology has given rise to many new applications, and many early applications have already been deployed in healthcare and logistics. In particular, several researchers have proposed smartphone-enabled wireless systems with wireless communication for meat quality monitoring to improve the fresh meat supply chain and ensure cost efficiency. Wireless/smartphone-based sensors for meat spoilage detection and quality control provide more reliable traceability throughout the supply chain. Instead of relying only on periodic inspections by government agencies and internal quality inspectors, manufacturers, distributors, and consumers can rely on these strategies to identify spoilage organisms and adjust storage environments to control food-borne hazards. Alternatively, this technology can be an effective method for obtaining expert opinions remotely, possibly even for further verification of relevant results [137].

One of the most important tools for ensuring meat safety is the use of various packaging solutions that extend shelf life and provide maximum convenience for consumers. Various types of packaging, including vacuum bags, aseptic containers, and modified eco-friendly packaging, are used in the food industry to extend shelf life, preserve freshness, and protect the food products from contamination [138].

Furthermore, AI-powered image analysis systems use image recognition and machine learning algorithms to thoroughly examine and authenticate packaging materials, labels, and codes on food products [139]. Their purpose is to ensure regulatory compliance and verify the accuracy of labeling information by identifying counterfeit packaging or labeling, ensuring consumer confidence. AI-powered machine vision systems play a significant role in improving product traceability, reducing the likelihood of inaccurate labeling, maintaining product quality, and enhancing consumer safety and satisfaction [140].

Automated systems with image recognition capabilities (AI Vision technologies) are used to confirm accurate packaging and labeling, thereby minimizing errors and ensuring compliance with regulatory standards [16]. Implementing AI Vision technology into packaging operations has the potential to transform the food sector by improving quality control, ensuring regulatory compliance, and providing consumers with quality products. Food companies are increasingly using personalized packaging to improve consumers' satisfaction and expand market covering [16].

Problems, challenges and limitations

AI and related technologies are creating a host of new complex interactions not only between humans and machines, between machines and machines [141], but also increasingly between machines and ecosystems, as well as with the Earth system as a whole. The introduction of AI and related technologies into the world of agriculture, agricultural processing, and resource management can be seen as adding new nodes and connections to these already complex socio-ecological and socio-technical systems [142].

There is growing interest and investment in the development and implementation of AI and related technologies in sectors critical to sustainable development, particularly the food industry. However, the efficiency of these technologies and the social, economic, and environmental impacts of their implementation unfold within a broader social, technological, and environmental context, making it difficult to forecast their implications in regards for distribution and sustainability [142].

In order to fully implement the potential of artificial intelligence and machine learning in the food industry, several obstacles must be overcome. The primary obstacle is the significant upfront financial investment required to implement these latest innovations. Purchasing and integrating advanced AI systems can be costly, especially for small and medium-sized businesses. Another major concern is the potential for cyberattacks and data breaches. Food industry businesses are more susceptible to cyberattacks that can steal sensitive data and cause operational disruptions as they become increasingly digitalized and networked. Reliable cybersecurity protection is essential to prevent such threats [16].

Although AI systems are often more accurate than traditional approaches, there are still challenges in maintaining their consistent performance and reducing false positives and negatives. Issues with scalability and compatibility with existing technologies and technical requirements can hinder the widespread use of AI technology [16]. For example, integrating AI technologies with existing food systems and processes can be challenging. Legacy systems may be incompatible with new AI solutions, leading to integration issues. Furthermore, ensuring the smooth operation of AI systems with existing hardware, software, and workflows requires careful planning and implementation. The application of AI requires transparency and interpretability of algorithms: many AI algorithms, especially deep learning models, are often considered "black boxes", meaning their decision-making processes are not easily interpreted. This lack of transparency can be problematic in the field of food safety, where understanding the rationale behind AI decisions is critical to trusting the resulting data and ensuring compliance. Scaling AI solutions to handle large volumes of data and adapt to changing conditions can be challenging. As food safety needs evolve and data volumes increase, AI systems must be able to scale and adapt to new data types and scenarios [143].

Another drawback is that managing and operating AI-based systems requires a trained staff team. Despite the time and cost involved, investing in training employees to use the latest technologies is crucial. Addressing security and ethical dilemmas is also crucial. For example, using AI in the food industry to increase automation could lead to potential layoffs among production and management staff. To gain public trust and acceptance, AI systems must comply with security regulations and ethical principles. While AI vision and machine learning have much to offer the food industry, their successful implementation and long-term sustainability depend on addressing issues such as cybersecurity risks, cost, accuracy, scalability, workforce skills, and ethical considerations [16].

When collecting and processing personal data, such as consumer reviews or health information, in food safety applications, privacy concerns may arise. The use of artificial intelligence and data analytics often involves collecting extensive data on individuals, which may include sensitive information. Mishandling or unauthorized access to this data can lead to privacy breaches and loss of consumers' trust. Food safety systems often store and process large volumes of sensitive data, including supply chain information and quality control parameters. Leakage or loss of this data can compromise food safety, disrupt operations, and damage the organization's reputation. Therefore, ensuring data security is crucial to preventing unauthorized access to information, data breaches, and cyberattacks [143].

The implementation of artificial intelligence and data collection technologies may have environmental consequences, such as increased energy consumption and growing volume of electronic waste. In the context of food safety, the use of complex sensors and computing resources may contribute to an increased overall environmental impact [144].

Conclusion

This article reviews the potential capabilities of artificial intelligence in the food industry, particularly in meat and poultry processing and livestock farming, and critically analyzes the challenges these technologies face and the innovative solutions they offer. The researches presented in the review shows that artificial intelligence possesses enormous potential for the digital transformation of the food industry, thus contributing to process improvement, higher product quality and safety, and more efficient resources management. At the same time, we also note the challenges associated with artificial intelligence, such as issues of data privacy and security, technical complexity and integration with classical (traditional) methods, as well as personnel qualifications. Using of artificial intelligence achieves technological, economic, and social benefits. The technological benefit is associated with: (1) increased accuracy and speed of production due to the human factor elimination; (2) reducing volume of rejection through automated quality control; (3) ensuring regulated food safety parameters (continuous monitoring of critical points). Economic effect — (1) reduction of operating costs due to reduction of manual labor, energy saving; (2) reduction of raw material losses due to optimization of ingredients use; (3) reduction of logistics costs (smart inventory management). Social effect — (1) product safety (control over storage and transportation conditions, prevention of food-borne diseases outbreaks); (2) creation of new professions (automation does not replace people, but transforms their role).

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EFFECT OF THE MORINGA LEAVES EXTRACT ON THE TENDERIZATION OF *LONGISSIMUS* MUSCLE IN INDONESIAN NATIVE GOATS

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Keywords: *Moringa leaves extract, goat meat, cooking loss, proteolytic enzymes, tenderness*

Abstract

Meat tenderization techniques often involve time-consuming processes or chemical additives that may raise health concerns or alter the taste of meat. Exploring alternative natural methods, such as utilizing proteolytic enzymes derived from moringa, presents an opportunity to address these limitations. Studies on moringa have been limited, especially those focusing on goat meat tenderization, leaving a gap in understanding how moringa affects this process. The research aims to bridge this gap by systematically examining how protease enzymes from moringa leaves can tenderize goat meat. The research was performed on goat meat samples (100 g of meat per sample) treated with the moringa leaves extract (prepared from 20 g of moringa leaves with 60 ml of distilled water) at different storage times (2, 24, and 46 hours), as well as a control sample without any additive. The results showed that the moringa leaves extract did not significantly alter pH values and increased cooking loss for the 24-hour treatment (44.3 %) and 46-hour treatment (39.8 %) compared to the control (36.8 %). Color analysis showed increased lightness (L) and redness (a*). The yellowness (b*) showed considerable difference between the control and the 24-hour samples compared with the 2-hour and 46-hour samples. The moringa leaves extract significantly reduced tenderness by lowering hardness, gumminess, resilience, and chewiness. This research advances environmentally friendly, renewable solutions in the food industry because the food industry produces significant quantities of meat by-products and waste during processing. This research will reduce waste by transforming tougher or less desirable meat cuts into more tender and valuable products.*

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Introduction

Meat tenderness refers to the texture and softness of meat when it is cooked and consumed, and is an essential characteristic of meat quality that significantly affects the eating experience [1]. It is defined as the ease with which a product's structure can be disintegrated by the combination of shear, compression, and grinding actions during mastication. Tenderness is one of the most important quality parameters in consumer perceptions of meat [2]. In the tenderization process, the degradation of structural proteins in meat and collagen reduces meat toughness [3]. The production of consistently tender meat is crucial for retaining consumer confidence in red meat and maximizing financial gain, as tender meat cuts fetch a higher premium than less tender cuts. Several factors contribute to meat tenderness, including pre- and post-slaughter factors, such as the cut of meat, the animal's age, breed, diet, and the cooking method used. Over the years, the meat industry has been continually searching for ways to improve meat tenderness, including ultrasound, high-pressure processing (HPP), wilting, electrocatalysis, and others. There is growing interest in exploring natural methods, such as the use of bacterial and plant enzymes, for meat tenderization. It has been shown that applying different types of bacterial enzymes, such as collagenolytic proteases derived from *B. subtilis* B13 and *B. siamensis* S6, to tenderize goat meat during wet aging decreased hardness, gumminess, and chewiness but increased springiness. Goat meat treated with these enzymes had shear force values that were 30 % and 26 % lower, respectively, than those of untreated samples [4]. Plant protease enzymes, such as papain, bromelain, ficin, actinidin, and zingibain, have been shown to improve the tenderness of meat due to proteolytic degradation [5]. Plant proteases can influence blood coagulation and may be used to treat digestive disorders [6]. Despite the benefits of using plant proteases in meat processing,

their application can present a challenge. If not appropriately used, they may over-tenderize meat, resulting in a mushy or undesirable texture. Plant proteases, depending on the specific enzyme used and the treatment duration, can affect meat flavor and aroma. Nevertheless, plant proteases have significant potential for various industrial applications.

Moringa is highly nutritious and contains essential amino acids, vitamins, and phenolic compounds. Moreover, it is widely distributed as a medicinal plant in tropical countries. *M. oleifera* (Moringaceae) is a tropical tree species with increasing utility, occurring naturally mainly in Pakistan and northern India. *M. oleife*ra is currently cultivated in Africa, South America, Asia, and the Middle East. It is of high Importance in the food pharmaceutical, cosmetic, agricultural industries. It has earned a few nicknames, such as 'the miraculous tree' and 'the tree of long life', and provides high-nutrient raw materials [7]. Studies on moringa have been limited, especially those focusing on goat meat, which are very scarce. Most existing research has focused on other types of meat and has also studied moringa as a supplement in animal diets. This left a gap in understanding how moringa affects the tenderness of goat meat. Thus, there is limited exploration of the biochemical mechanisms by which moringa contributes to meat tenderization, underscoring the need to understand the enzymatic activity and the impact of specific compounds in moringa that facilitate muscle fiber relaxation or collagen breakdown to provide valuable insights. Several studies have investigated the enzymatic properties of moringa protein and found that it contains proteolytic enzymes. Natural plant-derived extracts, such as those from *M. oleifera* leaves and seeds, have garnered interest as natural preservatives and functional additives, owing to their substantial antioxidant and antimicrobial properties, which can impede the proliferation of various pathogens, thereby enhancing food safety [8]. The presence of phytochemicals in the moringa, including flavonoids and other phenolics in its leaf extract, can hinder the growth of pathogenic microorganisms and extend the shelf life of food [9]. The study found that the moringa leaves extract exhibited potent antimicrobial activity against spoilage bacteria, including aerobic plate count and Enterobacteriaceae counts, and reduced counts of *E. coli*, *Salmonella enterica* serovar Typhimurium, and *Staphylococcus aureus* artificially inoculated into ground beef [9]. Nevertheless, this purified enzyme can be considered a promising agent, a cheap and safe source suitable for various industries [10]. Moringa extracts contain a wealth of bioactive compounds, including flavonoids, phenolic compounds, and antioxidants, which have garnered attention for their potential use in meat and meat products [11]. Mashau et al. [12] found that adding moringa leaves powder to ground beef improved its nutritional properties and inhibited lipid oxidation. Moringa leaves have been found to significantly enhance the tenderness of goat meat, especially in the *Longissimus* muscle, by making it more tender when using moringa leaves as a paste [13] Adding the moringa leaves extract improved tenderness in beef patties, with a 2 % extract level yielding the best results in terms of protein and fat content and sensory quality [14]. In addition to tenderness, moringa leaves also improve meat aroma without compromising color or taste. This was evident in the Bali beef, which was marinated with moringa leaves powder and received better overall acceptance from taste testers [15]. Moringa leaves are a treasure trove of nutrients and bioactive compounds, providing antioxidant and antimicrobial benefits that lead to healthier meat products [16]. Kenawi et al. [17] utilized moringa as a natural antioxidant to investigate its impact on the quality of buffalo meat products. It was demonstrated that the moringa leaves extract exhibited an inhibitory effect on bacterial growth and enhanced the acceptability of the meat product. Moringa extract acts as an antioxidant, helping maintain meat quality during storage and indirectly affecting its texture by preventing oxidative rancidity [18]. Bioactive compounds in moringa have antimicrobial and antioxidant properties that can enhance meat preservation and safety by reducing microbial growth and oxidative stress [16]. Moreover, other studies have demonstrated that moringa can help modulate the gut microbiota of broilers by promoting the establishment of beneficial microorganisms while inhibiting harmful pathogens, thereby further supporting meat quality and safety [19]. Mwankunda et al. found that meat samples treated with a 1.5 % crude extract of moringa leaves. had significantly improved pH, juiciness, texture, flavor, taste, and overall acceptability scores compared to the control and other treated samples [20]. Abubakar et al. [21] concluded that marinating African catfish with a combination of moringa leaves and ginger rhizome significantly improved the proximate and sensory qualities of the fish. Moringa leaves powder increased the protein content in meat, particularly in the thigh and breast muscles, while at the same time reducing fat levels in broilers [22]. The inclusion of moringa also lowered the cholesterol and triglyceride levels in meat, leading to a healthier meat product [19]. Using moringa leaves in meat processing could also help tackle food security and sustainability issues, thanks to its resilience and rapid growth, making it a promising option for improving meat quality [16]. The moringa leaves extract enhances meat quality by increasing the antioxidant activity and protein content, reducing fat content, altering pH levels, and improving consumer preference when unpleasant odors are removed, contributing to better nutritional and sensory properties [23].

Ultimately, research on improving the tenderness of goat meat using moringa leaves can significantly contribute to both academic knowledge and practical applications in the meat industry. Enhancing the quality of goat meat through natural additives such as moringa can increase farmers' productivity and profitability, supporting local economies and food systems. By improving the tenderness and overall quality of goat meat, moringa supplementation

can potentially lead to better consumer health outcomes. As a natural additive, moringa may reduce the reliance on synthetic tenderizers, thereby contributing to safer food products for consumers. Moringa is a natural additive that reduces the need for synthetic tenderizers or antibiotics, leading to safer food products for consumers. Moringa leaves can be sourced from local agricultural practices, reducing waste and promoting a circular economy within livestock production. The primary purpose of this research is to enhance the tenderness of goat meat using the natural proteolytic enzyme derived from moringa leaves. Moreover, the extraction of this enzyme could be economically viable and environmentally friendly, as it is derived from natural sources. The analysis yielded new data on how moringa affects various physical, chemical, and textural attributes, including pH, color, cooking loss, and textural properties such as hardness, chewiness, springiness, and overall mouthfeel, which are critical to consumer acceptance. The parameters studied include cooking loss, texture profile, acidity (pH), and color.

Objects and methods

Objects

The primary research material consisted of goat meat, collected from approximately one-year-old male goats, specifically the *Longissimus dorsi* muscle, which was taken between the 9th and 12th ribs on both the left and right sides, totaling 1200 g of muscle samples (6 pieces of *Longissimus dorsi* muscle, each was 200 g, each piece was divided into two pieces of 100 g to use as one replicate). These samples were collected from six goats. They were obtained from a meat shop after slaughter, transported to the lab, stored in the freezer (–19 °C) for one week, and then used. The study was conducted in the Animal Products Technology Laboratory, Faculty of Animal Sciences, and the Biochemical Laboratory, Faculty of Mathematics and Natural Sciences at Jenderal Soedirman University, Purwokerto, Indonesia. Moringa leaves were obtained from a farm near Jenderal Soedirman University.

The preparation of the moringa leaves extract

Twenty grams of moringa leaves (for each sample) were weighed, washed, and then ground using a mortar and pestle. The ground leaves were mixed with 60 mL of distilled water and stirred using a magnetic stirrer (Thermolyne Cimarec 3 hotplate stirrer, Model SP47235, Thermo Fisher Scientific, US) for 30 minutes at 4 °C. The mixture was then filtered using a cloth and centrifuged at 7000 rpm for 10 minutes (BIOBASE, Model BKC-TH23RII, BIOBSE Group, China). The supernatant was used to marinate meat samples. The process of the moringa leaves extract preparation is illustrated in Figure 1.

Protease activity

Protease activity was assayed using casein as a substrate. A total of 0.5 mL of casein solution was preincubated (Lab-Line, Model Aquabath, Lab-Line Instruments Inc, US) at 35 °C for 5 min, after which 0.1 mL of crude enzyme extract was added. The mixture was incubated at 35 °C for 30 minutes, and the reaction was stopped by adding 4.3 mL of 1 M HCl, resulting in a final volume of 4.9 mL. Samples were kept at 4 °C for 30 minutes and then centrifuged at room temperature for 10 minutes to remove undigested protein (LD-3 Electronic Centrifuge, maximum speed 4000 ppm, YJNGUI China). The absorbance of the supernatant was recorded at 275 nm using a Spectrophotometer (Shimadzu Corporation, Model UV-1280, Japan). Tyrosine was used as the standard, with the calibration curve described by the Equation.

$$y = 0.0232x - 0.0073, \quad (1)$$

where y is the absorbance; x is the tyrosine concentration (mg/L).

One protease unit (U) was defined as the amount of enzyme that releases one μmol of tyrosine per minute under the assay conditions.

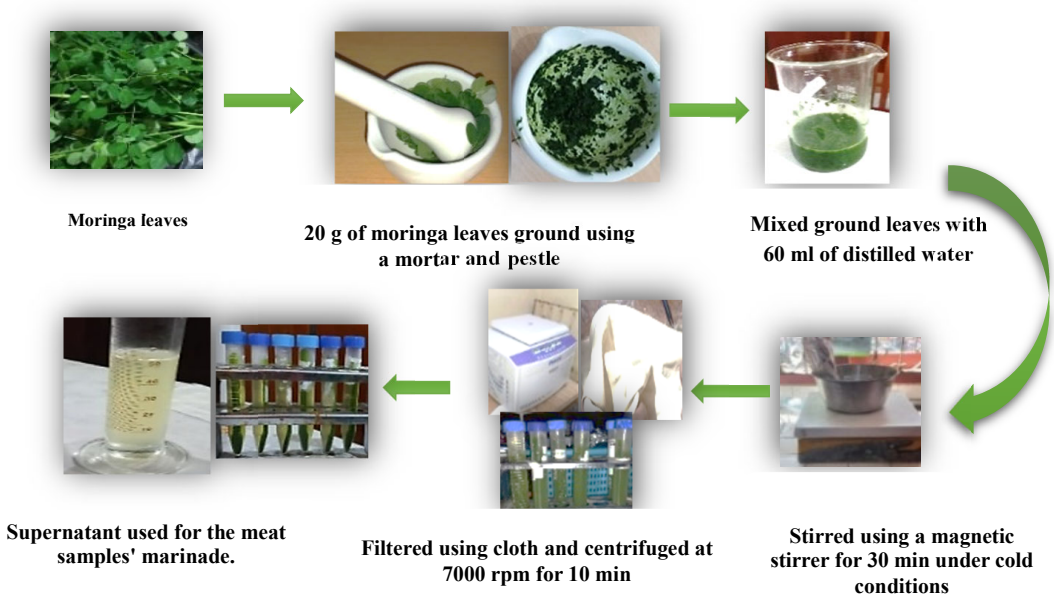

Figure 1. The process of preparing the moringa leaves extract

Phytochemical test (alkaloids, flavonoids, and tannins)

Phytochemical screening was carried out to identify alkaloids, flavonoids, and tannins in leaves extracts. Alkaloids were detected using Dragendorff's reagent, which yields a characteristic green, brown, or orange-red precipitate. Flavonoids were tested with a 5 % aluminum chloride solution, which produced a yellow to green coloration or a precipitate in the presence of flavonoid hydroxyl groups. Tannins were identified using ferric chloride ($FeCl_3$), which gives a dark green, purple, or black color when tannins are present.

Meat sample preparation

The meat samples were divided into four treatments (each 100 g) with three replications: T0 (control without additives), T1 (marinated for 2 hours), T2 (marinated for 24 hours), and T3 (marinated for 46 hours). The samples were marinated in the moringa leaves extract, ensuring that they were fully covered. They were stored in glass containers and put in the refrigerator at different times (2, 24, and 46 hours). The samples were analyzed for pH, color, cooking loss, and texture profile. The tenderization of the meat samples using the moringa leaves extract is shown in Figure 2.

pH determination

The measurement was performed by dipping a calibrated pH meter ((pH meter (Portable pH Meter HI8424, Hanna Instruments, USA) into a homogenized meat mixture. All measurements were performed at room temperature.

Cooking loss (CL)

Three cubes were cut from each sample, weighed, placed in labeled plastic bags, and subsequently cooked in a water bath (Memmert Water Bath WTB11, Memmert GmbH, Germany) at 80 °C for 10 minutes. Next, the cooked muscles were chilled, blotted dry, and weighed using a scale (AND GF Series, A&D Technology, Japan). The cooking loss percentage (CL%) for each sample was then calculated using the following formula, adapted from [24]:

$$CL(\%) = \frac{\text{Initial Sample Weight} - \text{Sample Weight After Cooking}}{\text{Initial Sample Weight}} \times 100, \quad (2)$$

Texture profile analysis

Texture profile analysis of meat was performed using a Food Texture Analyzer (TA-XT, Stable Micro Systems, Godalming, UK). An aluminum cylinder probe with a diameter of 40 mm was used. The experiments were conducted at room temperature under a 30 % compression ratio and crosshead speeds of 1.0 mm/s and 2.0 mm/s. Meat samples were cut into uniform pieces to ensure homogeneity and then placed between the probe and the base. Parameters measured included hardness, springiness, cohesiveness, chewiness, gumminess, resilience, firmness, and adhesiveness.

Color

The colorimetric evaluation of treated and control samples included the colorimetric parameters L* (lightness), a* (green to red coordinate), and b* (blue to yellow coordinate) using a Konica-Minolta CR-400 colorimeter (Konica-Minolta, Japan).

Statistical analysis

The obtained data were statistically analyzed using the SPSS program for Windows (Version 25) and tested using one-way ANOVA, followed by Duncan's multiple range test to compare means. Data are expressed as mean values ± standard deviation (SD). The accepted level of significance for all comparisons was $p < 0.05$.

Results

Protease activity

Protease activity assays demonstrated measurable casein hydrolysis by the leaves extract. The crude leaves extract produced an absorbance of 0.510 at 275 nm, equivalent to 22.30 mg/L tyrosine. After subtracting the control (0.126, 5.74 mg/L), the net release was 16.56 mg/L, corresponding to 0.449 µmol tyrosine. This yielded an activity of 0.015 U in 0.1 mL of enzyme extract, corresponding to 0.15 U/mL in the crude extract.

Phytochemical test

Qualitative phytochemical screening revealed that leaves extract of moringa tested positive for alkaloids, flavonoids, and tannins. Distinct color changes and

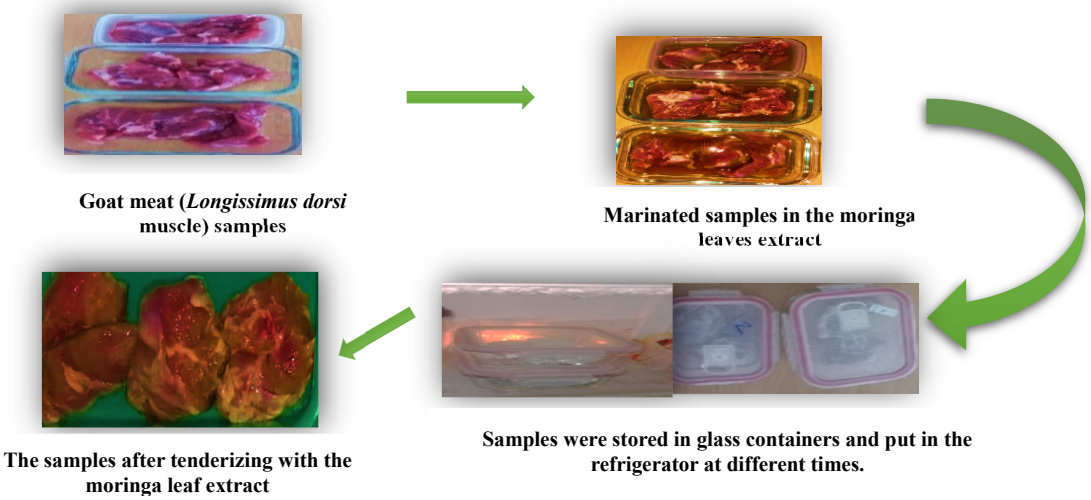

Figure 2. Tenderization process of goat meat samples with the moringa leaf extract

precipitate formation confirmed the presence of these secondary metabolites. These results are consistent with previous findings that moringa tissues contain abundant phytochemicals, which contribute to their antioxidant and antimicrobial potential.

Effects of the moringa leaves extract on the texture profile analysis of goat meat

Table 1 shows the texture profile analysis (TPA) parameters of the treatments (T0 (control), T1, T2, and T3) from *Longissimus* muscle treated with the moringa leaves extract and stored for different durations (0-hour [control], 2-hour, 24-hour, and 46-hour) at a temperature of 4 °C. There was a significant difference ($p < 0.05$) between the control and treated samples, in hardness, as the hardness decreased from (4720.3 ± 2354.28) in the control to (451.04 ± 301.75, 335.96 ± 122.92, and 1013.56b ± 739.11) in the samples treated for 2, 24, and 46 hours, respectively. There was a significant difference ($p < 0.05$) between the treated and control samples in gumminess, resilience, and chewiness, with lower values in the treated samples than in the control. There was no statistically significant difference between the treated samples and the control samples in springiness, cohesiveness, firmness, or toughness.

Effects of the moringa leaves extract on the pH and cooking loss of goat meat

Figure 3 and Table 2 show no significant differences in pH between the control samples compared to the 2-, 24-, and 46-hour treatments. Figure 4 and Table 2 show that the treated samples have increased cooking losses compared to the control samples, except for the 2-hour treatment. The cooking loss for the 24-hour treatment was significantly higher than that of the control and the other treatments, increasing from 36.8 % to 44.3 %.

Effects of the moringa leaves extract on the color of goat meat

Figure 5 shows a significant difference between the control and the treatments in lightness; however, no significant difference was observed among the treatments themselves. There was no significant difference in redness between the treated samples and the control. Also, there was no significant difference between the control and the 24-hour samples, nor between the 2-hour and the 46-hour samples, in terms of yellowness. However, there was a considerable difference in yellowness between the control and the 24-hour samples, as well as between the 2-hour and 46-hour samples.

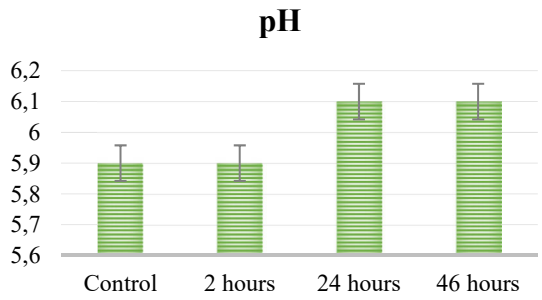

Figure 3. Effects of the moringa leaves extract on the pH of goat meat during different aging periods

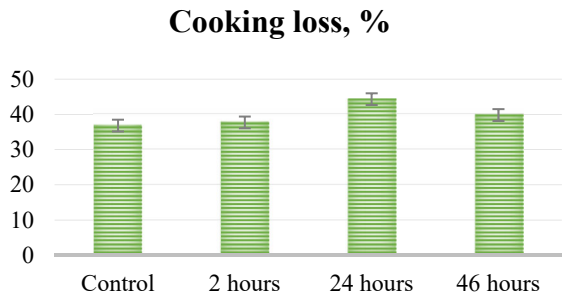

Figure 4. Effects of the moringa leaves extract on the cooking loss of goat meat during different aging periods

Table 1. Texture profile analysis (TPA) of goat meat treated with the moringa leaves extract during different aging periods

Item	Control	Tenderization period, hr.		
	0	2	24	46
Hardness, g	4720.31 ± 2354.28^{a}	451.04± 301.75^{c}	335.96 ± 122.92^{c}	1013.56 ± 739.11^{b}
Chewiness, mJ	1057.01± 670.87^{a}	120.92 ± 96.15^{b}	93.13 ± 55.40^{b}	170.80 ± 118.00^{b}
Firmness	3971.56 ± 885.95^{a}	4687.14 ± 2026.85^{a}	3220.38 ± 506.77^{a}	3663.77 ± 1919.00^{a}
Toughness	25448.59 ± 5561.73^{a}	27071.39 ± 13227.14^{a}	19019.55 ± 2222.62^{a}	19388.06 ± 8790.85^{a}
Adhesiveness, mJ	–47.17 ± –47.17 ^{b}	–15.02 ± 15.24^{a}	–26.44 ± 26.12^{ab}	–21.88 ± 13.14^{ab}
Gumminess, g	1886.87 ± 959.17^{a}	209.90 ± 130.00^{b}	162.09 ± 69.15^{b}	396.44 ± 309.90^{b}
Cohesiveness	0.40 ± 0.08^{a}	0.50 ± 0.10^{a}	0.47 ± 0.05^{a}	0.40 ± 0.09^{a}
Springiness, mm	0.54 ± 0.14^{a}	0.52 ± 0.12^{a}	0.56 ± 0.15^{a}	0.48 ± 0.10^{a}
Resilience	0.24 ± 0.06^{a}	0.20 ± 0.05^{ab}	0.16 ± 0.04^{b}	0.17 ± 0.03^{b}

Different letters (a, b, c) in the row indicate significant differences between means ($p < 0.05$).

Table 2. PH and cooking loss of goat meat treated with the moringa leaves extract during different aging periods

Item	Control	Tenderization period (hr.)		
	0	2	24	46
PH	5.9 ± 0.2^{a}	5.9 ± 0.7^{a}	6.1 ± 0.2^{a}	6.1 ± 0.4^{a}
Cooking loss (%)	36.8 ± 1.6^{b}	37.7 ± 4.2^{b}	44.3 ± 1.4^{a}	39.8 ± 6.1^{ab}

Different letters (a, b, c) in the row indicate significant differences between means ($p < 0.05$).

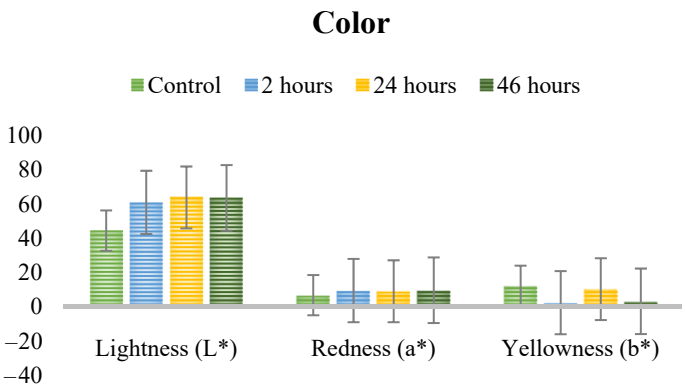

Figure 5. Effects of moringa leaves extracts on the color parameters of goat meat during different aging periods

Discussion

Protease activity

The protease from moringa leaves had a molecular weight of approximately 51 kDa and was classified as a cysteine protease based on its inhibition by $HgCl_2$ [25]. The proteases showed high specificity for casein, with additional activity on whey and gelatin. They were stable at temperatures between 40–60 °C and pH levels of 4–7 [25,26]. Future work should include protein quantification, kinetic studies, and characterization of pH and temperature optima. Together, these findings demonstrate that leaves of Moringa oleifera are promising sources of proteolytic enzymes and phytochemicals, supporting their potential applications in food processing, nutraceutical, and biotechnological industries.

Phytochemical test

M. oleifera, frequently referred to as the drumstick tree, is distinguished for its extensive phytochemical profile and protease activity, which are integral to its multifaceted medicinal attributes. The foliage and seeds of moringa are rich in alkaloids, flavonoids, and tannins, which are pivotal phytochemicals with a range of health-promoting effects. These bioactive compounds are recognized for their antioxidant, antimicrobial, and anti-inflammatory capabilities, rendering moringa a significant botanical resource in both traditional and contemporary medicinal practices. While moringa is lauded for its rich phytochemical composition and protease activity, it is essential to acknowledge the variability of these compounds, which depends on the extraction methodologies used. Such variability can significantly affect the effectiveness and potency of moringa-derived products, underscoring the need for standardized extraction and analytical protocols to ensure consistent outcomes. The presence of flavonoids, alkaloids, and tannins in leaf extracts highlights the multifunctional nature of moringa bioactive components, in agreement with the studies by Ogah et al. [27] and Arbab et al. [28], who found that preliminary phytochemical screening of leaves extracts revealed the presence of alkaloids, tannins, flavonoids, and saponins. Alkaloids found in the leaves of the moringa contribute to its pharmacological functions, including anti-inflammatory and analgesic properties [29,30]. The leaves of moringa are particularly rich in flavonoids, which are efficacious antioxidants that help scavenge free radicals, thereby alleviating oxidative stress and inflammation [31]. The tannins present in moringa are recognized for their astringent properties, which can facilitate wound healing and reduce inflammation [32]. They also exhibit antimicrobial properties, thereby enhancing moringa's efficacy in treating infections and promoting gastrointestinal health [29]. Additionally, the presence of various bioactive compounds contributes to its antimicrobial properties [33], antioxidant benefits, and may also influence enzyme stability or interactions with substrate proteins.

Effects of the moringa leaves extract on the texture profile analysis of goat meat

In the current study, statistical analysis showed that leaves extract significantly decreased ($p < 0.05$) the textural properties of hardness, gumminess, resilience, and chewiness; the other attributes, including springiness, cohesiveness, adhesiveness, and resilience, showed no significant differences between treated and control samples. Some of the studies suggest that moringa leaves can act as a natural tenderizer, improving the texture profile analysis (TPA) of goat meat. Mohamed et al. [13], stated provided that moringa leaves paste, when applied for 3 to 6 hours, leads to a substantial difference in textural properties such as hardness and gumminess in goat meat. The findings of Botinestean et al. [34] indicate that specific cuts of meat can exhibit resistance during mastication. This resistance can lead to oral discomfort, particularly for elderly consumers, which may deter them from consuming meat. Rahman et al. [35] demonstrated a significant increase in tenderness and overall acceptability when using *M. oleifera* leaves extract on goat meat nuggets, which agrees with the findings of the current study. Thus, moringa leaves can offer a solution to enhance meat tenderness and improve overall consumption experiences for this demographic. Tender steaks often come with a high price tag, limiting access for many consumers. The changes in hardness observed with moringa indicate its potential to enhance meat softness, thereby significantly improving the sensory experience during consumption. This finding aligns with Rizqiati et al. [36], who reported a marked increase in softness in raw samples treated with papain. Additionally, the observed changes in chewiness and firmness suggest that moringa facilitates the breakdown of meat fibers, making it easier to chew. Alterations in firmness also play a critical role in overall mouthfeel and the eating experience. This is supported by Kakash et al. [37], who found that marinating chicken thighs in kiwi protein for varying durations at 4 ± 1°C improved chewiness and reduced hardness. Their study highlighted that the duration of enzyme exposure significantly affected the degradation of meat proteins, similar to the effects noted with the moringa treatment. The 0.5 % concentration of protease from the Biduri

plant has been shown to significantly reduce compression and tensile strength values, indicating enhanced tenderness [38]. The tenderizing effect is attributed to proteolytic enzymes and other bioactive compounds in plants that interact with muscle proteins during marination, leading to their breakdown and enhanced tenderness [39]. Empirical evidence supports this, showing that the moringa leaves extract improves textural attributes, juiciness, and overall acceptability in ground beef [20]. Adding moringa oleifera not only improves texture but also enhances the nutritional content by raising protein levels and antioxidant activity in meat products [22]. Plant proteases, such as papain and bromelain, degrade muscle proteins, reducing shear force and imparting tenderness [40,41]. However, excessive proteolytic activity can quickly tenderize meat, leading to a mushy or unacceptable texture that consumers dislike [40]. This requires control of enzyme concentration and duration for optimal meat quality.

