



INFLUENCE OF PACKAGING TYPES ON THE AGING PROCESSES OF THE SEMI-FINISHED MEAT PRODUCTS

Natalia M. Revutskaya, Elena V. Mileenkova*, Karina V. Sivtseva

V. M. Gorbатов Federal Research Center for Food Systems, Moscow, Russia

Keywords: meat, aging, autolysis, proteolytic processes, vacuum, modified atmosphere packaging, storage time

Abstract

The thematic review is aimed at the integrated analysis of the aging process of various meat types in correlation with the applied method ("dry", "wet"), and a review of the packaging methods for proteolytic processes responsible for the formation of taste, aroma, color and texture of the finished food product. Analysis of scientific publications showed that the "dry" aging of meat creates a more pronounced sensory profile, but increases weight loss. "Wet" aging (vacuum aging) creates optimal anaerobic conditions for active proteolysis, thus ensuring a tender and juicy texture due to improved moisture-binding capacity. In the course of the study, unconventional approaches to raw meat aging were found, including the use fungal starter culture, mineral water, and wrapping in parchment paper to improve rheological properties. The modern packaging technologies, including vacuum packaging and the use of the modified atmosphere packaging, affects the dynamics of biochemical changes during meat aging, as well as oxidative and microbiological processes in semi-finished meat products. Packaging into a modified atmosphere packaging allows controlling the color characteristics and shelf life, but its effect dramatically depends on the composition of the gas medium. A high concentration of oxygen in MAP provokes oxidative spoilage, while its absence stabilizes the color and slows down the degradation of meat proteins. An analysis of domestic and foreign scientific articles showed the high relevance of the meat aging problem for the manufacturers in this industry. The authors noted that in order to achieve high consumer properties of semi-finished meat products, it is necessary to make reasonable choice of aging ways and packaging methods taking into consideration the context of the active development of innovative technologies and new types of packaging materials.

For citation: Revutskaya, N.M., Mileenkova, E.V., Sivtseva, K.V. (2025). Influence of packaging types on the aging processes of the semi-finished meat products. *Theory and Practice of Meat Processing*, 10(2), 189–198. <https://doi.org/10.21323/2414-438X-2024-10-2-189-198>

Funding:

The article was published as part of the research topic No. FGUS-2024-0001 of the state assignment of the V. M. Gorbатов's Federal Research Center for Food Systems of RAS.

Introduction

Meat quality is influenced by the effect of the factors combination before and after the slaughter of an animal. Factors that affect meat quality before slaughter include the animals' species, variety, breed, sex, age, and fatness, as well as the conditions of the animals' husbandry and feeding diet, method of transportation, pre-slaughter handling, and the method of knocking. Factors that influence meat quality after slaughter cover cooling, aging, packaging method, and storage conditions, including temperature and duration [1].

Together with the above-listed factors that form the foundation of raw meat materials characteristics, the further processing of raw meat to produce semi-finished meat products while maintaining high consumer qualities remains an important issue.

Semi-finished meat products are a diverse range of the food products produced from bone-in meat cuts or boneless meat in the shape of cut pieces or minced meat. These food products require subsequent cooking heat treatment until they are ready to serve.

According to the Federal State Statistics Service of the Russian Federation, the production volume of semi-finished meat products in Russia increased by more than 35 %, or from 3.66 up to 4.95 million tons, from 2019 to 2024¹. The growth of this food product segment on the domestic market is caused by its high demand among the consumers, including the market availability of these food products and easiness of their cooking. Currently, the manufacturers produce a wide range of semi-finished food products from various types of raw meat materials (beef, pork, lamb).

When choosing the semi-finished meat products, the consumers pay attention to the visual presentation (appearance, color, smell) of the product, its labeling (information on the composition, shelf life, storage conditions, manufacturer, etc.), and the price. To form a positive impression of the quality of the product, the manufacturer must ensure its safety throughout the entire shelf life.

¹ Official website of the Federal State Statistics Service of the Russian Federation Retrieved from <https://fedstat.ru/indicator/58636> Accessed May 3, 2025. (In Russian)

However, meat, being a highly perishable product, has a limited shelf life mainly because of oxidation and spoilage caused by microbial activity and enzymatic processes. After a certain period, this can lead to a significant decrease in the quality and safety of the product, which poses serious risks to the health of the consumers [2].

The aim of the study is to analyze domestic and foreign scientific works to study the influence of packaging types and aging methods on the proteolytic activity of muscle tissue in the production of the semi-finished meat products.

Objects and methods

This review is based on the analysis of scientific literature on meat and modern scientific publications devoted to the effect of packaging on enzymatic processes, physicochemical properties and sensory characteristics of meat during its aging. The sources were sought and selected from the international scientific databases, including Scopus, ScienceDirect, PubMed and ResearchGate, as well as the Russian databases: Elibrary and Cyberleninka. To make the search more specific, a descriptive method was used by the keywords: meat packaging, enzymatic processes, aging of meat, vacuum packaging, modified atmosphere packaging, proteolysis, calpains, cathepsins, protein degradation, protein oxidation, meat quality, meat tenderness, “dry” and “wet” aging, sensory properties.

The article also includes the materials obtained from the experimental studies and systematic reviews published between 2004 and 2025. Use of other sources, published earlier than the specified period is explained by their conceptual importance for the current literature review. The main selection criteria were the availability of data on the packaging types (vacuum packaging, modified atmosphere packaging, permeable polymer packaging materials), the dynamics of enzymatic activity development, changes in myofibrillar proteins (desmin, troponin T), as well as availability of information on the textural and sensor characteristics of meat.

Results and discussion

As long-term researches made by domestic and foreign scientists show, today the issue of meat aging remains highly relevant. First of all it is related to the increased demand for semi-finished meat products, the emergence of new breeds of slaughter animals, and the use of modern packaging technologies.

The level and nature of the development of autolytic processes after the slaughter of an animal have a significant impact on the quality of meat. Forming of the chemical composition of raw meat and its technological properties depends on the species, variety, breed, age, sex and fatness of the animal, as well as the conditions of its transportation, pre-slaughter care and fasting, as well as the method of knocking.

Also, the various biochemical processes occurring during the period of raw meat aging from the moment of slaughter until its processing are highly important [3].

The main methods of meat aging: dry and wet

The process of meat aging is quite complicated; it consists of numerous series of changes in the composition and condition of multiple components of meat [4].

