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

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- 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;
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ANTIOXIDANT ACTIVITY AND COLOR OF BEEF JERKY WITH KLUWEK

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Keywords: *jerky, kluwek, antioxidant, L*a*b*, cooking loss*

Abstract
*Spoilage that often occurs in jerky is generally caused by the fat oxidation process, either during the manufacturing process, heating, or storage, and can lead to health hazards. This study was conducted to examine the potential of kluwak as a natural antioxidant that can reduce the oxidation process, by exploring its effects on antioxidant activity and physical properties, namely L*a*b* color values and cooking loss of jerky with the addition of kluwak. Kluwak, originating from community gardens in Soppeng Regency, Makassar, Indonesia, was used as an additional ingredient in making ground beef jerky. The part of meat used was the thigh, obtained from a slaughterhouse, and different levels of kluwak — namely 0%, 2%, 4%, and 6%—were added. The research results show that the addition of kluwak to jerky increases antioxidant activity, as indicated by higher values in the antioxidant activity test using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, which signifies increased antioxidant capacity. In addition, the use of kluwak also showed a significant effect on the color parameters a* and b*, indicating positive changes in the visual appearance of the product. However, no significant effect was found for the L* color parameter, which measures brightness, and no difference was observed in the cooking loss of the jerky. In conclusion, kluwak can function as an effective natural antioxidant in reducing fat oxidation in jerky, while providing positive changes in product color without affecting brightness or cooking loss.*

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Introduction

Beef is a highly nutritious food commodity with an average content of 77.65% water, 14.7% fat, and 18.26% protein. It is commonly used in ground form or various processed forms in food menus [1]. The role of meat is very important, especially in meeting the community's need for animal protein. Meat is a food product derived from livestock that is rich in nutrients but highly perishable. Meat spoilage can be caused by physical, chemical, or biological contamination [2]. The high water and protein content in meat makes it prone to spoilage, which reduces its usability and shelf life. To overcome this, preservation or processing methods are applied [3].

Preserving and processing meat into various products aims to minimize quality degradation and extend shelf life, while also adding value to the final products [4]. *Dendeng*, or Indonesian beef jerky, is a well-known processed beef product, both domestically and internationally. It is generally prepared with different spice blends, resulting in varying aromas and flavors [5]. *Dendeng* is produced using drying technology to reduce moisture content, thus making the product safe and inhibiting bacterial growth and reproduction [6]. *Dendeng* is classified as an Intermediate Moisture Food (IMF), typically with an aw range of 0.60–0.90 and a moisture content of 10–50% [7]. The maximum allowable water content for beef jerky is 12%. The use of sugar can help reduce the water content in beef jerky, inhibit microbial growth, and extend its shelf life [3].

Through the drying process, jerky can be stored longer due to its lower water content compared to fresh meat. Jerky is a sheet-shaped product made from fresh or frozen meat that is cut or ground, then seasoned and dried [8]. In addition, the process of making *dendeng* also considers the homogeneous distribution of spices, which is a key factor in enhancing shelf life [9]. Ground beef jerky is a meat product made from ground beef that is seasoned, shaped into thin sheets, and dried [4]. In the production of ground beef jerky, spice absorption is better compared to sliced jerky. The grinding process improves the taste and texture of the jerky as the added seasonings are more evenly distributed throughout the product [10]. However, ground jerky is more susceptible to oxidation during processing.

Processing meat into jerky increases the formation of malondialdehyde (MDA), a secondary product of lipid oxidation [11]. Spoilage caused by fat oxidation leads to a reduction in nutritional value and deterioration of flavor in the final product [8]. Lipid oxidation negatively affects meat quality by altering its sensory attributes and nutritional composition [12]. The oxidation process during processing and storage is influenced by fat content, myoglo-

bin, oxidative enzymes, heat, light, and water activity [13]. Fat oxidation in meat produces off-flavor compounds, especially aldehydes [14]. The oxidation of myoglobin to metmyoglobin also causes discoloration, turning the meat brown [15].