Effects of the moringa leaves extract on the pH and cooking loss of goat meat

The current study found that there was no effect of the moringa leaf extract on the pH in the treated samples when compared to the control. This is the opposite of what was reported by Mohamed et al. [13], who found that the moringa leaf paste increased the pH of goat meat samples. The difference between two studies may be due to the difference in the amount of moringa leaves, as well as in the time and method of treatment applied in these studies. Das et al. [18], concluded that the moringa leaf extract did not significantly influence the pH of raw and cooked goat meat patties. This result is in close agreement with the current study. In addition, Moyo et al. [42] indicated that the dietary supplementation of moringa leaf meal did not significantly affect the pH of goat meat compared to different diets, such as grass hay or sunflower seed cake, at 24 hours post-mortem. Thus, these studies demonstrate that there is a possibility of improving meat quality sometimes without causing a significant change in pH as moringa leaves contain phenolic compounds, which have antioxidant activity, and enzymes that affect the tenderness of meat.

The results obtained in the current study show that even though the moringa leaves extract did not directly affect pH, it indirectly affected other nutritional qualities, such as color and texture, ultimately improving overall meat quality. However, the treated samples exhibited higher cooking losses than the control samples, except for the 2-hour treatment. The cooking loss for the 24-hour treatment was significantly higher than that of the control and the other treatments, increasing from 36.8 % to 44.3 %. These results indicate that marination time was a key factor in determining cooking loss, with the lowest values at 2 hours, peaking at 24 hours, and then declining at 46 hours. This is interpreted as resulting from extensive proteolysis at 24 hours, which weakens the meat water-holding capacity. At 46 hours, partial protein restabilization may occur, reducing further water loss. Applying moringa as a natural tenderizer not only can improve the texture but also can increase cooking yield, preventing moisture loss during cooking [13]. The stable pH range (5.9–6.1) observed in the present study, despite the addition of moringa extract, can be attributed to the inherent buffering capacity of the meat system, as well as to the concentration of moringa extract used, which may not be sufficient to significantly alter the pH of meat. Moringa extracts contain high concentrations of phenolic compounds and flavonoids [11]. Apriantini et al. [23] reported that the moringa leaves extract affected the pH of goat meat, leading to increased cooking loss. Das et al.[18] have not found a significant difference in cooking yield between the control and treated patties using the moringa leaves extract, indicating a minimal impact on cooking loss. On the other hand, despite the promising outlook for moringa in improving meat quality, excessive application would impair water-holding capacity and cooking yield [14]. The effects of moringa extracts on cooking loss can be compared with those of other plant-based additives to assess the specificity of these interactions. Studies on marination with Citrus aurantifolia juice showed variable effects on cooking loss in chicken breast meat, depending on concentration and marination time [43].

Effects of the moringa leaf extract on the color of goat meat

The chromatic characteristics of meat are a critical element in the meat industry, as they profoundly shape consumers' perceptions of product quality and thereby significantly influence purchasing decisions [44]. The use of the moringa leaves extract in this study resulted in increased lightness of the treated samples compared to the control. This result may be attributed to increased water loss, which improves light reflectance due to the proteolytic enzyme in moringa that enhances lightness by altering protein structure. In the study by Mohamed et al. [13], moringa leaves were not found to be effective in improving lightness, which is inconsistent with the current research. The antioxidative properties of moringa may also help maintain color stability during storage and reduce oxidative changes that cause discoloration[18]. In the present investigation, a discrepancy in redness was observed between the treated samples and the control group; however, this difference was not statistically significant. This phenomenon may be attributed to the antioxidants present in moringa, which could have preserved myoglobin stability, thereby averting discernible changes in redness. Feihrmann et al. [45] noted that moringa extracts did not significantly influence the visual coloration of beef samples, which aligns closely with the results of the current study. In this study, no statistically significant differences were observed between the control and the 24-hour samples, nor between the 2-hour and 46-hour samples, with respect to yellowness; however, a noteworthy difference was identified between the control and the 24-hour samples and between the 2-hour and

46-hour samples. Mwankunda et al. [20] found that the values of lightness (L*), redness (a*), and yellowness (b*) exhibited a significant reduction upon the application of moringa. In the study by Mohamed et al. [13], yellowness exhibited an increase, thereby enhancing the overall color profile. Comparable results were reported, showing that when using proteolytic enzymes from ginger and papaya, collagen and myofibrillar proteins, improved tenderness and the appearance of the goose breast cut became lightener likely due to the breakdown of darker muscle fibers [46]. Enzymes derived from ginger and papaya resulted in from the breakdown of proteins, reducing toughness and improved color in goat meat [5]. It is crucial to note that the documented color alterations did not adversely affect overall quality; instead, they may enhance consumer acceptance by increasing visual appeal. Although the moringa leaves extract demonstrated a beneficial impact on the color and quality of goat meat in this investigation, it is crucial to acknowledge that consumer acceptability will likely fluctuate based on additional sensory attributes, particularly flavor, which is also contingent upon the preparation methods, the quantity of moringa extract utilized, and the duration of storage.

Conclusion

The current research investigated the tenderizing efficacy of moringa leaves on Indonesian goat meat (Longissimus dorsi) across varying marination durations. This research has established that moringa leaves exhibit considerable potential as a natural tenderizer for goat meat and significantly enhance meat tenderness by markedly reducing specific texture attributes, while maintaining pH within acceptable limits. Aqueous leaves extracts, when applied for 2 hours, demonstrated substantial tenderizing effects. However, extending the marination duration to 24–46 hours increased cooking loss and, in some instances, negated the tenderness benefits due to excessive protein degradation. Overall, the moringa leaves extracts are promising, environmentally friendly, and health-conscious alternatives to synthetic tenderizers. This research concluded that the moringa leaves extract has a promising future in meat tenderization, given the optimal treatment period and the amount of the extract applied. This finding opens a broad field for further research into the optimal amount of the moringa leaves extract and treatment duration for tenderizing meat. In summary, taking into account the sustainability development goals of this study, investigating moringa as a sustainable alternative to synthetic tenderizers aligns with current trends toward natural food additives to attract interest from both consumers and producers focused on sustainable practices. In addition, given that goat meat is a staple in many cultures, particularly in developing regions, exploring practices of using moringa could provide culturally relevant insights into improving meat quality while respecting traditional methods. The findings from this study on the tenderization of goat meat can be extrapolated to other ruminants, broadening the impact on livestock management practices across species.

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THE RUMEN MICROBIOME: A BRIEF REVIEW

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Abstract

The rumen microbiome is a complex dynamic community of microorganisms that participate in digestion and provide an utmost impact on the cattle efficiency. Despite significant advancements in microbiome research, understanding the formation and management of the rumen microbiome still remains a significant scientific challenge. This topic holds both economic and environmental importance. The purpose of this literature review is to analyze and arrange structurally the current knowledge about the composition and functions of the rumen microbiome for further application of this knowledge in livestock farming. The article emphasizes that diet is an important factor that defines the composition and variability of the microbiome. This work demonstrates that the main functions of the rumen are provided by mutually coordinated groups of bacteria, methanogenic archaea, bacteriophages, protozoa, and fungi. The review covers various microbial groups in the rumen and their functions, as well as the factors that influence changes in the microbial community. Traditional methods of studying the rumen microbiome, based on culture-based techniques, have been significantly improved by the introduction of modern sequencing technologies. The review also explores the history of microbiome research and the "Hungate 1000 collection" project. This work demonstrates how metagenomics, metatranscriptomics, and metaproteomics have not only discovered numerous previously unknown microorganisms, but also provided insights into their functional roles. The systematization of knowledge presented in this review provides a comprehensive understanding of the rumen microbiome as a dynamic object for innovation targeted at improving the productivity, sustainability, and environmental safety of modern livestock farming.

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Introduction

The rumen microbiome is a complex and dynamic ecosystem that in major scope participates in the digestion and metabolism of ruminants. Its study is of utmost importance for improving livestock efficiency and reducing environmental impacts.

Recent researches have significantly expanded our understanding of the versatile diversity and functions of microorganisms in a rumen. For example, the rumen microbial community of cattle has been analyzed across various geographic locations [1] and periods (such as the late perinatal period [2]). The importance of protozoa in shaping rumen ecosystems and their influence on metabolism has been noted [3], and changes in composition of fungal cultures community have been examined with various diets [4] and among various cattle [5]. Also, a meta-analysis has identified the main microbiota of the rumen epithelium [6].

The rumen cavity is divided with numerous internal and external structures, thus forming five separate rumen bags: the cephalic, dorsal, caudal-dorsal cecum, ventral, and caudal-ventral cecum. These names refer to their locations. In combination with environmental factors, these distinct bags create distinct ecological niches within a rumen [6–8]. The ventral compartments contain the largest amount of liquid chyme components, while the dorsal compartments feature more gaseous space and solid feed particles. There is evidence of differences in the structure of specific microbial communities in different fractions of the rumen contents, which is associated with differences in the composition of these contents [9,10].

The rumen microbiome is a complex system of microorganisms that interact with each other in the same environment. These include bacteria, protozoa, archaea, fungi, and viruses [11]. The ruminants' rumen microbiota metabolizes a variety of feed components, including cellulose and starch, as substrates for growth. This process produces volatile fatty acids, which are the primary energy source (approximately 70 %) for the host animal and which are absorbed through the rumen epithelium. Furthermore, microbial growth in the rumen leads to the formation of microbial biomass, which serves as an important source of metabolizable protein for the host animal due to its high

concentration, optimal composition of amino acids, and good digestibility [12].

In recent decades researches of the rumen microbiome has focused primarily on bacteria, archaea, protozoa, and, in lesser extent, on fungi. Rumen viruses, which remained poorly studied for pretty long time, are now attracting increased scientific attention due to their potential applications in biotechnology and their possible impact on the ruminants' health and productivity [12].

Recent achievements in molecular techniques, including high-throughput sequencing and multi-omics approaches, have revolutionized our understanding of the rumen microbiome. These methods have revealed a greater diversity of microorganisms and allowed for a better understanding of their functional capabilities [13]. For example, studies have shown that the rumen microbiome can significantly influence feed efficiency, methane emissions, and milk composition in dairy cattle [14].

The rumen microbiome is very diverse and dynamic; it is influenced by various factors such as diet, age, and host animal's genetics. Understanding these interactions is crucial for developing rumen ecosystem management strategies to enhance cattle productivity and reduce impacts of environmental factors. For example, recent studies have been aimed to identification and isolating of potential probiotic strains from the rumen that are able to improve nutrient digestion and suppress pathogenic bacteria growth [15].

Researches of the rumen microbiome have enormous importance for the sustainable development of livestock production. They allow developing the new feeding strategies, improving animal health, and reducing greenhouse gas emissions. In the future, studying the rumen microbiome is expected to help develop more effective probiotics, methods for methanogenesis reducing, and to optimize using of feed resources.

Materials and methods

The literature review was composed on the basis of search of data published before 2025 in well-known scientific databases, such as PubMed, Web of Science, and ScienceDirect. A variety of keywords were used for the search, including "rumen", "rumen microbiome", "rumen bacteria", "ruminal phages", "rumen archaea", "rumen protozoa", "rumen methanogenic archaea", "rumen fungi", and "Hungate 1000". Then the literature from the scientific databases was thoroughly classified and reviewed for its relevance with the objectives of this study.

Evolution of rumen microbiome research technologies

Understanding the rumen microbiome is important for developing methods for producing food from ruminants. Rumen research technology has evolved over time, driven by technological and scientific progress. In the last century compiling of the Hungate 1000 collection[1] began worldwide, providing a better understanding of the microbiome picture. The creation of a shared catalogue now makes it possible to trace the genome of a cultured isolate without fearing of cross-species contamination or the integration of fragments from other species. Genome sequencing technologies have provided a broader view of protein functions, along with the potential to predict their structure. Sequencing has also enabled analysis and clearer evolutionary correlations, provided more data for taxonomy and classification, and enabled comparison of rumen functions, taxa, and metabolism across various countries [16].

As in many studies, rumen researches started small. In the 19th and 20th centuries, the majority of researches were biochemical and morphological ones. Researches were conducted all over the world and covered various areas of interest: from the study of acidity to the study of microorganisms. In 1939 Monroe and Perkins measured pH values of rumen components in cows with rumen fistula [17]. While studying the microbiome, in 1942 and 1943, Hungate conducted studies of the rumen when he discovered that some protozoa, such as *Diplodinium maggii, D. multivesciculatum, D. denticulatum* in the rumen digest cellulose [18,19]. Various ruminants were under research. In 1947, Gall and colleagues compared rumen bacterial communities in sheep and calves, and examined the differences in the bacterial population under different modes of feeding [20]. In 1947, Hungate [21] was already engaged in the cultivation of cellulose-digesting rumen microorganisms. In 1960, he published an article discussing the microbial ecology of the rumen [22], where he described the correlation between bacteria and protozoa.

A lot of previous researches were conducted through culturing and maintaining cultures, which process was able not only influence on certain microorganisms, but also alter them. However, culturing is necessary before sequencing, as sometimes it is not possible to isolate and culture a microorganism outside a body. It is known that not all rumen microorganisms are culturable.

Since the last century, with each study, data on the microbiome has expanded, and now we have a certain picture of a microbiome concept in which bacteria, fungi, protozoa, viruses, bacteriophages, and amoebae coexist. In 1995, the first bacterial genomes (human pathogens) of *Haemophilus influenzae* Rd [23] and *Mycoplasma genitalium* [24] were sequenced, and only eight years later, in 2003, the first sequenced and analyzed genome of a rumen microorganism — *Wolinella* (*Vibrio*) *succinogenes* DSMZ 1740 — was published [25].

When Illumina technology appeared (developed by the same name company, founded in 1998), the laboratories began using it for their researches purposes. The company Pacific Biosciences of California, Inc. also became known for its PacBio product, which also enabled genome sequencing. It was Illumina, along with PacBio, that formed the basis for the creation of the "Hungate 1000 Collection" catalogue. The availability of sequencing allowed defining

[1] "Hungate 1000 collection". Retrieved from www.hungate1000.org.nz Accessed Novemder 20, 2025.

the correlation of bacterial and archaeal communities with important rumen functions [6,26].

PacBio and Illumina can be used together in researches, such as the one conducted by Brede et al. [26], where they used the technologies to confirm complete recovery of the bacterial community after subacute rumen acidosis.

Since the early 2000s, the genome has been actively analyzed, and in 2018, the largest dataset — almost 500 genomes from the "Hungate 1000 Collection" — was published. Since then, the number of completed genome sequences has only grown. The project "Hungate 1000 collection" included genomes sequenced with the help of Illumina and PacBio technologies. The resulting collection included several genomes of prevalent species and isolates representing a variety of the cultured organisms. Although most samples were obtained from cattle, the collection also included genomes of bacteria isolated from non-ruminants, as these isolates are known to also inhabit the rumen [27]. As of 2018, the collection contained 27,755 putative genes engaged in carbohydrate breakdown, and scientists also expressed 90 candidate proteins. Of these data, 57 % were enzymatic, that showed positive activity toward cellulosic substrates. Also 15 uncultured microbial genomes were also included into the collection. In total, numerous findings and datasets have been collected that are valuable for analyzing correlations in the rumen microbiome [28]. Similar isolates were also detected in both rumen fluid samples and those collected from the human intestine. There were several of those isolates at the time of the study [29]. This suggested that these isolates possess similar functions for both organisms.

The comparative genomic analysis was conducted, and it revealed that most genes encoding growth hormone were acquired by rumen bacteria through horizontal gene transfer [30]. Meta-omics technologies have provided better understanding of metabolic activities, rumen community structure, and metabolic potential [31].

For functional assessment of the rumen microbiome, Wilkinson et al. in 2018 developed CowPI — a version of PICRUSt software [32]. CowPI predicts functional profiles that are compliant with assessments for both metagenomic and transcriptomic datasets. Today and further in the future the scientists will most likely use both the latest sequencing techniques and microbiological and biochemical methods developed in the last century.

Rumen microbiome

The rumen microbiome includes anaerobic bacteria, protozoa, archaea, fungi, and bacteriophages. Microbial activity in the first two sections of the digestive tract, the rumen and reticulum, satisfies most of the ruminants' energy needs and plays a key role in the feed materials breakdown [33].

Bacteria are the most abundant microorganisms reaching concentrations of 10^{10}–10^{11} cells/mL of rumen fluid [34]. The major bacterial phyla are *Bacteroidetes* and *Firmicutes*, followed by *Proteobacteria* [35]. At the genus level, *Prevotella*, *Ruminococcus*, *Butyrivibrio*, and others predominate [2].

There are data on the rumen bacterial community that prove that specific community members are associated with milk yield efficiency in dairy cows during two lactation cycles [36]. During the experiment, it was observed that the most common genera detected in cows were *Succiniclasticum* (2.28 %), *Coprococcus* (2.29 %), *Ruminococcus* (2.35 %), *Butyrivibrio* (2.38 %), *Prevotella* (40.15 %), and the most common phyla were *Tenericutes* (2.17 %), *Proteobacteria* (5.67 %), with the highest rates for *Firmicutes* (39.32 %), *Bacteroidetes* (49.42 %).

Studies were also conducted in which the predominant identified phyla were *Fusobacteria* (1.1 %), *Synergistetes* (1.5 %), *Actinobacteria* (3.5 %), *Proteobacteria* (11.1 %), *Bacteroidetes* (30.9 %), and *Firmicutes* (51.9 %).

Cattle do not have genes that encode enzymes necessary for the breakdown of carbohydrates and rupture of glycosidic bonds in plant cell walls (cellulose, hemicellulose, lignin, pectin, etc.), so rumen microorganisms play a crucial role in these processes. For example, anaerobic bacteria such as *Ruminococcus*, *Fibrobacter, Megasphaera*, *Streptococcus*, and *Escherichia* participate actively in cellulose breakdown [37].

Henderson et al. [38] studied microbial communities in camelids and ruminants taking into consideration their geographic location and diet. They found that similar bacteria and archaea dominated in most samples, while protozoan communities were more diverse. In over 90 % of studies, the main bacterial groups were *Clostridiales, Ruminococcus, Bacteroidales, Lachnospiraceae, Ruminococcaceae, Butyrivibrio,* and *Prevotella*. These groups comprise a core of the rumen bacterial microbiome, but some of them still remain poorly studied.

Protozoa can comprise up to 50 % of the rumen microbial biomass, but their study is challenged by the lack of reference genomic sequences and the complexity of standard methods of analysis.

Functional variations in the rumen microbiome arise from differences between microbial communities which run specialized functions in different parts of the rumen. The microbial population in the gastrointestinal tract not only affects feed efficiency and feed digestion, but also the condition of immune system and overall behavior of cattle [39]. An increase in pathogenic bacteria in the gastrointestinal tract can impair animal behavior and reduce feed intake. Microbial therapy of the gastrointestinal tract can improve the health and overall well-being of cattle. Three types of therapy are used to modulate the microbial community in the gastrointestinal tract: intestinal microbiota transplantation, probiotics administration, and using of this type of therapy [40].

There are three main microbial communities in the rumen: 1) planktonic (free-floating in rumen fluid), 2) fiber-attached, and 3) epimural (associated with the rumen wall) [41,42]. The differences and modes of interaction

between planktonic and fiber-attached communities have been extensively studied [42]. However, few studies have been conducted on rumen epimural communities. This may be due to their lower relative abundance and density compared to the rest of the rumen, as well as difficulties in sampling [42]. However, epimural communities, despite their lower abundance, perform specialized functions that are critical for maintaining a healthy rumen environment. These functions include host epithelial tissue recycling, oxygen uptake, and urea transport [6,43]. Epimural microorganisms localize along the rumen epithelium, allowing them to influence host gene expression through host-microbiome interactions [43–45].

Studies have shown that diet significantly influences rumen bacterial and archaeal communities. De Menezes et al. [46] found significant differences in bacterial communities between complete and pasture feeding, as well as between solid and liquid rumen contents. For example, higher abundance of members of the *Fibrobacteraceae* family was found in solid samples of total ration, and members of the propionate-producing *Veillonellaceae* family were found in samples from pasture-fed diets [46].

The metabolome of the rumen and reticulum is different, although both of them are the lower compartments of the rumen. Studies of the metabolome and metabolism in the five rumen compartments can contribute to improved cattle husbandry efficiency by providing the understanding how feed is processed in all compartments of the rumen.

The fermentation compartments and gastric compartments include the rumen, reticulum, abomasum, and reticulum-rennet. In a study of beef bulls, the majority of operational taxonomic units (hereinafter referred to as OTUs) related to tissue samples were found in the chyme. From 38 to 49 % of the OTUs found in the reticulum, rumen, abomasum, and reticulum-rennet of this bull were consistent across the samples of its tissue and chyme. The combination of physiological, anatomical, and microbial differences between rumen compartments highlights the need for analysis of the entire rumen to obtain a more comprehensive picture of the microbiome [47].

Despite significant progress, some aspects of the rumen microbiome still remain poorly researched. Key gaps in rumen microbiome data relate to the technical challenges of sequencing and analyzing eukaryotic genomes [1]. The role of eukaryotic microorganisms (protozoa, fungi) in the rumen ecosystem also requires further investigation.

Future research should aim to better understanding the interactions between various microbial communities, their impact on animal health and yield, and the development of microbiome modulation strategies to improve livestock husbandry efficiency.

Rumen bacteria

Bacteria are the dominant microorganisms in the forestomachs of the ruminants, with their total count reaching approximately 10^{10}–10^{11} cells/mL of rumen fluid, and their species diversity counts for over 200 species [34]. The composition of rumen bacteria is determined by a number of factors, including energy requirements, substrate preferences, and their tolerance to metabolic end products [48].

Previously, studies of rumen fluid in dairy cows were conducted during two lactation cycles [36]. The most common phyla were *Bacteroidetes* (49.42 %) and *Firmicutes* (39.32 %), and the most common genus was *Prevotella* (40.15 %). The study concluded that the rumen bacterial community is dynamic in terms of its diversity and composition, while specific community members are responsible for high and low milk yield [36]. Differences between the rumen microbiota and fecal microbiota can account for approximately 60 %. Most studies are conducted using the rumen rather than feces. In the other research of fecal microbiome, the majority of bacterial isolates were *Firmicutes* (51.9 %) and *Bacteroidetes* (45.8 %) [49].

Sequencing technologies have revealed that *Prevotella*, *Butyrivibrio*, and *Ruminococcus* are the most common bacteria in the rumen. Variations in host cattle diet provide a significant impact on the structure of the microbial community. In particular, dietary diversity has proved to increase microbial diversity [1].

Dairy cows primarily consume vegetation-based diets. There carbohydrates (starch and cellulose) serve as the main source of energy. Digestion of these carbohydrates occurs in the rumen due to digestive enzymes and microorganisms. These bacteria are in charge for the breakdown of cellulose and hemicellulose, which are the main components of plant fiber [50].

The capability to degrade cellulose depends largely on some factors like feed type, crop maturity, and the availability of cellulolytic bacterial communities [34]. Cellulose consists of β-1,4-linked glucose residues, whereas hemicellulose consists primarily of xylans, which are composed of β-1,4-linked xylose residues. Xylan is substituted with acetyl, arabinose, and glucuronic residues. The degradation of this complex plant polymer matrix requires the synchronous action of a wide range of hydrolytic enzymes [50].

During the study by Hungate in 1950 [51], cellulolytic bacteria were identified, the most common of which were classified as *Bacteroides succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*. Six years later another study identified another microorganism — *Butyrivibrio fibrisolvens* — but it was weak cellulolytic [52].

Fibrobacter succinogenes and *Ruminococcus albus* [53] bacteria demonstrated their efficiency in breaking down cellulose. Their ability to digest cellulose is much higher than that of other cellulolytic bacteria. This is likely due to the fact that *F. succinogenes* and *R. albus* feature several genes which encode enzymes involved in cellulose breakdown. The end products of fermentation produced by these cellulolytic bacteria are CO2, propionate, acetate, and butyrate. Additionally, lactic acid, succinic acid, hydrogen, ethanol, and formic acid are also fermentation products but are quickly utilized by other bacteria [53].

Strain of *R. albus 7*, and some others with bright yellow-lemon color of its colonies (on a complex medium with addition of 0.4 % cellulose) break down cellulose better than strain 8, which has white colonies [54].

Starch is an important component of ruminants' diets, and high-grain content diets significantly increase its presence in the rumen. Low amount of *Streptococcus bovis*, an amylolytic bacterium, is most often found in cows fed with high-forage diets or in cows adapted to grain-based diets for some time. However, its amount increases significantly in non-adapted cows fed with high-grain content diets [55].

S. bovis feature a lower optimal pH for growth than many other bacteria. The increase in its numbers after high-grain content diets is explained by a sharp increase in rumen glucose levels and the death of protozoa because of higher acidic environment created by such diets.

Some anaerobic bacteria are capable of obtaining energy by breaking down pectin. The most important pectolytic species include *Butyrivibrio fibrisolvens*, *Lachnospira multiparus*, and *Prevotella ruminicola*. These species are capable of breaking down pectin into oligogalacturonides, this way releasing large amounts of acetate, a volatile fatty acid important for cattle metabolism [56].

Lactic acid is formed from starch, and since it is not digested by the animal, it is absorbed through the rumen walls, thus resulting in increased lactic acid levels in the blood and pH decreasing. If an animal's diet changes too fast, volatile fatty acids accumulate in the rumen and negatively affect the microbiota and animal health. Those abrupt changes lead to a decrease in rumen pH and an increase in populations of *S. bovis* and *Lactobacillus* [34,57].

Ruminants are fed with citrus byproducts, which contain high amounts of pectin. They can serve as an alternative to grain-based feeds with high enzymatic activity, this way preventing overgrowth of *S. bovis* and associated rumen acidosis [34]. Furthermore, some studies suggest that the use of such products can improve feed efficiency for milk production [58].

Rumen bacteria play key role in the ruminants' digestion. Their diversity and functions are closely linked to the composition of an animal's diet. Understanding these correlations is crucially significant for optimizing ruminant nutrition and health, as well as for improving livestock husbandry efficiency.

Rumen fungi

Fungi are an important part of the rumen ecosystem because they serve as decomposers, for example, in the breakdown of fibrous feedstuffs. Recent studies have revealed a surprising abundance of both anaerobic and aerobic fungi within the rumen, with their population structure being significantly dependent on diet [4,59].

Rumen fungi (10^3–10^6 zoospores/ml of rumen fluid) are predominantly anaerobic and belong to the phylum *Neocallimastigomycota*, class *Neocallimastigomycetes*. This class includes six previously recognized genera (*Anaeromyces*, *Caecomyces*, *Cyllamyces*, *Neocallimastix*, *Orpinomyces*, and *Piromyces*) with twenty-one known species, as well as two newly discovered genera, *Oontomyces* and *Buwchfawromyces* [34].

Fungi in the rumen of the ruminants play a key role in the digestive process, particularly in the breakdown of complex plant polysaccharides. Anaerobic fungi belonging to the phylum *Neocallimastigomycota* are particularly efficient in producing enzymes such as cellulases, hemicellulases, and xylanases. These fungi are capable of completely degrading non-lignified plant walls and are more efficient at colonizing and degrading lignin-containing tissues in comparison with bacteria [60].

Recent study [61] demonstrated the availability of significant amounts of aerobic fungi in the rumen of buffaloes, with a predominance of ascomycetes (*Ascomycota*) and basidiomycetes (*Basidiomycota*) under various feeding conditions. These fungi, particularly those that form mycelium, are able to penetrate through rigid plant cell walls, this way facilitating the decomposition of resistant plant material [4].

Fungi in the rumen produce enzymes that break down plant cell walls, providing nutrient access for other microorganisms. Although bacteria are the most common microorganisms in the rumen, fungi also act as effective degraders. Certain fungal species are thought to play a significant role in improving feed conversion and productivity in ruminants [62].

Research has shown that the composition of rumen microorganisms can vary significantly depending on diet. Diets rich in fiber promote the growth of microorganism populations with high cellulolytic activity, while diets high in starch can promote the growth of other microbial communities.

The higher diversity of microbial communities is observed in free-range ruminants in comparison with domesticated breeds, which observation proves the influence of lifestyle and diet on rumen microbial populations [5]. Further research is needed to elucidate the mechanisms regulating the dynamics and ecological roles of both aerobic and anaerobic fungi in the rumen microbiota. Using omics approaches (genomic, transcriptomic, and proteomic) provides insight into the unique cellular processes, evolutionary history, and metabolic capabilities of these fungi [60].

Rumen fungi are an important component of the ruminant microbiome and play a key role in the breakdown of complex plant polysaccharides. Their ability to degrade lignocellulosic materials makes them indispensable in the digestive process in ruminants. Understanding the complex interactions between fungi and other rumen microorganisms, as well as the influence of various factors on fungal communities, can lead to improved nutrition and health of ruminants, as well as the optimization of feeding strategies for increased feeding productivity.

Rumen methanogenic archaea

Methanogenic archaea play a vital role in the rumen ecosystem of ruminants. These unique microorganisms possess specific metabolic processes that enable them to survive in the anaerobic conditions of the rumen. Methanogens are the only microorganisms capable of producing methane and exist at concentrations of 1010–1011 cells/mL of rumen fluid, constituting less than 4 % of the rumen microbial community [34,63,64]. Methane, produced by these organisms through microbial fermentation in the ruminants' digestive tract, is a potent greenhouse gas, thus making the research of methanogens important from both a scientific and environmental point of view [65].

Archaea are divided into two kingdoms: *Euryarchaeota*, consisting of methanogens and extreme halophiles, and *Crenarchaeota*, consisting of hyperthermophiles and nonthermophiles [34].

Current research confirms that the genus *Methanobrevibacter* dominates among the rumen methanogens, accounting for approximately 74 % of the total methanogen rRNA gene sequences. Members of the order *Methanomassiliicoccales*, along with the genus *Methanosphaera*, form the second-largest group, accounting for approximately 16 % of the sequences [33]. *Methanobrevibacter gottschalkii*, *Methanobrevibacter ruminantium*, the species of the genus *Methanosphaera*, and two members of the family *Methanomassiliicoccaceae* (formerly known as "Rumen cluster C") together account for approximately 90 % of the total rRNA sequences of rumen methanogens genes.

The primary function of most methanogens in the rumen is to reduce CO2 with the help of hydrogen gas (H_2) to produce methane (CH_4). This process plays a key role in maintaining low hydrogen levels within the rumen, which promotes the growth of other microorganisms and increases feed fermentation efficiency [34].

However, it is worth to note that there are exceptions as well. For example, *Methanosphaera stadtmanae* produces methane by reducing methanol with H_2 rather than CO_2. This species feature one of the most stringent energy metabolisms of all methanogenic archaea. This diversity of metabolic pathways highlights the complexity and adaptability of rumen microbial communities.

Development of strategies to reduce methane emissions from farm cattle is of great scientific and ecological importance. These strategies may include: (1) targeting methanogens or microorganisms that produce substrates for methanogenesis; (2) studying the correlations between methanogens and other rumen microorganisms; (3) using information obtained from sequencing the genomes of rumen microorganisms to develop vaccines, inhibitors, and other methods to reduce methane emissions; (4) breeding animals with low emissions; and (5) modifying the rumen microbial community [65]. These approaches are aimed to comprehensively address the issue of methane emissions in cattle farming.

The research [66] showed that selective breeding ruminants for low methane emissions can significantly impact their digestive physiology.

The Food and Agriculture Organization of the United Nations (FAO) estimates that in 2015, greenhouse gas emissions from livestock agricultural food systems amounted to approximately 6.2 billion tons of CO_2-equivalent, which is approximately 12 % of all anthropogenic greenhouse gas emissions [67].

Our current understanding of rumen methanogens is insufficient to develop effective strategies for reducing methane emissions while maintaining rumen function. Despite extensive research, the dominance and role of *Methanobrevibacter spp*. still remain unclear. To gain new knowledge, more culture isolation studies are required, particularly for poorly studied groups (e.g., *Methanomassiliicoccales*), as well as genomic data of better quality [68].

In light of projected global population growth and an expected 20 % increase in demand for animal products by 2050 [67], developing effective strategies to reduce greenhouse gas emissions from the global livestock sector is becoming a matter of paramount importance. A balance must be found between meeting growing food demand and minimizing the negative impact of livestock farming on the planet climate. This requires innovative approaches to managing agricultural systems and the implementation of new technologies capable of significantly reducing the carbon footprint of the livestock breeding industry.

Ruminal phages

As it was mentioned earlier, rumen microorganisms play a crucial role in the ruminants' vital functions. Bacteriophages (phages) are the most numerous and well-studied group of viruses in the rumen. Their density varies between 107 and 1011 particles/mL of rumen fluid. Bacteriophages are an integral part of the rumen microbiome, significantly influencing microbial dynamics, nutrient recycling, and overall rumen function. These viruses play a critical role in the complex rumen ecosystem by infecting bacterial populations and causing lysis of microorganisms [12,34]. In the rumen, bacteriophages infect bacteria, archaeophages are targeted to archaea, mycophages infect fungi, and protozoan viruses [12]. Morphological studies have identified phages from the *Myoviridae, Siphoviridae, Podoviridae*, and *Mimiviridae* families.

Viruses are simple infectious particles (virions or free viruses) consisting of genetic material (DNA or RNA in single or double strands) and a protein shell. Some viruses have an additional outer membrane, and are called as enveloped viruses. The totality of viruses in the environment is called the virome [29,69].

In the rumen, the density of free virus particles per milliliter varies in the range between 5×10^7 and 1.7×10^{10} [70,71]. In addition, viruses also parasitize other microorganisms, thereby modulating the microbiome and influencing the

cattle itself and its productivity [72]. However, viruses in rumen fluid still remain poorly studied, despite their direct impact onto the rumen ecosystem [73].

A study in both beef and dairy cattle examining the relationship between diet and the rumen virome and its impact on animal performance showed an indirect rather than a direct interaction between diet and the rumen virome, as diet influenced the host to varying degrees. It was also found that rumen viruses can regulate microbial strains and communities through antagonistic and mutualistic interactions. There is a correlation between the cattle yield efficiency and dietary changes that alter the rumen virome. The virome of beef cattle fed with highly concentrated feed is less diverse than that of cattle fed with medium-concentrated feed. The virome of dairy cattle is more diverse when fed with high-lipid and high-starch feed. However, virome diversity decreased in cattle fed in grazing grass-lands, compared to a total ration fed to the cattle [74]. Recently, a comprehensive rumen DNA virome profile was compiled using NGS and HiFi sequencing technology [73] to expand the rumen database, and the authors also proposed mechanistic pathways by which viruses may influence feed efficiency. This is hypothesized to occur either by disrupting host cells or by inducing metabolic changes in the host.

Yoshiaki Sato [75] analyzed the genomes of viruses from the rumen of Japanese Black cattle and found that the virome differed between cattle fed with low-fat and high-fat diets, as well as between cattle with low and high slaughter weights. Possible maternal transmission of rumen viruses to the cattle offspring may also influence beef cattle productivity [76].