Autolysis is one of the stages of aging and it is a set of biochemical processes in muscle tissue, including changes in the physical and colloidal structure of proteins exposed to the meat's own enzymes [5]. However, these processes are not limited to only the transformation of the protein component; they also cover fats, carbohydrates and other components. As a result, low-molecular compounds are formed that are actively involved in the formation of the smell and taste of meat during subsequent heat treatment. Moreover, during aging the meat acquires a tender texture and juiciness [6].

It should also be noted that during aging, partial dissociation (separation) of actomyosin into actin and myosin begins, as well as the transition of meat from muscular *rigor mortis* state to a relaxed state. Meanwhile, the number of hydrophilic centers of myofibrillar proteins increases, which increases the water-binding capacity (WBC) of muscle tissue. Due to the release of cathepsins from lysosomes, which most actively affect sarcoplasmic proteins, proteolytic activity increases in the muscles. At the same time, myofibrillar proteins are also exposed to limited proteolysis, which enhances tenderness of muscle tissue. Thus, according to the research of V. I. Solovyev, it was established that the stage of fibrillar proteins proteolysis exposed to cathepsins can be considered as the beginning of meat aging [7].

The quantity and quality of connective tissue, whose components' lability increases during the aging period, plays a significant role in changing the texture of meat. The effect of acids formed at this stage of autolysis enables loosening of the collagen bundles, weakening of intermolecular cross-links, and collagen swelling, which also lead to a more tender and juicy texture of the ready-to-eat food product [8–11].

At the present stage, two methods of meat aging have gained the greatest popularity in the world: “dry” one — in unpackaged form, and “wet” one — in vacuum packaging. For many centuries, after slaughter, skinning and evisceration, the animal carcass was stored in a dark and cool place. This method of meat aging can be considered as the forefather of the modern “dry” aging, which can provide meat with a special flavor. The traditions from the distant past have occupied one of the popular trends in the restaurant business, when meat is placed in aging chambers with a certain microclimate. To obtain a tender product with a pronounced aroma and taste, it is necessary to control the temperature, humidity and air speed, which can restrain the development of bacteria and mold. In addition, the key aspect is the correct placement of meat, minimizing its contact with any surfaces. In this regard, the most rational method is hanging the meat in the aging chamber.

Scientifically the process of “dry” aging of meat was first described in the early 1950s. At that time, the American butchers aged whole carcasses for more than a week to get

the tender and tasty meat. However, a decade later, they rejected this method due to significant weight loss (up to 20 %) and fading of the original product color. At the same time, it was noted that it is the evaporation of moisture that contributed to development of the most pronounced aroma and notable taste of the finished food product.

In the review article Dashdorj et al. [12] considered the influence of dry aging parameters on beef quality, including temperature, relative humidity and air flow rate, as well as the influence of packaging on microbiological safety and enzymatic processes. In this article attention was drawn to the fact that using the packaging materials with high vapor permeability allows maintaining the required level of dehydration, while preserving the activity of natural meat enzymes, such as calpains and cathepsins. This promotes the acceleration of proteolysis, the breakdown of connective tissue and the improvement of the texture (tenderness) of the product. During the dry aging process, profound changes in protein and lipid fractions are also noted, which changed lead to the formation of a peculiar nutty taste and concentrated aroma. At the same time, insufficient evaporation of moisture or its excessive loss can disturb the balance of enzymatic reactions, thus worsening the texture and sensory properties of meat. So, the correctly selected packaging and control of aging conditions are critically important for optimizing the enzymatic processes and achieving high quality of semi-finished meat products that have undergone “dry” aging [13,14].

The research of “dry” aging processes continues to attract the interest of the domestic and foreign researchers [15–19], since it is most in demand for aging the high-quality (marble) beef in the restaurant industry. Steaks obtained from such meat raw materials feature rich aroma and saturated taste, and the presence of fat veins provides the additional juiciness, which allows classifying them as premium quality food products.

The work of Korean scientists on semi-finished pork meat products obtained by the method of “dry” aging [20] is noteworthy. This study analyzed the effect of long-term (40 days) “dry” aging of pork loin on its physicochemical properties at a temperature of $2 \pm 1^\circ\text{C}$ and a humidity of 80 %. Changes in the content of free amino acids and dipeptides were studied using high-performance liquid chromatography (HPLC), as well as sensory characteristics (texture, aroma and taste) of meat. The results showed a significant build-up of free amino acids, such as glutamate, as well as dipeptides, which indicates the activity of endogenous proteases — cathepsins and calpains. Enzymatic breakdown of proteins contributed to an increase of meat tenderness, improved aroma and taste characteristics, which allowed the conclusion that the selected aging method is effective and increases the consumer appeal of the product.

Other scientific studies in the process of systematic review focused on the biochemical processes and transformations during the period from the beginning of the aging process to the final ready-to-eat food product after heat

treatment. It was found that one of the most common reasons for unsatisfactory meat quality for the consumer is the meat toughness. This phenomenon is caused by number of reasons, including the amount of intramuscular connective tissue, intramuscular fat and sarcomere length. An important factor is the degree of proteolysis of key proteins in muscle fibers that determines the final tenderness [21].

It has been noted that water-soluble compounds with low molecular weight and meat lipids formed during the aging process of meat are the important precursors for the taste of the ready meat dish [22].

The preference for the “dry” aging method was also noted in the work of other Korean scientists who studied the effect of various aging methods (“dry” and “wet”) on the quality of pork loin. The researchers determined the content of moisture, protein, fat, ash, values of myofibril fragmentation index (MFI), and protein solubility. It was noted that “dry” aging contributed to a more pronounced moisture content decrease and increased the level of proteins and fats. Proteolytic activity due to the action of endogenous enzymes (calpains, cathepsins) was expressed as MFI increase and shear force decrease. This ensured better tenderness and reduced weight loss during cooking, which made “dry” aging a preferable method for obtaining semi-finished meat products with improved their process and sensory characteristics [23].

The use of “wet” aging at low positive temperatures (not exceeding 4°C) is a common practice and is widely used in the meat industry for production of meat (including semi-finished meat products) with a long shelf life [24]. Due to the tightness of the packaging, moisture is retained, which provided positive effect on the texture of the finished product. In addition, “wet” aging of meat is accompanied by significant changes in the concentrations of such metabolites as creatine, hypoxanthine, carnosine, as well as various dipeptides and tripeptides. These compounds are related to the processes of proteolysis of myofibrillar proteins, as well as the oxidation of proteins and lipids. Enzymatic processes of muscle tissue protein breakdown, activated during storage, led to an increase in meat tenderness, an improvement in its water-holding capacity and a change in color, which is critically important for improving the consumers’ perception of meat and the properties of semi-finished meat products [25].