Free radicals formed during oxidation can damage meat molecules and accelerate spoilage. An imbalance between free radicals and antioxidants can cause oxidative stress, which may negatively impact health [15]. Free radicals generated during meat processing oxidize fatty acids, especially polyunsaturated fatty acids, through radical chain reactions [16]. The level of lipid oxidation in jerky can be reduced by adding ingredients or spices that contain antioxidants [8]. Antioxidants play a crucial role in preventing and treating various chronic diseases associated with oxidative stress [17]. Antioxidant compounds found in fruits and vegetables include vitamin C, vitamin E, β-carotene, and polyphenols [18]. Natural antioxidants in plants are generally phenolic or polyphenolic compounds, including flavonoids, cinnamic acid derivatives, coumarins, tocopherols, and polyfunctional organic acids [19].

Antioxidants can be derived from both natural and synthetic sources. Several studies have reported plant-derived bioactive compounds as natural antioxidants. Some natural sources of antioxidants include cloves, kluwak, cinnamon, cumin, and fennel seeds [20]. In addition to preventing rancidity, natural antioxidants in food also offer potential health benefits for consumers.

One of the traditional spices with antioxidant potential is kluwak (*Pangium edule* Reinw.). Kluwak contains flavonoids, vitamin C, iron ions, and β-carotene, all of which act as antioxidants [21]. Kluwak can serve not only as a flavor enhancer but also as a natural colorant. Color influences the quality and appeal of meat products, reflecting their biochemical, physiological, and technological status [22]. Food with visually appealing colors is more likely to attract consumers, making color an important indicator of food quality [23,24].

Kluwak fruit and seeds are commonly used in Indonesia as ingredients in ise' pangi and lope' pangi dishes, as well as in traditional cooking [25]. Kluwak is valued for imparting a blackish-brown color in cooking. The best time to use kluwak is when it is ripe, which can be identified by the sound produced when the seed is shaken [26]. The addition of kluwak to meat products such as beef sausages has been shown to increase antioxidant activity during storage [17]. However, there have been no previous reports on the use of kluwak seeds in jerky production. This gap forms the basis for the present study, which explores the use of kluwak (*Pangium edule* Reinw.) as an antioxidant and enhancer of the physical properties of jerky. Therefore, this study aims to determine the antioxidant activity and physical characteristics, including color (L*a*b*) and cooking loss, of jerky with the addition of kluwak at concentrations of 0%, 2%, 4%, and 6%.

Objects and methods

The objects of the study were beef samples obtained from the Manggala Slaughterhouse, Makassar City, South Sulawesi Province, Indonesia. Thigh cuts of Balinese cattle were immediately transported to the laboratory using a Modena MD20 A freezer (Modena, Italy) at a low temperature of 4 ± 1°C under strict aseptic conditions. Prior to processing, the meat was thawed in a refrigerator for 24 hours.

Kluwak was obtained from community gardens (Figure 1), and brown sugar was purchased from traditional markets in Soppeng Regency, Makassar City, South Sulawesi Province. The research samples were analyzed at the Meat and Egg Processing Technology Laboratory, Faculty of Animal Science, Hasanuddin University, Makassar.

Figure 1. Kluwak (primary data, taken from the kluwak tree garden in Soppeng district)

Other ingredients in this study, such as coriander, salt, pepper, garlic, and galangal, were obtained from the Tello Market in Makassar City, South Sulawesi Province, Indonesia. The materials tested included 0.01% butylated hydroxytoluene (BHT) as a synthetic antioxidant for comparison, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and methanol.

This research was conducted experimentally using a Completely Randomized Design (CRD), with four levels of kluwak treatment (P0: 0%, P1: 2%, P2: 4%, and P3: 6%) and an additional reference solution treatment (BHT) (P4), each with three replications. P0 was used as a control and P4 as a comparison for synthetic antioxidants.

To make fermented kluwak, kluwak seeds were washed first to remove dirt, then boiled for 1 hour, and then dried. The kluwak seeds were buried in the soil for 40 days [24]. After that, they were cleaned, and brownish kluwak seeds were obtained. Kluwak seeds were cracked, then the non-bitter kluwak flesh was taken by prying it from the shell, and the kluwak was ready to use. It was then mixed with other spices and applied to the ground beef.