Depending on their life cycle in the rumen, viruses can be divided into two types: temperate and lytic. Temperate viruses create a provirus by integrating their genetic material into the host genome. The provirus then becomes responsible for the symbiotic interaction with the host. Under the influence of a number of factors (physical or mechanical), or spontaneously, the prophage can then enter the lytic cycle. Lytic viruses act by infecting host cells, replicating, and causing cellular lysis. Subsequently, the progeny virions are released and infect other hosts [73]. Microbial lysis caused by the phage releases cellular components, such as proteins and enzymes, which can be used by other microorganisms or absorbed by the host animal. This process also promotes nutrient recycling, as the enzymes released during lysis enhance feed fermentation and improve nutrient absorption. Phages are involved in the control of microbial populations, regulating their numbers through "kill-the-winner" dynamics, where dominant bacterial species become the targets for their viral predators, thus allowing less competitive species to survive. Furthermore, phages facilitate genetic exchange, facilitating horizontal gene transfer between bacteria, which plays a crucial role in the adaptation and evolution of the rumen microbiome.

Phages can alter the rumen environment by influencing the composition and activity of microorganisms. For example, phage therapy has been proposed to reduce methane emissions in ruminants by targeting methanogenic archaea. Phage therapy in ruminants shows promise as an alternative to antibiotics, offering targeted solutions to key challenges in animal husbandry. This approach uses bacteriophages to address issues such as methane emissions, dysbiosis, and subclinical rumen acidosis. Studies have identified phage-derived lytic enzymes, such as PeiR from *Methanobrevibacter ruminantium*, which have the potential to reduce methane production by targeting rumen methanogens. Furthermore, phage therapy may help restore microbiome homeostasis in cases of dysbiosis and combat antibiotic-resistant microorganisms.

Rumen amoeba

Amoebas are single-celled eukaryotes found in a variety of environments, including the rumen of ruminants. Although generally less studied than other rumen microorganisms, they are an integral part of this complex ecosystem. Amoebas may represent an important reservoir for rumen bacteria, although their exact role is not fully understood. They are nonetheless part of the diverse microbial community in the rumen [34].

Similar to ciliates, amoebas in the rumen survive by engulfing bacteria through phagocytosis. This feeding behavior allows them to actively ingest bacteria as a protein source, which may influence overall protein dynamics in the rumen ecosystem [77].

Some bacteria can survive phagocytosis by protozoans, including amoebas, and survive as endosymbionts [34]. For example, *Campylobacter jejuni* has been shown to invade *Acanthamoeba polyphaga* and can replicate itself in the vacuoles. This relationship between bacteria and amoebae may have implications for the rumen and overall health of cattle, as *C. jejuni* and *C. fetus* can significantly impact fertility, immunity, and overall health condition of the cows [78,79].

Amoebae, along with other protozoa, may be involved in regulating prokaryotic diversity in the rumen [3]. Their predatory behavior could potentially mitigate the effects of competitive exclusion between bacterial taxa, thereby helping to shape the composition of the prokaryotic community.

The role of amoebae in the rumen is not as well studied as the roles of other microorganisms, like bacteria or ciliates. Further research is needed to understand their ecological significance and how they interact with other members of the rumen microbiome. Although amoebae are available in the rumen and is likely to play a role in its complex ecosystem, their specific contribution to rumen function and animal health still requires further studies [34].

Rumen protozoa

The most important protozoa in the rumen of ruminants are ciliates / infusoria, which comprise more than

50 % of the rumen's microbial mass. Based on morphological characteristics, ruderal protozoa are divided into two orders: endodiniomorphs (order: *Entodiniomorphida*) and holothrichs (order: *Vestibuliferida*). They are the biological indicators of proper rumen function. Ciliates break down the structural and non-structural carbohydrates, and participate in host digestive metabolism.

Depending on geography, diet, and a number of other factors, the protozoan profile of the rumen varies among the ruminants. In 2020, Cedrola et al. [80] analyzed the past and current studies of the rumen trichostomatids and in order to expand the phylogeny proposed using evolutionary models, such as the method of branch lengths (MBL). The same authors conducted a taxonomic review of ciliates among Brazilian livestock in Brazil and described a new morphotype of entodinid ciliates *Entodinium caudatum* m. *orbonuclearis* [81]. They found that urea supplementation did not affect the density of rumen trichosomatid ciliates, their relative abundance, or their diversity.

Cellulolytic activity of rumen-inhabiting protozoa was identified. Cellulolytic protozoa such as *Eudiplodinium maggie*, *Ostracodinium album,* and *Epidinium caudatum* are protozoa that degrade cellulose by absorption [50]. The study in 2003 of the rumen protozoan *Polyplastron multivesiculatum* revealed that these protozoa can digest fiber [82]. Devillard et al. isolated the xylanase XYN10B from GH10 from rumen protozoa and described its enzymatic properties [83]. It was also suggested that some rumen protozoans may have acquired genes for cellulases and xylanases through horizontal transfer. In a study by Andersen et al. [84], it was found that although some protozoan species are capable of digesting starch, they were less common in animals that produce little methane. Cysteine peptidases were also found to be beneficial for nitrogen assimilation in ruminants [85].

Omics studies have shown that predation of the protozoa *En. caudatum* includes endocytosis, phagosomes, lysosomes, and cells motility [86]. Compared to isotrichids, endodiniomorphids were found to feature higher predatory activity. This is indicated by the increased nitrogen concentration in the rumen and the higher abundance of endodinimorphs in goats compared to the bovine cattle being analyzed [87].

In vitro studies of the rumen have demonstrated the possibility of suppressing protozoan activity using specific lysozyme and peptidase inhibitors without disrupting fermentation processes [88]. The implementation of proteomics, genomics, and transcriptomics technologies can help identify specific targets for effective management of protozoa activity in the rumen.

Factors influencing the rumen microbiome

There are many factors that influence the rumen microbiome, including age, diet, genetics, geographic location, breed variety, and sex. Understanding and managing these factors is crucial for ensuring efficient nutrients utilization by the ruminants via influencing the microbial population in the rumen [1,62].

Dairy and beef cows have different nutritional needs, which are reflected in their diets and, consequently, their microbiomes. Dairy cows require the diets that optimize milk yield and milk quality. Their gastrointestinal tract is tailored to efficient processing of high-fiber diets. Changes in feed intake can alter the composition of the rumen microbiome and general production efficiency during lactation. Beef cows, in their turn, are targeted to weight gain and ensuring meat quality. Their gastrointestinal tract is optimized to convert feed into muscle mass. Similar to dairy cows, the rumen microbiota of beef cattle can vary depending on the diet type, thus impacting growth rate and feed conversion efficiency [89].

Although the rumen microbiome varies across various diets, it is able to adapt to these changes and remain efficient. Certain rumen microbiota species in dairy cows are associated with improved feed efficiency. Some anaerobic microbes promote cellulose breakdown, which improves nutrients absorption and overall productivity [90–92].

Researches confirm the influence of diet on the rumen microbiome. For example, one study in beef cattle found that *Prevotella* species count was higher in a group with inefficient consumption of residual feed. Feeding the cows with low-forage diet resulted in decreased abundance of *Fibrobacter succinogenes*, and increased abundance of *Entodinium* and *Prevotella* spp. [93].

The buffalo rumen fungal community is dominated by ascomycetes, with amount varies depending on diet, thus indicating a significant influence of feed composition on rumen fungal diversity and functionality.

Conclusion

The rumen microbiome is a complex and dynamic ecosystem that influences livestock health. The achievements in molecular techniques have significantly expanded our understanding of the diversity and functions of rumen microbial community participants. This knowledge is key for development of strategies to enhance livestock yield performance, improvement of feed efficiency, and mitigation of environmental impacts such as methane emissions. Future researches should focus on clarification of the complex interactions between the host and various microbial communities, and exploring the potential of rumen-derived probiotics for sustainable livestock husbandry. As our understanding of the rumen microbiome keeps on growing, it will undoubtedly lead to innovative approaches in livestock nutrition and management, thus contributing to more efficient and environmentally friendly ruminant livestock production systems.

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LACTIC ACID BACTERIA: TWO SIDES OF THE SAME COIN

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Abstract
This review systematizes current data on the dual role of LABs. On the one hand, they are the basis for biopreservation as a source of bacteriocins and organic acids and serve as probiotics in fermented products. On the other hand, psychrotrophic strains of Lactobacillus, Leuconostoc, Carnobacterium, and Weissella are adapted to refrigerated storage and modified atmosphere packaging conditions, making them key spoilage agents in meat products, causing acidification, gas production, and slime formation. A particular problem is their ability to form resistant biofilms on processing equipment, leading to cross-contamination. The paper substantiates the need to move from generalized approaches to precise, strain-specific control. An effective risk management strategy should integrate modern methods of molecular monitoring (metagenomics, MALDI-TOF MS) to trace contamination sources; the development of targeted sanitation procedures against biofilms; and the implementation of biological control methods using antagonist cultures. It is concluded that the future of sustainable LAB use lies in an integrated approach that maximizes their beneficial potential for biopreservation and food fortification while simultaneously employing advanced scientific methods to mitigate the associated spoilage risks.

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Introduction

The growing consumer demand for "clean label" products (free from chemical preservatives) brings the modern food industry to a fundamental dilemma regarding lactic acid bacteria (LABs). The reduction in the use of traditional preservatives in the production of natural deli meat products, prepared salads, and fermented foods increases the vulnerability of products to microbiological spoilage, which often occurs without obvious external signs [1]. Furthermore, LABs are becoming the key agents determining both the safety and spoilage of such products, which shapes their dual role.

On the one hand, LABs serve as valuable probiotics and the basis for biopreservation, inhibiting pathogens (e.g., *Listeria monocytogenes*) through the production of organic acids, bacteriocins, and other antimicrobial compounds. They are widely used in the production of fermented foods (kefir, yogurt, sauerkraut, sourdough bread), where they not only improve sensory and nutritional properties but also naturally extend shelf life. Research also confirms the antiviral and antifungal activity of their metabolites.

On the other hand, certain LAB strains may cause spoilage, especially in meat and finished products with limited chemical preservatives. For example, *Lactobacillus, Leuconostoc, Carnobacterium*, and *Weissella* species can produce diacetyl, which imparts an "oily" flavor, emit gases that cause packaging to bulge, or form biogenic amines that are hazardous to consumers [1]. Of particular concern are psychrotrophic strains adapted to refrigerated storage and capable of forming resistant biofilms on equipment, leading to persistent cross-contamination.

Modern research confirms that the spoilage or beneficial potential depends on the specific strain rather than on the species. For example, *Lactobacillus sakei* may exhibit both antimicrobial activity and act as a spoilage agent depending on its biotype [2]. This highlights the critical need to move from generalized approaches to strain-specific risk analysis. Modern methods, including metagenomics, metabolomics, and molecular typing, allow not only the identification of "problematic" strains but also the prediction of their behavior in various products and storage conditions.

The aim of this review is to summarize current data on the dual role of LABs in the food industry, emphasizing their ability to act both as biopreservatives/probiotics and as food spoilage agents, particularly in the meat industry. The review aims to systematize knowledge about strain-specific properties, mechanisms of beneficial and adverse effects, and modern control methods, as well as to analyze the pathways of LAB dissemination and persistence in the production environment.

Objects and methods

A systematic review of the scientific literature (1996 to 2023) was conducted using Google Scholar, PubMed, Web of Science, Research Gate, Springer Link, ScienceDirect, Taylor & Francis, and Scopus, as well as Google and other search engines. A total of 105 relevant publications were identified and analyzed using keywords related to lactic acid bacteria, their beneficial properties, and food spoilage.

Classification and characteristics of lactic acid bacteria

Lactic acid bacteria (LABs) are a functionally distinct but phylogenetically diverse group of Gram-positive microorganisms united by a key physiological trait: the ability to produce lactic acid as the primary end product of carbohydrate fermentation [3]. These bacteria are chemoorganoheterotrophs, obtaining energy exclusively through substrate-type phosphorylation during fermentation. Morphologically, the group is represented by both bacillary and coccoid forms. Common features of LABs include Gram-positive staining, do not form endospores (with rare exceptions), immobility in most species [4], and catalase negativity. They demonstrate increased tolerance to acidity, which is their key competitive advantage [5]. LABs have complex nutritional requirements, requiring the presence of amino acids, B vitamins, and minerals [6,7]. In nature, they are widespread in nutrient-rich ecological niches: on plants, in dairy products, fermented substrates, and in the gastrointestinal tract of humans and animals [8]. Their ability to produce organic acids allows them to effectively suppress the growth of competing and potentially pathogenic microflora, which determines their key role in biopreservation practices [9].

The traditional classification of LABs established at the beginning of the 20th century was based on a complex of morphological, physiological, and biochemical characteristics. A fundamental and still valid criterion is the division into homofermentative and heterofermentative types depending on the hexose fermentation pathway [3]. Homofermentative LABs (e.g., the genera *Lactococcus, Streptococcus, Pediococcus*) break down glucose via the glycolytic pathway (Embden-Meyerhof-Parnas), and the end product is predominantly lactic acid. In contrast, heterofermentative LABs (e.g., the genera *Leuconostoc, Weissella, Oenococcus*) use the phosphoketolase pathway, resulting in the formation of lactic acid, ethanol or acetic acid, and carbon dioxide from one glucose molecule [3]. Other important phenotypic criteria include optimal growth temperature, the ability to ferment certain carbohydrates, and salt tolerance [10,11]. However, phenotypic identification is often insufficiently reliable for differentiating closely related species, which has led to a radical revision of LAB taxonomy with the development of molecular genetic methods. Thus, based on DNA hybridization data and phylogenetic analysis, the heterogeneous genus *Streptococcus* was divided into several independent genera: *Streptococcus, Enterococcus,* and *Lactococcus*. A major revision of species initiated in 2020 also affected the largest genus *Lactobacillus*, which was divided into 25 new genera (e.g., *Lacticaseibacillus, Limosilactobacillus*) based on whole-genome sequencing [12].

Therefore, modern taxonomy and identification of LABs are based on a polyphasic approach integrating phenotypic data with the results of genetic analysis [12]. Key methods include 16S rRNA gene sequencing, which is the "gold standard" for species identification, genomic fingerprinting methods such as restriction fragment length polymorphism (RFLP) analysis and randomly amplified polymorphic DNA (RAPD) analysis, as well as whole genome sequencing (WGS), which provides the most comprehensive information for taxonomy and the study of metabolic potential [13].

The application of these methods has allowed to form a modern taxonomic structure and clearly define the key LAB genera of industrial significance. According to the modern classification, the core of the LAB group is the order *Lactobacillales*. Bacillary bacteria of the genus *Lactobacillus* (and newly described genera) are extremely diverse in their metabolic properties and are of great importance in the production of fermented products and as probiotics [3,8,12,14]. Mesophilic homofermentative cocci *Lactococcus lactis* are a key starter microorganism in cheese manufacturing [3]. Thermophilic homofermentative cocci *Streptococcus thermophilus* are one of the essential components of yogurt starter [3]. Obligately heterofermentative cocci *Leuconostoc mesenteroides* are important in the initial stages of vegetable fermentation, and *Oenococcus oeni* is specially adapted to survive in wine and is responsible for biological stabilization [3,11]. Homofermentative cocci of the genus *Pediococcus*, which form tetrads, are highly acid-resistant and are used in the fermentation of plant and meat raw materials [9]. The genus *Bifidobacterium* belonging to the phylum *Actinobacteria* stands apart. Although their metabolic pathways and phylogenetic position are distinct, they are often considered together with LABs in applied contexts [8].

Lactic acid bacteria are the basis of numerous food biotechnologies. Their role is far beyond simple acidification. Through proteolytic and lipolytic activity, they shape the flavor profile of cheeses, fermented meat, and plant products [3]. Bacteriocins produced by many strains serve as natural biopreservatives [9,13]. A special area of research is the use of LABs as probiotics, live microorganisms that benefit the host's health [8,14,15]. Probiotic strains must possess a combination of properties: resistance to gastric juice and bile acids, adhesion to intestinal epithelium, antagonistic activity against pathogens, clinically proven efficacy and safety [14,15]. Thus, lactic acid bacteria represent a classic example of the interplay between fundamental science and practice. The evolution of their classification from phenotypic descriptions to genome-focused approaches has opened up new opportunities for targeted

screening, selection, and safe use of these microorganisms in the food industry, medicine, and agriculture [5,12].

Bioactive compounds of lactic acid bacteria: diversity, mechanisms of action, and application in food technology

LAB bioactive compounds as multifunctional agents

Lactic acid bacteria (LABs) are an integral part of food biotechnology, not only due to their primary enzymatic activity but also due to their ability to produce a wide range of bioactive compounds [16]. These metabolites synthesized during fermentation make a key contribution to the safety, quality, texture, shelf life, and health benefits of final products. The main classes of bioactive compounds produced by LABs are peptides, exopolysaccharides (EPS), bacteriocins, organic acids, and enzymes [16]. The profile and quantity of the compounds produced vary depending on the bacterial species and strains, substrate, and fermentation conditions, opening up vast opportunities for the targeted creation of products with desired technological and functional properties. Thus, LABs act as powerful biotransformers and biofactories of valuable substances.

Main classes of bioactive compounds and their functions in food products

Bioactive peptides formed as a result of proteolysis of raw material proteins (e.g., milk casein) are the most significant from a functional point of view are. These peptides exhibit a variety of physiological activities, including antihypertensive, antioxidant, immunomodulatory, and opioid effects, which determines the prophylactic potential of fermented products [16]. Exopolysaccharides (EPS) secreted by LABs into the environment play a dual role: they improve the rheological properties of products, acting as natural thickeners and stabilizers, and also exhibit prebiotic and immuno-stimulating effects, promoting the growth of beneficial intestinal microbiota [16,17]. Organic acids, primarily lactic and acetic acids, are the main biopreservation agents lowering the pH and creating an unfavorable environment for the development of pathogenic and opportunistic microflora [18]. The accumulation of acids also contributes to the denaturation of proteins and the formation of the characteristic texture of products such as yogurt or cottage cheese. Furthermore, the enzymatic activity of LABs (proteases, lipases, amylases) leads to profound transformations of the food matrix: increased availability of nutrients, accumulation of free amino acids and fatty acids, formation of flavor compounds, and reduction in antinutrients or allergens in raw materials [17]. The combined action of these compounds significantly increases the nutritional and functional value of the original substrates.

Bacteriocins: mechanisms of action and application strategies in biocontrol

A special place among bioactive metabolites is occupied by bacteriocins, peptides synthesized in ribosomes and exhibiting antimicrobial activity primarily against closely related bacterial species [19]. In the food industry, they are considered promising natural biopreservatives for combating spoilage and pathogens (e.g., *Listeria monocytogenes, Staphylococcus aureus*) [20]. Their key advantages include safety for humans (many have Qualified Presumption of Safety (QPS) status), biodegradability, and efficacy at low concentrations [21]. QPS status implies overall safety across all potential uses, and the assessment incorporates four key principles: taxonomy, scientific knowledge, safety profiles, and expected end use [22]. Combination strategies are used to expand the spectrum of activity, particularly against Gram-negative bacteria. The combined use of bacteriocins with ethylenediaminetetraacetic acid (EDTA) is one of the most common strategies currently used to sensitize Gram-negative bacteria. EDTA promotes the destruction of the bacterial outer membrane, enhancing the activity of the bacteriocin against Gram-negative microorganisms, especially *Salmonella enterica* subsp. *typhimurium, Enterobacter aerogenes, Shigella flexneri, Citrobacter freundii, Escherichia coli* O157: H7, *Pseudomonas aeruginosa,* and *Arcobacter butzleri* [23]. Interestingly, small amounts of EDTA, ranging from 10 to 20 mM, are usually sufficient to induce sensitization to bacteriocins [23]. Combination with essential oils (thymol, carvacrol) allows for a reduction in the working concentrations of both agents, minimizing the impact on the sensory properties of the product [24,25]. A study of *Buyssa* essential oil against *Salmonella enterica* subsp. *typhimurium* and *Staphylococcus aureus* showed that the inclusion of nisin significantly reduced the oil concentration required to inhibit both bacteria [26]. The combined use of bacteriocins with mild physical processing methods (high pressure, moderate heat) is being actively studied [27]. The use of protective cultures producing bacteriocins is an environmentally friendly alternative to synthetic preservatives [28,29].

Antiviral potential of LAB metabolites

In addition to antibacterial activity, LAB metabolites demonstrate potential for inhibition of viruses. This effect is studied primarily in the context of its impact on host health, since viruses do not replicate in food products [30]. Mechanisms of antiviral action may include: direct interaction of bacterial cell components or their metabolites with viral particles; blocking of viral receptors on target cells; and modulation of the body's systemic immune response [30,31]. Studies have shown the activity of some lactobacilli strains and their metabolites against rotaviruses, noroviruses, and caliciviruses [30,31], as well as against the human immunodeficiency virus (HIV) [32]. Until now, information on the ability of lactic acid bacteria to kill viruses has focused on their immunomodulatory effect on the host immune response rather than on the direct antiviral action in foods. However, it is hypothesized that the presence of LABs in food indirectly protects consumers from viral infections by blocking receptor sites on host cells and neutralizing viral spread, or by enhancing the immune

system to counteract viral infections after food consumption [31,32]. Consumption of LAB-containing foods may enhance the immune response, indirectly increasing the body's resistance to viral infections [31,33].

Antifungal activity of LABs and its application in biopreservation

The antifungal activity of LABs, directed primarily against filamentous fungi and yeasts, is of great practical interest for the biopreservation of mold-prone foods [34]. The main agents of this activity are organic acids, low-molecular-weight metabolites, and volatile compounds (diacetyl, acetoin) [34,35]. Many strains of the genera *Lactobacillus, Pediococcus,* and *Leuconostoc* effectively inhibit the growth of toxigenic molds such as *Aspergillus, Penicillium,* and *Mucor* [34,36]. Volatile organic compounds capable of diffusing in the airspace of packaging are particularly promising for protecting product surfaces [35]. The antifungal effect is often the result of synergy between several compounds and interactions between different bioprotective cultures, which increases efficiency [37]. The antifungal process may involve interactions between microbial metabolites produced by various bioprotective cultures [38].

Prospects for the use of bioactive compounds from lactic acid bacteria

Bioactive compounds from lactic acid bacteria form a multifaceted and highly effective arsenal of tools for the modern food industry [16]. Their unique ability to simultaneously address the challenges of biopreservation [18,20,27], texture improvement [16,17], enrichment of products with valuable metabolites [16,17], and the manifestation of preventive (probiotic) properties [18,29] makes LABs indispensable agents for the creation of functional foods. Further research should focus on in-depth understanding of the molecular mechanisms of action, optimization of production conditions for target metabolites, and the development of innovative synergistic approaches. Maximizing the potential of these natural biocatalysts will facilitate the development of food technologies within the framework of current concepts of "clean label," personalized nutrition, and sustainability.

Fermentation: biochemical foundations, historical evolution and modern aspects

Fermentation is one of the oldest biotechnological methods used by humanity for the transformation and preservation of food raw materials. It is essentially a process of anaerobic or microaerophilic metabolism, during which microorganisms (bacteria, yeast, fungi) convert carbohydrates and other substrate components into alcohols, organic acids, gases, and a range of aromatic compounds [39]. These biochemical changes have three key functions: significantly increasing shelf life by suppressing the growth of pathogenic and spoilage microflora (biopreservation), improving sensory properties (taste, aroma, texture), and increasing the nutritional value and digestibility of the product through the preliminary breakdown of complex compounds and the synthesis of vitamins.

The history of fermentation dates back over 13,000 years, as evidenced by archaeological finds such as the remains of fermented beverages in stone mortars from the Natufian period [40]. The transition to sedentary agriculture in the Neolithic gave a powerful impetus to the development of these practices. In China, as early as 9,000 to 8,700 years ago, there was a ritual tradition of brewing beer from rice, Job's tears, and tubers, with molds apparently used to saccharify the starch, a principle underlying the production of modern East Asian sourdoughs [41]. In the Middle East, the fermentation of milk led to the development of cheese prototypes. The preservation of plant and animal products by lactic acid fermentation was independently mastered in various regions of the world: from the Roman Empire and Slavic territories to the Himalayas and the Korean Peninsula. A classic example is sauerkraut (and its spicy Korean version, kimchi), which not only ensured long-term storage of plants but, as was later discovered, due to its high content of vitamin C and probiotics, played an important role in the prevention of scurvy and maintaining health [42]. Today, the world's diversity of fermented foods, many of which are part of cultural heritage, numbers in the thousands [43].

Fermented dairy products: from traditional starters to targeted probiotics

Lactic acid bacteria (LABs) are the primary fermentation agents in the dairy industry. Over 260 species of these bacteria are known, performing complex transformations of raw materials. The key process is the fermentation of lactose to form lactic acid, which leads to casein coagulation and curd formation. At the same time, the proteolytic and lipolytic activity of LABs breaks down proteins and fats, generating peptides, free amino acids, and fatty acids that determine the flavor, aroma, and texture of the final product [44].

The evolution from spontaneous fermentation by adding the final product to the use of standardized starter cultures has revolutionized the industry. Modern biotechnology enables the selection of strains with specific properties: high acid production rates, specific flavor-forming properties (diacetyl and acetoin synthesis), and bacteriophage resistance. A particular area of research is the development of probiotic dairy products. Probiotic strains such as *Lactobacillus* spp. and *Bifidobacterium* spp. must not only exhibit targeted beneficial effects (e.g., pathogen antagonism, immune modulation), but also maintain high viability throughout the entire production process and product shelf life, which represents a distinct technological challenge [45].

Fermented plant-based foods: biodiversity and innovation

Fermentation is an effective method for preserving perishable fruits, vegetables, and grains, not only extending their shelf life but also enriching them with nutrients

and bioactive substances [46]. The surface of plant-based raw materials harbors autochthonous microbiota, including low numbers of LABs, but during fermentation, specific consortia are formed. The dominant genera are often *Leuconostoc, Weissella, Pediococcus, Lactococcus,* and *Lactiplantibacillus* (formerly *Lactobacillus*) [47].

Classic products such as sauerkraut demonstrate a staged process: an initial heterofermentative phase involving *Leuconostoc mesenteroides* is followed by a homofermentative phase dominated by *Lactiplantibacillus plantarum*, which ensures optimal acidity and stability. Olive fermentation is a more complex symbiotic process involving LABs and yeasts, with *L. plantarum* and *L. pentosus* playing a key role [48].

A current trend is the development of a market for functional plant-based soft drinks, such as fermented juices. These are positioned as natural, healthy alternatives for consumers with lactose intolerance or milk protein allergies. The use of specially selected probiotic strains (e.g., *Lactobacillus* spp.) allows for the combination of the benefits of plant-based raw materials (vitamins, polyphenols, and fiber) with the beneficial properties of live cultures [49]. Research is focused on identifying new adapted strains, optimizing processes to preserve nutrients, and studying the *in vivo* effects of such products.

Fermentation in bakery: symbiosis of yeasts and lactic acid bacteria

Sourdough bread technology is based on the synergy of yeast and LABs. Yeast (*Saccharomyces cerevisiae*) is responsible for alcoholic fermentation, releasing carbon dioxide to leaven the dough. LABs, a complex of over 70 species (with a predominance of now-reclassified genera such as *Lactiplantibacillus, Fructilactobacillus*, and others), carry out lactic acid fermentation [50,51].

This symbiosis imparts fundamentally different properties to bread. Acidification of the environment (lowering the pH) improves the rheological properties of the dough, slows staling, and inhibits the growth of mold and *Bacillus cereus*, the bacterium that causes "potato disease" of bread. LAB metabolites, lactic and acetic acids, diacetyl, and esters, create a unique flavor profile. In addition, bacterial proteolytic activity increases the availability of amino acids, including glutamate, enhancing the umami flavor, and helps reduce the content of potentially harmful compounds such as acrylamide [52,53]. Traditional Chinese cereal fermentation methods for steamed bread also rely on complex microbial communities to enrich the product [51].

Fermentation of meat and fish products: biopreservation and flavor development

Fermentation of meat and fish is primarily a controlled biopreservation process, an alternative to the use of chemical preservatives. The primary mechanism is the rapid acidification of the substrate through the activity of LAB starter cultures (most commonly *Latilactobacillus sakei* and *Latilactobacillus curvatus*). The accumulation of lactic acid reduces the pH to values (usually below 5.0) unacceptable for the development of most pathogenic and spoilage bacteria, such as *Salmonella* spp. and *Staphylococcus aureus* [54].

Regional differences in technology (e.g., more acidic Northern European products versus less acidic Mediterranean ones) are due to the use of different strains, temperature regimes, and salt concentrations (2–6 %) [55]. In addition to preservation, fermentation makes a decisive contribution to the development of sensory characteristics. The enzymatic activity of LABs and associated microflora (micrococci, coagulase-negative staphylococci, yeasts) leads to profound proteolysis and lipolysis. This causes softening of muscle fibers and the accumulation of free amino acids, peptides, and volatile aromatic compounds creating the characteristic flavor and aroma of dry-cured delicacies [54].

Fish fermentation, one of the oldest methods of fish preservation, also aims to stabilize the product through acidification and enzymatic hydrolysis. This process helps preserve valuable nutrients, including proteins and omega-3 fatty acids, and creates unique traditional products with distinctive flavors [56].

Negative effects of LABs in the food industry

Food spoilage

Lactic acid bacteria (LABs) occupy an ambivalent position in the meat industry, acting simultaneously as agents of targeted biopreservation and as the primary spoilage agents of chilled products. This duality is due to their exceptional ecological plasticity and metabolic diversity. The current understanding of this problem has become more complex with the broadening of plant-based meat analogs. While possessing favorable physicochemical properties for LABs (neutral pH, high moisture), they have become a new niche for their development, including potentially hazardous strains. In light of recent in light of recent research (after 2020), this dual role appears even more complex and is determined not only by species but also by strain-specific metabolic profile, which is being actively studied in the context of clean label and functional food development.

The ecological source and adaptation of LABs in the production environment are the key to understanding the problem of recurrent spoilage. Sources of contamination include both primary raw materials and stable populations within factories. Cross-contamination between raw materials and finished products (e.g., sliced ham) occurs via equipment surfaces and air [57,58]. Psychrotolerant LAB species, which not only survive but also actively grow at refrigerated storage temperatures (0–8 °C), are a particular problem. These species successfully colonize production facilities, adapt, and form stable biofilms, leading to systematic contamination of the final products [58–61]. Similar complex ecosystems, where starter and spoilage

microorganisms coexist, are also characteristic of other food industry sectors [62]. Finished meat products packaged in modified atmosphere or vacuum, which inhibit the growth of competitive aerobic microflora, create ideal conditions for the dominance of psychrotrophic LABs, which cause spoilage [63–65].

An important contemporary aspect is the taxonomic revision of the LAB group. In 2020, phylogenetic analysis led to the division of the heterogeneous genus *Lactobacillus* into 25 new genera, including *Latilactobacillus, Lactiplantibacillus,* and *Levilactobacillus*. This division reflects profound genetic and metabolic differences and explains why closely related organisms may exhibit contrasting technological properties. For example, *Latilactobacillus sakei* may be both a dominant spoilage agent and the basis for protective cultures. Understanding this taxonomy is critical for the accurate identification of contaminants and the selection of beneficial strains. Modern analysis also shifts focus to the bioactive potential of LABs beyond classical lactic acid fermentation. In addition to bacteriocins [66–68], their ability to synthesize other antimicrobial agents (organic acids, hydrogen peroxide, reuterin), texture-improving exopolysaccharides, and even neuroactive substances such as gamma-aminobutyric acid (GABA) is being actively studied, opening up prospects for the creation of functional foods.

The taxonomic diversity of LABs associated with meat spoilage is extremely high and geographically determined. Species belonging to new genera derived from *Lactobacillus* (e.g., *Latilactobacillus sakei, Latilactobacillus curvatus*) are among the most common spoilage agents, causing severe acidification, off-flavor, and slime in various types of meat stored under vacuum or in modified atmosphere [69–77]. The strictly psychrotrophic *Lactobacillus algidus*, first isolated from vacuum-packed beef, poses a particular problem for markets with long-term refrigerated storage [78]. Members of the genus *Leuconostoc*, particularly *L. gelidum* and *L. gasicomitatum*, are responsible for gas and acetic acid production, leading to package bulging, oily odor, and slime formation [59,79–85]. *Carnobacterium* spp. are frequently found in meats with low oxygen levels and play a dual role [80, 86–88]. *Weissella* spp. and *Lactococcus* spp. (e.g., *L. piscium*) are also significant spoilage agents, with the latter often dominating toward the end of shelf life [71,74,80,81,89–92]. Certain species of the genus *Enterococcus* have also been detected in spoiled meat [93,94].

Innovative research in recent years has expanded our understanding of the mechanisms for antagonism of protective cultures. In addition to the classical mechanisms of competitive exclusion and bacteriocin diffusion [66–68], new modes of interaction have been discovered. For example, recent studies have identified a contact-dependent mechanism of pathogen inhibition, in which direct physical contact with the cell of an antagonist bacterium (such as *Lactococcus carnosus*) is required to kill a target cell (e.g., *Listeria monocytogenes*). This mechanism is mediated by a specialized protein, bacteriolysin, and opens up new prospects for targeted biopreservation. Furthermore, the use of LABs to combat fungal spoilage and mycotoxins is actively developing. Many strains are capable of producing antifungal metabolites and adsorbing mycotoxins, making them promising "green preservatives" for protecting a wide range of food products.

The geographic and temperature distribution of spoilage agents shows clear pattern. In cold climates and in global supply chains, obligate psychrotrophic species pose a major problem [78,80,91,92]. Their cold adaptation and high competitiveness make them serious spoilage agents. A key problem is that standard microbiological tests at 30 °C often completely ignore these psychrotrophic populations, leading to underestimation of quality and overestimation of shelf life [95]. Control of these LABs is difficult due to their tolerance to cold, vacuum, modified atmosphere [61,64,83,96,97], and even sodium nitrite [98]. The resistance of many strains to sanitization is aggravated by their ability to form biofilms [99]. Research confirms that psychrotrophic species of *Leuconostoc* and *Lactococcus* are the dominant problem at the end of shelf life of packaged chilled products, demonstrating their persistence [92,100].

Thus, the negative impact of LABs in the meat industry is the result of a complex interaction of factors: the adaptability of psychrotrophic species, the inadequacy of standard control methods [95], the selective action of modern packaging technologies [92], and the formation of stable biocenoses in production. Solving this problem requires a paradigm shift. Prospects lie in the field of precision microbial management: the use of modern molecular methods for monitoring, in-depth study of metabolomics and strain-specific mechanisms of action (including recent ones, such as contact-dependent antagonism), and the development of integrated solutions. These include the creation of "microbial consortia" with targeted properties, combining LABs with other gentle technologies (gentle physical methods, active packaging) within the hurdle technology concept, and thoroughly assessing the safety of each candidate strain, including the absence of antibiotic resistance genes. This approach will not only reduce spoilage but also create safe meat products with an extended shelf life and improved functional properties.

LAB prevalence in the food industry

The prevalence and environmental persistence of lactic acid bacteria (LABs) in the food industry represent a complex problem beyond simple contamination of raw materials. LABs are integral commensals of the production environment, demonstrating high plasticity and the ability to form resident populations on processing equipment, indoor surfaces, and in engineering systems. This environmental adaptation reinforced by resistance to a number of environmental factors and the ability to form biofilms creates a significant risk of recurrent contamination of finished products, which directly threatens the

quality and shelf life, especially for pasteurized and chilled products such as cooked sausages [101]. The key genera of contaminants in the meat processing industry are *Lactobacillus* (particularly *L. sakei, L. curvatus*), *Leuconostoc* (*L. gelidum, L. carnosum*), *Carnobacterium,* and *Weissella* (*W. viridescens*), each of which is associated with specific defects, ranging from acidification and slime formation to color changing and packaging bulging.