Vacuum packaged meat features a prolonged shelf life in comparison with the aerobic method, it is easy to transport and store. However, despite a number of advantages, this method has its negative sides. For example, the aroma and taste of the finished product are less pronounced, and the moisture-holding capacity of such meat is lower than with “dry” aging. In addition, a sharp pressure drop during the vacuuming process contributes to the separation of meat juice in the package, which is an environment for the development of microorganisms.

In addition to the most popular methods of meat aging, there are a number of other approaches to aging meat raw materials, among which it is worth noting the use of fungal

starter culture that form a protective mold in order to obtain a delicate texture, rich aroma and taste [26]. In addition, it is possible to note so called aqua-aging method using mineral water, as well as additional wrapping meat into parchment before placing it into vacuum-sealed packaging during wet aging, which is widely used in *haute cuisine* all over the world.

In addition to the above, the duration of meat aging depends on many other factors, including the age of the animal, its fatness, the type of meat, and the temperature. For example, the aging of meat of higher fatness requires more time than meat of lower fatness and from the young animals [4,27].

Also, accelerated aging of meat is facilitated by elevated ambient temperatures. For example, beef ages in 10–14 days at 0 °C, in 6 days at 8–10 °C, and in 4 days at 16–18 °C. Pork and lamb aged faster at 0 °C — it takes 10 and 8 days, respectively [9].

Accelerated aging method (accelerated aging — AA) in vacuum packaging and a water bath (temperature 49–54 °C) demonstrates a significant effect on the enzymatic processes in low-grade beef, in particular the activity of cathepsins. The study at Louisiana State University (USA) showed that cathepsins B and L remain active even at a temperature of 55 °C, thus providing degradation of tropomyosin T (protein of cardiac and skeletal muscles) and dissolution of collagen, including both soluble and insoluble fractions. Particularly active proteolysis was observed at 49 °C for 3 h, where the highest levels of enzyme activity were recorded. Accelerated aging increased meat tenderness, as measured by a reduction in Warner-Bratzler shear force. It also reduced microbial contamination and promoted the formation of additional myofibrillar protein degradation. However, this process resulted in moisture loss and lowered juiciness, which was reflected in the sensory evaluation. Thus, the AA method can be considered as an effective approach for improving texture and functional properties of meat semi-finished products, especially when using vacuum packaging, which activates heat-stable proteolytic enzymes under moderate heat exposure [28,29].

In their turn the American scientists from the University of Idaho ran a scientific research, which consisted of determining the aging effect for various storage periods (2, 3, 4, 14, 28 and 42 days) on the activity of calpain-1 and -2 in steaks from the *longissimus dorsi* and *semimembranosus* muscle of beef, vacuum-packed and frozen at a low negative temperature (minus 75 °C). During the research, it was established that both enzymes contribute to improving the tenderness of beef during storage. However, after 14 days, calpain-1 was inactivated, while calpain-2 continued its action until the end of the experiment [30].

The comparative analysis of “wet” and “dry” aging of beef showed that the choice of packaging and storage conditions provides key impact on enzymatic processes intensity, texture and sensory characteristics of meat. Vacuum packaging provides a more pronounced reduction

in toughness due to the activation of autolytic enzymes such as calpains and cathepsins, especially for the first two weeks of storage. Meanwhile, the long-term storage (more than 21 days) shows, that the efficiency of further softening decreases, and comes to certain plateau. The advantage of “wet” aging is stable tenderness and high water-holding capacity explained by the destruction of proteins with high molecular weight (titin, nebulin) and the formation of gel-like structures that reduce moisture loss. At the same time, dry aging promotes development of more pronounced taste and aroma, but is accompanied by increased weight loss and the risk of microbial spoilage. However, the additional use of protective cultures (for example, *Lactobacillus sakei*) under vacuum storage conditions additionally increases the microbiological safety of the product, thus reducing the activity of *lactobacilli* and *listeria*. So, the properly selected packaging system in combination with controlled storage conditions allows for targeted management of enzymatic transformations, forming a preset quality profile in the semi-finished meat products [31,32].

In addition to use of microbiological preparations in the technological practice of the meat industry, enzyme preparations are also actively used that can specifically affect the proteins of muscle and connective tissue, increasing the tenderness, juiciness and water-holding capacity of semi-finished products. Proteolytic enzymes — papain, bromelain, chymotrypsin and ficin — show a pronounced effect in the processing of hard (collagen-containing) areas of the carcass, providing aid in their softening. Vacuum packaging creates favorable conditions for realizing enzymatic potential. Minimizing oxygen access slows down undesirable oxidative processes and simultaneously enhances autolytic and exogenous proteolysis processes. However, the efficiency of enzymatic processing directly depends on compliance with the technological parameters: temperature, duration, dosage of enzymes. Thus, the optimal combination of packaging technology and enzymatic processing allows for the sustainable use of meat raw materials, intensification of aging and stabilization of the quality of semi-finished meat products [33–36].

Meat aging in various types of packaging

Packaging plays a key role in ensuring the preservation of consumer properties of semi-finished meat products throughout the entire shelf life. The choice of packaging method sets the conditions for the enzymatic processes and meat stability during long-term storage. There are three main methods of packaging meat products in the industry:

- aerobic packaging;
- vacuum packaging;
- modified atmosphere packaging (MAP)

Polymer packaging is important in ensuring the quality and safety of semi-finished meat products, including the regulation of enzymatic processes during their storage and aging. It is worth noting that today various companies offer

a wide range of polymeric materials designed for packaging of semi-finished food products [37].

In its turn the vacuum packaging helps reduce the activity of aerobic microorganisms, slows down oxidation processes and creates favorable conditions for enzymatic autolysis, in particular proteolysis, which increases the tenderness of meat. At the same time, the packaging must have low gas and vapor permeability, especially in relation to oxygen, since the residual O_2 content above 0.15 % can give a start for undesirable change of meat color. The use of vacuum ensures the preservation of the sensory properties of beef for up to 60 days and promotes the uniform process of autolytic changes. The types of vacuum packaging like heat-shrinking packaging and skin packaging not only stabilize the physicochemical characteristics of the food product, but also increase the appealing and attractiveness for the consumer due to quality of visual representation. In addition, active and intelligent packaging containing antioxidants, antimicrobial agents and freshness sensors opens up wide prospects for precise control of enzymatic and microbiological processes that significantly affect the aging of semi-finished meat products [38].