The ground meat, processed using a meat grinder (Type TC-12C, Gea Getra, China) with a 6 mm plate hole size, was then weighed and grouped based on its treatment. After that, fine spices were added to 250 g of meat according to each treatment: 3% salt (7.5 g), 34% brown sugar (85 g), 2.5% coriander (6.25 g), 1.5% garlic (3.75 g), 0.3% galangal (0.75 g), 0.3% pepper (0.75 g), tamarind (0.25 g), and kluwak at 0%, 2%, 4%, 6% (0 g, 5 g, 10 g, 15 g) [15]. Beef jerky without the addition of kluwak (0%) was added with BHT

at 0.01% of the meat weight (250 g). The mixture was then evenly blended using a food processor (Braun, Germany) and stored for 24 hours in a refrigerator Model MD10 W (Modena, Italy) [27].

After that, the dough was molded using a 3 mm thick mold, and the jerky was dried using a mold made from acrylic to maintain the desired thickness as preferred by consumers in Makassar City, Indonesia. Then, it was dried using a food dehydrator (Getra, China) by air-drying (at a temperature of 70 °C for 4 hours) so that the outer layer of the meat dried first. Heating was continued (at a temperature of 70 °C for 2 hours) by rotating the tray so that the heat could be evenly distributed [8,28,29]. The dried jerky was cooled at room temperature in the oven and then analyzed.

Antioxidant activity analysis

Testing was carried out using the method used by [29]. The sample extraction ratio to methanol was 1:5 for homogenized and modified foods [30]. A total of 0.4 ml of beef jerky extract was reacted with 3.6 ml of DPPH (with a concentration of 0.1 mM). The mixture was then incubated at 37 °C for 30 minutes. Pure methanol was used as a reference material in the calibration of the SHIMADZU UV-1800 UV–VIS spectrophotometer (Shimadzu Corporation, Japan). The absorbance value of the solution was measured using a UV–Vis spectrophotometer at a wavelength of 517 nm [29]. The amount of the antioxidant activity was calculated using the formula:

$$\text{DPPH inhibition } (\%) = \\ = \frac{\text{Absorbance of DPPH-absorbance of sample}}{\text{Absorbance of DPPH}} \times 100\%. \quad (1)$$

*L*a*b* color of jerky*

The color testing of beef jerky was carried out using the CIE Lab method using a color reader or TES-135A Color Meter Color Analyzer Portable (TES Electrical Electronic Corp, Taiwan) and included L (brightness), a* (redness) and b* (yellowness) colors [31]. The "L" value indicates the brightness level from 0 to 100, with 0 indicating black and 100 indicating white. The "a" value reflects the red and green colors from –80 to 100. A negative "a" indicates green, a positive "a" indicates red. The "b" value indicates yellow and blue colors from –70 to +70. A negative "b" indicates yellow, a positive "a" indicates blue [31].

Cooking loss

Cooking loss is a major indicator of the nutritional value of meat and is related to the amount of water bound in the cells between muscle fibers [32]. To determine cooking loss of ground beef jerky cooked in the oven, meat samples were weighed before and after cooking. Cooking loss (CL) was calculated using the formula:

$$\text{CL } (\%) = \frac{W_1 - W_2}{W_1} \times 100\%, \quad (2)$$

where: W_1 = Weight of sample before cooking; W_2 = Weight of sample after cooking.

Statistical analysis

The data obtained from the research were analyzed by statistical data processing using the MS Excel and IBM SPSS Statistics 24 computer programs, the analysis of variance or ANOVA method. Analysis was continued with Tukey's advanced test with a 5% confidence interval or ($P < 0.05$) [32].

Results and discussion

Antioxidant activity

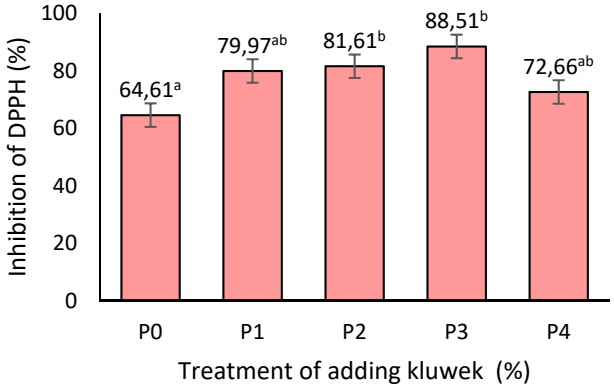

Figure 2. Bar chart of the average antioxidant activity of ground beef jerky with the addition of kluwak (P0: 0%, P1: 2%, P2: 4%, and P3: 6%) and addition of BHT reference solution (P4)

Note: Different superscripts in the figures indicate significant differences ($P < 0.05$).