The primary source of LABs is the raw material, i.e. fresh meat with an initial contamination level that may reach 10^4-10^5 CFU/g. However, to ensure the microbiological stability of products, secondary contamination and microflora circulation control within the facility is critical. Modern studies using molecular typing methods, such as [62,102], clearly demonstrate the phenomenon of cross-contamination by establishing the genetic identity of strains isolated from the surfaces of processing equipment (e.g., cutting tables) and strains responsible for spoilage of finished sausages. This transfer occurs through several key channels.

Air is one of the most significant and dynamic vectors of spread. Aerosols generated during washing, sanitization, cutting, and slicing serve as a vehicle for viable LAB cells. Bacterial concentrations in the air of production areas may be significant, and general ventilation and air conditioning systems facilitate their migration between different departments, including technologically isolated workshops. A striking example is the detection of *Weissella viridescens* in the air of refrigerated rooms, where this specific spoilage microorganism enters from primary processing areas [62,103,104]. Modern studies of the air environment in meat processing plants confirm that the qualitative and quantitative composition of microbial bioaerosols, including the proportion of LABs, serves as a reliable indicator of the sanitary condition of both individual zones and the entire production facility, allowing for the identification of critical risk points before mass product spoilage occurs.

The surfaces of equipment, tools, walls, and floors, especially in conditions of high humidity and the presence of organic residues, serve as primary reservoirs for the adhesion and subsequent formation of biofilms by resident LAB strains. Areas difficult to access for mechanical cleaning, as well as joints, seals, and drain systems, pose a particular hazard. Personnel, mobile process equipment (trolleys, containers), and inadequately disinfected post-treatment water also act as active carriers of contaminants between relatively clean and dirty areas.

Effective management of these risks requires a shift from reactive control to a preventive strategy based on a comprehensive approach. This includes regular monitoring not only of products but also of critical points in the production environment using modern methods such as quantitative PCR (qPCR) and metagenomic analysis, which enable the rapid identification and tracking of target indicator microorganisms, including key spoilage LABs. Based on monitoring data, sanitization programs (HACCP) should be developed and continuously adapted, taking into account the need to disrupt biofilms and target specific resident populations. Zoning of production flows is also an important element, minimizing the contact of raw materials, semi-finished, and finished products, as well as cross-contamination through personnel and air. This systematic approach, integrating continuous environmental monitoring, validated sanitation procedures, and targeted management of processes, allows for not simply reacting to spoilage incidents, but proactively suppressing the circulation of LABs in the production environment, which is the key to the consistent quality and safety of meat products.

Modern approaches to monitoring and controlling the resident LAB populations

Traditional microbiological control methods based on selective swabs are often insufficiently sensitive to detect and identify specific contaminant strains that form biofilms. Modern strategies are shifting toward the use of molecular methods, such as high-throughput sequencing (metagenomics) and MALDI-TOF mass spectrometry, which enable not only detection but also comparative phylogenetic analysis of microflora from various points along the production chain. This makes it possible to map the distribution of a specific undesirable strain and identify the true source of persistent contamination.

Combating persistent LAB biofilms requires a revision of traditional sanitation protocols. In addition to optimizing cleaning and disinfection regimens to address the need to disrupt the biofilm's extracellular polymer matrix, the use of biocontrol and biopreservation tactics is a promising approach. This involves the targeted colonization of production surfaces or the introduction of competing, safe strains of LABs or their metabolites (bacteriocins) into the product, which will suppress the development of unwanted spoilage microflora [102]. Another relevant area is the study and application of combinations of natural antimicrobial agents, such as essential oils (citrus, clove) and their active components (carvacrol, eugenol), which have demonstrated synergistic effectiveness against LABs and may be used within the framework of the "clean label" concept for surface treatment or in packaging materials [101,103,104].

Thus, the problem of LAB prevalence in the food industry has transformed from a simple issue of raw material contamination into a complex task of managing the microbial ecology of the enterprise. An effective solution lies at the intersection of precise microbiological monitoring, the use of modern molecular tools for strain tracking, the development of innovative sanitization protocols targeting biofilms, and the implementation of proactive biological control strategies. This integrated approach allows not only for the mitigation of consequences but also for the proactive management of microbial risks, ensuring consistent food quality and safety.

Conclusion

The review highlights the fundamental ambivalence of lactic acid bacteria (LABs) in the modern food industry, particularly in the context of the "clean label" trend. This duality manifests itself in their ability to act both as indispensable biopreservatives, fermentation agents, and probiotics, and as key microbial spoilage agents, particularly in the chilled meat sector.

On the one hand, LABs are a powerful biotechnological tool. Their ability to produce a complex of bioactive compounds, i.e. organic acids, bacteriocins, and exopolysaccharides, underlies natural methods for extending shelf life and creating functional foods. The introduction of LAB-based protective cultures offers an environmentally friendly alternative to synthetic preservatives and meets consumer demand.

On the other hand, the high ecological plasticity and adaptive potential of certain LAB strains make them a serious challenge. Psychrotrophic representatives of the genera *Lactobacillus, Leuconostoc, Carnobacterium,* and *Weissella*, adapted to refrigerated storage and modified atmosphere packaging, are dominant spoilage agents. Their ability to form persistent populations and biofilms on equipment leads to persistent cross-contamination and significant economic losses.

A key conclusion is the need to move from generalized approaches to precise, strain-specific microbial management. Modern molecular identification and typing methods (metagenomics, MALDI-TOF MS) allow not only the accurate identification of contaminants but also the tracking of their spread throughout the enterprise. Solving this problem requires a comprehensive strategy, including:

1. Improved monitoring of the production environment with an emphasis on identifying psychrotrophic populations and biofilms;
2. Development and validation of targeted sanitation procedures effective against complex microbial communities;
3. Active implementation of biocontrol methods based on the use of safe antagonist strains or their metabolites;
4. Hurdle technology, where the use of LABs as protective cultures is combined with gentle physical processing methods and active packaging.

Thus, the future of the effective use and control of LABs in the food industry lies in a balanced approach that recognizes and utilizes their beneficial potential while simultaneously applying advanced scientific methods to minimize the associated risks. This will ensure consistent quality, safety, and extended shelf life of products while reducing the use of chemical preservatives.

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CALCULATION OF HYDRAULIC LOSSES IN A PIPELINE TAKING INTO CONSIDERATION THE DEPENDENCE OF MINCED MEAT DENSITY AND RHEOLOGICAL PARAMETERS ON PRESSURE

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Keywords: *minced meat, transportation, viscosity, pressure, pipes, hydraulic losses*

Abstract
The article highlights the development of a method for calculating hydraulic losses in interoperation transportation systems taking into account the dependence of minced meat density and rheological parameters on pressure. These dependencies were taken from the experimental data of the well-known monograph written by A. V. Gorbatov. Minced meat density, its flow index, and texture index decrease along the pipeline axis together with the pressure level decreasing. As a result, the specific pressure losses caused by friction decrease from the inlet to the outlet of the pipeline. The Cauchy problem was formulated to determine the excessive pressure at the pipeline inlet. This pressure is necessary to determine the required pumping pressure and accordingly select the pumping equipment. The solution of the differential equation was found numerically for different values of the determining parameters. The range of parameter variation was the same as in the above-mentioned monograph: moisture content of minced meat 1.86–2.70 kg/kg, excessive pressure up to 1 MPa, internal pipeline diameter 55–80 mm, temperature 3–23 °C, mass flow rate of minced meat — up to 4 kg/s. The percentage by which the pumping pressure calculated taking into consideration the dependence of the minced meat properties on pressure (the full calculation) was determined to be greater than the value calculated without considering this dependence (the simplified calculation). Under these conditions, the error of the simplified calculation compared to the full calculation can exceed 50 %. In all cases, as hydraulic losses increase, so does the required correction to the calculated pumping pressure. The dependence of the correction factor on the pumping pressure calculated using the simplified (traditional) method was plotted, ignoring the dependence of the density and rheological parameters of the minced meat on pressure. This dependence provides for an approximate estimation of the required increase in the pumping pressure found via the simplified method.

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Introduction

When designing production lines for production of sausages and other minced meat food products, a rational choice of interoperation transport system is crucial. This article examines the problem of calculating the pressure drop ΔP along a pipeline length in those systems. This issue is covered in a special section of the well-known monograph written by A. V. Gorbatov [1]. This monograph specified that rheological parameters and the density of minced meat, ρ, play significant role in calculating of ΔP value.

As is known, the rheological properties of minced meat are well described by the power-law fluid model up to certain values of ω [2,3]:

$$\tau = K \times \omega^m, \quad (1)$$

where: τ — is shear stress, Pa; m — is dimensionless flow index; K — is liquid texture index, Pa·s^m; ω — is velocity shear (gradient), s^{-1}.

For example, in [2], based on the results of an experimental study, a diagram is presented in coordinates (τ–ω), which shows that formula (1) can be used to calculate the shear stress in the analyzed minced chicken meat when the velocity shears from zero to approximately 45 s^{-1}.

The research [1] presents the results of a comprehensive set of experimental and theoretical studies of dependence of density values ρ, K, m of minced meat on various factors, including composition, temperature, humidity, fat content, etc. Subsequently, many of the results and conclusions [1] were used in the studies of other researchers and in practice. These results were confirmed and further developed.

Wide range of studies has been devoted to the effect of various additives on the rheological properties of minced meat. For example, [3] presents the results of an experimental study of shear stress in minced meat systems in cases of adding flour (chickpea, flaxseed, buckwheat, and

rice flour). The dependence of the effective viscosity of the minced meat on velocity shear is presented. The least rate of structural breakdown was detected in the sample with chickpea flour addition, while the highest rate was found in the sample with buckwheat flour.

Authors of the study [4] experimented with the frozen minced meat samples with fat content of 2 %, 10 %, and 18 %, the samples were defrosted via various methods (defrosting in a refrigerator at an ambient temperature of +4 °C, defrosting under running cold water (+4 °C), and ohmic defrosting at various voltages). The viscoelastic properties were determined using rheological tests (tests for oscillation and creep/reconditioning tests). The values of elasticity modulus while storage, elasticity loss modulus, complex elasticity modulus, loss angle tangent modulus, dynamic viscosity modulus, and complex viscosity modulus of the minced meat samples increased along with increasing fat content. With increasing frequency, the elasticity modulus of the minced meat samples increased, but the dynamic viscosity values decreased.

The article [5] describes an experiment to develop minced meat recipes for poultry-based semi-finished food products. The authors sought for optimization of recipe ingredients compatibility in order to create a balanced meat system. Modelled recipes included meat of various cattle and poultry species, offal, and dairy products. The rheological properties of the obtained minced meat systems were studied.

Rheological properties, microstructure and stability of meat emulsions, stabilized with surfactants were discussed in [6]. Research has shown that Pickering emulsions are the potential filler particles for increasing the moisture stability of meat gels, as well as the fat substitutes for protecting lipids from oxidation.

The article [7] studies texture properties, colloidal interactions and rheological properties of meat emulsified systems with flax flour and tomato powder addition. The cooked sausages that contained 3 % of each additive showed the highest values of hardness and cohesive capacity in comparison with the reference samples. These regularities were confirmed by the higher values of elasticity parameter.

Li et al. [8] studied the effect of adding peanut, corn, soybean, and sunflower oils on the gelling and rheological properties of chicken meat-based emulsions. Vegetable oils improved emulsion stability, increased effective viscosity, and increased elasticity. The addition of sunflower oil had the greatest effect.

A study [9] examined the effect of insoluble tiger nut dietary fiber on the rheological properties and protein digestibility of low-fat meat emulsions. The results showed that this additive (3 % by weight) increased the elastic modulus and improved the processing properties of low-fat pork meat emulsions. Moreover, these properties were better than those of the high-fat reference emulsions.

An experimental study of the properties of emulsions made from four types of meat (fish, beef, sheep, and pork) was conducted in [10]. Rheological parameters were measured under deformation and vibration conditions. The samples made from fish and mammals vary significantly in their rheological properties and microstructural characteristics. Pork-based samples were found to possess the highest strength and the most compact gel structure.

Zheng et al. [11] studied the properties of minced chicken breast samples in which amidated pectins were used as natural lipid substitutes. These samples featured higher viscosity and gelling ability in comparison with the reference sample. After heat treatment, no significant differences in color and texture profile characteristics were found in any of the samples.

The authors [12] investigated the influence of chicken slaughter by-products (chicken legs and heads) on the chemical and rheological properties of minced meat. Chicken legs and heads were prepared as a ground mass and as a dry powder. Six types of samples were prepared with the addition of ground mass and powder made from chicken legs and heads in various proportions, legs and heads together and separately. It was found that adding this powder significantly increased the moisture-binding capacity and yield strength of the minced meat.

The article [13] is devoted to the study of the effect of temperature (4–50 °C) and sodium bicarbonate (0.4 %) on the solubility, protein aggregation and rheological properties of low-salt chicken meat emulsion. Along with increasing temperature the solubility and effective viscosity initially increased, reaching maximum values at 30 °C, and then they decreased. It was determined that the combination of sodium bicarbonate with a temperature of about 30 °C can change the structure of chicken protein under low salt content conditions.

The effect of high pressure (up to 80 MPa) and heat treatment (80 °C, 30 min) with the addition of modified protein on the rheological properties and taste of a pork emulsion product with reduced phosphate content was investigated in [14]. The results showed that the addition of such an additive, coupled with high pressure and heat treatment, significantly improved the stability, textural characteristics, and taste of the products. The authors believe that these improvements are associated with rheological and structural changes in the meat emulsions and recommend them for the production of meat products with reduced phosphate content.

There are not so many studies on the rheological properties of minced meat transported through pipelines. Some innovations and improvements have been made to the calculations of pressure drop along the pipeline in order to prevent it [15–18]. Thus, according to the data of [15], in the measured range of shear velocities, the texture flow index does not depend on the pipe diameter. To calculate the value of K of minced meat in the pipeline, an empirical formula was obtained. The effect of temperature on the rheological parameters of meat mixtures was analyzed in a number of works [17,18]. The authors of the article [18] showed that in the hydraulic calculation of pipeline systems assigned for minced meat transportation, it is necessary to take into ac-

count the characteristics of the pumps and determine the parameters (flow rate, pumping pressure, spent power, efficiency) at the operation point of the pumping unit.

The change in excessive pressure P is a well-known feature of pipeline systems used for interoperation transportation: it varies from the highest value at the inlet down to the lowest value at the outlet. In [1], the results of an experimental study of the dependence of the density and rheological parameters of minced meat on pressure were presented. Empirical formulas for those dependencies were proposed for "Doctorskaya" sausage mince and "Russian" sausages mince (hereinafter referred to as RSM). The phenomenon of increase in the density and viscosity of minced meat systems along with increasing pressure was confirmed in studies conducted by Golovanets et al. [19].

In all the above-mentioned studies, the dependence of density and rheological parameters of minced meat on pressure was not considered in hydraulic losses calculations. The approach [1] to the hydraulic calculation of process pipelines was retained: the density and rheological parameters were assumed to be constant all along the pipe axis.

The purpose of this article is to develop a method for calculating hydraulic losses in interoperation transportation systems, taking into consideration the dependence of minced meat density and rheological parameters on pressure. The relevance of this study is based on the need to improve mathematical models and calculation methods for pipeline transportation of minced meat for development of digital clones of production processes.

Research tasks:

1. Assessment the experimental data [1] sufficiency for their use in hydraulic calculations of minced meat pipeline transportation;
2. Development of a method for calculating hydraulic losses in a pipeline, taking into consideration the dependence of minced meat density and rheological parameters on pressure;
3. Exploring the impact of various factors (moisture content of minced meat, mass flow rate, pipeline diameter, temperature) on the error of calculation results without taking into consideration the dependence of minced meat density and rheological parameters on pressure.

Objects and methods

A system for interoperation transportation of minced meat through cylindrical pipes is the object of this research. The subject of the research is the effect of pressure on minced meat density and rheological properties on hydraulic losses during minced meat movement through the pipe.

This article uses the findings of dependence of density on pressure [20] observed in three samples of "Russian" sausages mince (RSM), minced in a mincer with mesh diameter of 3 mm of holes. Sample of RSM-1 has a fat content $\varphi = 0.1826$ kg/kg, moisture content $U = 2.06$ kg/kg; RSM-2: $\varphi = 0.1544$ kg/kg, $U = 2.55$ kg/kg; RSM-3: $\varphi = 0.127$ kg/kg, $U = 2.70$ kg/kg.

In [20] an empirical formula is given for calculating the density of sausage mince, obtained as a result of generalizing experimental data:

$$\rho = F_1(\varphi, U, p) = \\ = 1037 - (290 \cdot \varphi + 10{,}5U) + 22 \times \lg(p \times 10^5), \quad (2)$$

where: ρ — is density of minced meat, kg/ m^3; φ — is fat content, kg/kg; U — is moisture content, kg/kg; p — is dimensionless excessive pressure (excessive pressure referenced to atmospheric pressure): $p = P/P_A$.

Formula (2) was obtained for a wide range of pressures ($p = 0.1 - 16$). The error in calculating the density as per (2) does not exceed 4 %.

Formula (2) shows that density dependence not only on excessive pressure but also on the fat content and moisture content of the minced meat was obtained. In this article the authors confine themselves to the dependence of density on p for the determined RSM samples:

$$\rho = F_{2i}(p) = \rho_{0i} + 22 \times \lg p, \quad (3)$$

where: the first term in (3) is calculated using the known values of φ and U: for RSM-1 $\rho_{01} = 1072.4$ kg/m^3, for RSM-2 $\rho_{02} = 1075.4$ kg/m^3, for RSM-3 $\rho_{03} = 1081.8$ kg/m^3; index 22 in formula (3) is dimensional (kg/m^3).

It is necessary to note that density changes only slightly with an increase in dimensionless excessive pressure from $p = 0.1$ to $p = 10$, and amounts to no more than 4.2 % for three RSM samples being considered.

For m and K parameters of sausage mince given in [21] empirical formulas are given. For RSM they can be written in the following way:

$$m = 0{,}732 \times (1 + 0{,}091 \times \lg(1 + p) + 0{,}0017 \times t, \quad (4)$$

$$K = 64 \times (1 - 0{,}02 \cdot t) \times \\ \times \exp[-10{,}8 \times (W - 0{,}48) \times (1 - 0{,}173 \times \lg(1 + p))], \quad (5)$$

where: $W = U(1 + U)$ — is relative humidity of RSM, kg/kg; t — temperature, °C.

The dependence of rheological parameters on pressure is more significant than that of density. As the dimensionless excessive pressure changes from zero to ten, the value of m increases by approximately 9 %, and K increases by more than 45 %.

In [1] the limits of formulas applicability are indicated: (1) — is applicable up to $p = 16$, (4) and (5) — up to $p = 10$. In industrial conditions they try to use systems of interoperation transportation of minced meat with a pumping pressure of less than 1 MPa ($p = 10$). It is known that higher pressure can lead to overgrinding of minced meat and its quality worsening [1]. Pumping equipment is selected to provide a relevant level of pumping pressure [22, 23]. Therefore, the experimental data and the analytical dependencies of the density and rheological parameters of RSM on excessive pressure proposed on their basis from the article [1] are quite sufficient for calculating the hydraulic losses along the length of the pipeline used in industrial conditions for transporting minced meat. Therefore, the first task of the research is solved.

We assume that RSM flow in the pipeline occurs in laminar mode. Pressure losses along the pipeline during laminar fluid flow (including non-Newtonian fluid) can be calculated using the well-known Darcy-Weisbach formula:

$$\Delta P = \frac{\lambda}{2} \cdot \frac{L}{D} \cdot \rho \cdot V^2, \lambda = \frac{64}{\mathrm{Re}}, \quad (6)$$

where: λ — is the index of friction losses along the pipeline length; L — is pipeline length, m; D — is the pipeline internal diameter, m; ρ — is the liquid density, kg/ m^3; V — is average (in cross-section) velocity of mince flow in the pipeline, m/s; Re = ρ · D · V/μ — Reynolds number; μ — is the index of dynamic viscosity of the liquid, Pa·s.

But when transporting a power-law fluid, in particular, minced meat, instead of the usual Reynolds number Re, its analogue for a power-law fluid Re_{PL} should be substituted into formula (6), as in [18,24]:

$$\mathrm{Re}_{PL} = \frac{V^{2-m} D^m \rho}{8^{m-1} K \cdot \left(\frac{3m+1}{4m}\right)^m}, \quad (7)$$

Where: Re_{PL} _ is the analogue of Reynolds number for a power-law fluid; V — is the average flow velocity in the pipeline; m — is flow index; D — is the pipeline internal diameter; ρ — is minced meat density; K — is the index of minced meat texture.

This article takes into consideration the fact that the minced meat density does not remain constant, and decreases along the length of the pipeline together with the pressure [1]. Therefore, we will calculate the velocity based on the mass flow rate G (kg/s), which remains constant: $V = G/(\rho \cdot S)$, where $S = 0.25 \cdot \pi \cdot D^2$ is the cross-sectional area of the pipeline, m^2. By substituting (7) into (6) and dividing by L, the value of specific pressure losses along the length of the pipeline is obtained:

$$I = \frac{\Delta P}{L} = \frac{2^{3 \cdot m+2}}{D^{m+1}} \cdot K \cdot \left(\frac{3 \cdot m+1}{4 \cdot m}\right)^m \cdot \left(\frac{G}{\rho \cdot S}\right)^m. \quad (8)$$

In previously published studies [14,17,18], the values of m, K, and ρ were assumed to be constant along the pipeline length. Therefore, to calculate pressure losses, the value I was simply multiplied by L.

According to formulas (3)–(5), m, K, and ρ depend on the dimensionless excessive pressure p, temperature t, fat content φ, and moisture content W. We assume that in the problem of determining pressure losses, the values φ, W, t, G, D, and L are the parameters. They do not change for the given pipeline and for the sample of minced meat. Then, the value of the specific pressure loss is a function of only one argument — the dimensionless excessive pressure: $I = f(p)$.

To determine the pressure loss along the length of a pipeline when transporting a given sample of minced meat, it is necessary to find a solution to Cauchy problem:

$$\frac{dp}{dY} = f(p), p(0) = p_0, \quad (9)$$

where: Y — is coordinate measured along the pipe axis from the outlet to the inlet, m; p_0 — is dimensionless excessive pressure at the outlet of the pipe.

Next, we assume that the pressure at the outlet is atmospheric, therefore $p_0 = 0$.

The Cauchy problem (9) in general has no analytical solution, therefore a numerical finite-difference method was used, implemented in the Mathcad environment with the help of Given-Odesolve operators combination.

The obtained value of the dimensionless excessive pressure at the pipeline inlet p(L) is the sought-after value of pressure loss along the pipeline length Δp_1. Thus, the second task of the research is solved — a method for calculating hydraulic losses in a pipeline was developed taking into consideration the dependence of minced meat density and rheological parameters on pressure.

For comparison, a simplified calculation was performed, determining the value of $\Delta p_0 = I_0 \cdot L$, where the value I_0 was calculated using formula (8). Moreover, m, K, ρ were considered as constant, equal to the corresponding values at atmospheric pressure.

The error in calculating hydraulic losses with and without considering the dependence of minced meat parameters on pressure (full calculation and simplified calculation) was calculated via the formula:

$$\varepsilon = 100 \cdot \left(\Delta p_1 / \Delta p_0 - 1\right). \quad (10)$$

As shown, while increasing excessive pressure, the density of RSM changes notably less than does the viscosity. Therefore, a calculation of the hydraulic losses Δp_2 was performed under the condition that the values of m and K depend on the excessive pressure, and ρ along the pipe axis remains constant, equal to its value at atmospheric pressure. The calculation error introduced by the density constancy was estimated using formula (11).

$$e = 100 \cdot \left(\Delta p_1 / \Delta p_2 - 1\right). \quad (11)$$

Results and discussion

Figure 1 shows the results of calculations via formula (8) for the dependence of specific hydraulic losses on excessive pressure at six values of RSM mass flow rate. The increase in I with increasing G corresponds to the well-known physical effect of the influence of dynamic pressure on hydraulic losses. Thus, an increase of G from 0.3 to 4 kg/s at $P = 0$ led to an increase of I by 6.7 times, and at $P = 1$ MPa — by 8 times. The increase of I along with excessive pressure growth is a feature of the proposed method. According to Figure 1, at $G = 4$ kg/s, if the excessive pressure rises from zero to 1 MPa, the value of I will increase by 95.4 %. At $G = 0.3$ kg/s, it will increase by 63.2 %.

The physical reason for this phenomenon is that increasing pressure leads to an increase in the minced meat viscosity. The higher the viscosity of the mass, the greater the hydraulic losses. It is necessary to note that while the minced meat moves through the pipeline, the excessive

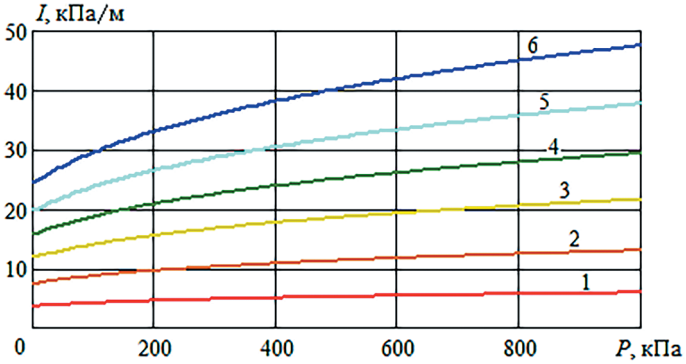

Figure 1. Results of calculating the dependence of specific hydraulic losses on excessive pressure for RSM at 3 °C, W = 0.673, D = 60 mm and different values of mass flow rate: 1 — G = 0.3 kg/s, 2 — G = 0.8 kg/s, 3 — G = 1.5 kg/s, 4 — G = 2.2 kg/s, 5 — G = 3 kg/s, 6 — G = 4 kg/s

pressure decreases from the inlet to the outlet. Consequently, the viscosity and specific pressure losses decrease.

The results of solving the Cauchy problem (9) are numerically presented in the Figures 2 and 3. In all calculations the pipe length was assumed to be constant, L = 20 m. The X coordinate was measured from the pipe inlet: $X = L - Y$. For each set of parameters, the pressure change along the pipe axis was calculated twice: taking into account the dependence of the density and rheological parameters of the FRS on excessive pressure (solid lines in the Figure 2) and without taking this dependence into account (dashed lines in the Figure 3). The latter calculation should be called as simplified.

The dashed lines in the Figure 2 correspond to the hydraulic calculation for a power-law fluid flow, where density and rheological parameters are not dependent on excessive pressure. Therefore, they linearly decrease along the pipe axis. As G increases, hydraulic losses increase. In the Figure 2, the greater G — the higher the dashed lines are located, due to the dependence of density and rheological parameters on pressure. Their deviation from straight lines is noticeable, especially at high mass flow rates.

The RSM density along the pipe axis decreases slightly: by 1.4 % at G = 0.3 kg/s and by 5.5 % at G = 4 kg/s. Therefore, the increase in the RSM flow velocity from the pipe inlet to the outlet is small. Under the similar conditions, the texture index decreases by 8.6 and 27.9 %, respectively. Both of these latter changes contribute to increase in the Reynolds number along the pipe axis. Figure 3 shows the results of its calculation using formula (7). The calculations were performed taking into account the pressure drop along the pipe axis, found using differential equation (9).

According to Figure 3, the value Re_{PL} increases from 22.0 at the pipe inlet up to 41.8 at the outlet (89.5 % increase) at G = 4 kg/s. At G = 0.3 kg/s, this increase goes from 1.39 up to 1.59 (14.1 % increase). This indicates that the flow regime in the pipeline remained laminar under all studied conditions. Therefore, using the formulas (6) and (7) is copmletely justified.

All calculations via the proposed method were then performed with varying one of the following factors: pipe-

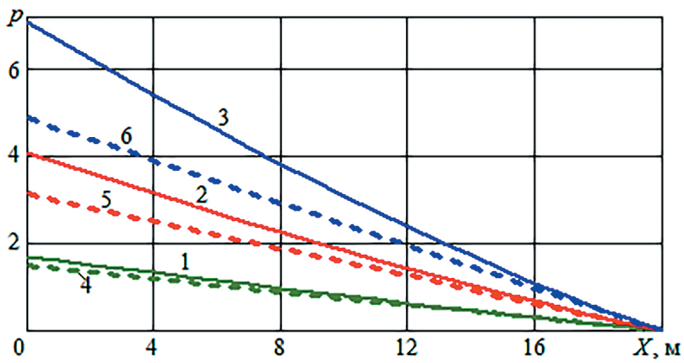

Figure 2. Results of calculating the dimensionless excessive pressure along the pipe axis taking into consideration (lines 1, 2, 3) and not taking into consideration (lines 4, 5, 6) the dependence of the RSM density and rheological parameters on pressure at 3 °C, W = 0.673, D = 60 mm and different values of mass flow rate: 1 and 4 — G = 0.8 kg/s, 2 and 5 — G = 2.2 kg/s, 3 and 6 — G = 4 kg/s

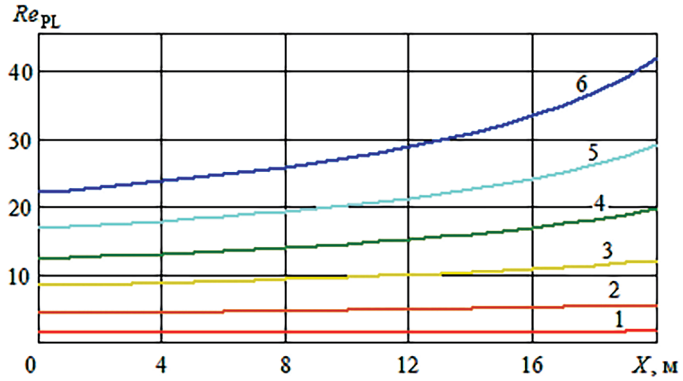

Figure 3. Results of calculating the Reynolds number analogue along the pipe axis at 3 °C, W = 0.673, D = 60 mm and different values of mass flow rate: 1 — G = 0.3 kg/s, 2 — G = 0.8 kg/s, 3 — G = 1.5 kg/s, 4 — G = 2.2 kg/s, 5 — G = 3 kg/s, 6 — G = 4 kg/s

line diameter, RSM moisture content, and temperature. For comparison, the Figure 4 shows the dependence of the dimensionless pressure drop across a pipe of a given length L for two diameters, taking into account the dependence of the density and rheological parameters of RSM on excessive pressure (solid lines for $\Delta p_{1)}$ and without this dependence (dashed lines for $\Delta p_{0)}$. It is evident that the solid lines extend significantly above the dashed lines. Moreover, this difference increases along with increasing RSM mass flow rate.

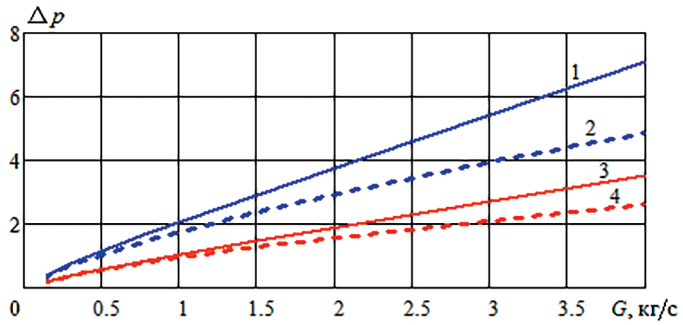

Figure 4. Dependence of the dimensionless pressure drop on RSM mass flow rate at 13 °C, U = 2.06 kg/kg and two diameter values: 1 and 2 — D = 55 mm, 3 and 4 — D = 68 mm; 1, 3 — calculation taking into consideration the dependence of RSM density and rheological parameters on excessive pressure ($\Delta p_{1)}$; 2, 4 — not taking into consideration (Δp_0)

The proposed method for calculating pressure drop during pipeline transportation of minced meat is significantly more complicated than the traditional hydraulic calculation method, because it involves the numerical solution of differential equation (9). The feasibility of its application is evaluated in reference to the pressure drop calculation error value, ignoring the dependence of RSM density and rheological parameters on excessive pressure. The results of calculating this error using formula (10) with varying values of individual factors are presented in the Figures 5 and 6.

In all cases, increasing RSM mass flow rate increases the simplified calculation error. This is caused by increase in dynamic pressure due to G increase. Changing the internal diameter of the pipeline significantly affects the calculation results. Increasing D reduces the dynamic pressure and decreases the simplified calculation error. In the Figure 5, for G = 4 kg/s and D = 55 mm, the simplified calculation error is 52 %, while for a diameter of 80 mm, it decreases down to 17 %.

Increasing temperature leads to a decrease in RSM viscosity, which leads to a decrease in the specific pressure loss calculated with the formula (8). As a result, the error in the simplified calculation decreases, but not as much as with increasing diameter. In Figure 6, at G = 4 kg/s and a temperature of 3 °C, it is 45.6 %, while at 23 °C, it decreases to 33.8 %.

As the moisture content increases, the viscosity of RSM decreases. This also reduces the specific pressure loss and the simplified calculation error. For example, at G = 4 kg/s and U = 1.86 kg/kg, this error amounts to 49.4 %, at U = 2.06 kg/kg, it decreases down to 45.6 %, and at U = 2.70 it decreases to 34.7 %.

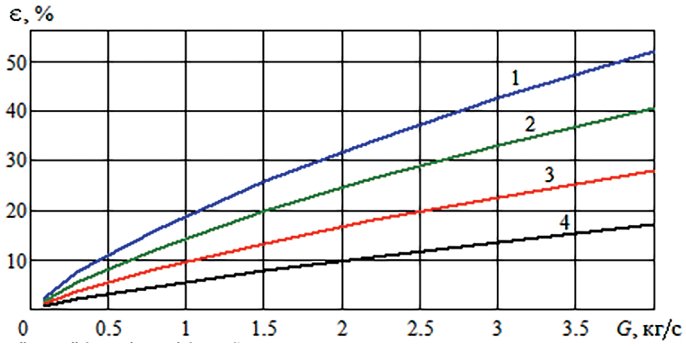

Figure 5. Dependence of the error of the simplified calculation of the pressure drop on RSM mass flow rate at 13 °C, U = 2.06 kg/kg and for 4 diameters of pipe: 1 — D = 55 mm, 2 — 60 mm, 3 — 68 mm, 4 — 80 mm

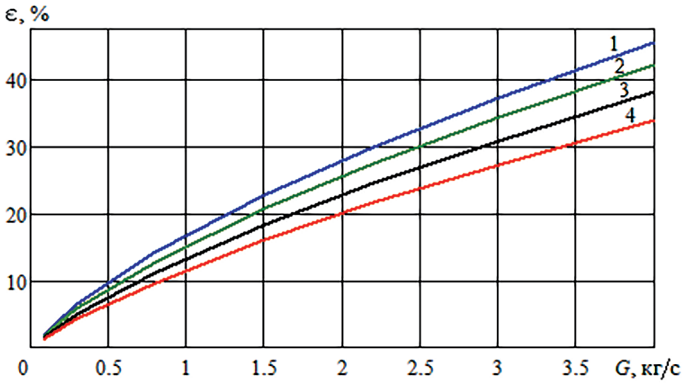

Figure 6. Dependence of the simplified calculation error on RSM mass flow rate at D = 60 mm, U = 2.06 kg/kg and for 4 temperatures: 1 — 3 °C, 2 — 10 °C, 3 — 17 °C, 4 — 23 °C

Figure 7 shows how the error calculated using formula (11) depends on the mass flow rate of RSM. The conditions used are the same as in Figure 5. It can be seen that assuming RSM density as constant, equal to its value at atmospheric pressure, leads to overestimation of the pressure loss. This is because, in the model used, mince density decrease directly leads only to an increase in RSM flow velocity within the pipeline. As a result, hydraulic losses increase. However, this increase does not exceed 4 % over the entire range of parameters studied.

Note that the error in the simplified calculation ε increases with increasing values of the factors that lead to increased hydraulic losses. To analyze this phenomenon it is necessary to plot another graph.