It should be noted that aging in vacuum packaging creates favorable conditions for proteolysis without oxygen access, which helps preserving moisture, reduces microbiological risks and provides a food product with a soft texture. At the same time, aging without packaging or aging in air-permeable bags contributes to more pronounced aromatic changes due to oxidative processes, but is accompanied by significant weight loss [39–41].

Aging of meat raw materials in vacuum packaging has a significant effect on the enzymatic processes taking place, thus promoting the active breakdown of proteins into peptides and free amino acids. As a result of such biochemical changes, the functional and technological indicators of meat improve: moisture-binding and fat-retaining capacity increase, the structure of muscle tissue improves, and the ultimate shear stress decreases. Studies have shown that the optimal mode for aging beef in half carcasses includes 6 days of natural aging, followed by vacuum packaging and aging for 6 days at a temperature of $4 \pm 2^\circ\text{C}$. This approach ensured the production of chopped semi-finished products with high sensory characteristics (tasting score of 8.3–8.4 points), reduced losses during heat treatment and increased juiciness and tenderness of the product. A particularly pronounced effect was noted for the beef of premium and first grade quality, while long-term storage of the samples with a high content of connective tissue in vacuum packaging led to worsening of the semi-finished product quality [42].

Scientists from Texas Tech University (USA) noted that the temperature and duration of “wet” aging using a vacuum have a pronounced effect on the formation of texture and taste of semi-finished meat products due to the modulation of enzymatic processes and metabolic activity of microflora. The study found that the best sensory as-

sessments of juiciness, tenderness and taste were achieved when beef was aged for 42 days at a temperature of minus 2°C or 4°C . At the same time, 56-day storage of meat at 4°C featured the greatest development of volatile compounds — ethanol, acetic and heptanoic acids, as well as substances of microbial and thermal origin, such as methional and 2-methylbutanal. These compounds are associated with the creating of sour, earthy and liverish foreign tastes, which intensify during long-term storage. The main mechanism of accumulation of the final aroma is the active growth of microflora under conditions of moderately elevated temperature (4°C), while lower temperatures (-2°C) allow to slow down undesirable processes, while maintaining the efficiency of proteolysis (including degradation of desmin and troponin T). Thus, the choice of temperature conditions and storage time in vacuum is critically important for preserving the desired sensory and technological characteristics of semi-finished meat products [43].

Scientists from the University of Costa Rica [44] confirmed that the packaging method (aerobic and anaerobic) has a significant effect not only on the enzymatic processes occurring during storage, but also on the microbiological stability of minced rabbit meat. The studies showed that vacuum packaging significantly slows the growth of psychrotrophic and lactic acid bacteria (LAB), reduces the lipid oxidation index (TBARS), and limits the build-up of total volatile nitrogen (TVB-N) associated with proteins degradation. Vacuum packaging conditions the microbial threshold values associated with spoilage were reached only by the 11th day of storage at a temperature of 4°C . At the same time, in aerobic packaging, visible signs of spoilage appeared already on the 5th day of storage. The researchers explained this fact by the fact that vacuum packaging conditions, bacteria with low proteolytic activity (LAB) predominate, while in an aerobic environment, aerobic psychrotrophic microorganisms that actively destroy protein structures dominate. Moreover, the studies found that the decrease in pH in vacuum packaging is due to the growth of lactic acid bacteria. At the same time, in aerobic packaging, an increase in pH was observed due to the breakdown of proteins with the formation of amines. Thus, the authors of this work concluded that vacuum packaging not only increases the shelf life of semi-finished meat products, but also helps control enzymatic processes, reducing the likelihood of oxidative and microbial spoilage, especially in products sensitive to lipid instability, like rabbit meat.

The influence of packaging on enzymatic processes and the quality of semi-finished meat products is especially pronounced when using modified atmosphere (MAP) technology. Therefore, we will further consider the features of this packaging method in more detail. The use of this technology allows you to vary the composition of the gas mixture, which can slow down oxidation processes, prevent the proliferation of bacteria and preserve the attractive appearance of the product depending on the type of meat used and the desired shelf life.

The research ran by Brazilian scientists [45] has shown that packaging beef under anaerobic atmosphere with carbon monoxide (CO) content of 0.2 % — 0.4 % provides more pronounced tenderization and proteolysis, accompanied by myofibril fragmentation and ultrastructural changes in muscle tissue. In contrast to vacuum packaging, the use of CO-MAP allows maintaining a bright and stable red color of meat due to stable carboxymyoglobin formation, while reducing the rate of protein oxidation and improving the sensory properties of the food product. In contrast, packaging the meat with high oxygen content (75 %) provokes increased oxidation of lipids and proteins, negatively affecting the tenderness of meat and its aroma, despite the initial improvement in color characteristics. Thus, the use of a small amount of carbon dioxide in the composition of MAP can be recommended as an effective method for stabilizing the quality of meat products during aging, especially when processing raw materials from older cattle.

Modern technologies of meat packaging in MAP provide a wide range of tools for managing enzymatic and oxidative processes that determine the quality of semi-finished meat products during storage and display in self-service store windows. Different gas mixture compositions — from a highly oxygenated environment (80 % O₂) to an oxygen-free combination of N₂ and CO₂ — have different effects on the enzyme activity, color, water-holding capacity and sensory properties of meat. In particular, a high oxygen content promotes the formation of bright oxymyoglobin, but at the same time provokes the oxidation of lipids and proteins, reducing the tenderness and juiciness of the product. On the contrary, vacuum packaging and MAP with a low O₂ content (including the addition of carbon monoxide (CO)) allow to slow down oxidative processes, preserve the activity of calpains and proteolysis cytoskeletal proteins that increase water retention and tenderness of meat. In addition, active packaging with antioxidants, antimicrobial substances, and O₂ — absorbers can specifically regulate the microbiological and enzymatic state of the product, increasing color stability, taste, and shelf life. Thus, the integration of MAP with the enzymatic characteristics of meat raw materials opens up opportunities for targeted quality control of semi-finished products depending on the type of muscle, storage conditions, and consumer preferences [46].