As can be seen from the bar diagram above, the average antioxidant activity of beef jerky with the addition of kluwek (P1: 2%, P2: 4%, and P3: 6%) ranged from 79.97% to 88.51%, while it was 64.61% in beef jerky without the addition of kluwek (P0: 0%) and 72.66% in the samples with 0.01% BHT. These results show that there was an increase in antioxidant activity of 15.36% to 23.9%. This indicates that kluwek can increase the antioxidant activity of beef jerky. The data above demonstrate that the antioxidant levels followed this descending order: P3 (6% kluwek) > P2 (4% kluwek) > P1 (2% kluwek) > P4 (0.01% BHT) > P0 (control, without kluwek). Several previous studies also confirmed that the addition of kluwek can increase the antioxidant activity in meat products [17].

The results of the analysis of variance show that the addition of kluwak had a significant effect ($P < 0.05$) on the antioxidant activity of jerky. Further results from the Tukey test indicated significant differences in antioxidant activity. This is thought to be due to the beta-carotene, flavonoid, and vitamin C content in kluwak, which function as antioxidants [21]. Antioxidant compounds present in spices inhibit lipid oxidation reactions, thereby reducing the formation of malondialdehyde [11]. The antioxidant compounds in kluwak have the ability to capture free radicals, making them a potential alternative to synthetic antioxidants [33]. Therefore, treatment P3 (6% kluwak) produced the best effect compared to P2 (4% kluwak), P1 (2% kluwak), P4 (addition of BHT), and P0 (control, without kluwak). This occurs because kluwak contains antioxidants that can reduce the negative impact of oxidants in the body

by donating an electron to the oxidant compound, thereby reducing its activity [34]. Kluwak contains beta-carotene, which functions as an antioxidant by protecting and maintaining the integrity of cell membranes against free radicals, thus indirectly preventing lipid peroxidation in cell membranes [35].

Research on the antioxidant compounds in kluwak and processed meat products containing kluwak has been reported by several researchers. Kluwak contains alkaloids (2.69 ppm), tannins (16.0 ppm), flavonoids (1.23 ppm), and cyanide (122.7569 ppm) [36]. In addition, the antioxidant activity of beef sausage with kluwak fermented for 0 and 40 days was 32.43 ± 8.14% and 34.39 ± 6.94%, respectively, and the addition of kluwak at levels of 1%, 2%, and 3% was shown to increase antioxidant activity [17]. Other studies also suggest that kluwak can function as a natural antioxidant. For example, the addition of 4% kluwak seeds was found to help maintain the quality of patin fish after storage at room temperature for 4 days [20].

The results of this study, along with findings from several other studies mentioned above, show that processed meat products without the addition of kluwak still exhibit antioxidant activity derived from the ingredients and spices used in their preparation. In addition, kluwak, which is traditionally used as a spice in various dishes and herbal remedies, is also beneficial for boosting immunity, maintaining overall health, preventing cardiovascular disease, and functioning as a natural antioxidant [37].

Color

L Value (brightness)*

The results of the color measurement of jerky (Table 1) showed that the addition of kluwak did not have a significant effect ($P > 0.05$) on the L* value (brightness) of the jerky. The brightness values of jerky with the addition of kluwak ranged from 21.58 to 22.14, while the control sample without kluwak (0%) had a brightness value of 21.37. This indicates that the addition of kluwak slightly reduces the brightness of jerky, making the color appear darker or more blackish. The low brightness in jerky with kluwak is caused by the presence of tannin compounds in kluwak, which result in a darker product. This is supported by phytochemical test results, which showed that water and ethanol extracts of kluwak reacted with $FeCl_3$ to produce a blackish green color, due to the formation of complex compounds between tannins and $FeCl_3$ [22].

Additional ingredients in jerky also affect its color, such as brown sugar. The higher the sugar content, the lower the protein level, which influences the occurrence of the Maillard reaction (a reaction between amino acids and the carbonyl groups in sugar) and leads to the formation of a brown color [38]. Jerky is generally brown or blackish due to the Maillard reaction that occurs during the drying process [30]. The brown color may also be influenced by tannins, which are yellowish to light brown in color and, when added to processed meat products, can intensify the brown color, making it darker and more intense [39].