Δp_0 was chosen as the argument, calculated without taking into account the dependence of RSM density and rheological parameters on excessive pressure. The groups of points are numbered. Each group includes 6 points, which were obtained at six values of RSM mass flow rate from 0.3 to 4 kg/s. Groups of points 1–4 were calculated by varying the pipeline diameter, and 5–8 — by varying the temperature. It is evident that all of the indicated points practically lie on the same line. In the range Δp_0 = 0–4, the dependence in Figure 8 is close to a directly proportional dependence: $\varepsilon = 10\Delta p_0$. Only at $\Delta p_0 > 4$ does the indicated line deviate slightly downwards from the straight line.

According to the Figure 8, if the pressure loss according to the simplified calculation is $\Delta p_0 > 1$, then the simplified calculation error is $\varepsilon > 10$ %; if $\Delta p_0 > 6$, then $\varepsilon > 50$ %. This error is unacceptable for engineering calculations. The conducted assessment allows us to conclude that the third objective of the study has been fulfilled.

The Figure 8 enables estimation for how much greater the hydraulic losses are in the extended calculation than in the simplified one. With putting $\varepsilon = 10\Delta p_0$ into formula (10), the approximate estimation of the required dimensionless pumping pressure is obtained:

$$\Delta p_1 \approx \Delta p_0 \cdot (1 + 0{,}1 \cdot \Delta p_0). \qquad (12)$$

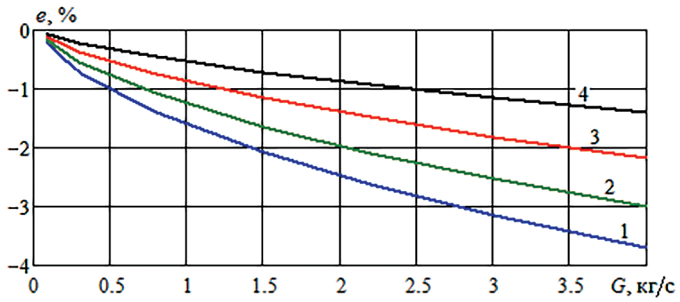

Fig. 7. Dependence of the simplified calculation error e on RSM mass flow rate at 13 °C, U = 2.06 kg/kg and for 4 diameters of pipe: 1 — D = 55 mm, 2 — 60 mm, 3 — 68 mm, 4 — 80 mm

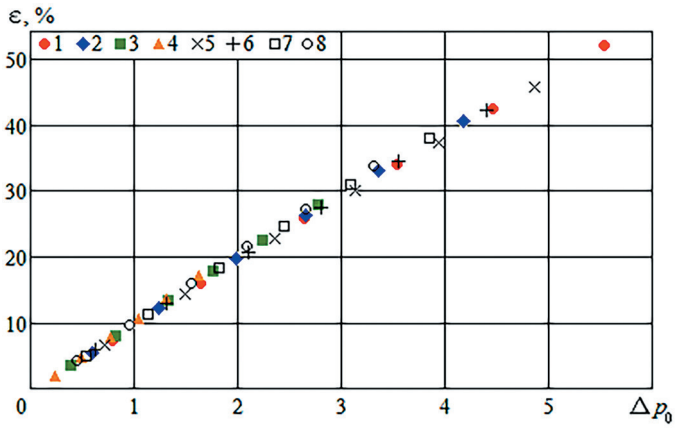

Figure 8. Dependence of the simplified calculation error on the dimensionless value of the pressure drop, calculated without taking into consideration the dependence of RSM density and rheological parameters on the excessive pressure at U = 2.06 kg/kg: 1, 2, 3, 4 — t = 13 °C; 1 — D = 55 mm, 2 — 60 mm, 3 — 68 mm, 4–80 mm; 5, 6, 7, 8 — D = 60 mm; 5 — t = 3 °C, 6 — t = 10 °C, 7 — t = 17 °C, 8 — t = 23 °C

The practical application of the Figure 8 and formula (12) is as follows. Under the assumption that while feeding the RSM the value $\Delta p_0 = I_0 \cdot L = 3$ (0.3 MPa) is obtained, where the value of I_0 is calculated via simplified method, according to formula (8), without taking into consideration the dependence of RSM density and rheological parameters on excessive pressure. Then, taking into consideration the dependence of RSM density and rheological parameters on excessive pressure, the hydraulic losses will be approximately 30 % greater, $\Delta p_1 \approx 3.9$ (0.39 MPa). It is this pumping pressure that should be taken into account when selecting the appropriate pumping equipment.

It should be noted that the Figure 8 was obtained for the representation of dependence of RSM density and rheological parameters on pressure. For the other types of minced meat the correlation between Δp_0 and ε (and therefore between Δp_0 and Δp_1) may quantitatively differ.

Conclusion

Thus, the purpose set out in the article has been achieved, and all research tasks have been solved. The developed method for calculating hydraulic losses in a pipeline, taking into consideration the dependence of minced meat density and rheological parameters on pressure, includes the following main steps:

1. Determination of the analytical dependence of minced meat density and rheological parameters (flow index m and liquid texture index K) on pressure according to the results of experimental studies — the formulas (3)–(5) should be referred to.
2. Derivation of the formula for specific pressure losses caused by friction along the length of the pipeline being researched (designed), taking into consideration the dependence of m and K on pressure — the formula (8) should be referred to.
3. Mathematical formulation of the Cauchy problem, including a differential equation for dimensionless excessive pressure and its preset value at the pipeline outlet.
4. Solving the Cauchy problem and determining the dimensionless excessive pressure at the pipeline inlet. This value is the required pumping pressure, which (along with the specified flow rate) is referred to in order to select the relevant pump.

The analysis showed that the results of previously conducted and published studies on the relationship between density and rheological parameters of "Russian" sausage mince (RSM) are sufficient to solve the tasks of this article. However, a full calculation for other mince systems will require similar experimental studies.

The error in the full calculation of hydraulic losses (dimensionless pressure losses $\Delta p_{1)}$ using the Cauchy problem (9) is determined by the accuracy of the used approximations and does not exceed 4–5 %. However, the error in the simplified calculation ($\Delta p_{0,}$ ignoring the dependence of density and rheological parameters on pressure) can reach 50 % under certain conditions. This error is unacceptable for design engineering calculations. Therefore, it is necessary to use the calculation method developed in this article. This method is significantly more labor-consuming than the traditional hydraulic calculations, because it requires numerical solving the Cauchy problem. Therefore, an approximate estimate of hydraulic losses increase due to the effect of pressure on the minced meat parameters was proposed.

On the example of minced meat produced for the "Russian" sausages, the influence of temperature, humidity, mass flow rate, and pipeline diameter on the calculation results was studied. In all cases increasing in hydraulic losses leads to increasing the correction required for the calculated pumping pressure. The dependence of ε on the pumping pressure was calculated using a simplified method, which ignores the dependence of the minced meat density and rheological parameters on pressure. In this context ε value represents a correction to the result of the simplified calculation of the pumping pressure, and it can be used for approximate assessment. The percentage by which the pumping pressure calculated with the dependence of the minced meat properties on pressure exceeds the value calculated without taking this dependence into consideration was determined. Formula (12) enables an approximate estimating of Δp_1 by Δp_0 without solving differential equation (9).

It should be noted that the numerical values of corrections obtained in this article are valid for the minced meat used in "Russian" sausages. For other minced meat systems those values may significantly differ. Moreover, it is necessary to take into consideration the hydraulic losses due to local spots of resistance, primarily at the pipeline bends.

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DEVELOPMENT AND EVALUATION OF LOW-FAT CHICKEN BURGERS USING CHICKEN FEET MEAT AS A FUNCTIONAL FAT REPLACER

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Keywords: *chicken burger, chicken feet, low fat, fat replacer, healthy food*

Abstract
The increasing demand for healthier meat products has encouraged the development of innovative fat replacers in processed foods. This study evaluated the use of chicken feet meat, a collagen-rich poultry by-product, as a functional alternative to animal fat in chicken burger formulations. Two formulations were prepared: a control containing 20 % animal fat and a reformulated sample in which fat was replaced with 20 % chicken feet meat. Proximate composition, physicochemical characteristics, oxidative stability, microbial quality, and sensory attributes were assessed. Results demonstrated a significant reduction of fat (19.37 % → 4.81 %) and caloric value (249 → 131 kcal/100 g) in the reformulated burgers, accompanied by higher protein (15.18 % → 18.05 %), collagen (0.25 % → 1.12 %), and moisture contents (61.43 % → 71.84 %). Technologically, the reformulated product exhibited lower cooking loss, improved water-holding capacity, and a firmer texture. Microbiological analyses confirmed product safety during 90 days of frozen storage, with slightly lower bacterial counts and thiobarbituric acid (TBA) values indicating enhanced stability. Sensory evaluation demonstrated significantly higher scores for color, odor, taste, texture, and overall acceptability compared with the control. Furthermore, the reformulation offered a significant economic advantage by reducing raw material costs. These findings indicate that chicken feet meat is a cost-effective and sustainable fat replacer that enhances the nutritional profile, improves functional properties, and maintains consumer preference in chicken burgers. Beyond its health benefits, the valorization of chicken feet supports waste reduction and contributes to more sustainable poultry processing systems.

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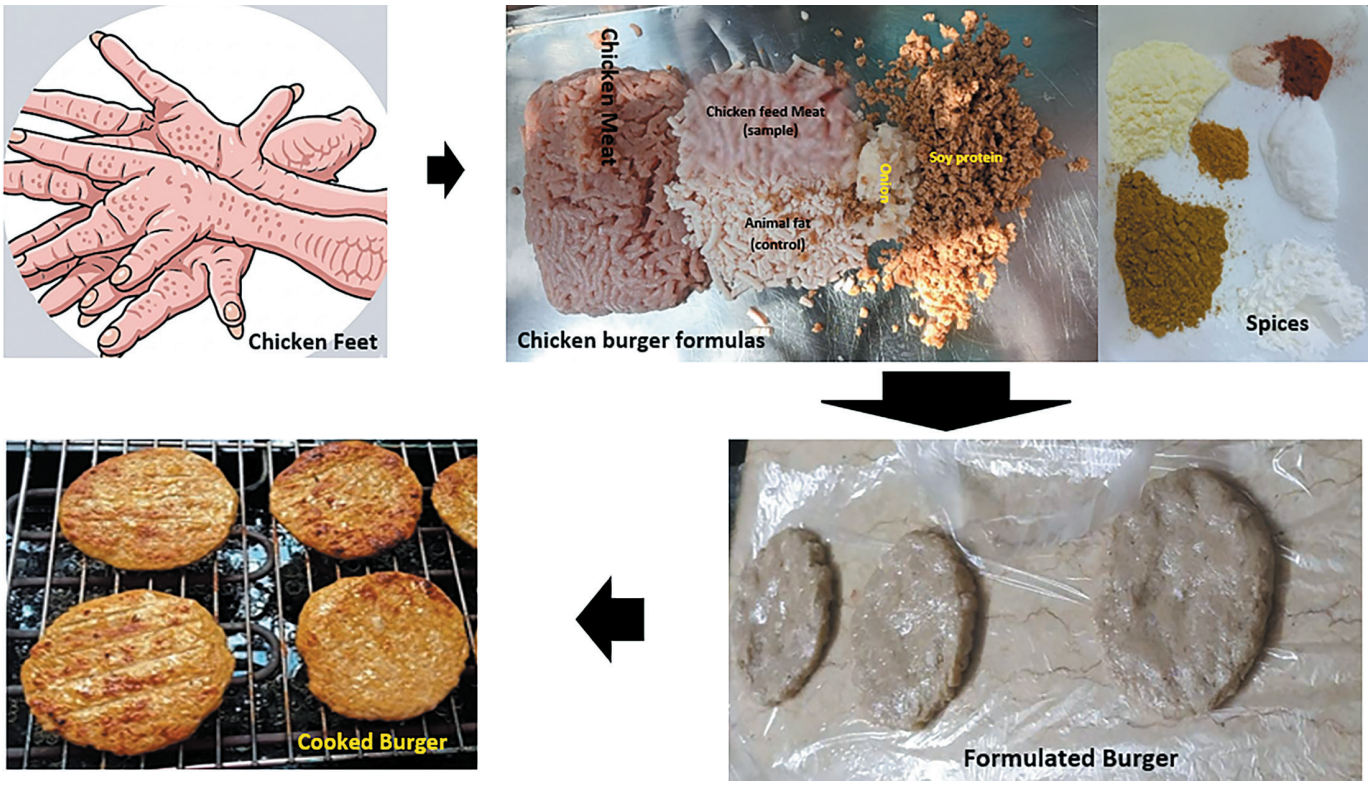
Graphical Abstract



Introduction

Chicken burgers have emerged as a globally popular food product, primarily due to their convenience, affordability, and high protein content, making them a staple in modern dietary habits. However, like many processed meat products, they are typically formulated with relatively high levels of animal fat — often exceeding 20 % of the formulation — to ensure desirable organoleptic properties. While fat contributes positively to juiciness, flavor, and texture, its excessive intake is strongly associated with obesity, cardiovascular disease, and other chronic health conditions, a link emphasized by recent global health guidelines [1]. Therefore, the development of low-fat chicken burgers that retain desirable sensory and technological properties is an important task for food scientists [2].

Numerous fat replacers have been explored to address the dual challenge of preserving sensory quality while improving nutritional profiles. Plant-based ingredients, such as olive oil, avocado, and flaxseed oil, are commonly employed to enhance the fatty acid composition by introducing beneficial unsaturated lipids, while animal-based substitutes like fish oil and poultry fat are utilized to boost flavor complexity. However, despite their nutritional and technological advantages, these alternatives often face significant limitations, including high formulation costs, susceptibility to oxidative rancidity during storage, and variable consumer acceptance due to potential textural deviations or off-flavors. Consequently, this has driven growing interest in utilizing sustainable, low-cost by-products — rich in bioactive compounds and dietary fibers — that can effectively improve product functionality while simultaneously addressing environmental concerns and promoting healthy meat formulations [3–5].

Chicken feet, a widely available poultry-processing by-product, are rich in collagen and proteins with unique gelling and water-binding properties. Collagen not only improves textural characteristics but also provides bioactive health benefits, including supporting joint health, bone density, and skin elasticity. Although collagen has been used in various food applications, limited research has investigated the direct use of chicken feet meat as a fat replacer in poultry-based products [6,7].

This knowledge gap highlights an opportunity to valorize chicken feet as a functional ingredient in meat processing. By incorporating chicken feet into chicken burger formulations, it may be possible to reduce fat content, improve nutritional value, and maintain consumer-preferred sensory qualities, while simultaneously contributing to waste minimization in the poultry industry. Accordingly, the objective of this study was to evaluate the effect of replacing 20 % of fat with chicken feet meat on the chemical composition, physicochemical characteristics, microbial stability, and sensory acceptability of chicken burgers. The findings aim to provide evidence for the development of healthier and more sustainable meat formulations.

Objects and methods

Objects

Fresh chicken breasts were procured from a local market and minced using a domestic meat mincer. A standardized spice mixture, salt, fresh onions, starch, powdered milk, and soybean were also sourced from the local market. Chicken feet were obtained from the same source and subjected to preliminary processing to ensure quality and safety. They were first soaked in water containing salt and vinegar to eliminate undesirable odors. Subsequently, they underwent a brief boiling treatment for 5 minutes to inactivate enzymatic activity and prepare them for further processing.

Chicken burger preparation

Chicken burger formulas (control and sample) were prepared by mixing well-minced chicken breast with different ingredients, which are presented in Table 1.

Table 1. Composition of formulated chicken feet burger

Ingredients	Control, %	Sample, %
Chicken breasts	60	60
Beef suet (kidney fat)	20	0
Chicken feet meat	0	20
Soybean flour	10	10
Minced onion	5	5
Salt	1.7	1.7
Spices	1.3	1.3
Milk powder	1	1
Starch	1	1

Chemical composition

Chemical composition (fat, protein, moisture, and collagen) of boiled chicken feet meat and samples of chicken burgers was determined using Food Scan™ Pro meat analyzer [8]. Ash content, representing the total mineral residue remaining after complete combustion of organic matter, was determined using the international standards [9]. Samples were subjected to dry ashing in a muffle furnace at 500–600 °C. The organic matter was incinerated in the presence of oxygen, resulting in the release of CO_2 and N_2, along with volatilization of water and organics. The residual ash was weighed and expressed as a percentage of the original sample mass.

Cooking loss

Cooking loss (%) was determined according to [10]. After grilling each sample, cooking loss (%) was calculated as follows:

$$\text{Cooking loss } \% = \frac{F - G}{F} \times 100\,, \qquad (1)$$

where: F — fresh burger sample weight (g); G — grilled burger sample weight (g).

Water holding capacity

The water holding capacity (WHC) of the samples was assessed using the filter paper press method, as described by [11]. A 0.3 g chicken burger sample was placed on

ashless filter paper (Whatman No. 41) and subjected to a 1 kg weight for 10 minutes. The pressure created two distinct zones on the paper; their surface areas were quantified using a planimeter. The outer zone, representing exuded water, indicated WHC, while the inner zone reflected tissue plasticity. The WHC was calculated by deducting the area of the inner zone from the outer zone and reported in square centimeters (cm^2).

Thiobarbituric acid (TBA)

The quantification of thiobarbituric acid (TBA) was performed according to the protocol established by [12], with minor modifications. Briefly, 1 mL of the sample homogenate was combined with 2 mL of a stock reagent. This stock solution, containing 0.37 % TBA, 15 % TCA, and 0.25 N HCl, was gently warmed to 75 °C in a water bath SW22, (Julabo, Germany) to ensure the complete solubility of TBA. The reaction mixture was subsequently incubated in a boiling water bath for 15 minutes to allow for the development of a pink pigment. After cooling under running tap water and centrifuging at 2000 rpm for 15 minutes, the absorbance was recorded at 532 nm using a Unico UV-2000 spectrophotometer (Dayton, NJ, USA). TBA values were reported as mg of malonaldehyde per kg of chicken burger samples.

Tenderness and color

Tenderness was evaluated by measuring the shear force (N) using an Instron Universal Testing Machine (Model 2519–105, USA). Six replicates were analyzed for each sample, with the crosshead speed set to 200 mm/min [10]. Color measurements were obtained using a Chroma meter (Konica Minolta, Model CR 410, Japan), which was calibrated with a white tile and light trap according to the manufacturer's instructions. Color values were expressed using the CIE L*, a*, b*, C, and H color system [13], with five spectral readings recorded for each sample. Lightness (L*) ranged from 0 (dark) to 100 (light), while redness (a*) values ranged from positive (reddish) to negative (greenish). Yellowness (b*) values were also estimated, ranging from positive (yellowish) to negative (bluish). Furthermore, Chroma and Hue angle were calculated.

Microbial load

Microbial load was quantified using the Total Plate Count (TPC) method to assess product safety and hygienic quality. Enumeration was conducted via the pour plate technique using Plate Count Agar (PCA). The inoculated plates were incubated in an inverted position at 30 °C for 24–48 hours, and colony-forming units (CFUs) were subsequently enumerated. The microbiological analyses were performed under controlled ambient conditions (22.5 °C; 42.1 % relative humidity), following the methodology described by [14].

Sensory evaluation

A structured sensory evaluation was conducted to assess the organoleptic properties and hedonic preference comparing a conventional chicken burger (control) with a reformulated low-fat prototype. A panel of twenty trained assessors evaluated the freshly grilled samples immediately after thermal processing. The evaluation focused on five key attributes: color, aroma, taste, texture, and overall acceptability. Quantification was performed using a 9-point hedonic scale (1 = 'dislike extremely'; 9 = 'like extremely'). To ensure standardized testing conditions and mitigate carryover effects, panelists were provided with individual utensils and scorecards, with water supplied for palate cleansing between successive samples [10,15,16].

Statistical analysis

All quantitative data were statistically analyzed using one-way analysis of variance (ANOVA) to determine significant differences among treatment groups. This method allowed for the comparison of mean values across the control and low-fat chicken burger samples. Differences were considered statistically significant at ($p < 0.05$). The ANOVA analysis provided a robust statistical basis for evaluating consumer acceptance scores, chemical composition, and microbiological data across multiple replicates [17].

Results and discussion

Chemical composition of boiled chicken feet

The proximate analysis of boiled chicken feet (Table 2) elucidates a nutritional profile highly conducive to application in meat product formulation, particularly for fat replacement and texture modification. The data highlights a significant presence of structural proteins and moisture that collectively suggest this by-product can serve as a functional ingredient capable of mimicking the technological roles of fat [18]. The most notable finding is the substantial collagen content (5.54 %), a fibrous protein that plays a pivotal role in forming thermally irreversible gels upon heating, which is critical in low-fat formulations where fat reduction often leads to undesirable hardness and dryness; indeed, this high collagen content supports the ability to maintain structural integrity and juiciness. This corroborates observations by Araújo et al. [7], who demonstrated that collagen gel from chicken feet significantly improved water-holding capacity (WHC) in chicken sausages, and Sousa et al. [19], who noted that up to 50 % of fat could be replaced using collagen without compromising sensory quality. Furthermore, the protein content (15.62 %) and high moisture level (66.14 %) observed in this study reinforce this potential. The high moisture is indicative of the tissue's ability to bind water, an essential function for yield and succulence, while the protein fraction acts as a natural binder to enhance matrix cohesion, aligning with Huff-Lonergan and Lonergan [20] on protein-water interactions. Additionally, Kim et al. [21] reported that chicken feet gelatin increased processing yield and succulence in semi-dried jerky, further supporting these findings. Regarding the moderate natural fat content (12.52 %) and calculated caloric value (176 Kcal/100g), these figures suggest

a promising profile for "light" meat products where the intrinsic fat contributes to mouthfeel and palatability without necessitating exogenous fat addition. This is consistent with Araújo et al. [22], who highlighted the nutritional and textural benefits of poultry connective tissue, and Mohammed et al. [23], who emphasized the value of chicken secondary products in enhancing nutritional quality. Ultimately, the chemical composition of boiled chicken feet, characterized by its unique balance of collagen, moisture, and protein, provides a robust scientific basis for its utilization as a fat replacer and functional additive in chicken burgers [24].

Table 2. Chemical composition of boiled chicken feet

Parameters	Boiled chicken feet
Collagen, %	5.54 ± 0.42
Fat, %	12.52 ± 0.06
Moisture, %	66.14 ± 0.16
Protein, %	15.62 ± 0.25
*Carbohydrates, %	0.18
Calories (Kcal /100 g)	176

* Calculated by difference; the experimental values (means and SD for $n = 3$).

Chemical composition and some physical properties of chicken burger formulas

Proximate composition and nutritional implications

The proximate analysis in Table 3 demonstrates a significant compositional shift in the reformulated chicken burger, primarily driven by the substitution of conventional animal fat with chicken feet collagen. The most pronounced change was the substantial reduction in fat content, decreasing from 19.37 % in the control to 4.81 % in the sample. This reduction aligns with the global recommendations of the World Health Organization (WHO) regarding limiting saturated fat intake to prevent cardiovascular diseases (CVDs) and supports the concept of producing healthier meat products with fat replacers [25]. Consequently, the caloric value of the product was reduced by nearly half (from 249 to 131 kcal/100 g), categorizing the reformulated product as a functional, low-calorie meat alternative.

The significant increase in collagen content (0.25 % to 1.12 %) is a direct result of the chicken feet incorporation, a by-product rich in connective tissue. Similar findings were reported by Araújo et al. [7], who noted that adding collagen gel extracted from chicken feet significantly increased the protein content and reduced fat in chicken sausages. Furthermore, the increase in moisture content from 61.43 % to 71.84 % can be attributed to the enhanced water-binding capacity of the collagen gel. This hydrophilic nature of collagen allows it to trap water molecules within the protein matrix, preventing exudation and improving juiciness, a phenomenon also observed by Pavanello et al. [26] in chicken burgers produced with hydrolyzed collagen as a partial fat substitute.

The protein content also increased significantly (15.18 % to 18.05 %), enhancing the nutritional density of the burger. The slight increase in ash content (0.31 % to 0.40 %) may be attributed to the mineral content present in the chicken feet, such as calcium and phosphorus, which are naturally higher in bone and cartilage tissues compared to pure muscle meat.

Functional properties, cooking loss and water holding capacity (WHC)

The functional properties of the reformulated burger showed marked improvements. Cooking loss was significantly lower in the sample (18.85 %) compared to the control (26.29 %). This improvement in cooking yield is a critical economic factor for the meat industry. The reduction in cooking loss is likely due to the formation of a stable gel network by the collagen and the increased protein matrix, which immobilizes water and fat, preventing their expulsion during thermal processing. These results corroborate the findings of Araújo et al. [7], where sausages formulated with chicken feet collagen gel exhibited superior water holding capacity and reduced cooking loss compared to standard formulations.

Water holding capacity (WHC), assessed via the filter paper press method, also indicated better performance in the sample (0.61 cm^2) compared to the control (0.92 cm^2). In this method, a smaller area of fluid release indicates superior water retention. This enhancement suggests that the collagen gel effectively replaces the lubricating and water-binding role of the removed animal fat, preventing the texture from becoming dry or gritty, which is a common defect in low-fat meat products. This observation aligns with Schmidt et al. [27], who developed reduced-fat chicken sausages with improved functional properties.

Textural profile (shear force)

The shear force analysis revealed that the reformulated burger was slightly firmer than the control (2.99 N vs. 2.42 N). This increase in hardness is a common outcome of fat reduction, as fat acts as a lubricant that softens the meat matrix. However, the increase in shear force in this study is likely also associated with the cohesive properties of the added collagen gel, which forms a rigid protein network upon heating. Kim et al. [21] reported that shear force in chicken jerky decreased with the addition of gelatin due to its plasticizing effect at high concentrations; however, in the current formulation, the gel content appears to reinforce the structure, providing a "bite" or firmness that is desirable in burgers. Similarly, Choe et al. [28] noted that the quality characteristics of reduced-fat sausages depend heavily on the type of fat replacer used. The pH values remained stable and within the optimal range for meat product stability (pH ~6.0), indicating that the reformulation did not adversely affect the acid-base balance required for protein functionality.

Color profile

Instrumental color evaluation (Table 4) showed a significant decrease in lightness (L*) in the reformulated sample (56.16) compared to the control (59.04). This darkening effect is typically expected in low-fat meat products

because fat globules reflect light, contributing to a lighter appearance. Replacing beef suet with darker chicken feet and collagen-rich connective tissue results in a darker surface. However, the non-significant differences in redness (a*) and yellowness (b*) suggest that the core color pigment (myoglobin) was not significantly affected, preserving the visual appeal of the cooked meat product.

Lipid oxidation stability

TBA values showed a slight, non-significant reduction in the reformulated sample (0.22 mg MDA/kg) compared to the control (0.28 mg MDA/kg). While not statistically significant, this downward trend suggests that the collagen inclusion might possess mild antioxidant properties or that the reduction in total fat content provided fewer substrates for lipid oxidation. This is consistent with Araújo et al. [7], who reported that chicken feet collagen gel treatments had lower TBARS values and higher antioxidant activity compared to standard sausages during storage.

Finally, the reformulation of chicken burgers using chicken feet meat was successful in producing a low-fat, high-protein, and low-calorie product with superior functional properties. The improvements in cooking yield and water retention, coupled with acceptable textural and color profiles, suggest that chicken feet collagen is a viable and sustainable fat substitute in meat processing, offering a solution to valorize poultry by-products while meeting consumer demand for healthier meat options.

Table 3. Chemical composition and some physical properties of control and low fat chicken burger

Parameters	Control	Sample
Collagen, %	0.25 ± 0.07^{b}	1.12 ± 0.14^{a}
Fat, %	19.37 ± 0.07^{a}	4.81 ± 0.02^{b}
Moisture, %	61.43 ± 0.10^{b}	71.84 ± 0.07^{a}
Protein, %	15.18 ± 0.04^{b}	18.05 ± 0.11^{a}
Ash, %	0.31 ± 0.11^{a}	0.40 ± 0.12^{a}
* Carbohydrates, %	3.46	3.78
Calories (Kcal /100 g)	249	131
Cooking loss, %	26.29 ± 0.23^{a}	18.85 ± 0.35^{b}
WHC, cm^2	0.92 ± 0.20^{a}	0.61 ± 0.10^{b}
pH	6.03 ± 0.09^{a}	6.12 ± 0.08^{a}
Shearing force, N	2.42 ± 0.24^{a}	2.99 ± 0.44^{a}
TBA, mg MDA/kg	0.28 ± 0.05^{a}	0.22 ± 0.07^{a}

* Calculated by difference; WHC: Water Holding Capacity (cm^2); TBA: Thiobarbituric Acid; The experimental values (means and SD for $n = 3$) with small letter are significantly different ($P \leq 0.05$).

Table 4. Color values of control and low fat chicken burger

Parameters	Control	Sample
L*	59.04 ± 0.38^{a}	56.16 ± 0.30^{b}
a*	2.76 ± 0.21^{a}	2.94 ± 0.10^{a}
b*	23.74 ± 0.85^{a}	22.35 ± 0.79^{a}
C	23.90 ± 0.82^{a}	23.13 ± 0.56^{a}
H	83.36 ± 0.68^{a}	82.48 ± 0.31^{a}

The experimental values (means and SD for $n = 3$) with small letter are significantly different ($P \leq 0.05$).

Microbiological evaluation

Hygienic quality and safety assessment

The microbiological profile presented in Table 5 serves as a critical indicator of the hygienic conditions maintained during processing and the stability of the product during frozen storage. A primary safety criterion in meat products is the absence of coliform bacteria, which act as hygienic markers and potential indicators of fecal contamination. The non-detection of coliforms (ND) in both the control and the reformulated sample throughout the 3-month storage period underscores the high standard of hygiene employed during the manufacturing process. Also, Sheng et al. [29] emphasized the importance of monitoring microbial contamination in cooked chicken feet products, reinforcing the need for strict hygienic protocols when utilizing these by-products.

The total bacterial count (TBC)

The total bacterial count (TBC) was monitored to evaluate the shelf-life potential and microbial stability of the burgers under frozen storage conditions (–18 °C). Freezing acts as a hurdle technology that typically arrests or strongly retards microbial growth below about –10 °C, as *psychrotrophic* and *psychrotolerant* bacteria predominately remain metabolically inactive at such temperatures. However, certain *psychrotolerant* and psychrophilic bacteria can survive for extended periods and may undergo limited multiplication during handling or thawing episodes at mild refrigeration temperatures (around 4–7 °C), where growth of *psychrotrophic* microorganisms is still possible. In the current study, a gradual increase in TBC was observed in both groups over the 3-month storage period. This trend is likely attributable to the survival of cold-tolerant microorganisms and limited growth during thawing at 4 °C and subsequent handling, rather than continuous proliferation within the freezer. Despite this increase, TBC at the end of 90 days (6.7×10^5 CFU/g for the control and 5.6×10^5 CFU/g for the reformulated sample) remained below the typical spoilage threshold of 10^7 CFU/g, indicating that the products maintained an acceptable microbiological quality throughout storage, in line with reported data for frozen chicken meat products where TBC often ranges from 10^5 to 10^7 CFU/g depending on initial contamination and processing conditions [30,31].

An inhibitory or stabilizing effect of the reformulation is evident from the consistently lower TBC observed in the reformulated sample compared to the control at all sampling intervals (zero, one, two, and three months). At the initial stage, the reformulated sample recorded 2.7×10^2 CFU/g, compared to 3.4×10^2 CFU/g in the control. This difference persisted, culminating in the treated sample showing a lower load (5.6×10^5 CFU/g) than the control (6.7×10^5 CFU/g) by the third month. This suggests that the lower fat content (~5 % vs ~19 %) and the specific protein matrix of the chicken feet collagen may have reduced the availability of nutrients for *lipolytic*

psychrotolerant bacteria and slightly limited their survival during repeated thawing–handling cycles. The reduction in total fat also limits the protective effect of lipid droplets against ice crystal damage, yet the overall microbial load remained within safe limits [30–34].

The microbiological data confirm that the reformulated chicken burger with chicken feet meat is safe for consumption and complies with hygienic standards. The absence of coliforms and the lower TBC compared to the control indicate that the processing conditions were hygienic and that the reformulation may contribute to a marginal improvement in microbial stability during frozen storage, in agreement with previous studies showing that formulation adjustments in reduced-fat poultry products are important for maintaining microbiological stability. These results support the practical application of this formulation as a safe, shelf-stable meat product, although the observed increase in counts emphasizes the importance of controlled thawing and handling practices to minimize microbial growth.

Table 5. Microbiological analysis of control and chicken feet burger

Targeted microbial group	Coliform CFU/g		Total bacterial count CFU/g	
	Control	Sample	Control	Sample
Zero time	N.D.	N.D.	3.4×10^2	2.7×10^2
After 1 month	N.D.	N.D.	4.5×10^3	3.8×10^3
After 2 months	N.D.	N.D.	5.3×10^4	5.1×10^4
After 3 months	N.D.	N.D.	6.7×10^5	5.6×10^5

* N.D.: not detected

Sensory evaluation

Overcoming the "low-fat" sensory barrier

Sensory evaluation is the ultimate determinant of a product's market viability, particularly when formulating low-fat meat products, as the removal of fat often leads to undesirable changes in texture, juiciness, and flavor. In this study, the reformulated sample, where 20 % of the animal fat was replaced with chicken feet meat, achieved significantly higher mean scores ($p \leq 0.05$) than the control in all evaluated attributes. This finding is of particular importance because it demonstrates that the functional fat replacer not only maintained but actively enhanced the organoleptic profile compared to the conventional high-fat formulation. These results contradict the common industry challenge where fat reduction typically correlates with lower consumer acceptance, confirming the success of the formulation strategy.

Texture and mouthfeel enhancement

One of the most critical challenges in producing low-fat meat products is maintaining a tender and juicy texture. The reformulated sample received a significantly higher texture score (8.8) compared to the control (8.4). This sensory improvement aligns with the proximate composition data, which showed a substantial increase in moisture content (71.84 %) and collagen content (1.12 %) in the reformulated burger. The high water-holding capacity (WHC) and the gel-forming ability of collagen derived from chicken feet likely prevented the product from becoming dry or rubbery during cooking. Instead, the collagen-protein matrix acted as a lubricant and moisture binder, mimicking the mouthfeel typically provided by animal fat. This supports the findings of Kim et al. [21] who reported that the addition of chicken feet gelatin to chicken jerky resulted in a significant decrease in shear force (increased tenderness), and Pavanello et al. [26] reinforced that hydrolyzed collagen improves the texture of reduced-fat chicken burgers.

Color, odor, and taste profiles

The superior color score of the reformulated sample (8.8 vs. 8.2) suggests that the browning reaction and visual appearance were optimized by the replacement. While instrumental color analysis (Section 3.2) indicated a slight reduction in lightness (L*), panelists evidently perceived the reformulated sample as more aesthetically appealing, possibly due to a richer, more "cooked" appearance resulting from the Maillard reaction facilitated by the protein-rich chicken feet.

Regarding odor and taste, the reformulated sample outperformed the control (8.6 and 8.8, respectively). This suggests that the inclusion of chicken feet meat did not introduce any off-flavors or "gamey" odors, which are often concerns when utilizing meat by-products. On the contrary, the enhancement in taste scores may be attributed to the retention of volatile flavor compounds within the improved gel network or the inherent flavor profile of the cartilaginous tissue. Kim et al. [21] found no significant differences in flavor scores when adding chicken feet gelatin to jerky, while Abedelmaksoud et al. [32] noted that additives in nuggets could maintain sensory acceptability up to certain limits. Choe et al. [28] also found that using skin and fiber mixtures as fat replacers maintained acceptable sensory profiles. In the present study, the chicken feet replacement appears to have synergistically enhanced the flavor profile, potentially compensating for the loss of fatty acids, which usually contribute to flavor richness in meat.