It should be noted that the enzymatic and structural changes that occur in the muscle tissue of an animal after slaughter play a key role in the formation of the water-holding capacity of semi-finished meat products. Research by scientists from the University of Iowa (USA) has shown that the activity of endogenous calpains promotes proteolysis cytoskeletal proteins such as desmin, synemin and vinculin. This weakens the bonds between the myofibrils and the sarcolemma. This prevents excessive contraction of the muscle cell and reduces moisture loss. At the same time, conditions that promote calpain activation — optimal pH,

reducing environment and temperature conditions — are critical for the implementation of the proteolytic potential. In contrast, oxidative processes that occur at high level of oxygen inhibit calpain activity and disrupt enzymatic degradation of proteins, which leads to a decrease in juiciness and deterioration of meat texture. Thus, packaging technologies that minimize oxidation (e. g. vacuum packaging or low-oxygen MAP) create favorable conditions for the implementation of enzymatic tenderization and preservation of the water-holding capacity of meat, especially in the first 24 hours after slaughter, when proteolysis has the greatest impact on product quality [47].

In a study by Jaspal et al. [48], the effect of three packaging types and methods: vacuum packaging (VP), modified atmosphere packaging (MAP: 80 % O₂ + 20 % CO₂) and conventional polyethylene packaging (PE) on the quality characteristics of water buffalo meat during the aging process (21 days) was analyzed. The authors found that vacuum packaging provided the best conditions for enzymatic processes. For example, the Warner-Bratzler shear force (WBSF) values of the VP-packed samples decreased by more than 28 %, indicating a significant improvement in tenderness due to active proteolysis. In the MAP group, the decrease in WBSF was less pronounced and amounted to 17 %, while in the PE group, virtually no changes were observed.

In addition, at the end of the shelf life, vacuum-packed meat featured the lowest moisture loss during cooking (23.90 % versus 26.75 % in MAP and 29.45 % in PE), which is related to the preservation of protein structure exposed to enzymes action. Despite the fact that MAP maintained higher lightness (L) values throughout the entire storage period (e. g. 42.40 versus 39.53 in VP on the 21st day), it also increased lipid oxidation, which was expressed in an increase in the oxidative spoilage rate (TBARS) to 1.57 mg malondialdehyde / kg versus 1.32 mg / kg in vacuum packaging. Taking into account the obtained results, the authors concluded that vacuum packaging made it possible to most effectively maintain enzymatic activity, improving texture and slowing down the development of oxidative changes compared to other packaging methods [48].

The scientists from China came to the similar conclusion in the work [49] about the preference for vacuum packaging. In the process of comparative studies of various packaging methods (aerobic, vacuum packaging and modified atmosphere packaging), it was found that beef samples packaged using a vacuum featured greater preservation of free thiol groups, lower content of carbonyl compounds and higher activity of μ -calpain compared to the samples packaged in air-permeable packaging or packaging with a modified atmosphere packaging. The authors noted that the use of a vacuum ensured a more active proteolysis of desmin and better tenderness of beef, and also contributed to the creation of favorable conditions for slowing down the proteins oxidation and maintaining the enzymes activity that help improving the meat texture.

Li et al. [50] studied the effect of vacuum packaging and different oxygen concentrations (40 %, 60 %, and 80 %) in MAP packaging on protein oxidation and pork color during storage at 4 °C. The results showed that carbonyl groups are formed during storage regardless of the packaging type. However, an increase in oxygen concentration leads to higher protein carbonylation and worsening of meat texture. Meanwhile, sarcoplasmic proteins happened to be more susceptible to oxidation than myofibrillar proteins. The results showed that a small amount of oxygen (40 %) in MAP packaging contributes to obtaining fresh pork of the desired color, while high oxygen content leads to more pronounced protein oxidation with an insignificant improvement in the color characteristics of meat.

The influence of different packaging methods on proteolysis, accumulation of free amino acids and development of beef flavor during aging are reflected in experimental studies by scientists from Texas Tech University [51], Colorado State University [52], and researchers from the Warsaw University of Life Sciences [53]. Their studies compared four types of packaging: vacuum packaging, high-oxygen MAP, carbon monoxide packaging (CO-MAP) and traditional PVC packaging. It was found that meat packaged in MAP with high oxygen concentration was characterized by minimal degradation of desmin, the highest rigidity values and more pronounced development of flavors that do not belong there (bitter, sour, fishy), which is related to increased oxidative processes and decreased activity of enzymatic proteolysis. In contrast, vacuum packaging led to increased proteolysis, accumulation of free amino acids associated with meat flavor development (umami, bloody, roasted profile) and improved textural characteristics of the product. These results indicate that anaerobic storage conditions (e. g. vacuum packaging) help preserve enzymatic activity in muscle tissue, allowing for optimal flavor and tenderness development in beef during aging.

In the work of Chinese scientists to study the influence of different types of packaging (breathable film, vacuum, MAP (80 % O₂ + 20 % CO₂)) on protein oxidation, calpain activity, proteolysis desmin and protein solubility were studied in beef lumbar and semimembranosus muscles during aging (10 days) at 4 °C. The results showed that the inhibition of calpain activity in samples packed in permeable film and MAP is presumably closely related to protein oxidation, which further reduces the level of desminization in comparison with the vacuum packaging [54].

Enzymatic processes that take place in meat raw materials during aging are closely related to the formation or, vice versa, destruction of protein aggregates, which significantly affects the texture, tenderness and juiciness of semi-finished meat products. Thus, in the work of the Russian scientists [55], it was established that when storing meat in vacuum packaging a gradual formation of protein ag-

gregates occurs against the background of autolytic processes, which is enhanced by access to oxygen after opening the package. At the same time, it was established that in an atmosphere with high level of oxygen, aggregation of heavy myosin chains is observed, associated with oxidative cross-linking of proteins, which leads to a decrease of water-holding capacity, deterioration in consistency and increase meat toughness. Those processes are able to reduce the efficiency of proteolytic enzymes, including μ -calpain, and inhibit the development of the desired texture. The authors came to conclusion that packaging that provides low level of oxygen, especially in combination with vacuum, is preferable in terms of minimizing the undesirable aggregation and preservation of the endogenous enzyme systems activity that promote meat tenderization.