Color a (redness)*

The results of the measurement of the a* color value of dendeng (Table 1) showed that the a* value of dendeng with the addition of kluwak ranged from 4.89 to 7.33, while without the addition of kluwak (0%) it was 8.28. These results indicate that the addition of kluwak had a significant effect ($P < 0.05$) on the a* value of dendeng. As the concentration of kluwak increased, the a* value tended to decrease, although it remained within the reddish color range. This is due to the presence of flavonoid and tannin compounds in kluwak, where flavonoids contribute to reddish coloration and tannins produce a blackish green tone. In dye extraction from kluwak, a red, yellow, or orange color appearing in the amyl alcohol layer indicates the presence of flavonoid compounds, while the formation of a dark blue or blackish green color indicates the presence of tannins [26,40].

Kluwak seeds contain tannins and flavonoids, which can serve as natural alternatives to synthetic dyes such as chocolate brown FH (used in fashion products) and chocolate brown HT food (used in food products) [25]. Dendeng is generally light brown to dark brown in color due to the Maillard reaction, which involves a reaction between the carbonyl groups of reducing sugars and the amino groups of proteins and amino acids [39].

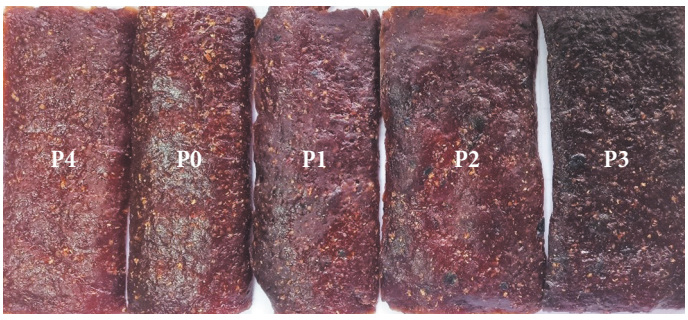

Figure 3. Ground beef jerky with added kluwek.
(Source: primary data (personal documentation, 2024)

Note: The color of jerky with the addition of kluwak (P0: 0%, P1: 2%, P2: 4%, P3: 6%, and P4: addition of BHT comparison solution).

Table 1. Color of ground beef jerky with the addition of kluwak

Parameter	P0	P1	P2	P3	P4
L*	21.37 ± 0.43	22.14 ± 1.12	21.79 ± 1.93	21.58 ± 0.74	22.10 ± 0.73
a*	8.28±1.72^{ab}	7.33±0.77^{ab}	5.86±0.89^{ab}	4.89±0.57^{a}	9.15 ± 2.18^{b}
b*	6.21±1.64^{b}	5,348±0.26^{ab}	3.98±0.93^{ab}	3.29±0.48^{a}	5.27±0.39^{ab}

Note: Different superscripts in the same column indicate significant differences ($P < 0.05$). L* (brightness) = 0 (black) – 100 (white); a* (redness) (a = 0 – 80 for red, a = 0 – (–80) for green); b* (yellowness) (b = 0 – 70 for yellow, b = 0 – (–70) for blue).

Color b (yellowness)*

The average b* value of jerky color with and without the addition of kluwak (0%) ranged from 3.29 to 6.21. The addition of kluwak had a significant effect ($P < 0.05$) on the b* color value of jerky (Table 1). The b* value decreased as the concentration of added kluwak increased. This is because kluwak contains more tannin compounds than flavonoids, where tannins play a role in producing a dark blue color, while flavonoids contribute to a yellow hue.

In general, jerky has a dark or dark brown color [41]. The yellow color in meat is caused by low levels of pigments such as myoglobin and hemoglobin. The amount of marbling fat in the meat also influences the yellowish color of stored meat due to the presence of beta-carotene [4].

Cooking loss

Table 2. Cooking loss of jerky with the addition of kluwak

Treatment	Cooking loss (%)
P0	55.38 ± 2.92
P1	56.06 ± 0.96
P2	55.44 ± 1.31
P3	54.62 ± 0.53
P4	55.38 ± 1.37

The addition of kluwak to jerky in amounts of 0–6% did not have a significant effect ($P > 0.05$) on cooking loss, which ranged between 54.62% and 56.06% (Table 2). The cooking loss of jerky with the addition of 6% kluwak tended to be lower. Low cooking loss in meat products can positively affect their quality. Meat with lower cooking loss is considered to have relatively better quality because fewer nutrients are lost during cooking [40,42].