Overall acceptability

The ultimate measure of success, overall acceptability, was significantly higher in the reformulated sample (8.7) than in the control (8.4). This confirms that the health benefits (lower fat, higher protein) did not come at the expense of sensory pleasure. The data validates the hypothesis that chicken feet meat is not merely a low-cost filler but a value-adding ingredient that improves product quality. As concluded by Araújo et al. [7], the technological and bioactive properties of chicken feet collagen allow for the production of healthier meat products without compromising, and indeed potentially improving, the sensory characteristics desired by consumers. The sensory evaluation confirms that the reformulated chicken burger is superior to the conventional counterpart in terms of color, odor, taste,

texture, and overall acceptability. The successful integration of chicken feet meat as a fat replacer overcame the textural defects typical of low-fat products, resulting in a juicier, more palatable, and healthier meat option with high consumer potential.

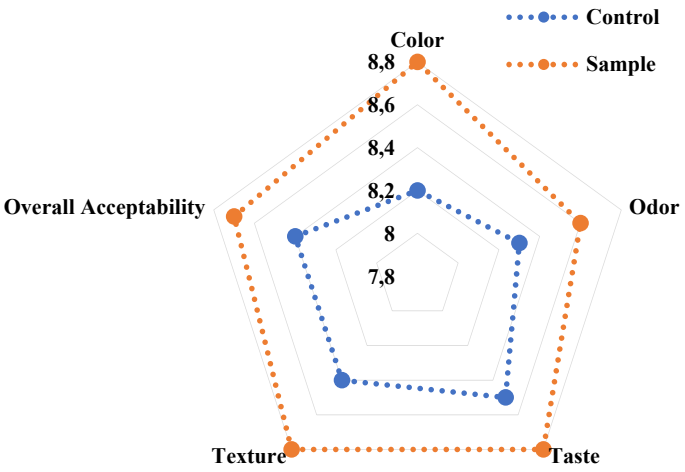


Figure 1. Organoleptic evaluation of control and chicken feet burger

TBA of control and samples during frozen storage (–18 °C) for 90 days

Progression of lipid oxidation during frozen storage

Lipid oxidation is a primary determinant of meat product quality, leading to rancidity, discoloration, and nutritional degradation. The thiobarbituric acid (TBA) values presented in Figure 2 and Table 8 reveal a progressive accumulation of malondialdehyde (MDA) in both formulations over the 90-day frozen storage period. This gradual increase, despite the low-temperature storage (–18 °C), confirms that oxidative reactions continue at a slowed pace over time. The mechanism is driven by the propagation of free radical chains within the lipid fraction and the interaction of these radicals with oxygen and proteins.

Superiority of the reformulated formulation can be seen from the fact that the reformulated chicken burger sample consistently exhibited significantly lower TBA values compared to the control ($p < 0.05$) throughout the entire storage duration. By the end of the storage period (Day 90), the control sample reached 0.84 mg MDA/kg, whereas the reformulated sample showed significantly lower values of 0.64 mg MDA/kg. This reduction in TBA values suggests that the reformulation strategy significantly enhanced the oxidative stability of the product. The superior resistance to oxidation in the reformulated sample can be attributed to two synergistic factors. First, the substantial reduction in total fat content (from ~19 % to ~5 %) reduced the substrate available for lipid peroxidation, thereby limiting the generation of secondary oxidation products. Second, the inclusion of chicken feet meat introduces functional bioactive compounds, specifically collagen and gelatin, which possess inherent antioxidant properties. Previous literature supports this mechanism. Nuñez et al. [33] and Araújo et al. [7] demonstrated that collagen and gelatin from animal sources can retard lipid oxidation in meat matrices. This antioxidant activity is often attributed to the ability of collagen-derived peptides to scavenge free radicals and chelate pro-oxidant metal ions [34,35], thereby interrupting the oxidative chain reaction. Pavanello et al. [26] and Rather et al. [24] further corroborated that such protein-based formulations effectively stabilize meat products against oxidative deterioration during storage.

Safety and rancidity thresholds

Notably, despite the increase in TBA values over time, all samples remained well below the critical threshold of 2 mg MDA/kg, which is generally considered the limit for the onset of perceivable rancidity in meat products [7]. This confirms that both the control and the reformulated burger maintained acceptable flavor profiles throughout the 90-day frozen storage period. However, the consistently lower TBA values in the reformulated sample suggest a longer window of sensory stability and a lower risk of off-flavor development compared to the high-fat control.

Economic and sustainability implications, cost-effectiveness and commercial viability

Beyond the chemical and sensory improvements, the economic analysis highlights the industrial relevance of this formulation strategy. The reformulated chicken burger demonstrated a distinct economic advantage, with raw material costs reduced from 180 L.E/kg in the control to 150 L.E/kg in the reformulated sample. This reduction of 30 L.E per kilogram (approximately 16.7 % cost savings) is significant in the highly competitive meat processing sector, where profit margins are often dictated by raw material costs. By substituting expensive animal backfat with lower-cost chicken feet, a by-product typically undervalued in the primary meat industry, the formulation offers a commercially viable solution for producing premium "healthier" meat products without the associated premium cost structure.

Valorization of poultry by-products

The economic advantage is intrinsically linked to environmental sustainability. Chicken feet constitute a significant volume of poultry slaughterhouse waste. The current study demonstrates a successful "waste-to-value" application, transforming chicken feet into a functional ingredient that enhances nutritional profile and oxidative stability. This valorization supports circular economy principles by reducing the environmental burden of waste disposal while creating a high-quality, sustainable food ingredient [23,24,36]. Finally, the reformulation of chicken burgers with chicken feet meat not only yields a product with superior nutritional quality, functional properties, and oxidative stability but also presents a compelling economic case for industrial adoption. The significant reduction in production costs, coupled with the valorization of a poultry by-product, positions this formulation as a sustainable, healthy, and economically advantageous alternative to conventional high-fat chicken burgers.

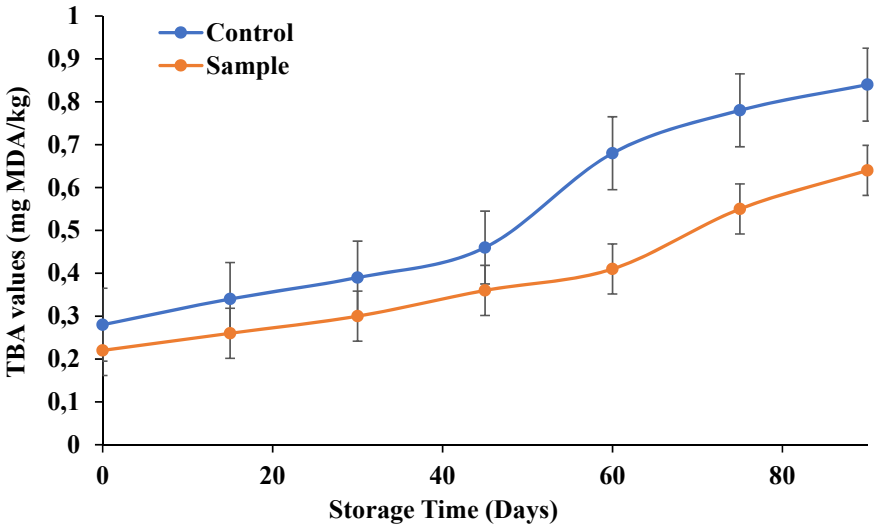


Figure 2. TBA values (mg MDA/kg) of control and low-fat chicken burgers during frozen storage (–18 °C)

Conclusion

This study successfully demonstrated the viable application of chicken feet meat as an innovative and functional fat replacer in chicken burger formulations. The substitution of animal fat with chicken feet resulted in a significant improvement in the nutritional profile, characterized by a marked reduction in total fat and caloric content alongside a substantial increase in protein and collagen levels. From a technological standpoint, the reformulated product exhibited superior functional properties, including enhanced water holding capacity, reduced cooking loss, and favorable textural firmness, which translated into improved juiciness and mouthfeel. Critically, sensory evaluation confirmed that the reformulated burger outperformed the control in all organoleptic attributes — color, odor, taste, texture, and overall acceptability — indicating that the health benefits were achieved without compromising, and in fact enhancing, sensory quality. Furthermore, the product demonstrated good microbiological safety with lower bacterial counts and enhanced oxidative stability, as evidenced by lower TBA values throughout frozen storage. The economic analysis further highlighted the cost-effectiveness of the formulation, reducing raw material costs by valorizing poultry by-products. Collectively, these findings validate chicken feet meat as a sustainable, functional, and economical alternative to animal fat, offering a significant contribution to the development of healthier, high-quality processed meat products.

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ECONOMIC LOSSES DUE TO REPRODUCTIVE DISORDERS DURING FOOT AND MOUTH DISEASE OUTBREAK IN JEMBER REGENCY, INDONESIA

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Keywords: *FMD, reproductive disorders, abortion, economic losses, cattle, disease*

Abstract

Jember Regency as one of the regencies with the largest beef cattle population in East Java, recorded a high number of FMD cases. In the beef cattle farming business, reproduction is a key factor in the success of beef cattle breeding, therefore this study aims to determine how much impact FMD has on abnormalities in the cattle reproductive system so that it can be known what reproductive disorders occur, the factors that most influence abortion during FMD and the potential economic losses for farmers and business actors engaged in this field in Jember Regency. This study is a quantitative descriptive study aimed at determining the incidence of post-FMD reproductive disorders, identifying the main factors influencing abortion, and estimating the economic losses resulting from abortion and reproductive disorders based on the increase in days open and calving intervals. The results of the study showed that reproductive disorders after FMD were found in 56 % of studied animals with the largest percentage being ovarian hypofunction at 27 %. The incidence of abortion during FMD outbreaks reached 12 % and the most influential factor in the incidence of abortion was FMD virus infection. The economic losses caused by abortions were estimated at IDR 210,840,000 (approximately USD14,000), while the losses due to reproductive disorders calculated on the basis of increased days open and calving intervals were estimated at IDR 1,015,200,000 (approximately USD67,700). Based on the results of this study, it is necessary to further study service per conception, conception rate and calving rate so that the value of the livestock's reproductive efficiency is known more precisely.

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Introduction

Foot and mouth disease (FMD) is an acute and highly contagious viral disease affecting cloven-hoofed animals such as cattle, buffalo, sheep, goats, and pigs [1]. The disease is characterized by the formation of vesicles and erosions in the oral cavity, nostrils, teats, and interdigital spaces. These clinical manifestations have been widely reported in previous studies on FMD pathogenesis and clinical

presentation [2]. FMD can cause major economic losses due to decreased production and become an obstacle in the trade of animals and their products [3].

Based on data from the integrated National Animal Health Information System in 2022, the number of FMD incident reports was 192,169 in 38 regencies/cities in East Java, with the number of recoveries being 187,712, the number of deaths being 3,965 and the number of forced slaughter being 2,483. Jember Regency is one of the regencies with the highest number of FMD cases in East Java with a total of 14,292 cases, with the number of recoveries being 14,123, the number of deaths being 154 and the number of forced slaughter being 15 [4].

East Java Province is the center of beef cattle in Indonesia. Data from the Badan Pusat Statistik (BPS), the Statistics Indonesia Agency, indicate that East Java consistently has the largest beef cattle population in Indonesia. In 2021, the population reached approximately 4.94 million head, and subsequent reports in 2022–2023 confirm that East Java remains the primary national center for beef cattle production. According to the data on the population of beef cattle in East Java, Jember Regency is one of the regencies with the largest population of beef cattle with a total of 274,162 heads or equivalent to 5.56 % of the total population in East Java. From Integrated National Animal Health Information System data, it is known that Jember Regency is also a regency that sends quite a lot of livestock outside the province. FMD is the most feared contagious animal disease in the world because it causes enormous economic and social losses [5].

As Knight-Jones and Rushton et al. [3] stated the impact of FMD in a region can be direct or indirect. The losses caused by Foot and Mouth Disease (FMD) include reduced milk production (up to 25 % annually), decreased growth rates in beef cattle (resulting in 10–20 % longer time to reach market weight), reduced draft power capacity (up to 60–70 % in the first month after infection), decreased fertility (with abortion rates reaching up to 10 %) and delayed conception, increased mortality in young animals (20–40 % in sheep and pigs), culling of chronically affected livestock, disruption of domestic trade and livestock management systems, loss of export opportunities, and the costs associated with disease control and eradication programs. In developed countries, FMD is also one of the most feared diseases because it has an impact on livestock population and productivity as well as significant economic losses. Various eradication measures taken include slaughtering cattle that show clinical symptoms (depopulation), caution in obtaining meat, destroying milk production, carrying out mass disinfection, emptying farms for 6 months, and carrying out quarantine measures with a radius of 15–25 km [6].

Indonesia is actually very suitable for eradication because it consists of islands, but many neighboring countries are not free from FMD. In accordance with the guidelines of the World Organisation for Animal Health (WOAH), formerly known as the Office International des Epizooties (OIE), the government has implemented adjustments to its FMD control strategy. These modifications include strengthening post-outbreak surveillance, conducting reproductive status examinations in cows that have recovered from FMD, and improving the management of reproductive disorders. These measures are intended to accelerate herd recovery, reduce production losses, and support the long-term eradication of FMD by ensuring that recovered animals do not become a source of prolonged productivity decline or potential disease persistence [7].

Attention and assistance are required from the government to help smallholder farmers due to the difficulties experienced during and after the FMD outbreak. Reproductive performance is a critical determinant of success in beef cattle farming [8]. Therefore, the aim of this study was to evaluate the impact of FMD on reproductive disorders in beef cattle in Jember Regency. Specifically, this study aimed to identify the types of reproductive abnormalities occurring during the FMD outbreak, determine the main factors associated with abortion cases, and estimate the resulting economic losses for farmers and stakeholders involved in the beef cattle sector.

Objects and methods

Time and location of research

The sampling location was livestock in the Jember Regency Animal Health Center area that reported FMD cases in the area of several sub-districts, namely Ambulu, Arjasa, Balung, Jelbuk, Jenggawah, Jombang, Kencong, Kalisat, Ledokombo, Mumbulsari, Puger, Silo, Sukowono, Tempurejo and Wuluhan. Jember Regency is a regency in East Java Province which is astronomically located between 6° 27′ 29″ to 7° 14′ 35″ East Longitude and 7°59′ 6″ to 8° 33′ 56″ South Latitude (Figure 1).

Figure 1. The research location is in the districts of Ambulu, Arjasa, Balung, Jelbuk, Jenggawah, Jombang, Kencong, Kalisat, Ledokombo, Mumbulsari, Puger, Silo, Sukowono, Tempurejo and Wuluhan, Jember Regency, East Java, Indonesia (This figure was generated using MapChart.net)

Jember Regency has territorial boundaries, namely Bondowoso Regency and Probolinggo Regency in the north, the Indonesian Sea in the south, Banyuwangi Regency in the east and Lumajang Regency in the west. The implementation of questionnaire data collection with interviews and the following per rectal examinations on the female cows to determine their reproductive status were carried out on May 1 — September 30, 2023.

Research design

The research method used in this study is quantitative descriptive using a survey approach. Beef cattle data were collected from several Animal Health Centers in several sub-districts of Jember Regency that have been recorded as having reported FMD cases. The types of data used in this study are primary and secondary data. Primary data were obtained directly from respondent monitoring through interviews and filling out a previously compiled questionnaire. The respondents in this study were beef cattle breeders in Jember Regency. After obtaining the questionnaire data, the study was then continued with a per rectal examination to determine the reproductive status of the cattle. Secondary data were obtained from various related agencies such as data from the Integrated National Animal Health Information System of the Directorate General of Animal Husbandry and Animal Health, the Central Statistics Agency of Jember Regency, the East Java Provincial Animal Husbandry Service, the Jember Regency Food Security and Animal Husbandry Service and several relevant publications obtained through journals, research results, the internet and reference books.

Population

The population used in this sample was 274,162 cows and the drawing of areas for samples in this study was in accordance with the recommendations of the Ministry of Agriculture and the Food Security and Livestock Service of Jember Regency. The study was carried out in the area consisted of 15 sub-districts in Jember Regency with the predefined sampling criteria, namely that a farmer has one female cow that has at least one incisor tooth (two years old) and has been infected with FMD, so 251 samples were obtained. Thus, the data taken included 251 female beef cattle infected with FMD that were owned by smallholder farmers as the initial data sample to be analyzed.

Clinical examination

Clinical examinations included assessment of general reproductive health, pregnancy diagnosis, and identification of reproductive disorders. Rectal palpation of the reproductive organs was carried out to assess the structure of the ovaries and the condition of the uterus. Cattle were diagnosed as pregnant if there was uterine development. On the other hand, if the uterine development was not observed but there was a corpus luteum (CL) or dominant follicle (DF) in the ovary that could be palpated, it was stated that these were normal cycle cattle. Ovarian cysts were defined as one or more follicle-like structures with a diameter of >25 mm. Ovarian structures were evaluated by rectal palpation. Ovaries were classified as inactive when no palpable follicles ≥10 mm and no corpus luteum (CL) were detected during the examination [9].

Variables

The variables analyzed in this study included breed type, age, vaccination history, duration of recovery from FMD, return to estrus after FMD, pregnancy status after FMD, and reproductive disorders observed in female cattle following FMD infection.

Parameters

The parameters measured in this study were the type of reproductive disorders, factors that influence the occurrence of abortion, and the economic losses caused by them. The types of reproductive disorders include anestrus, cysts (both follicular cysts and luteal cysts), uterine disorders such as pyometra, and metritis, cervical disorders (cervicitis), urovagina. The percentage of pregnant and non-pregnant cattle, non-pregnant cattle experiencing reproductive disorders, types of reproductive disorders, and inactive ovaries were calculated by dividing the total number of cattle multiplied by 100. Data analysis in this study was presented in the form of numbers and percentages using descriptive statistical methods. The most influential factors in the occurrence of abortion in pregnant cows affected by FMD were analyzed by neural network analysis using the Statistical Product for the Service Solution (SPSS) program. The value of economic losses was determined by calculating the total costs incurred by farmers due to abortion in pregnant cows and reproductive disorders based on indicators in the assessment of reproductive efficiency in reproductive management of female beef cattle, namely days open and calving interval.

Data analysis

Losses due to abortion were calculated by estimating the economic impact of disease events according to De Vries [10]. Meanwhile, losses associated with prolonged days open and extended calving intervals were estimated by calculating the additional costs incurred, as described by Rushton [11].

Results and discussion

Distribution of beef cattle affected by FMD

The distribution of beef cattle affected by FMD in Jember Regency, East Java Province, Indonesia, based on breed, age, vaccination history, recovery duration, interval to return to estrus after FMD, pregnancy rate, reproductive examination results, and abortion incidence, is presented in Tables 1–8.

Table 1. Beef cattle breeds affected by FMD

Breed	Percentage
Limousin	69.72 % (175/251)
Ongole crossbreed	4.78 % (12/251)
Simmental	25.50 % (64/251)

As shown in Table 1, the majority of FMD-affected cattle were Limousin (69.72 %), followed by Simmental (25.50 %) and Ongole crossbreeds (4.78 %).

The predominance of Limousin cattle among affected animals is likely related to the population structure in the study area, where Limousin crossbreeds are widely maintained due to their favorable growth performance and economic value. Therefore, the higher proportion of affected Limousin cattle in this study may reflect their greater representation in the local population rather than increased breed susceptibility.

In Indonesia, Limousin cattle are commonly developed as crossbreeds with local and exotic breeds, contributing to their widespread distribution in smallholder farming systems [12]. Previous studies have shown that reproductive performance, including conception rate, is more strongly influenced by management practices, nutritional status, and reproductive protocols than by breed differences alone. For instance, Yendraliza et al. [13] reported comparable conception rates between Bali and Limousin cattle under similar estrus synchronization protocols.

These findings suggest that the higher proportion of affected Limousin cattle observed in this study is more likely associated with herd composition and management systems rather than inherent breed-related susceptibility to FMD [14].

Table 2. Age of beef cattle affected by FMD

Age	Percentage
< 3 years	0.80 % (2/251)
> 5 years	23.51 % (59/251)
3–5 years	75.70 % (190/251)

As presented in Table 2, most affected cattle in this study were 3–5 years old (75.70 %), followed by cattle older than 5 years (23.51 %), while animals younger than 3 years accounted for only 0.80 % of cases.

In Jember Regency, the majority of cattle are within the 3–5 years age group, which also accounted for the highest percentage of FMD-affected animals in this study. This finding is likely related to the population structure, as cattle aged 3–5 years represent the most active reproductive and productive segment of the herd and constitute the largest proportion of animals exposed during the outbreak [15]. Therefore, the higher number of cases in this group may reflect population structure and management practices rather than age-specific susceptibility. Similar patterns have been reported in previous studies, where the predominance of FMD cases reflects the demographic composition and management systems of the cattle population [16].

Age is an important factor influencing reproductive performance in cattle. Increasing age is associated with physiological and hormonal changes that may affect estrus expression, ovulation, conception rate, and pregnancy maintenance, potentially leading to reduced reproductive efficiency [17].

In addition, age and parity have been reported to influence reproductive efficiency indicators such as services per conception (S/C). Higher age and parity are often associated with increased S/C values, indicating decreased reproductive efficiency [18]. Therefore, age-related physiological changes should be considered as a contributing factor when evaluating reproductive disorders in cattle affected by FMD.

Table 3. History of FMD vaccination of beef cattle with FMD

Frequency	Percentage
0	21.91 % (55/251)
1	37.05 % (93/251)
2	25.90 % (65/251)
3	15.14 % (38/251)

The results in Table 3 show that most cattle received only one dose of FMD vaccination (37.05 %), while 21.91 % had never been vaccinated. Only a small proportion (15.14 %) received three doses. These findings indicate that vaccination coverage in the study area was not yet optimal, particularly regarding booster administration.

FMD has significant economic impacts on livestock production, including reduced productivity and decreased market value of animals and their products [6]. Vaccination is therefore an essential strategy to control the spread of FMDV by inducing protective immunity and reducing virus transmission.

Various types of FMD vaccines have been developed and are used globally, including inactivated vaccines formulated against different viral serotypes [19]. The type of vaccine used, dosage, and vaccination schedule may vary depending on national control programs, manufacturer recommendations, and outbreak conditions.

In general, FMD vaccines are administered to clinically healthy animals as a preventive measure, since vaccination is intended to stimulate immunity in susceptible populations rather than to treat infected animals [20]. In the study area, vaccination was implemented as part of a national emergency response program, which typically includes primary vaccination followed by booster doses. The interval between doses may vary; however, previous studies have suggested that the second dose is commonly administered several weeks after the first, followed by subsequent boosters at longer intervals depending on control strategies and epidemiological conditions [21,22].

Table 4. Recovery time from FMD in beef cattle

Recovery	Percentage
> 6 month	7.17 % (18/251)
1–3 month	68.92 % (173/251)
4–6 month	23.90 (60/251)

As shown in Table 4, most cattle (68.92 %) recovered within 1–3 months after FMD infection, while a smaller proportion required 4–6 months (23.90 %) or more than six months (7.17 %). These findings indicate variability in

recovery duration, which may be influenced by factors such as management practices, nutritional status, severity of infection, and supportive treatment.

Clinical recovery in cattle is generally characterized by the return of normal appetite and behavior, as well as the healing of lesions in the mouth, feet, and other affected tissues. However, the course of the disease and recovery outcomes may vary depending on the age and physiological condition of the animals [23].

High mortality has been reported in young calves, and in some cases this has been associated with acute myocarditis caused by FMD virus infection. Previous studies have indicated that sudden death in calves may occur due to myocardial damage, even in the absence of severe clinical signs [24].

In addition to its direct health effects, FMD can have long-term impacts on reproductive performance. Prolonged recovery periods may delay the return to estrus, thereby extending the interval between calving and conception [25]. Under normal conditions, the ideal calving interval in beef cattle is approximately 12 months [26,27]. However, cows experiencing extended recovery periods following FMD infection may exhibit longer calving intervals, which can reduce reproductive efficiency and negatively affect herd productivity.

Table 5. Return to estrus in beef cattle affected by FMD

Re-estrus	Percentage
1–3 month	25.10 % (63/251)
4–6 month	52.19 % (131/251)
> 6 months	22.71 % (57/251)

As shown in Table 5, most cows (52.19 %) returned to estrus within 4–6 months after FMD infection, while 22.71 % required more than six months to resume estrus. Only 25.10 % returned within 1–3 months. These findings suggest that FMD infection may contribute to delayed resumption of ovarian activity in a substantial proportion of animals.

The estrous cycle plays a crucial role in regulating ovulation and the establishment of pregnancy in cattle [28,29]. Under normal conditions, the estrous cycle ranges from approximately 18 to 24 days, with an average of 21 days [30]. Disruptions in estrous cyclicity may negatively affect ovulation, conception, and overall reproductive performance.

Delayed return to estrus following FMD infection may be associated with physiological stress, systemic illness, and alterations in hormonal balance. In addition, factors such as age, nutritional status, and body condition may further influence the recovery of reproductive function. Therefore, impaired estrus expression and delayed ovarian activity can contribute to reduced fertility and lower efficiency of reproductive management, including artificial insemination.

Table 6. Pregnancy rates in beef cattle affected by FMD

Pregnancy	Percentage
Pregnant	7.97 % (20/251)
Not pregnant	92.03 (231/251)

As shown in Table 6, the pregnancy rate of cows after FMD infection was very low (7.97 %), while the majority of animals (92.03 %) were not pregnant. This finding indicates a substantial decline in reproductive performance following FMD outbreaks.

The low pregnancy rate may be associated with a prolonged anestrous period in cows affected by FMD [26], which delays the resumption of ovarian activity and reduces the opportunity for successful conception. In addition, silent estrus has been reported in FMD-affected cattle [31], further complicating estrus detection and reducing the effectiveness of breeding programs. Consequently, farmers may fail to identify the optimal timing for insemination.

Overall, prolonged recovery, disruption of normal reproductive cycles, and impaired ovarian function following FMD infection may collectively contribute to reduced conception rates and decreased reproductive efficiency in affected cattle.

Table 7. Reproductive diagnosis results in beef cattle with FMD

Reproduction diagnosis	Percentage
Pregnant	7.97 % (20/251)
Clinically normal reproductive status	36.25 % (91/251)
Persistent corpus luteum (PCL)	1.20 % (3/251)
Delayed puberty	1.99 % (5/251)
Endometritis	16.33 % (41/251)
Ovarian hypofunction	27.49 % (69/251)
Ovarian hipoplasia	1.59 % (4/251)
Placental retention	3.19 % (8/251)
Follicular cyst	3.98 % (10/251)

The results presented in Table 7 indicate that ovarian hypofunction was the most frequently observed reproductive disorder in beef cattle affected by FMD in Jember Regency, accounting for 27.49 % (69/251) of the diagnosed cases.

Ovarian hypofunction is commonly associated with inadequate nutritional intake, which can impair hormonal regulation of the reproductive cycle [32,33]. In FMD-affected cattle, decreased appetite due to oral and foot lesions may lead to insufficient nutrient intake, thereby affecting energy balance and reproductive hormone activity [34]. Poor nutritional status has been identified as a major contributing factor to ovarian hypofunction in cattle [35]. In addition, body condition plays an important role in reproductive performance, as cows with low body condition scores are more likely to experience impaired ovarian activity [36].

Previous studies have also reported ovarian hypofunction as one of the most common reproductive disorders in cattle populations. For example, Utomo et al. [37] reported that ovarian hypofunction accounted for 14.55 % (136/935) of reproductive disorder cases. Clinically, cows with ovarian hypofunction typically show inactive ovaries with no palpable follicles or corpus luteum.

In the present study, endometritis was the second most common reproductive disorder, accounting for 16.33 % (41/251) of cases. Endometritis is an inflammatory condition

of the uterus, commonly associated with bacterial infection, particularly by *Escherichia coli* and *Trueperella pyogenes* [38]. Affected cows may exhibit abnormal vaginal discharge, often characterized by foul-smelling mucus. This condition can impair uterine function and reduce fertility if not properly managed [39].

Overall, reproductive disorders in beef cattle are multifactorial and may be influenced by nutritional status, disease conditions, and management practices, including postpartum care [40].

Table 8. The incidence of abortion in beef cattle affected by FMD

Abortion	Percentage
No	87.65 % (220/251)
Yes	12.35 % (31/251)

The results presented in Table 8 show that the incidence of abortion among female beef cattle affected by FMD in Jember Regency was 12.35 % (31/251).

Foot-and-mouth disease (FMD) outbreaks are known to cause significant production losses, including impaired reproductive performance. Although the primary manifestations of FMD involve epithelial tissues, systemic effects such as fever, stress, and metabolic disturbances during infection may indirectly contribute to reproductive failure, including abortion [41].

In addition, transplacental transmission of FMD virus has been reported, which may lead to fetal infection and abortion. Ranjan et al. [42] demonstrated the presence of FMD virus in multiple fetal tissues, indicating vertical transmission and its role in fetal mortality. Severe pathological findings, including myocarditis and hemorrhagic lesions, have also been observed in aborted fetuses, supporting the role of viral infection in reproductive failure.

Furthermore, elevated body temperature during infection may disrupt normal cellular processes, including protein synthesis, and impair placental function, thereby increasing the risk of abortion [43]. Physiological stress associated with disease conditions may further compromise reproductive performance and pregnancy maintenance [44].

Economic losses due to abortion during FMD

Losses due to abortions and an increase in days open and calving intervals can be calculated using estimates of economic losses due to disease events according to De Vries [10].

From the research results, the following data was obtained:

- Number of FMD cases 251.
- Total livestock population 251 heads.
- Proportion of animals affected by FMD

$$(\mathrm{Ip}) = \frac{\text{Number of FMD cases}}{\text{Livestock population}} = \frac{251}{251} = 1.$$

- Miscarriage rate (Ag) = 12 % or 0.12
- Average calf price (Ha) based on survey in animal market = IDR 7.000.000

Economic losses due to abortion in the research population sample can be calculated using the following formula [10]:

$$\begin{aligned}\text{Abortion Loss} &= \mathrm{Ag} \times (\mathrm{Ip} \times \mathrm{P}) \times \mathrm{Ha} \\ &= 0.12 \times (1 \times 251) \times 7.000.000 \\ &= 0.12 \times 251 \times 7.000.000 \\ &= 210.840.000\end{aligned}$$

If the formula is simulated in the beef cattle population in Jember district, the following results are obtained:

- Data on the Number of FMD Case Reports in Jember Regency: 14.292 with the number of infected productive females reaching 10.004.
- Beef Cattle Population Data in Jember Regency: 274.162 heads
- Proportion of animals affected by FMD $(\mathrm{Ip}) =$

$$= \frac{\text{Number of FMD cases reports}}{\text{Livestock population}} = \frac{10.004}{274.162} = 0{,}0365$$

- Miscarriage rate (Ag) = 12 % or 0.12
- Average calf price (Ha) based on price survey at animal market in Jember = IDR 7.000.000

$$\begin{aligned}\text{Abortion Loss} &= \mathrm{Ag} \times (\mathrm{Ip} \times \mathrm{P}) \times \mathrm{Ha} \\ &= 0.12 \times (0.0365 \times 274.162) \times 7.000.000 \\ &= 0.12 \times 10.004 \times 7.000.000 \\ &= 8.403.360.000\end{aligned}$$

The analysis of economic losses caused by abortion during the FMD outbreak in beef cattle in Jember Regency demonstrated a substantial economic impact, reaching IDR 210,840,000. This finding reflects the proportion of abortion cases observed in the present study (12 % of total FMD cases).

Economic losses due to abortion have a significant impact on cattle farmers, as the loss of pregnancy directly reduces the number of calves produced and delays herd replacement and expansion [45]. In addition, abortion may increase the risk of subsequent reproductive disorders, such as retained placenta and endometritis, which can lead to additional treatment costs and prolonged recovery periods [43].

Furthermore, reduced reproductive performance and increased reproductive disorders may contribute to slower herd population growth, posing a challenge to the sustainability and productivity of beef cattle farming systems [46].

Economic losses due to reproductive disorders

Losses due to post-FMD reproductive disorders can be seen through the following data:

- Length of recovery from FMD (LS) with an average value = two months
- The duration of estrus return after recovery from FMD (LE) with an average value = five months
- Length of time for a cow to be ready for insemination (SI) = LS + LE = two + five = seven months
- The cost of livestock maintenance per head per month is calculated by adding the components: feed costs (greens + concentrate) + other operational costs (Table 9).

Labor costs are calculated based on the daily hourly wage in Jember Regency, which is IDR 10.000

- Additional costs (EC) incurred due to delays in the time when the cow is ready for insemination can be calculated using the following formula:

$$EC = SI \times BP$$
$$= 7 \times 1.000.000$$
$$= 7.000.000$$

- Cost of reproductive disorder treatment per head per treatment = IDR 100,000,0 with treatment carried out twice so that the average cost of reproductive disorder (OB) treatment = IDR 200.000.

Economic losses due to reproductive disorders that occur in the research population sample can be calculated using the following formula:

$$\text{Infertility losses} = (EC + OB) \times (\text{sample population of cattle affected by FMD} \times \% \text{ number of cases of reproductive disorders})$$
$$= (7.000.000 + 200.000) \times (251 \times 56\,\%)$$
$$= 7.200.000 \times 141$$
$$= 1.015.200.000$$

If the formula is simulated in the beef cattle population in Jember district, the following results are obtained:

$$\text{Infertility losses} = (EC + OB) \times (\text{population of cattle affected by FMD} \times \% \text{ number of reproductive disorder cases})$$
$$= (7.000.000 + 200.000) \times (10.004 \times 56\,\%)$$
$$= 7.200.000 \times 5.602$$
$$= 40.334.400.000$$

Table 9. Cost components of maintaining beef cattle experiencing FMD

Component	Cost/tail (IDR)	Cost per month (IDR)
Labor	10.000	300.000
Green fodder	15.000	450.000
Concentrated feed (1.5 kg/head/day)	5.000	150.000
Other operations		100.000
Total		1.000.000

Economic losses due to post-FMD reproductive disorders in the study population were estimated at IDR 1.015.200.000. This substantial loss is associated with the high proportion of cattle experiencing reproductive disorders after FMD infection, which reached 56 % of the observed population.

Post-FMD reproductive disorders are generally multifactorial and not limited to a single condition [26]. These disorders may lead to delayed resumption of estrus, which subsequently affects the timing of conception, prolongs days open, and extends the calving interval. In the present study, the combined duration of recovery and return to estrus indicates a considerable delay in reproductive activity.

Prolonged reproductive recovery increases production costs, particularly due to extended feeding and maintenance requirements during non-productive periods [47]. In addition, reduced fertility following FMD infection further contributes to economic losses by lowering reproductive efficiency and overall herd productivity.

Conclusion

Post-FMD reproductive disorders in beef cattle in the study population in Jember Regency reached 56 %, with ovarian hypofunction being the most frequently observed disorder (27 %), followed by endometritis (16 %).

Abortion was also identified as one of the reproductive consequences observed during the FMD outbreak, contributing to additional production losses. The economic losses associated with abortion in the study population were estimated at IDR 210.840.000 (approximately USD 14.000).

Furthermore, economic losses due to post-FMD reproductive disorders in productive female beef cattle were estimated to reach IDR 1.015.200.000. IDR (approximately USD 67.700) These losses are primarily associated with delayed resumption of estrus, which leads to postponed conception, prolonged days open, and extended calving intervals. Consequently, farmers incur increased feed and operational costs due to longer non-productive periods.