Conclusion

The analysis of domestic and foreign literature has shown that the problem of meat aging for many decades keeps being relevant for the industry producers. Biochemical processes that take place in muscle tissue being exposed to its own enzymes lead to changes in the sensory properties of the food product.

It has been defined that the modern types of packaging provide a significant impact on the enzymatic, microbiological and functional-technological characteristics of semi-finished meat products. This is particularly important in forming consumer-appealing parameters such as flavor, taste, and mouthfeel (texture).

The review of scientific publications showed the advantage of the “wet” aging (vacuum aging), as this method creates more favorable conditions for proteolysis which is responsible for the final rheological properties of the finished food product. At the same time, the “dry” aging method ensures brighter sensory profile, although with significant weight loss.

Meanwhile, use of MAP technology with various compositions of gas media is able to provide various effects on enzyme activity, color, WBC and sensory properties of semi-finished meat products.

In particular, high concentration of oxygen (80 % O₂) leads to bright red color manifestation, but provokes proteins and oxidation lipids, thus reducing the tenderness and juiciness of the meat product. In its turn, an oxygen-free medium (N₂ and CO/CO₂) inhibits the development of aerobic microorganisms and slows down oxidation processes.

The obtained results do not permit drawing clear conclusions about the absolute advantages of using one or another method of raw meat aging, and do not determine the most optimal choice of packaging method, as these issues still keep being relevant for the modern scientific research in the context of the active development of innovative technologies and new types of packaging materials.

REFERENCES

- Pandey, S., Kim, S., Kim, E. S., Keum, G. B., Doo, H., Kwak, J. et al. (2024). Exploring the multifaceted factors affecting pork meat quality. *Journal of Animal Science and Technology*, 66(5), 863–875. <https://doi.org/10.5187/jast.2024.e56>
- Sani, M.A., Zhang, W., Abedini, A., Khezerlu, F., Shariatifar, N., Assadpour, E. (2024). Intelligent packaging systems for the quality and safety monitoring of meat products: From lab scale to industrialization. *Food Control*, 160, Article 110359. <https://doi.org/10.1016/j.foodcont.2024.110359>
- Zharinov, A.I., Kuznetsova, O.V., Tekutyeva, L.A. (2023). Modern technology of sausage production. Vladivostok: Far Eastern State University, 2023. (In Russian)
- Anfemov, A.N., Lavrova, L.P., Manerberger, A.A., Mirkin, E. Yu. (1959). Meat and meat products technology. Moscow: Pischepromizdat, 1959. (In Russian)
- Meat industry. Encyclopedic dictionary. Moscow: V. M. Gorbato Federal Research Center for Food Systems, 2024. (In Russian)
- Kudryashov, L.S. (1992). Meat aging and salting. Kemerovo: Kuzbassuzdat, 1992. (In Russian)
- Solov'ev, V.I. (1966). Meat aging. Moscow: Food industry, 1966. (In Russian)
- Hammond, P. A., Chun, C. K., Wu, W., Welter, A. A., O'Quinn, T. G., Magnin-Bissel, G. et al. (2022). An investigation on the influence of various biochemical tenderness factors on eight different bovine muscles. *Meat and Muscle Biology*, 6(1), Article 13902. <https://doi.org/10.22175/mmb.13902>
- Lisitsyn, A.B., Lipatov, N.N., Kudryashov, L.S., Aleksakhina, V.A., Chernukha, I.M. (2004). Theory and Practice of Meat Processing. Moscow: V. M. Gorbato Meat Research Institute, 2004. (In Russian)
- Koulicoff, L.A., Chun, C.K.Y., Hammond, P.A., Jeneske, H., Magnin-Bissel, G., O'Quinn, T.G. et al. (2023). Structural changes in collagen and aggrecan during extended aging may improve beef tenderness. *Meat Science*, 201, Article 109172. <https://doi.org/10.1016/j.meatsci.2023.109172>
- Wang, L., Li, J., Teng, S., Zhang, W., Purslow, P.P., Zhang, R. (2022). Changes in collagen properties and cathepsin activity of beef *M. semitendinosus* by the application of ultrasound during post-mortem aging. *Meat Science*, 185, Article 108718. <https://doi.org/10.1016/j.meatsci.2021.108718>
- Dashdorj, D., Tripathi, V.K., Cho, S., Kim, Y., Hwang, I. (2016). Dry aging of beef: Review. *Journal of Animal Science and Technology*, 58, Article 20. <https://doi.org/10.1186/s40781-016-0101-9>
- Perry, N. (2012). Dry aging beef. *International Journal of Gastronomy and Food Science*, 1(1), 78–80. <https://doi.org/10.1016/j.ijgfs.2011.11.005>
- Hulánková, R., Kameník, J., Saláková, A., Závodský, D., Borilova, G. (2018). The effect of dry aging on instrumental, chemical and microbiological parameters of organic beef loin muscle. *LWT*, 89, 559–565. <https://doi.org/10.1016/j.lwt.2017.11.014>
- Gurinovich, G.V., Patrakova, I.S., Khrenov, V.A. (2022). Effect of dry maturation time and the curing composition on proteins in high quality beef. *Food Processing: Techniques and Technology*, 52(1), 98–107. <https://doi.org/10.21603/2074-9414-2022-1-98-107> (In Russian)
- Gurinovich, G.V., Patrakova, I.S., Khrenov, V.A., Patshina, M.A., Shevchenko, A.I. (2023). Effect of dry aging on beef muscle proteins. *Food Processing: Techniques and Technology*, 53(3), 621–629. <https://doi.org/10.21603/2074-9414-2023-3-2462> (In Russian)
- Gurinovich, G.V., Hrenov, V.A., Patrakova, I.S., Kudryashov, L.S. (2022). Study of the oxidative changes in beef upon dry aging. *Vsyo o Myase*, 5, 54–57. <https://doi.org/10.21323/2071-2499-2022-5-54-57> (In Russian)
- Berger, J., Kim, Y.H.B., Legako, J.F., Martini, S., Lee, J., Ebner, P. et al. (2018). Dry-aging improves meat quality attributes of grass-fed beef loins. *Meat Science*, 145, 285–291. <https://doi.org/10.1016/j.meatsci.2018.07.004>
- Kim, Y.H.B., Kemp, R., Samuelsson, L.M. (2016). Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. *Meat Science*, 111, 168–176. <https://doi.org/10.1016/j.meatsci.2015.09.008>
- Lee, C.W., Lee, J.R., Kim, M.K., Jo, C., Lee, K.H., You, I. et al. (2016). Quality improvement of pork loin by dry aging. *Korean Society for Food Science of Animal Resources*, 36(3), 369–376. <https://doi.org/10.5851/kosfa.2016.36.3.369>
- Kemp, C.M., Sensky, P.L., Bardsley, R.G., Buttery, P. J. Parr, T. (2010). Tenderness — An enzymatic view. *Meat Science*, 84(2), 248–256. <https://doi.org/10.1016/j.meatsci.2009.06.008>
- Khan, M.I., Jo, C., Tariq, M.R. (2015). Meat flavor precursors and factors influencing flavor precursors — A systematic review. *Meat Science*, 110, 278–284. <https://doi.org/10.1016/j.meatsci.2015.08.002>
- Kim, J. -H., Kim, J.-H., Yoon, D.-K., Ji, D.-S., Jang, H.-J., Lee, C.-H. (2018). A comparison of dry and wet aging on physicochemical and sensory characteristics of pork loin with two aging times. *Food Science and Biotechnology*, 27, 1551–1559. <https://doi.org/10.1007/s10068-018-0418-x>
- Zhang, R., Ross, A.B., Yoo, M.J.Y., Farouk, M.M. (2021). Metabolic fingerprinting of in-bag dry- and wet-aged lamb with rapid evaporative ionisation mass spectroscopy. *Food Chemistry*, 347, Article 128999. <https://doi.org/10.1016/j.foodchem.2020.128999>
- Yu, Q., Cooper, B., Sobreira, T., Kim, Y.H.B. (2021). Utilizing pork exudate metabolomics to reveal the impact of aging on meat quality. *Foods*, 10(3), Article 668. <https://doi.org/10.3390/foods10030668>
- Lee, Y.E., Lee, H.J., Kim, C.H., Ryu, S., Kim, Y., Jo, C. (2022). Effect of *Penicillium candidum* and *Penicillium nalgiovense* and their combination on the physicochemical and sensory quality of dry aged beef. *Food Microbiology*, 107, Article 104083. <https://doi.org/10.1016/j.fm.2022.104083>
- Ijaz, M., Jaspal, M.H., Hayat, Z., Yar, M.K., Badar, I.H., Ullah, S. et al. (2020). Effect of animal age, postmortem chilling rate, and aging time on meat quality attributes of water buffalo and humped cattle bulls. *Animal Science Journal*, 91, Article 13354. <https://doi.org/10.1111/asj.13354>
- Jeneske, H., Chun, C. K., Hene, S., Koulicoff, L. A., Aufdemberge, H., Vipham, J. L. et al. (2023) Evaluating the effect of accelerated aging at different temperature and time points on beef quality and enzyme activity of lower quality beef cuts. *Kansas Agricultural Experiment Station Research Reports*, 9(1), Article 19. <https://doi.org/10.4148/2378-5977.8429>
- Jeneske, H.J., Chun, C.K.Y., Koulicoff, L.A., Hene, S.R., Vipham, J., O'Quinn, T.G. et al. (2024). Effect of accelerated aging on shelf-stability, product loss, sensory and biochemical characteristics in 2 lower quality beef cuts. *Meat Science*, 213, Article 109513. <https://doi.org/10.1016/j.meatsci.2024.109513>