The non-significant effect is likely due to the meat storage method during transportation, which used ice packs to maintain the meat's temperature [43].

Based on the research results, the relationship between cooking loss and beef pH shows that the more acidic the meat, the lower its cooking loss. Cooking loss refers to the loss of water and soluble nutrients, which are components that influence meat texture and tenderness. This loss typically ranges from 15% to 40% during cooking and significantly affects the eating quality of the meat [44]. Meat shrinkage can be influenced by several factors, including muscle fiber structure, cut length, meat weight, and cooking time [45]. Meat with a cooking loss percentage of less than 40% is considered to have better quality, as it retains more nutrients during cooking compared to meat with higher cooking loss percentages [46].

Conclusion

This study generally found that kluwak has oxidation resistance, functions as a natural antioxidant, and is effective in inhibiting oxidation in ground beef jerky products, which have a high risk of oxidation that can lead to product spoilage and pose potential health risks if consumed. The addition of kluwak to jerky has been shown to increase antioxidant activity, as indicated by higher antioxidant values in the antioxidant activity test using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, which signifies increased antioxidant capacity. In addition, the use of kluwak also showed a significant effect on the color parameters a* and b*, indicating positive changes in the visual appearance of the product. However, no significant effect was found for the color parameter L*, which measures brightness, and no differences were observed in the cooking loss during the jerky preparation process. Based on these findings, there is a need to promote the use of natural antioxidants such as kluwak in jerky products to maintain product quality and protect consumer health.

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METHODS OF DETECTING THE VETERINARY DRUGS RESIDUES AND THE WAYS OF REDUCING THEIR CONTENT IN FOOD PRODUCTS. REVIEW

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Abstract
The review was prepared in order to systematize the knowledge obtained in the recent years by the scientists from all over the world in the field of veterinary drugs application in the animal husbandry and the ways of management of their content in food products. It includes information on almost all currently known groups of antibiotics applied in animal husbandry, it considers the ways to reduce their amount in raw materials and / or the finished products, and describes the methods and procedures used to detect the residues of the veterinary drugs in raw materials and food products. It is not possible to run modern animal husbandry without the veterinary drugs. The global application of the veterinary drugs in animal husbandry exceeds 12 thousand tons, most of which are antibiotics for the infectious diseases treatment or prevention. Using the antibiotics in rearing the farm animals has led to the problem of their residues and / or metabolites in the raw materials and finished food products, which is why these food products cannot be considered safe for human consumption. The build-up of antibiotics in animal tissues depends on the group of the veterinary drug being used, and on the type of an animal. The content of residual amounts of some groups of antibiotics can be reduced by heat treatment of the meat. However, heat treatment can lead to the formation of new compounds that are potentially dangerous for the human health. Various analytical methods are used to determine the content of residual amounts of veterinary drugs in the food products, including enzyme immunoassay, chromatographic methods, biosensors and microbiological methods. The methods reviewed here for detecting the residual amounts of antibiotics in food products have their own advantages and disadvantages. In general, modern methods can currently detect the residues in food products of all known groups of antibiotics, used in animal husbandry, but it is necessary to keep on working on their improvement.

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Introduction

The application of veterinary drugs is an integral part of modern animal husbandry. Without the veterinary drugs the industry just would not produce the required volumes today. The research [1] conducted approximately 70 years ago put the start for the large-scale application of antibiotics. Since its beginning, this practice has become a backbone of animal husbandry, thus causing a transformational shift in the agricultural practices all over the world. The arising of antibiotic therapy has caused the dramatic increase in livestock quantity and a made revolution in animal nutrition. Growing global demand for animal protein has driven a shift to more intensive livestock production systems. These systems rely heavily on antimicrobial drugs designed to maintain animal health, to sustain relatively high levels of efficiency, and to ensure the economic viability of the industry. Thus, the historical progression of antibiotics application in animal nutrition underlines its key role in satisfying the growing needs of modern agriculture.