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TISSUES CHEMICAL COMPOSITION AND QUALITY IN BROILER CHICKENS WHEN USING AN ADAPTOGENIC COMPLEX

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Keywords: *Broilers, chemical composition of meat, bones, quality*

Abstract
To study the effect of a newly developed adaptogenic complex on the chemical composition of muscle and bone tissues, as well as on the quality and process characteristics of the broiler chickens breast and thigh muscles, an experiment was conducted on 3 groups of the broiler chickens in the physiological ward of the Federal Research Center for Animal Husbandry named after Academy Member L. K. Ernst (n = 40, N = 120) (1 control group and 2 experimental groups) under conditions of increased stocking density. The poultry from the experimental groups received the DHQEC complex with their diet (the 2nd experimental group started receiving it from the 22nd day of life, i.e., from the day of the onset of simulated overcrowded environmental conditions; the 3rd experimental group started receiving it from the 7th day of their life). On the day 34th (n = 10, N = 20) and 52nd (n = 10, N = 20) of age, the samples of the breast and thigh muscles and the tibia bones were collected. The chemical composition, as well as several quality and process properties of the meat were determined. The administration of DHQEC for 34 days of the poultry life contributed to an increase in fat content in the breast muscle from 0.74 % in the control group up to 1.03 % and 1.17 % in the experimental groups, respectively; an increase in the pH of muscle tissue; an increase in the water-holding capacity (WHC) of the breast ($p < 0.01$) and thigh ($p < 0.01$) tissues; and elevated levels of reduced glutathione (at $p < 0.05$ and $p < 0.01$) and superoxide dismutase (at $p < 0.01$) compared to the control group values. In the liver of the poultry that received DHQEC, an increase in the level of water-soluble antioxidants was observed, whereas in the cardiac muscle, conversely, a decrease was noted. At the 52nd day of age, the trend of differences between the groups persisted. A significant difference was found in phosphorus content (it was lower in the control group) ($p < 0.05$) and magnesium content (it was lower in the 3rd experimental group) ($p < 0.05$), which may be associated with the impact of stress and its mitigation by the DHQEC complex. The most pronounced effect of the complex was observed when it was introduced into the diet from the 7th day of the poultry life. The obtained data open broad prospects for the inclusion of the DHQEC complex into broiler chicken diets, particularly during periods of stress exposure from the first days of life.

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Introduction

The increase in meat production volumes in the Russian Federation and globally is driven, among other factors, by advances in breeding achievements in the development of modern fast-growing poultry crosses. For instance, 50 years ago, a carcass weight of 2.5 kg was achieved by the age of 3 months, whereas today such live body weight in broiler chickens can be obtained as early as 5–7 weeks of age [1]. Worldwide, poultry meat production is increasing, which is mainly associated with advances in genetics and breeding technologies [2]. This has led to a significant increase in growth rates, meat yield (especially breast meat), and feed conversion efficiency.

Intensive growth, as well as the conditions of feeding and housing technology, negatively affect the quality of poultry meat products, which is manifested in the emergence of various pathologies and the deterioration of process properties. The main mechanism influencing meat quality is the occurrence of oxidative stress in the cells and tissues of the body, which results from reduced immunity and impaired health [3]. For example, studies have shown that oxidative stress can increase shear force and decrease the pH value in the breast meat of the broilers [4].

A way to reduce the negative impact of stress of various etiologies on animal health and, consequently, on product quality is the use of various nutritional factors — adaptogens and their complexes [5,6]. Adaptogens are defined as natural compounds or plant extracts aimed at increasing the adaptability and survival of living organisms exposed to stress conditions [7]. The term “adaptogen” was first introduced by Nikolai Lazarev in 1947, when the effect of 2-benzylbenzimidazole against damage to the nervous

system and its ability to increase nonspecific resistance to adverse environmental conditions were described [8]

Plant extracts as feed additives not only contribute to enhancing resistance to diseases but also positively influence zootechnical performance — growth parameters and feed conversion. It has been noted that plant-based adaptogens can improve product safety by reducing the content of drug residues in the organism [9]. For example, adding *Ampelopsis grossedentata* extract to poultry diets promotes the digestion and absorption of feed nutrients and increases average daily weight gain and total weight gain [10].

Adaptogens included in the diets of both animals and humans are capable not only of modulating the immune system and various physiological processes but also of influencing reproductive function. Although the latter fact has not been extensively studied, positive effects of natural adaptogens on spermatogenesis and folliculogenesis have been reported, either through direct mechanisms or via the suppression of oxidative stress and inflammation. A significant role in this process is attributed to the regulation of neuroendocrine-immune interactions [11].

Despite the wide range of biological effects of adaptogens on living organisms, our interest is focused on their influence on the composition, quality, and safety of livestock and poultry products, particularly meat. Since these products are an integral part of the human diet and affect the health and well-being of the primary consumer.

In our earlier studies, the use of melanin in the diet of broiler chickens at a dose of 1.42 mg/kg body weight at 45 days of age contributed to an increase in the antioxidant content in the breast and thigh meat and an increase in the activity of antioxidant enzymes in the breast meat [12].

Essential oil extracted from *Lippia origanoides* improved bone mineralization in the *tibia* bones of the chickens subjected to cyclic heat stress by increasing calcium absorption and reducing bone resorption [13]. A similar positive effect on the improvement of bone microstructure and mineral density was observed by researchers when thymol was included in chicken diets [14], which was explained by an increase in osteoblast activity and a decrease in osteoclast activity [15].

Betaine supplementation increases bone mineral density and improves bone microstructure, which may prevent fractures and enhance skeletal health by increasing the digestibility of minerals such as calcium and phosphorus [16].

The combined use of adaptogens is the most appropriate approach. For example, it has been reported that the inclusion of a herbal mixture based on ginseng and artichoke into chicken diets led to improved breast muscle quality, increased redness of the breast meat, enhanced water-holding capacity, and reduced abdominal fat. The authors attributed the observed changes in the experimental group precisely to the synergistic effect of the components. Thus, the redness of the breast meat is explained by improved muscle oxygenation under the influence of ginseng, while the reduction in abdominal fat is associated with the lipid-lowering effect of both components of the complex [17].

A complex of feed additives with eco-antioxidants, based on beet powder and grape pomace, applied in poultry diets, contributed to a reduction in the amount of abdominal fat in the carcasses [18].

An adaptogen complex was developed, comprising the reference antioxidant dihydroquercetin (DHQ), obtained by extraction from the shredded wood or bark of Dahurian larch (*Larix dahurica*) ("Ekostimul-2", Ametis JSC, Russia; DHQ content 72–73 %), along with vitamins E and C [19].

In previous experiments on monogastric animals (piglets), the DHQEC complex contributed to the activation of metabolic processes and antioxidant defense, as well as to an increase in the adaptive capacity of the organism under intensive fattening technology conditions [20].

The purpose of this study was to investigate the effect of an adaptogenic complex on the chemical composition of muscle and bone tissues, as well as on the quality and technological characteristics of the pectoral and femoral muscles of broiler chickens of the domestic cross Smena 9.

Objects and methods

The experiment was conducted on 3 groups of the broiler chickens of the domestic cross Smena-9 in the physiological ward of the Federal Research Center for Animal Husbandry named after Academy Member L. K. Ernst (n = 40, N = 120) (1 control group and 2 experimental groups). As the main ration for chickens of all groups, full-fledged compound feeds were used, appropriate to the growing periods: from start up to 11 days of life — starter compound feed was used, after that grower (12–26 days), and finisher (27–52 days) feeds were used. The feeds were purchased from Mayskie Prostory LLC, Sergiev Posad city. The poultry were kept in the cages; all microclimate parameters complied with the requirements for this poultry cross[1]. The stocking density of birds in both the control group and experimental groups was increased by 10 % starting from the 21st day of life compared to the recommended values in the above-mentioned Guidelines. This was necessary to create simulated overcrowding stress conditions for the poultry. Stocking density was adjusted using movable plywood partitions as the birds grew (weekly). All three chicken groups did not differ in housing conditions, but differed in adding of 0.025 % of the developed nutritional antioxidant complex DHQEC to the diets of the broiler chickens in the experimental groups. Moreover, the additive was introduced into the diets of the chickens in the 1st experimental group from the onset of stocking density stress (from the 22nd day), and into the 2nd experimental group starting from an

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earlier age, from the moment of the poultry transfer from brooders to cages (from the 7th day of life).

At the 34th (n = 10, N = 20) and 52 (n = 10, N = 20) day of age, the chickens were slaughtered and samples of the breast muscle, thigh muscle, and *tibia* bone were collected from the right side of the carcass. The following chemical composition parameters of the pectoral and femoral muscles were determined: dry matter (GOST 33319-2015[2]), fat (GOST 23042-2015[3]), and ash (GOST 31727-2012 (ISO 936:1998)[4]). Crude protein content was calculated. The concentrations of calcium, phosphorus, and magnesium in the bone were determined according to the appropriate methodology[5]. Meat quality parameters were determined according to the following methods: meat pH — by GOST 31476-2012[6]; GOST R 57879-2017[7]; water-holding capacity (WHC) — by samples pressing according to Grau and Hamm method, as modified by Volovinskaya; the amount of water-soluble antioxidants (AWSA) — with a Tsvet-Yauza-01-AA device using the amperometric method. This parameter was determined not only in the breast and thigh meat but also in the cardiac muscle and liver. The activity of glutathione peroxidase, catalase, and the concentration of reduced glutathione were measured using commercial Elabscience kits by a Photometer Immunochem-2100 device.

Studies carried out with approval by the bioethical commission (No. 3, May 27, 2022). The experiments were carried out in accordance with the requirements of the Federal Law of the Russian Federation[8], the Declaration of Helsinki[9], the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes[10].

Mathematical and statistical processing of the results was done using Microsoft Office Excel 2003, STATISTICA 10 (Statistica 13RU, StatSoft, USA) with the methods of variance and factor analysis, using the Dunnett's test and Tukey's test, t-test. The differences believed as statistically significant at $p < 0.05$, highly significant at $p < 0.01$; $p < 0.001$.

Results and discussion

Chemical composition of muscle tissue, namely the content of fat, protein, and moisture, serve as criterion for determining the nutritional value of raw material. It is generally considered that thigh meat contains lower protein content than breast meat in both crosses. Thigh meat features higher intramuscular fat content but lower protein content compared to breast meat.

Table 1 presents the chemical composition of thigh and breast meat, as well as the mineral composition of the *tibia* bone at the 34th day of age. It should be noted that the most substantial intergroup differences are observed in the fat content of breast meat. Thus, feeding the developed complex contributed to a significant increase in fat content in the breast from 0.74 % in the control group up to 1.03 % and 1.17 % in the experimental groups No. 2 and No. 3, respectively. Other researchers have also drawn attention to the effect of plant adaptogens on lipid metabolism in the body. For instance, the addition of *Eucommia* leaf extract to piglet feed regulated the distribution of free amino acids and fatty acids in pork, thereby contributing to improved meat quality [21].

Table 1. Chemical composition of meat and bones of the broilers at the 34th day of age, % ($M \pm$ SEM, n = 10)

Parameter	Group			p-value
	1-Control	2-Experimental	3-Experimental	
Breast meat				
Moisture	74.72 ± 0.19	74.93 ± 0.22	74.66 ± 0.14	0.31
Protein	23.34 ± 0.25	22.84 ± 0.20	23.01 ± 0.15	0.35
Fat	0.74 ± 0.12^{C}	1.03 ± 0.07	1.17 ± 0.10^{A}	*0.001*
Ash	1.20 ± 0.04	1.20 ± 0.05	1.16 ± 0.03	0.32
Calcium	0.06 ± 0.001	0.06 ± 0.001	0.05 ± 0.002	0.40
Phosphorus	0.18 ± 0.003	0.15 ± 0.003	0.16 ± 0.003	0.40
Magnesium	0.026 ± 0.0011	0.026 ± 0.001	0.029 ± 0.001	*0.04*
Thigh meat				
Moisture	74.34 ± 0.29	73.61 ± 0.41	73.57 ± 0.44	0.16
Protein	19.16 ± 0.23	19.25 ± 0.28	19.21 ± 0.08	0.91
Fat	5.40 ± 0.33	6.07 ± 0.42	6.19 ± 0.47	0.20
Ash	1.1 ± 0.04	1.1 ± 0.04	1.03 ± 0.02	0.07
Calcium	0.05 ± 0.001	0.05 ± 0.003	0.05 ± 0.006	0.12
Phosphorus	0.15 ± 0.003	0.15 ± 0.003	0.15 ± 0.003	0.50
Magnesium	0.04 ± 0.02	0.02 ± 0.001	0.02 ± 0.0001	0.45
Bone				
Ash	11.26 ± 0.60	10.17 ± 0.49	9.38 ± 0.50	0.06
Calcium	3.84 ± 0.24	3.43 ± 0.19	3.17 ± 0.16	0.08
Phosphorus	1.74 ± 0.09	1.58 ± 0.08	1.44 ± 0.08	0.07
Magnesium	0.12 ± 0.009	0.11 ± 0.006	0.11 ± 0.01	0.04

Note: the differences are significant at: Significant differences according to Tukey's test compared to the group 1 (control): A — $p < 0.05$, AAA — $p < 0.001$. Significant differences according to Tukey's test compared to the group 2 (experimental): B — $p < 0.05$, BB — $p < 0.01$, BBB — $p < 0.001$. Significant differences according to Tukey's test compared to the group 3 (experimental): CC — $p < 0.01$, CCC — $p < 0.001$.

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The difference is significant between the control group and the experimental group that received DHQEC from an earlier age. A tendency toward an increase in magnesium level can also be noted in the 3rd experimental group compared to the control group parameters ($p = 0.04$). This parameter also tends to change in the 3rd experimental group compared to the control group, but in the direction of a decrease (from 0.12 to 0.11 % at $p = 0.04$).

Analyzing the quality parameters, including the antioxidant parameters of chicken meat at age of 34 days (Table 2), it can be noted that the use of the adaptogenic complex contributes to an increase in the pH of muscle tissue, water-holding capacity (WHC) of the breast ($p < 0.01$), WHC of the thigh ($p < 0.01$), reduced levels of glutathione (at $p < 0.05$ and $p < 0.01$), and superoxide dismutase (SOD) (at $p < 0.01$) compared to the values in the control group. It should also be noted that when the adaptogenic complex was introduced at the onset of the stress factor, a decrease in catalase activity in the breast meat was observed compared to the control group ($p < 0.05$), whereas with longer-term feeding of DHQEC, an increase in catalase activity was noted ($p < 0.05$). This demonstrates an increased expenditure of enzymatic antioxidant defense factors in response to simulated stress under short-term adaptogenic feeding. With prolonged use of DHQEC (the 3rd experimental group), the organism of the chickens adapts to environmental conditions and, conversely, promotes the accumulation of antioxidant enzymes in muscle tissue. This demonstrates an increase in the adaptive capacity of the organism of intensively growing chickens under simulated environmental conditions.

In studies by other authors, the adaptogenic quercetin, fed to the broiler chickens at a dose of 0.2–1 g/kg of feed, also contributed to the adaptation of the organism exposed to stress conditions. The authors attribute the mechanism of action of this adaptogen to the activation of antioxidant enzymes, either through the stimulation of transcription factors that enhance the antioxidant defense status. Feeding quercetin at the prescribed dosages promotes increased expression of antioxidant defense genes [22], a decrease in malondialdehyde (MDA) concentration and an increase in glutathione activity [23], including under conditions of oxidative stress [24].

The addition of 2 g/kg of cinnamon bark powder to the diet of broiler chickens leads to an improvement in the physicochemical characteristics of poultry meat [25]. As a result of the other study, it was found that the addition of the adaptogen sanguinarine to the diet can increase pH levels and improve the tenderness and flavor of chicken breast and thigh meat. The authors also noted changes in blood lipid metabolism markers in the poultry, in particular, a decrease in triglyceride levels and higher concentrations of high-density and low-density lipoproteins, which influenced fat deposition [26]. Fermented Ginkgo biloba leaves added into the diet can promote lipid metabolism, reduce lipid peroxidation, and improve the meat quality of the broilers [27].

The pH value determines the process characteristics, quality, and consumer properties of meat, and directly influences such characteristics as tenderness, water-holding capacity (WHC), color, juiciness, and shelf life. For example, pH is determined by the postmortem formation of lactic acid during glycolysis. The longer the meat is stored, the lower its pH becomes, and the more the activity of proteolytic enzymes is inhibited, which in its turn determines meat tenderness [28]. A decrease in pH also affects the rate of denaturation of myosin and actin in muscle fibers, thus reducing their hydrophilic function [29]. In connection with the important biological role of pH in the formation of meat quality, regulation of this parameter is crucial in poultry farming and processing. Proper preslaughter handling and minimization of stress during slaughter (appropriate transportation, careful treatment, rest) play a very important role in pH management. Feed antioxidants and adaptogens can also contribute not only to minimizing stressful situations [30, 31], but also to the

Table 2. Quality characteristics of broiler meat at the 34th day of age ($M \pm SEM$, $n = 10$)

Parameter	Group			p-value
	1-Control	2 -Experimental	3-Experimental	
Breast pH-45	6.10 ± 0.02	6.18 ± 0.04	6.22 ± 0.05	0.12
Breast pH-24	5.49 ± 0.04	5.59 ± 0.07	5.70 ± 0.07	0.42
Thigh pH-45	5.91 ± 0.08	6.03 ± 0.09	6.07 ± 0.04	0.38
Thigh pH- 24	5.65 ± 0.04^{ACCC}	5.67 ± 0.06^{CCC}	5.91 ± 0.03^{AAABBB}	0.0004
AWSA in the breast, mg/g	0.090 ± 0.04	0.084 ± 0.006	0.089 ± 0.006	0.75
AWSA in the thigh, mg/g	0.213 ± 0.01	0.207 ± 0.01	0.209 ± 0.013	0.98
AWSA in the liver, mg/g	0.941 ± 0.06	0.834 ± 0.07	0.860 ± 0.08	0.48
AWSA in the heart, mg/g	0.272 ± 0.03	0.289 ± 0.03	0.289 ± 0.02	0.82
TAS, mM/L	0.109 ± 0.02	0.116 ± 0.02	0.160 ± 0.02	0.80
Reduced glutathione in breast meat, μM/g	67.24 ± 2.49^{BCCC}	91.63 ± 3.38^{A}	101.33 ± 3.65^{AAA}	0.005
SOD in breast meat, U/g	10.65 ± 2.11^{CC}	13.69 ± 0.38^{CC}	22.18 ± 0.62^{AABB}	0.0006
Catalase in breast meat, U/g	20.07 ± 2.75^{BC}	17.38 ± 2.77^{AC}	34.16 ± 5.31^{A}	0.03
WHC of breast meat, %	58.33 ± 0.45^{BBBCCC}	63.23 ± 1.50^{AAAC}	67.46 ± 1.11^{AAAB}	0.000003
WHC of thigh meat, %	56.96 ± 1.31^{BBBCCC}	62.95 ± 0.97^{AAA}	65.60 ± 0.86^{AAA}	0.00007

Note: the differences are significant at: Significant differences according to Tukey's test compared to the group 1 (control): A — $p < 0.05$, AAA — $p < 0.001$. Significant differences according to Tukey's test compared to the group 2 (experimental): B — $p < 0.05$, BB — $p < 0.01$, BBB — $p < 0.001$. Significant differences according to Tukey's test compared to the group 3 (experimental): CC — $p < 0.01$, CCC — $p < 0.001$.

reduction of oxidative stress [32]. The rate of post-slaughter cooling also plays a role in pH regulation, as it can slow down glycolysis, thereby reducing the rate of pH decline. Temperature, humidity, and other parameters during the transportation and storage of poultry meat also play an important role in stabilizing pH levels [33].

Water-holding capacity (WHC) is a crucial factor determining meat juiciness and tenderness. It is considered that approximately 90 % of water is bound to proteins within muscle tissues, located inside the cell between actin and myosin, while the remaining 10 % is located between myofibrils [34]. It is known that a number of factors influence WHC. For example, there is a close relationship between WHC and pH. Changes in pH reduce the number of active sites available for water-protein binding. When pH reaches isoelectric point values (where the number of positive and negative charges is equal), proteins are unable to bind to charged groups of water [35]. This leads to a decrease in WHC. Another factor influencing WHC is energy deficiency during meat aging. This results in a reduction of WHC due to the aggregation of actin complexes within the muscles and a decrease in the space between myofibrillar proteins. On the other hand, WHC itself affects the color of broiler breast muscles, as changes in water content within the muscles alter light reflection on their surface [36]. One of the main factors influencing WHC is apoptosis, which can disrupt the structural integrity of muscle cells, leading to a decrease in water-holding capacity [37]. It is known that oxidative stress damages cell membranes and proteins, leading to a decrease in the water-holding capacity of muscle tissue. Therefore, the increase in WHC of both the breast and thigh muscles of broiler chickens fed with the antioxidant complex may indicate an enhancement of the body's antioxidant defense, with longer-term administration of DHQEC exerting the most significant effect.

For example, supplementing the broilers' diet with vitamin E has been shown to increase the brightness of breast muscle [38], and to increase muscle pH [39].

At the day 52 of age (Table 3), the muscle pH level in the chickens of the 3rd experimental group was higher than that in the 2nd experimental group ($p < 0.05$), indicating a positive and cumulative effect of adaptogens on meat quality parameters. The most pronounced effect was observed when the complex was used in the diet from day 7 of the poultry life. The highest moisture content was found in the breast meat of the chickens from the 3rd experimental group (74.33 %, $p < 0.01$ compared to the 2nd group). A significant difference was observed between the control and the 3rd experimental groups in phosphorus content (lower in the control) ($p < 0.05$) and magnesium content (lower in the 3rd experimental group) ($p < 0.05$), which may be associated with the impact of stress and its mitigation by the DHQEC complex.

In the liver of birds receiving DHQEC, an increase in the level of water-soluble antioxidants (AWSA) was observed ($p < 0.001$) compared to the control group and the 2nd experimental group. In the cardiac muscle, conversely, feeding the adaptogen complex resulted in a significant decrease in the accumulation of water-soluble antioxidants. It is known that the liver is an important and central

Table 3. Chemical composition of meat and bones of broilers at the 52nd day of age, % (M ± SEM, n = 10)

Parameter	Group			*p*-value
	1-Control	2-Experimental	3-Experimental	
Breast meat				
Moisture	73.26 ± 0.22^{CC}	73.87 ± 0.18	74.33 ± 0.25^{AA}	*0.016*
Protein	24.34 ± 0.16^{C}	23.78 ± 0.14	23.42 ± 0.27^{A}	*0.04*
Fat	1.02 ± 0.15	1.19 ± 0.09	1.09 ± 0.08	0.44
Ash	1.19 ± 0.01	1.17 ± 0.01	1.16 ± 0.02	0.44
Calcium	0.056 ± 0.002	0.058 ± 0.05	0.056 ± 0.002	0.85
Phosphorus	0.168 ± 0.03^{C}	0.160 ± 0.002	0.171 ± 0.004^{A}	*0.027*
Magnesium	0.031 ± 0.001^{C}	0.030 ± 0.001	0.027 ± 0.001^{A}	*0.029*
Thigh meat				
Moisture	73.11 ± 0.80	74.59 ± 0.36	74.63 ± 0.28	0.15
Protein	20.21 ± 0.21	19.75 ± 0.15	19.74 ± 0.15	*0.01*
Fat	5.65 ± 0.64	4.64 ± 0.35	4.61 ± 0.25	0.11
Ash	1.03 ± 0.02	1.02 ± 0.01	1.02 ± 0.01	0.41
Calcium	0.052 ± 0.004	0.049 ± 0.003	0.048 ± 0.001	0.78
Phosphorus	0.141 ± 0.004	0.139 ± 0.003	0.150 ± 0.005	*0.03*
Magnesium	0.024 ± 0.0003	0.025 ± 0.001	0.023 ± 0.001	0.28
Bone				
Ash	10.36 ± 0.47	10.65 ± 0.52	10.10 ± 0.56	0.46
Calcium	3.50 ± 0.19	3.50 ± 0.18	3.40 ± 0.20	0.45
Phosphorus	1.37 ± 0.07	1.38 ± 0.07	1.46 ± 0.0	0.11
Magnesium	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.83

Note: the differences are significant at: Significant differences according to Tukey's test compared to the group 1 (control): A — $p < 0.05$, AAA — $p < 0.001$. Significant differences according to Tukey's test compared to the group 2 (experimental): B — $p < 0.05$, BB — $p < 0.01$, BBB — $p < 0.001$. Significant differences according to Tukey's test compared to the group 3 (experimental): CC — $p < 0.01$, CCC — $p < 0.001$.

coordinator of the body's antioxidant defense. Its role involves the synthesis, storage, metabolism, and distribution of key antioxidant defense factors throughout the organism. Various liver dysfunctions negatively affect the overall oxidative status [40]. It can be assumed that liver tissues, playing a key role in the implementation of antioxidant defense, accumulate the relevant substances. Evidence of this, as well as an indirect indication of increased adaptive capacity of the organism, is the increase in the total antioxidant status of meat (from 0.14 mM/L in the control to 0.188 and 0.208 mM/L in the 2nd and 3rd experimental groups, respectively, at $p = 0.10$).

In another study, the addition of a herbal preparation consisting of a mixture of the following plant species: *Ichnocarpus frutescens, Terminalia chebula, Sida cordifolia, Terminalia arjuna, Phyllanthus emblica, Tephrosia purpurea, Fumaria indica, Andrographis paniculata, Azadirachta indica, Tinospora cordifolia, Achyranthes aspera, Boerhavia diffusa, Solanum nigrum, Citrullus colocynthis, Eclipta alba, Aphanamixis polystachya u Phyllanthus niruri* exerted a positive effect on chicken growth and liver protection [41]. The application of a nutritional adaptogen complex based on ginger powder and ginger essential oil reduced the level of malondialdehyde (MDA) in liver and blood serum samples [42]. Supplementation of broilers diet with a mixture containing 5 % carvacrol, 3 % cinnamaldehyde, and 2 % capsicum oleoresin at a dose of 100 mg/kg of feed exerted a positive effect on the concentration of antioxidants in the liver [43].

Conclusion

The chemical composition of broiler chicken muscle tissue determines its nutritional value, while quality parameters — pH and WHC — determine the process characteristics, quality, and consumer properties of the meat. Intensive growth, as well as the conditions of feeding and housing technology, negatively affect the quality of poultry meat products, which is manifested in the emergence of various pathologies and the deterioration of process properties. Stress of various etiologies affecting the chicken's organism also influences the quality of the resulting meat products. One strategy for improving the quality of meat raw materials is using of various adaptogens in the nutrition of intensively growing broiler chickens.

The DHQEC complex we developed, based on the synergistic effect of the referenced antioxidant DHQ and vitamins E and C, contributes to an increase in breast fat content, an increase in muscle tissue pH, an increase in the water-holding capacity (WHC) of the breast and thigh, the elevated levels of reduced glutathione and superoxide dismutase in muscle tissue, and the accumulation of water-soluble antioxidants in the liver. The most pronounced effect is observed when the complex is used in the diet starting from the 7th day of the poultry's life. The obtained data open broad prospects for the inclusion of the DHQEC complex in broiler chicken diets, particularly during periods of stress exposure from the first days of life.

Table 4. Quality characteristics of broiler meat at the 52nd day of age (M ± SEM, n = 10)

Indicator	Group			p-value
	1-Control	2-Experimental	3-Experimental	
Breast pH –45	5.55 ± 0.06	5.65 ± 0.04	5.68 ± 0.06	0.31
Breast pH 24	5.44 ± 0.11^{C}	5.70 ± 0.10	5.78 ± 0.04^{A}	*0.033*
Thigh pH –45	5.95 ± 0.03^{C}	6.05 ± 0.03	6.07 ± 0.02^{A}	*0.04*
Thigh pH 24	5.52 ± 0.06	5.69 ± 0.05	5.77 ± 0.04	*0.003*
AWSA in the breast, mg/g	0.149 ± 0.01	0.145 ± 0.01	0.134 ± 0.01	0.74
AWSA in the thigh, mg/g	0.196 ± 0.02	0.212 ± 0.01	0.229 ± 0.01	0.56
AWSA in the liver, mg/g	0.121 ± 0.02^{BBBCCC}	0.658 ± 0.03^{AAA}	0.703 ± 0.04^{AAA}	*0.000001*
AWSA in the heart, mg/g	0.455 ± 0.02^{BBBCCC}	0.312 ± 0.02^{AA}	0.308 ± 0.02^{AAABB}	*0.00001*
TAS, mM/L	0.140 ± 0.02	0.188 ± 0.03	0.208 ± 0.15	0.10
Reduced glutathione in breast meat, μM/g	58.49 ± 14.53	85.54 ± 6.73	117.39 ± 26.75	0.28
SOD in breast meat, U/g	11.80 ± 0.94	13.96 ± 2.14	15.35 ± 2.68	0.34
Catalase in breast meat, U/g	22.16 ± 3.22	24.39 ± 2.62	28.52 ± 0.33	0.37
WHC of breast meat, %	56.29 ± 0.75	58.42 ± 1.03	58.67 ± 0.62	0.19
WHC of thigh meat, %	55.32 ± 1.48^{BC}	61.38 ± 1.39^{A}	63.41 ± 1.17^{A}	*0.0005*

Note: the differences are significant at: Significant differences according to Tukey's test compared to the group 1 (control): A — $p < 0.05$, AAA — $p < 0.001$. Significant differences according to Tukey's test compared to the group 2 (experimental): B — $p < 0.05$, BB — $p < 0.01$, BBB — $p < 0.001$. Significant differences according to Tukey's test compared to the group 3 (experimental): CC — $p < 0.01$, CCC — $p < 0.001$. Significant differences according to Tukey's test compared to the group 4 (experimental): D — $p < 0.05$, DD — $p < 0.01$, DDD — $p < 0.001$.

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ANALYSIS OF PROMISING ELECTROPHYSICAL METHODS FOR FOOD PRODUCTS THAWING

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Abstract
This article summarizes the results of research published in scientific publications on application of innovative food thawing methods and their impact on quality of the food product. The thawing processes for food systems were scrutinized, which involve high hydrostatic pressure, ultrasound, electromagnetic waves of various frequencies, and electric fields. It has been established that gradient-free methods that use electromagnetic wave energy are the most effective. They reduce the duration of thawing processes, reduce the risk of microbial contamination, and help preserve the quality of food systems. The selection of the optimal thawing method should be based on a systemic approach and should consider multiple factors, including the type of food product, its geometric shape and dimensions, chemical composition, as well as the requirements to the final product quality and economic feasibility considerations.

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Introduction

The Strategy for improving food quality in the Russian Federation until 2030, approved by the Government of the Russian Federation on June 29, 2016 under No. 1364-r, sets the purpose of improving the quality of the population life and stimulating the development of production and market circulation of high-quality food products. Particular attention should be paid to maintaining the quality and ensuring the consumer safety of highly perishable food products [1,2]. Storing food products at low temperatures is one of the most effective preservation methods, as it slows down the biochemical and microbiological processes that take place in the food product [3,4].

During refrigeration processing and storage the food products can be in the following thermal states: chilled, supercoolled, subfreezing and frozen [5,6].

Microbial growth, as well as enzymatic processes occurring at low positive temperatures, significantly limit the shelf life of chilled foods, including meat, fish, seafood, and others. Therefore, freezing is a key preservation technology, as it simultaneously maintains the due quality and microbiological safety of foods, thus making them suitable for both industrial processing and direct consumption after their thawing.

Currently, approximately 2.8 million tons of frozen fish and fish products are provided to the market [7] and approximately 125.000 tons of frozen meat are imported from abroad [8]. Almost all of these raw materials require thawing for subsequent technological processing [9]. In this regard, thawing is becoming increasingly important as a technological process that affects the quality of food products due to the inevitable physicochemical changes [10].

During freezing, the molecular activity of water is significantly reduced, whereas thawing reactivates the water molecules in the food product. It should be noted that thawing is a longer process than freezing, even under the same temperature gradients. This is due to the fact that the water formed on the surface of the product has lower thermal conductivity compared to the ice layers formed during freezing [11,12].

Using of frozen meat raw materials in various technological processes involves thawing them in such a way as to approximate to the original quality of the raw material that used to be before thawing [13,14]. This is a complex task, as long-term storage is accompanied by processes that lead to color changes, proteolytic changes in protein structures, oxidation of lipids and intramuscular fatty acid composition, and to moisture loss during thawing [15–17].

Thawing can affect changes in food quality parameters, such as the formation of voids in the product structure, changes in texture, and changes in flavor. Some changes, like moisture migration and texture softening, are immediately apparent, while other changes, like oxidative and microbiological spoilage, become noticeable only during subsequent storage after thawing [18–22].

Changes in meat quality properties during thawing depend on the thawing method [23], duration [24], and temperature conditions of the thawing process [25]. It is necessary to select heat exposure conditions and methods that will maximally restore the original properties of the product.

Assessing changes in meat quality during thawing is based on comprehensive studies of various parameters: color, texture, water holding capacity, water losses during thawing, and the microbiological and chemical stability of the food product. A significant parameter to be observed is active acidity, which is directly related to the growth of microorganisms that cause food spoilage and protein denaturation. Volatile basic nitrogen and thiobarbituric acid reactive substances (TBARS) allow assessing the degree of lipid oxidation and nitrogen compound formation, which is crucial for determining meat quality [26–29].

Thawing losses are a key indicator of food quality after frozen storage. This parameter is directly related to damage to cellular structures and tissues caused by both the freezing process itself and subsequent thawing. Minimizing thawing losses is crucial for maintaining food quality, as emphasized by a number of researchers in their studies [26,30,31]. For meat in particular, thawing losses are caused not only by the mechanical action of ice crystals on muscle fibers but also by the denaturation of myofibrillar proteins, which leads to changes in the concentration of dissolved substances in the unfrozen phase during the freezing process [32]. These changes disrupt the protein structure, reducing the capability of muscle fibers to retain water and, consequently, increasing moisture loss during their thawing.

Based on the method of exposure to energy, the thawing processes can be divided into two major groups [3,33]. The first one covers the classical methods (gradient), based on the exposure of the food product to heat using warm air, irrigation, or immersion into a liquid medium [3,11]. These methods involve the transfer of heat to the surface of food products from an air or water environment and the internal dissipation of heat within the food product through thermal conductivity. They are quite economical and easy to operate, but have a number of significant drawbacks. The most modern methods in this group are: thawing in water, in air, in condensing saturated water vapor under a vacuum or at increased hydrostatic pressure [34,35].

The second is the methods of exposure to energy through application of the physical fields: constant and alternating electric fields [36–38], electromagnetic (EM) fields of a certain frequency (radio frequency, microwave and infrared heating) [39–41], as well as using infrasound or ultrasonic effects [42,43].

Objects and methods

Within this analysis a search for scientific information was conducted in the database of the electronic library eLIBRARY.ru, in the foreign websites of the International Institute of Refrigeration, scientific journals of the academic publishing house Elsevier — Science Direct and Springer Science.

The search for references sources for their systematization and analysis was conducted using the following keywords: thawing processes of the animal origin products, electrophysical effects, ultrasonic effects, electromagnetic fields, high hydrostatic pressure, electrostatic and pulsed electric fields, ohmic heating, radio frequencies, microwaves, and quality parameters. We analyzed reference sources in Russian and foreign languages, published in scientific journals and conference materials collections devoted to the analysis topic. In total, over 200 reference sources were analyzed and grouped by subject area. Articles were selected based on a preliminary analysis of titles, abstracts, and keywords. This review includes 150 publications from 2000 to 2024. Reference sources are predominantly foreign and indexed.

Exclusion criteria covered the publications published before 2000 and researches devoted to non-food materials, including application of supplementary coolants agents in addition to cold itself, applied in the form of biological, chemical and other effects.

Full texts of the articles that matched the search criteria were analyzed, with most of the sources used published between 2016 and 2024.

Traditional thawing methods

Thawing in liquid media

Thawing via an aqueous medium features high speed, but yet has the following disadvantages. This method is associated with significant losses of soluble substances, which negatively impacts food product quality, particularly water holding capacity and its structure. Prolonged exposure to water also increases the probability of microbiological contamination due to the accumulation of microorganisms in the water. To prevent this, it is necessary to use sealed containers or packaging to prevent contamination of the food product and its contact with the cooling medium. Furthermore, thawing in water without forced medium circulation leads to the formation of an insulating defrosted layer on the surface, which impedes effective heat exchange and, in some cases, leads to refreezing of the surface layers [44].