30. Colle, M.J., Doumit, M.E. (2017). Effect of extended aging on calpain-1 and -2 activity in beef longissimus lumborum and semimembranosus muscles. *Meat Science*, 131, 142–145. <https://doi.org/10.1016/j.meatsci.2017.05.014>
31. Gorbunova, N.A. (2012). Modern trends in beef aging researches. *Vsyo o Myase*, 6, 56–58. (In Russian)
32. Lepper-Bllie, A.N., Berg, E.P., Buchanan, D.S., Berg, P.T. (2016). Effects of post-mortem aging time and type of aging on palatability of low marbled beef loins. *Meat Science*, 112, 63–68. <https://doi.org/10.1016/j.meatsci.2015.10.017>
33. Botinestean, C., Gomez, C., Nian, Y., Auty, M.A.E., Kerry, J.P., Hamill, R.M. (2018). Possibilities for developing texture-modified beef steaks suitable for older consumers using fruit-derived proteolytic enzymes. *Journal of Texture Studies*, 49(3), 256–261. <https://doi.org/10.1111/jtxs.12305>
34. Semenova, A.A., Kuznetsova, T. G., Seliverstova, O. A., Salikova, M. N., Spirina, M. E., Bukhteeva, Yu. M. (2024). Improving the functional and technological properties of minced pork using a proteolytic enzyme. *Theory and Practice of Meat Processing*, 9(3), 212–219. <https://doi.org/10.21323/2414-438X-2024-9-3-212-219>
35. Abril, B., Bou, R., García-Pérez, J.V., Benedito, J. (2023). Role of enzymatic reactions in meat processing and use of emerging technologies for process intensification. *Foods*, 12(10), Article 1940. <https://doi.org/10.3390/foods12101940>
36. Akkaya, E., Cetin, O. (2024). Effects of aging methods combined with enzyme treatments on the quality parameters of beef striploins. *International Journal of Gastronomy and Food Science*, 38, Article 101056. <https://doi.org/10.1016/j.ijgfs.2024.101056>
37. Revutskaya, N.M., Nasonova, V.V., Mileenkova, E.V. (2018). Packaging of semi-finished products: key factors determining the stability of quality. *Vsyo o Myase*, 3, 20–23. <https://doi.org/10.21323/2071-2499-2018-3-20-23> (In Russian)
38. Semenova, A.A., Nasonova, V.V., Revutskaya, N.M., Trifonov, M.V. (2018). Achievement and future developments of polymer materials for meat and semi-finished products. *Food Processing: Techniques and Technology*, 48(3), 161–174. (In Russian)
39. Lisitsyn, A.B., Semenova, A.A., Kozyrev, I.V., Mittelshtein, I.M., Siniehkina, A.I. (2017). Forming the beef quality during aging. *Vsyo o Myase*, 5, 5–10. (In Russian)
40. Li, X., Babol, J., Bredie, W.L.P., Nielsen, B., Tománková, J., Lundström, K. (2014). A comparative study of beef quality after ageing longissimus muscle using a dry ageing bag, traditional dry ageing or vacuum package ageing. *Meat Science*, 97(4), 433–442. <https://doi.org/10.1016/j.meatsci.2014.03.014>
41. Xu, L., Liu, S., Cheng, Y., Qian, H. (2021). The effect of aging on beef taste, aroma and texture, and the role of microorganisms: A review. *Critical Reviews in Food Science and Nutrition*, 63(14), 2129–2140. <https://doi.org/10.1080/10408398.2021.1971156>
42. Charniauskaya, L., Gordynets, S., Napreenko, V., Kusonskaya, T., Yakhnovets, Zh. (2022). Studying the process of maturation of trimmed beef for the production of semi-finished chopped products with improved consumer properties. *Current Issues of Meat and Dairy Raw Materials Processing*, 17, 235–245. (In Russian)
43. Hernandez, S., Woerner, D.R., Brooks, J.C., Wheeler, T.L., Legako, J.F. (2023). Influence of aging temperature and duration on flavor and tenderness development of vacuum-packaged beef Longissimus. *Meat and Muscle Biology*, 7(1), Article 15710. <https://doi.org/10.22175/mmb.15710>
44. Redondo-Solano, M., Guzman-Saborio, P., Ramirez-Chavarria, F., Chaves, C., Quesada, Y.A., Araya, A. (2021). Effect of the type of packaging on the shelf life of ground rabbit meat. *Food Science and Technology International*, 28(9), Article 108201322110037. <https://doi.org/10.1177/10820132211003705>
45. Dos Santos-Donado, P.R., Donado-Pestana, C.M., Tanaka, F.A.O., Venturini, A.C., Delgado, E.F. et al. (2021). Effects of high-oxygen, carbon monoxide modified atmospheres and vacuum packaging on quality of *Longissimus thoracis et lumborum* steaks from Nelore cows during ageing. *Food Research International*, 143, Article 110226. <https://doi.org/10.1016/j.foodres.2021.110226>
46. McMillin, K.W. (2008). Where is MAP Going? A review and future potential of modified atmosphere packaging for meat. *Meat Science*, 80(1), 43–65. <https://doi.org/10.1016/j.meatsci.2008.05.028>
47. Huff-Lonergan, E., Lonergan, S.M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science*, 71(1), 194–204. <https://doi.org/10.1016/j.meatsci.2005.04.022>
48. Jaspal, M.H., Badar, I.H., Ghani, M.U., Ijaz, M., Yar, M.K., Manzoor, A. et al. (2022). Effect of packaging type and aging on the meat quality characteristics of water buffalo bulls. *Animals*, 12(2), Article 130. <https://doi.org/10.3390/ani12020130>
49. Fu, Q.-q., Liu, R., Zhang, W.-g., Li, Y.-p., Wang, J., Zhou, G.-h. (2015). Effects of different packaging systems on beef tenderness through protein modifications. *Food Bioprocess Technol*, 8, 580–588. <https://doi.org/10.1007/s11947-014-1426-3>
50. Li, S., Guo, X., Shen, Y., Pan, J., Dong, X. (2022). Effects of oxygen concentrations in modified atmosphere packaging on pork quality and protein oxidation. *Meat Science*, 189, Article 108826. <https://doi.org/10.1016/j.meatsci.2022.108826>
51. Vierck, K.R., Legako, J.F., Kim, J., Johnson, B.J., Brooks, J.C. (2020). Determination of package and muscle-type influence on proteolysis, beef-flavor-contributing free amino acids, final beef flavor, and tenderness. *Meat and Muscle Biology*, 4(1), Article 26. <https://doi.org/10.22175/mmb.10933>
52. Foraker, B.A., Gredell, D.A., Legako, J.F., Stevens, R.D., Tatum, J.D., Belk, K.E. et al. (2020). Flavor, tenderness, and related chemical changes of aged beef strip loins. *Meat and Muscle Biology*, 4(1), Article 28. <https://doi.org/10.22175/mmb.11115>
53. Moczowska, M., Półtorak, A., Montowska, M., Pospiech, E., Wierzbicka, A. (2017). The effect of the packaging system and storage time on myofibrillar protein degradation and oxidation process in relation to beef tenderness. *Meat Science*, 130, 7–15. <https://doi.org/10.1016/j.meatsci.2017.03.008>
54. Fu, Q.-q., Ge, Q.-f., Liu, R., Wang, H.-o., Zhou, G.-h. and Zhang, W.-g. (2017). Influence of modified atmosphere packaging on protein oxidation, calpain activation and desmin degradation of beef muscles. *Journal of the Science of Food and Agriculture*, 97(13), 4508–4514. <https://doi.org/10.1002/jsfa.8316>
55. Chernukha, I.M., Kovalev, L.I., Mashemtseva, N.A., Kovaleva, M.A., Vostrokhova, N.L. (May 14–16, 2018). Detection of protein aggregation markers in meat raw materials and finished products. Collection of the papers of the international symposium under the general editorship of A. Yu. Prosekov. Kemerovo, 2018. (In Russian)