Every year around the world about 12 thousand tons of antibiotics are used in animal husbandry, about 75% of which are used to cure the infectious diseases, for preventive purposes or for growth stimulation [2]. It has led to numerous problems in the food production system, one of which is the residual amounts of antibiotics, as well as their possible metabolites. The issue of residual amounts of antibiotics is stated in almost all countries of the world. The degree of this issue depends on the legislation of the country and the prohibition to use of certain veterinary drugs, valid in its territory. However, no matter how strict the legislation is on application of antibiotics, no country is able solve the issue of residual amounts of antibiotics in the food products. And the antibiotics are also detected in almost all types of food products of animal origin: antibiotics are found in milk, meat of productive animals and

poultry, eggs. The worst-case scenario (with antibiotic residues from animal products and microbial resistance genes) is assumed in many low-income and middle-income countries where there is either little or no relevant health regulation and supervision, and where there is disordered marketing and intense use of veterinary antimicrobials [3].

EU ban on using the antibiotic growth promoters due to microbial resistance genes became a turning point in the livestock industry. Since then, other countries have followed this example, including China that did the same in 2020. This is caused with the positive correlation between the intensity of antibiotics application and all the associated consequences of antibiotics use in animal products and their circulation the environment. Despite this ban, many farmers in many countries keep on using antibiotics, often in breach of any rules, regulations and permissions for their use. The excessive using of antibiotics in animal husbandry is the reason of the issue of antibiotic residues in the animal products, which is often impossible to get rid off completely. The nutritional value of animal products plays a key role in maintaining human health and well-being. But application of veterinary drugs can lead to antibiotics traces in the food products, which can negatively affect the consumers' health.

The authors set several purposes in this review of the scientific literature devoted to the methods for detecting residues of veterinary drugs, which purposes are caused by several important factors.

First, control of veterinary drug residues in food is critical to ensure its safety for the consumers. Veterinary drugs can be used to treat animals and improve their productivity. However, some of these drugs substances may persist in meat, milk, eggs, and other animal products even after slaughter or harvesting. These residues may pose a threat to human health via allergic reactions, toxic effects, or other adverse actions.

Second, food preparation methods can significantly affect the concentration and the form of veterinary drug residues. For example, cooking is able to decompose some compounds, while other processing methods, such as marinating or smoking, can change the chemical composition of the drug contained. The understanding of the way that various cooking processes affect veterinary drug residues is essential to develop efficient methods of food quality assurance.

Third, there is necessity to develop new and improved analytical methods for detection and quantification of veterinary drugs residues. The traditional analytical approaches pretty often require expensive equipment and highly qualified personnel, which demands limit their application in everyday practice. The development of the simpler, faster and more cost-saving methods will improve monitoring of food safety and will reduce risks for the consumers. Thus, the review of literature on this topic will contribute to a better understanding of the existing challenges and opportunities encountered in the field of monitoring of veterinary drug residue in food, and will help to define the directions for further research and development in this important area.

Objects and methods

In order to understand the scope of the issue and to define the ways to cope with it, an extensive analysis of peer-reviewed scientific papers written in English, Arabic, Turkish and Spanish was conducted. The articles selected from the initial search were manually screened by their abstracts and by extracting information from the full text. Initial search key words included "antibiotic residues", "antibiotics and poultry", "antibiotic residues and milk", "antibiotics and animal products", "antibiotics in ruminants" and "antibiotic residues effects". Scopus, Science Direct, Google Scholar, Academia, ResearchGate and Wiley Online were used to search for literature.

Groups of antibiotics used in animal husbandry

Antibiotics belong to several classes: β-lactams, cephalosporins, chloramphenicol, sulfonamides, macrolides, aminoglycosides, quinolones, fluoroquinolones, lincosamides, tetracyclines (Figure 1). The important β-lactam antibiotics include ampicillin, penicillin G, cloxacillin,