Thawing in air

Among the methods of thawing in air two main approaches can be distinguished: thawing with natural air circulation and forced air circulation. These methods are cost-effective, but are characterized by a long process time and a significant occupied area. To increase their efficiency, the air is saturated with moisture, which helps reduce mass loss during thawing, but this leads to an increased rate of microorganism growth and a deterioration in the sensory properties of the food products [45–50]. Although thawing in a refrigeration chamber at a temperature of 4.0 °C is safe, it has significant drawbacks: a long time to achieve equilibrium between the internal temperature of the product and the temperature of the refrigeration chamber, as well as high moisture losses due to damage to cellular structures. These limitations demand further development of thawing

technologies that ensure an optimal balance between process time, quality, and food safety.

Thawing at reduced pressure

One of the thawing technologies, that is of interest for meat processing plants, is the thawing of bulky, large-piece, or crushed raw meat materials in a saturated steam environment under reduced pressure [33,51]. Analysis of the research results of domestic [52,53] and foreign scientists has shown that this method features a range of significant advantages over the other method [54–56]. For example, compared to the thawing in air method, vacuum defrosters take up less space, allow for a 30–50 % reduction in duration, and the vacuum environment has a beneficial effect on the sanitary condition of the raw materials, preventing the growth of bacterial contamination [51].

An additional positive effect can be achieved by combining the process of thawing meat raw materials with its massaging in a defroster chamber [33,52], which ensures uniform heating and reduces losses. In addition, thawing and salting meat raw materials in a vacuum increases the hydration of proteins and their water holding capacity, which helps reduce the loss of meat juice [52,53]. In the works of Russian authors devoted to the food raw materials thawing, the main patterns of the process are shown [57,58] and rational modes for its implementation are proposed depending on the thawing methods [59–63].

The thawing methods presented above — the use of air and water working environments, despite their simplicity and economic availability, have significant limitations that affect the quality of products [35,64,65].

The reduced pressure thawing method is widely used in the meat industry, but it cannot be scaled up to the other food industries.

High hydrostatic pressure (HHP) thawing

This technology is implemented at pressures from 100 to 800 MPa [66]. Thawing under IOP was first studied by Takai R. et al. [67], who demonstrated that this method allows thawing food products at the temperatures below 5.0 °C at a high speed, thus preserving the physicochemical properties of the food products, like color and texture. HHP technology involves packaging products under vacuum, placing them into a chamber filled with water or another liquid, in which a pressure of 100 to 800 MPa is created, which contributes to the destabilization of the crystalline structure of ice, ensuring the transition of ice to the liquid phase at temperatures below 5.0 °C. This method not only reduces the duration of thawing, but also reduces the risk of microorganism growth.

Despite its advantages — i.e. high heat exchange rates and reduced risk of bacterial contamination [68] — HHP thawing has not become widespread in the food industry due to the complexity of the technology and the need for specialized equipment.

Table 1 presents the results of studies of the thawing process via HHP. The abbreviations for physical values designations used in Tables 1–6 are given before the list of references.

The results presented in the Table 1 were obtained in the processes of thawing various products using HHP within the pressure range from 0 to 200 MPa, in comparison with the control samples defrosted at atmospheric pressure, at the same values of working environment temperatures.

Studies have shown that thawing via HHP method significantly reduces the thawing time compared to thawing in air or water at atmospheric pressure. This is explained by the fact that pressure increase reduces down the melting point of ice, which leads to increasing the temperature difference between the defrosted product and the heating medium,

Table 1. Results of studies of the process of thawing food products with HHP method

Product type, sample size	Conditions of the experiment	Result	References
Longissimus dorsi muscle of pig (rectangular samples with dimensions of 150×100×40 mm)	p = 70, 140, 210 MPa; t_{av} = 20.0 °C	Reduced mass loss compared to thawing in air at 4.0 °C and water at 20.0 °C. Microstructure damage and increased protein denaturation with increasing pressure. The lowest losses and structural damage are observed at 140 MPa.	[69]
Chicken breasts (samples weighing approximately 220 g)	p = 100, 150, 200 MPa; t_{av} = 10.0 °C	Reduction of losses compared to thawing in water at p = 0.1 MPa, t = 10.0 °C	[70]
Fish — Atlantic salmon (samples weighing from 0.54 to 0.70 g)	p = 100, 150, 200 MPa; t_{av} = 20.0 °C	Reduction of the thawing process duration compared to thawing in water at p = 0.1 MPa, t = 20.0 °C	[71]
Fish — sea bream (samples weighing 100 ± 10 g)	p = 100, 150, 200 MPa; t_{av} = 20.0 °C	Reduced thawing time and losses, preservation of quality and texture, and reduced lipid oxidation. Lowest losses at p = 100 MPa compared to thawing in water at p = 0.1 MPa, t = 20.0 °C.	[72]
Fish — salmon (samples weighing about 400 g)	p = 100, 150, 200 MPa; t_{av} = 10.0 °C	Reduction of losses during thawing compared to thawing in water at p = 0.1 MPa, t = 10.0 °C, reduction of microbiological contamination	[73]
Fillets of various types of fish	p = 100, 150, 200 MPa; t_{av} = 10.0 °C	Reduction of losses during thawing compared to thawing in water at p = 0.1 MPa, t = 10.0 °C	[74]
Seafood (scallops)	p = 100, 150, 200 MPa; t_{av} = 10.0 °C	Reduction of losses during thawing compared to thawing in water at p = 0.1 MPa, t = 10.0 °C, increase in losses during cooking	[75]
Mango (samples with dimensions of 20×20×10 mm, weighing about 100 g)	p = 75, 100, 125 MPa; t_{av} = 20.0 °C	Reduced thawing time, minimal color change; increased loss of vitamin C	[76]

and, consequently, to heat exchange intensification. Furthermore, a decrease in the melting point of ice (by 5–7 K) allows running the thawing process at lower temperatures, which contributes to the preservation of product quality, texture and color, and a reduction in spoilage due to the inhibition of microorganism growth and a decrease of enzymatic processes activity [76,77]. However, in some cases an increase in weight loss during thawing has been noted, as well as an increase of vitamin C loss in fruit.

When conducting HHP thawing, the key factors influencing the process duration are the dimensions and geometric shape of the food product. Large and heterogeneous food products require longer processing times. Disadvantages of this technology also include the high cost of equipment and, for some food products, changes in protein structure and color [72,78]. Furthermore, HHP thawing requires technique justification of the process parameters, especially when dealing with the food products of complex shapes or heterogeneous structures.

Electrophysical methods of thawing

Thawing with ultrasonic field exposure (hereinafter referred to as USE)

Ultrasonic waves are used in thawing food products in liquid media as a way to intensify the heat exchange process between the heating medium and frozen products [79], such as pork, chicken breasts, and mango pulp [80,81]. This effect is associated with the destruction of the continuity of the liquid medium under the influence of ultrasonic waves — the formation and collapse of vapor-gas bubbles. This leads to generation of local shock waves and the emergence of acoustic flows of liquid, which contribute to the destruction of the boundary layer on the surface of the defrosted food product [40]. The efficiency of USF depends on the geometric dimensions and structure of the product, the frequency of oscillations and the energy flux density, the temperature and viscosity of the liquid medium.

In [65], the authors state that, compared to meat samples defrosted at room temperature, the weight loss of pork, beef, and lamb using USF thawing was approximately 43–45 % lower. Research by many authors has noted that USF thawing contributed to a reduction in losses, a decrease in the oxidation of proteins and lipids in meat, and an increase in the content of bound and immobilized water. Table 2 presents the results of studies by various authors on using of USF for food products thawing.

Studies on food thawing were conducted within the frequency range from 20 to 50 kHz and emitter power from 200 to 600 W. The authors stated that at higher power local overheating may occur, which contributes to increased oxidative degradation and loss of meat juice [83,90]. Research by many authors noted that USF thawing contrib-

Table 2. Results of studies of the thawing process of packaged food products with USF method

Type of the product, sample size	Conditions of the experiment	Result	References
Yak meat (samples measuring 100×100×50 mm, weighing 600 g)	P = 200, 400 and 600 W; f_{USF} = 20 kHz; t_{av} = 25.0 °C	Reduction of thawing duration, reduction of losses, preservation of color and microstructure of the product; the greatest effect is achieved at P = 400 W	[42]
Longissimus dorsi muscle of pig (samples with dimensions of 120×60×35 mm, weighing 160 g)	P_{spec} = 0.2; 0.4 W/cm^2*; f_{USF} = 25 kHz; t_{av} = 15.0 °C	Does not provide a significant effect on the change in microbiological parameters and textural properties compared to thawing in water at t = 15.0 °C	[82]
Longissimus dorsi muscle of pig (samples with dimensions of 120×60×35 mm, weighing 160 g)	P_{spec} = 0.2; 0.4; 0.6 W/cm^2* f_{USF} = 25 kHz; t_{av} = 15.0 °C	Features significant reduction in duration, preservation of microstructure and color; does not provide a significant effect on the change in losses compared to thawing in air and water at t = 15.0 °C	[80]
Sea cucumber (samples weighing 20.56 ± 1.78 g)	P = 200 W; f_{USF} = 43 kHz; t_{av} = 20.0 ± 1.0 °C	Reduction of thawing duration, preservation of quality parameters compared to thawing in water at t = 20.0 °C and air at t = 4.0 °C	[84]
Cuttlefish (samples weighing 1100 ± 50 g)	P = 200 W; f_{USF} = 53 kHz; t_{av} = 25.0 ± 1.0 °C	Increased moisture holding capacity; preservation of quality parameters compared to thawing in a microwave and in air at t = 4.0 °C	[85]
Fish (samples weighing 215 ± 25 g)	P = 200 W; t_{av} = 20.0 ± 1.0 °C	Preserving the same quality parameters as when thawing in water at t = 20.0 °C with reducing of duration	[86]
Fish — silver carp (samples weighing 180 ± 10 g and length (185 ± 5 mm)	P^1_{spec} = 0.135 W/ml**; f_{USF} = 28 kHz; t_{av} = 25.0 °C	Reduced thawing duration, color preservation, and reduced lipid oxidation, with no significant effect on texture compared to thawing in air and water at 25.0 °C	[87]
Mango (samples weighing 200 g)	P^1_{spec} = 0.037; 0.074 and 0.123 W/ml**; f_{USF} = 28 kHz; t_{av} = 4.0 and 25.0 °C	Reduction of thawing duration, preservation of organoleptic properties compared to thawing in water at t = 4.0 °C	[81]
Soybean seeds (soybeans) samples weighing 100 g	P_{spec} = 3.18; 6.54; 8.69 and 11.37 W/ cm^2 *	The best preservation of ascorbic acid, textural hardness, features the minimal moisture loss at P_{spec} = 8.69 W/ cm^2	[88]
Dough	P^1_{spec} = 60 W/l**; f_{USF} = 40 kHz; t_{av} = 25.0 °C	Improved rheological properties, increased specific volume and homogeneity of the internal structure of the dough compared to thawing in water, air and microwave	[89]

* P_{spec} — is the specific power relative to the surface area of the generator plates.

** P^1_{spec} — is the specific power, related to the volume of the working medium in the ultrasonic bath.

uted to shortening of the thawing process, a reduction in weight loss, and a decrease in the oxidation of proteins and lipids in meat. Research presented in [14,40,91] showed that optimization of USF thawing power and frequency significantly ensures the preservation of the original quality properties of animal origin products.

However, the noted advantages of USF thawing technology require careful analysis, since excessive radiation power can lead to the destruction of cellular structures, oxidation of lipids and destruction of pigments, which negatively affects the texture and sensory properties [55]. A number of authors note that at high powers local overheating can occur, which contributes to an increase in oxidative degradation of lipids and loss of meat juice [90]. At present, it is difficult to conduct an analysis when comparing the results obtained by different authors, since various frequency and power ranges, USF duration, geometric dimensions, product shape and packaging materials are used in the studies. There is no any unified system for calculating specific power values. Additional scientific work is required using unified methods for organizing experimental studies, monitoring process parameters and processing experimental data.

Thawing with electrostatic field (hereinafter referred to as ESF) method

The use of ESF method offers a more efficient technology for thawing food products in air, while preserving their quality and reducing processing time. The mechanism of action is based on the generation of electron wind, created by a corona discharge in a needle-plate electrode system. The corona discharge ionizes the air between the electrodes, creating turbulent electron wind flows that improve heat transfer from the defrosted product. Furthermore, the electric field promotes the orderly arrangement and movement of ions within the food product, thus improving heat transfer efficiency [92].

The thawing rate depends on the electric field voltage, the distance between the needle electrodes, and their position in reference to the product [70]. The thawing process via ESF can be conducted using either alternating or direct high-voltage current. There are various designs of electrode systems (needle, multi-point, plate), which are targeted at increasing the efficiency of thawing processes [93].

ESF allows reducing the duration of the thawing process and minimizing the loss of meat juice [94]; for example, research by Qian et al. [95] showed that applying 2.5 kV to frozen beef samples reduced the thawing time by 42 % and decreased meat juice loss down by 20 %.

These results were confirmed in [96], where the authors used ESF at 20 kV to defrost rabbit meat, reducing the thawing duration by 60 % and decreasing thawing losses by 30 %. ESF is being actively studied as a non-thermal method for processing food products, including pork, chicken, tuna, carp, shrimp, and some fruits [37,97]. This method has been shown to contribute to the preservation of quality properties — it reduces lipid oxidation and impedes development of microbial spoilage.

Research conducted by He et al. [98] showed that the use of ESF (10 kV) during pork thawing reduced the processing time and slowed down protein denaturation. After 5 days of storage after thawing, an increase of volatile basic nitrogen values from 10.64 to 16.38 mg/100 g was noted in the product exposed to ESF, while in the control group this value reached up to 19.87 mg/100 g, which proves a positive effect of ESF on the shelf life of the product. It was noted that an increase in the intensity of the corona discharge due to a decrease in the inter-electrode gap, led to a decrease in protein solubility and intensification of lipid oxidation [37,92].

Thawing with pulsed electric field (hereinafter referred to as PEF)

Electric fields are widely used in the food industry, in extraction, drying, and preservation processes. Pulsed electric field processing, based on the application of short-term high-voltage pulses (microsecond duration), accelerates mass and heat transfer [99,100] and allows for a significant reducing of the process duration compared to classical technologies [101,102].

For example, the use of pulsed electric fields for thawing pork at a voltage of 10 kV [37,103] made it possible to reduce the duration of the process, as well as to reduce the damage to muscle proteins, preserve their gelling properties, and improve their water holding capacity. Similar results were obtained during the thawing of duck meat; pulsed electric fields contributed to the slowing down of the denaturation of myofibrillar proteins, while preserving the emulsifying and gelling properties [100]. These data confirm that pulsed electric fields thawing can be considered an innovative method that helps to preserve the quality of meat products and reduce losses during processing. Table 3 presents the results of studies on using the electrostatic and pulsed electric fields for thawing food products process.

Research shows that ESFs and IEFs are effective methods of applying heat during food thawing, helping to preserve quality and reduce processing losses. The proposed technical solutions primarily involve using high-voltage needle electrodes and flat grounded electrodes. The products to be thawed are placed between the electrodes. Research shows that using ESFs and IEPs significantly reduces thawing duration, preserves quality, and reduces losses compared to thawing in water or air without an electric field exposure. The authors define optimal electric field voltages that maximize the preservation of the original properties of food systems. Increasing the electric field voltage above 4 kV/cm has been shown to reduce quality.

Despite the low cost of the equipment, these technologies have not found widespread industrial application and still remain in the laboratory research stage. Ensuring the safety of such systems in processing facilities with high relative humidity in the food processing area so far remains an unresolved issue.

Table 3. Results of studies of the thawing process via electrostatic field (ESF) and pulsed electric field (PEF)

Type of thawing	Type of product, sample size	Conditions of the experiment	Result	References
ESF	Beef (samples size 35×35×35 mm, weight 42 G)	The distance between the electrodes is 100 m, between the needles is 60 mm; U = 12, 16, 20, 24 and 28 kV; t_{av} = 25.0 °C	Reduction of thawing duration, maintaining quality indicators compared to thawing at U = 0 kV	[102]
ESF	Pork tenderloin (samples weighing approximately 35 g)	The distance between the electrodes is 60 mm, between the needles 30 mm; U = 4, 6, 8, 10 kV	Increased shelf life, reduced thawing time, no changes in quality indicators during thawing at U = 0 kV	[98]
ESF	Pork tenderloin (samples with dimensions of 20×50×50 mm, weighing 50 g)	The distance between the electrodes is 60 mm, between the needles 30 mm; U = 8, 10, 12, 14 kV	Reduced thawing duration, high temperature field uniformity; increased lipid oxidation compared to thawing at U = 0 kV	[38]
ESF	Pork tenderloin (samples with dimensions of 50×50×10 mm, weighing 45.3 ± 2.5 g)	The distance between needle electrodes is 50 mm; U = 10 kV	Increased water holding capacity and preservation of meat tenderness, reduced denaturation of myofibrillar protein compared to thawing in water and air	[37]
ESF	Chicken breast (samples with dimensions of (20×20×20 mm)	The distance between the needle electrodes is variable; U = from 4.5 to 18 kV E = 1.5; 2.25; 3 kV/cm	Reducing the thawing duration, maintaining quality indicators; optimal results were observed at E = 2.25 kV/cm	[92]
ESF	Chicken thighs (190g samples, 28 mm thick)	The distance between the needle electrodes is 20 mm; U = 20 kV	Reducing thawing duration and reducing losses during thermal processing	[104]
ESF	Rabbit meat (samples with dimensions of 30×30×20 mm)	The distance between the electrodes is 50 mm; between the needles = 20 mm; U = 15, 20, 25 kV	Reduction of thawing duration, at U = 20 kV the best preservation of quality indicators, moisture-holding capacity and texture	[96]
ESF	Beef (samples with dimensions of 40×40×20 mm)	The distance between the electrodes is 40 mm; between the needles — 26, 52, 78 mm; U = 10 kV	Decrease in quality indicators with an increase in the number of electrodes	[105]
ESF	Tuna (samples with dimensions of 20×40×40 mm)	Distance between electrodes — 30, 45, 60 mm; U = from 4.5 to 14 kV; E = from 1.25 to 3.5 kV/cm; t_{av} = 20.0 °C	Decreased thawing duration, with increased voltage, increased fat oxidation and thiobarbituric acid levels	[106]
ESF	Tuna (samples with dimensions of 20×40×40 mm)	Distance between electrodes — 30, 45, 60 mm; U = from 4.5 to 14 kV; t_{av} = 20.0 °C	Reduction of thawing duration, reduction of losses, preservation of quality indicators compared to thawing in air at t = 20.0 °C	[107]
ESF	Fish — carp (samples with dimensions of 40×30×15 mm, weighing 25.3 g)	The distance between the electrodes is 40 mm; the distance between the needles is 30 mm; U = 6 and 12 kV; t_{av} = 23.0 °C	Reduction of thawing duration, reduction of losses, preservation of quality indicators compared to thawing in water t_{cp} = 15.0 °C and air t = 23.0 °C	[108, 111]
ESF	Fish — mackerel (rectangular samples, size not specified)	U = 15, 25, 35 and 45 kV; t_{av} = 20.0 °C	Reduction of thawing duration, reduction of losses, preservation of quality indicators compared to thawing in air at U = 0 kV, t = 20.0 °C	[109]
ESF	Tuna (samples with dimensions of 20×40×40 mm)	The gap between the electrodes is 30, 45, 60 mm; U = 4.5; 6; 7.5; 10.5; 13.5; 14 kV; t_{av} = 20.0 °C	Reduction of the thawing duration compared to thawing in air at U = 0 kV, t = 20.0 °C; the shortest duration at U = 10.5 kV and a gap of 30 mm	[110]
ESF	Shrimp (10g samples)	U = 15, 30, 45 kV; t_{av} = 20.0 ± 1.0 °C	Reduction of thawing duration, maintaining quality indicators compared to thawing in air at U = 0 kV, t = 20.0 °C	[93]
ESF	Tofu (samples with dimensions of 35×35×35 mm)	The distance between electrodes is from 8 to 12 m; U = from 4 to 28 kV; t_{av} = 20.0 ± 1.0 °C	Reduction of the thawing duration compared to thawing in air at U = 0 kV, t = 20.0 °C	[97]
PEF	Peking duck meat (samples with dimensions of 50×50×30 mm, weight 50 g)	The distance between electrodes is 55 mm; U = from 5.5 to 22 kV; f = 50 Hz; E = from 1 to 4 kV/cm; t_{av} = 12.0 °C	Reduction of the thawing duration compared to thawing in air at U = 0 kV, t = 12.0 °C	[112]
PEF	Fish — Atlantic salmon (samples with dimensions of 50×40×20 mm, weighing 10 g)	The distance between electrodes is 50 mm; U = 5.5 kV; E = 1.0 kV/cm; f = 50 Hz; t_{av} = 10.0 °C	Reduction of thawing duration, reduction of losses, preservation of quality indicators compared to thawing in air at U = 0 kV, t = 10.0 °C	[99]

Ohmic thawing (hereinafter referred to as OT)

This method of electric thawing has been considered for use in the food industry in recent years due to its technological advantages over the traditional methods. The basic principle of the technology is the passage of an electric current through a food product, where the energy is converted into heat due to the internal resistance of the material. This ensures volumetric heating and a more uniform heat distribution compared to convective or microwave thawing [113,114]. As a result, thawing significantly reduces processing time, preserves product quality, reduces microbial growth on the surface, and improves energy consuming efficiency [115,116].

An important advantage of OT is using of alternating current, which eliminates negative effects compared to the using of direct current, which involves electrolysis, leading to the degradation of food components and corrosion of electrodes [68]. One of the key advantages of OT is the preservation of the physicochemical properties of food products, including color, texture, and lipid stability, by minimizing mechanical damage [117].

Research of Çokgezme et al. [118] showed that the sample's shape, voltage gradient, surface profile, and electrode contact with the food product determine the energy and economic characteristics of the process. It was noted that electrodes with needle-shaped and pyramidal surfaces provide better adhesion and minimize overheating [119].

Indiarto and Rezaharsamto [120] noted in their studies that high fat content in meat reduces electrical conductivity. Selecting optimal voltage values helps minimize changes in product quality, preserve texture and moisture-binding capacity [118,121–123]. Experimental studies conducted by Fattahi and Zamindar [117] indicated the need to ensure high-quality contact between the electrodes and the food product for uniform heat distribution. According to [121], thawing of minced meat and fish using OT method reduces meat juice loss and protein denaturation.

Table 4 presents the results of studies on OT using for various food products.

OT is presented as a technology for thawing food products, including meat and fruit. Analysis shows that this technology can be effective in both air and liquid (brine) thawing. It has been established that the concentration of salts in the brine can have a significant impact on the efficiency of the heating process, which helps to reduce the thawing duration and losses [117,128]. It has been noted that the chemical composition of the product affects changes in electrical conductivity [126], which in turn causes non-uniformity in the distribution of temperature fields across the volume of the defrosted object under the influence of an electric field. Non-uniformity leads to the formation of overheated or unthawed spots of the product [116,119].

Table 4. Results of studies of the thawing process with ohmic thawing method

Type of product, sample size	Conditions of the experiment	Result	References
Beef tenderloin (samples with dimensions of 50×100 mm, weighing 200–250 g)	$U = 50$ V	Reduction in thawing duration and loss of meat juice compared to thawing in air at $t = 20.0$ °C and 3.0 °C	[124]
Beef — biceps muscle (samples with dimensions of 25×25×50 mm)	$E = 10, 20, 30$ V/cm; $f = 50$ Hz	Reduction of textural and histological changes compared to thawing in air at $t = 25.0$ °C	[125]
Beef — minced meat (samples with dimensions of 130×60×20 mm)	E = 10, 13, 16 V/cm; $t_{av} = 4.0 \pm 0.5$ °C	Dependence of electrical conductivity changes within the temperature range from minus 18.0 to minus 1.0 °C was established. Decrease in electrical conductivity was noted along with increasing fat content.	[126]
Pork (samples with dimensions of 20×40×100 mm)	Thawing in brine; $U = 0, 20, 40, 50$ V	Color preservation, reduced fat oxidation compared to thawing in brine without OT using	[127]
Beef — biceps muscle (samples with dimensions of 25×25×50 mm; 25×50×50 mm; 50×50×50 mm)	$E = 10, 20, 30$ V/cm; $f = 50$ Hz	Reducing the thawing duration did not have a significant effect on juice loss and color characteristics compared to thawing at $t = 25.0$ °C.	[115]
Beef — minced meat (samples with dimensions of 130×40×30 mm; 130×60×20 mm; cylinder $h = 13$ cm; $d = 3.91$ cm)	Three types of electrodes. $E = 10, 13, 16$ V/cm; $t_{av} = 4.0$ °C	The lowest power consumption was obtained at $E = 16$ V/cm using pyramidal electrodes.	[118]
Tuna (samples with dimensions of 30×30×30 mm)	Thawing in brine. $E = 40, 50, 60$ V/cm; $t_{av} = 4.0$ °C	Reduced thawing duration, preservation of protein structure compared to thawing in air and water	[128]
Tuna (samples with dimensions of 30×30×30 mm)	Thawing in brine. $U = 40–60$ V; $t_{av} = 4.0$ °C	Significant reduction in thawing duration and loss. Increased fat oxidation compared to thawing in air and water.	[117]
Surimi — minced fish (samples with dimensions of 56×50×55 mm)	Thawing in brine. $U = 20$ V; $f = 60$ Hz	High uniformity of temperature distribution in the product, increased thawing speed compared to thawing in air at $t = 0.0$ °C	[129]
Spinach puree (vessel with dimensions of 30×50×100 mm, weight 125 g)	The distance between electrodes is 100 mm; $E = 10$ and 15 V/cm	Reduction of the thawing process duration by 70–80 % compared to thawing process at $t = 4.0$ °C	[130]

Despite the advantages of OT, the technology features implementation challenges related to ensuring reliable contact between the electrodes and the food product. This requires the use of electrodes tailored to the shape and structure of various products [118]. Researchers focus attention on optimization of the process parameters (electric field voltage, working environment temperature, brine concentration) and adapting the electrode design to the specific product. This method enables thawing of both unpackaged and packaged food products, provided the following conditions are met: the packaging must withstand high temperatures, possess good dielectric properties, and ensure close contact between the product and the electrodes.

Thawing with EM fields method

Thawing in the high frequency range (hereinafter referred to as HF)

Microwave methods employ electromagnetic radiation that causes dielectric heating and are a promising approach to developing food thawing equipment. Significant number of studies [131–135] are devoted to investigating thawing processes at frequencies of 27.12 MHz, which falls within the high-frequency range of waves according to GOST 24375-80[1].

The mechanism of HF action is based on the dipole interaction of polar molecules in an alternating electromagnetic field. This leads to the conversion of EM energy into thermal energy directly in the food product, thus increasing the efficiency and reducing thawing process duration [68,133].

Volumetric heating is particularly useful for large-piece samples, such as bulky blocks of meat, fish, and seafood, as it ensures uniform temperature distribution and preserves the original quality. Thawing technologies at 27.12 MHz frequencies have been implemented in many countries, including France, Turkey, Italy, and Japan. Their efficiency has been proven compared to traditional and ultra-high-frequency technologies.

When using high-frequency heating during the thawing of products with a high fat content, uneven temperature is observed throughout the volume of the product due to the higher heating rate of fat tissue, which leads to more intense oxidation of lipids and denaturation of proteins [136,137].

Table 5 presents the results of studies of radiofrequency thawing of food products with the high-frequency range.

Thawing in ultra-high frequency (hereinafter referred to as UHF) range

The study of thawing at frequencies of 915 and 2.450 MHz relates to the UHF range in accordance with GOST 24375–80[1]. These technologies have found wide application in both industrial and domestic settings. They offer a number of advantages: they are energy efficient, easy to operate, and highly productive [55]. UHF in meat thawing ensures a reduction in processing time, a reduced risk of bacterial contamination [142], and the preservation

[1] COST 24375-80. "Radio communication. Terms and definitions". Moscow: Publishing House of Standards, 1980. — 58 p. Retrieved from https://docs.cntd.ru/document/1200015766 Accessed November 17, 2025 (In Russian)

Table 5. Results of studies of thawing food products process in the high-frequency range

Type of product, sample size	Conditions of the experiment	Result	References
Ground beef (containers with dimensions of 190×125×27.5 mm)	The distance between electrodes is from 90 to 190 mm; f = 27.12 MHz; P = 6 kW; conveyor belt speed varies from 1 to 60 m/h	Experimental data on the distribution of temperature fields in the product at different gaps between the electrodes were obtained.	[136]
Chicken breasts (weighing 225 g)	Distance between electrodes 65, 75, 85 mm; f = 27.12 MHz; P = 10 kW	Reduction of meat juice loss, preservation of structure compared to thawing in air at t = 4.0 °C	[41]
Beef (block with dimensions of 200×200×100 mm)	f = 27.12 MHz; P = from 200 to 600 W	Reduction of thawing duration, increase in heating uniformity compared to thawing in air at t = 4.0 °C	[40]
Beef (block with dimensions of 200×200×200 mm; 100×100×100 mm)	f = 27.12 MHz; P = 400 and 500 W	Reduction of moisture loss compared to thawing in air at t = 4.0 °C	[135]
Beef (samples with dimensions of 160×102×60 mm; 220×140×60 mm; 285×190×60 mm)	The distance between electrodes is 115 mm; f = 27.12 MHz; P = 3 kW	The influence of the geometric shape of the samples on the uniformity of temperature distribution was established	[138]
Fish — tilapia fillet (samples with dimensions of 140×70×15 mm)	The distance between electrodes is 100, 120 and 140 mm; f = 27.12 MHz; P = 600, 800 and 1000 W	Reduction of thawing duration, preserving quality parameters compared to thawing without HF	[50]
Fish — tilapia fillet (sample length 188 ± 10 mm)	The distance between electrodes is 100, 120 and 140 mm; f = 27.12 MHz; P = 300, 600 and 900 W	Reduction of thawing duration, improvement of structural and mechanical properties compared to thawing without HF	[139]
Minced fish (samples with dimensions of 250×150×50 mm)	The distance between electrodes is 140, 160, 180, 200 and 240 mm; f = 27.12 MHz; P = 6 kW; t_{av} = 20.0 °C	Best quality performance was achieved with 160 mm electrode spacing	[140]
Salmon (samples with dimensions of 100×80×25 mm)	f = 40.68 MHz; P = 400 W; t_{av} = 10.0 °C	Reduction of thawing duration, reduction of lipid oxidation and protein denaturation compared to thawing in water and air at t = 10.0 °C	[141]

of nutritional properties and organoleptic indicators [70]. A number of studies have noted that thawing at these frequencies causes uneven heating and localized overheating of the product due to the low penetration depth of microwaves [55]. When processing bulky large-sized chunks of products, the issue becomes more acute, since the thermal gradient increases the unevenness of heating, which negatively affects the quality and safety of food products and hinders large-scale implementation in industrial processing.

To prevent thermal unevenness, measures are proposed to optimize the sizes of products chunks, develop special design solutions for wave distribution, and arrange the frequency of on/off cycles of wave generators.

In addition, studies are being conducted on the combined effects of UHF with other technologies. For example, Cai et al. [79] investigated the combination of UHF and USF for thawing fish (perch) and showed that the combined method made it possible to increase the uniformity of temperature distribution and maintain the stability of protein structures.

Similar results were obtained in the work of Cao et al. [143], who used a combination of microwaves and magnetic nanoparticles during the thawing of sea red bream fillets, which improved the uniformity of heat distribution and preserved the stability of secondary and tertiary protein structures. These combined methods features significant potential; however, their practical implementation is hindered by high technological complexity and economic costs.

Table 6 presents the results of studies on using the UHF range for thawing food products.

Analysis shows that a large body of experimental research on thawing food systems is conducted within 27.12, 915, and 2.450 MHz frequency ranges. Thawing at 27.12 MHz ensures uniform heating, but is significantly inferior in performance to the systems operating at 915 and 2.450 MHz. However, the latter frequency requires special conditions to ensure the safety of operating personnel and is characterized by high capital costs. Experience of its application demonstrates the key contradiction between systems operating at these frequencies: heating rate (performance) and the uniformity of temperature distribution across the volume of the heated product (quality).

Tables 5 and 6 show that 27.12 MHz technologies provide better temperature uniformity, preservation of the original product structure, and reduced weight loss after thawing compared to the frequency of 2.450 MHz. Thawing systems that operate at 2.450 MHz frequency ensure high throughput, but provide a negative impact on product microstructure and quality, while localized overheating occurs, especially for the products with complex geometric dimensions and shapes.

An alternative solution to this problem could be the using of a new range of radio frequencies — particularly high ones, which would combine the advantages of defrosters operating in the HF and UHF ranges [149,150].

To improve the performance of heating systems, it is proposed to use a higher frequency up to 150 MHz. Within this frequency range, the specific volumetric heating power is several times higher than at 27.12 MHz, and the radiation penetration depth into frozen muscle tissue is greater than at 2.450 MHz reaching over 30 cm, thus ensuring uniform heating of virtually any frozen product of animal origin.

Conclusion

Thawing technologies that involve high hydrostatic pressure, ultrasonic and ohmic heating are characterized by a high rate of heat exchange compared to convective methods and methods of conductive heat transfer, but they require application of specialized expensive equipment and the justification of rational processing modes taking into account the geometric shape and heterogeneity of the food products structure.

Using of electrostatic field and pulsed electric field during food thawing helps maintain quality and reduce processing losses. However, their using in industrial settings is restricted due to safety risks for service personnel.

Table 6. Results of studies of the thawing process using the effect of EM field within UHF range on food products

Type of product, sample size	Conditions of the experiment	Result	References
Chicken thighs, wings, breasts (100 g samples)	$P = 120$ W; $f = 2.450$ MHz	When thawing in UHF, no significant difference in acrylamide accumulation was detected compared to thawing in air at $t = 4.0$ °C and water at $t = 20.0$ °C	[144]
Fish fillet — Nile perch (samples with dimensions of 30×30×20 mm)	$P = 400$ W; $f = 2.450$ MHz	Reduction of thawing duration, reduction of bacterial contamination, reduction of negative impact on the microstructure of the product compared to thawing in water at $t = 20.0$ °C	[145]
Cuttlefish (samples weighing 1100 ± 50 g)	$P = 400$ W; $f = 2.450$ MHz	Negative impact on quality parameters	[85]
Fish — mackerel (samples weighing 215 ± 25 g)	$P = 400$ W; $f = 2.450$ MHz	Reduced thawing duration, uneven heating	[86]
Potatoes (6mm thick slices)	$P = 1250$ W; $f = 2.450$ MHz	Negative impact on quality parameters compared to thawing in air	[146]
Unfermented wheat dough (samples weighing 60 g)	$P = 1000$ W, $f = 2.450$ MHz	Negative impact on rheological properties compared to thawing in air at $t = 25.0$ °C	[147]
Dough (samples weighing 120 g)	$P = 100$ and 300 W; $f = 2.450$ MHz	Uneven temperature distribution and decreased survival of yeast cells compared to thawing in air at $t = 20.0$ °C	[148]

Thawing methods with electromagnetic waves are the most promising area of research in the development of efficient industrial defrosters. They reduce thawing duration, reduce the risk of microbial contamination, and preserve food quality. However, at high frequencies (915 MHz, 2.450 MHz), they fail to provide a uniform field of temperature. Researches of using VHF (100–200 MHz), which combines the advantages of defrosters operating both HF and UHF frequencies, is now a topic of scientific and practical interest for developing a new generation of defrosters.

The technological selection of the optimal thawing method should be based on a systemic approach that takes into consideration the type of food product, its geometric shape and dimensions, chemical composition, quality requirements for the final product and the economic feasibility component.

Accepted abbreviations and designations:

p — hydrostatic pressure, Pa;
P — electrical power, W;
f — frequency of electromagnetic field, Hz;
f_{USF} — frequency of ultrasonic field exposure, Hz;
U — electric field voltage, V;
E — electric field strength, V/cm;
t_{av} — temperature of the working environment, °C.

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