AUTHOR INFORMATION

Natalia M. Revutskaya, Researcher, Department of Scientific, Applied and Technological Developments, V. M. Gorbатов Federal Research Center for Food Systems. 26, Talalikhin Str., Moscow, 109316, Russia. Tel.: +7–495–676–95–11 ext. 305,
E-mail: n.revuckaya@fncps.ru
ORCID: <https://orcid.org/0000-0002-8898-9305>

Elena V. Mileenkova, Senior Researcher, Department of Scientific, Applied and Technological Developments, V. M. Gorbатов Federal Research Center for Food Systems. 26, Talalikhin Str., Moscow, 109316, Russia. Tel.: +7–495–676–95–11 ext. 322,
E-mail: e.mileenkova@fncps.ru
ORCID: <https://orcid.org/0000-0001-5745-305X>
* corresponding author

Karina V. Sivtseva, Junior Researcher, Department of Scientific, Applied and Technological Developments, V. M. Gorbатов Federal Research Center for Food Systems. 26, Talalikhin Str., Moscow, 109316, Russia. Tel.: +7–495–676–95–11 ext. 131,
E-mail: k.bakaeva@fncps.ru
ORCID: <https://orcid.org/0000-0002-3444-1483>

All authors bear responsibility for the work and presented data.

All authors made an equal contribution to the work.

The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

The authors declare no conflict of interest.