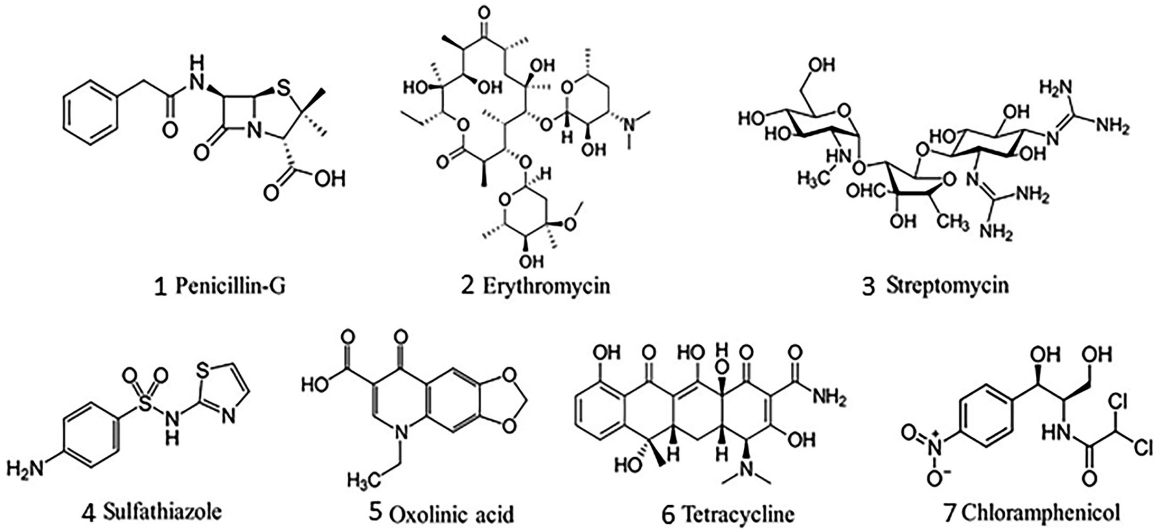

Figure 1. Chemical structure of the typical antibiotics from various families: 1 — penicillin G from β-lactams, 2 — erythromycin from macrolides, 3 — streptomycin from aminoglycosides, 4 — sulfathiazole from sulfonamides, 5 — oxolinic acid from quinolones, 6 — tetracycline from tetracyclines and 7 — chloramphenicol from amphenicols

dicloxacillin, and cephalexin. The variety of quinolones have been approved for their application in animal husbandry, including ciprofloxacin, enrofloxacin, marbofloxacin, danofloxacin, difloxacin, sarafloxacin, flumequine, norfloxacin, flumequine, oxalic acid and oxolinic acid [4].

Other antibiotics, like oxytetracycline, chlortetracycline, gentamicin, neomycin, streptomycin, sulfadimethoxine, erythromycin and bacitracin, amoxicillin, sulfamethazine, are approved for use in poultry, swine, ruminants and pseudoruminants, and can be administered orally (via water or feed), by injection or transfusion.

One more issue related to the use of antibiotics is the frequent use of broad-spectrum drugs, which include several antibacterial components. They are wide-spread in the developing countries and are available in various forms such as liquids, powders, packaged in sachets, plastic bottles, and glass bottles. Despite the various brand names, the commercial antibiotics are based on common active agents, and their formula consists of a combination of several antibiotics (Table 1).

Table 1. Antibiotics/antibiotic groups used in livestock farms

Group	Antibiotic
Quinolones	Ciprofloxacin, Enrofloxacin, Marbofloxacin, Danofloxacin, Difloxacin, Sarafloxacin, Norfloxacin, Flumequin,
Penicillins	Amoxicillin and Penicillin G
Cephalosporins	Cephalexin, Ceftiofur
Tetracyclines	Doxycycline, Oxytetracycline, Chlortetracycline, Tetracycline
Aminoglycosides	Streptomycin, Gentamicin, Amikacin
Macrolides	Tylosin, Erythromycin
Sulfonamides	Sulfadiazine, trimethoprim-sulfamethoxazole
Lincosamides	Lincomycin
Levomycetin	Chloramphenicol
Bacitracin	In the form of zinc bacitracin

Distribution of antibiotics in the animal products

According to the analyzed literature, the antibiotics distribute unevenly in animal tissues and, accordingly, in the animal products, which is explained by the animal's metabolism. This fact influences the subsequent use of obtained raw materials for the food production, as well as it influences the food processing methods. The distribution of antibiotics can vary significantly among various components of animal products. Depending on the group of antibiotics, their "migration" among the animal tissues, their accumulation and excretion occurs differently. For example, a study [5] showed that the amount of oxytetracycline and tetracycline found in the sheep kidneys was greater in comparison with their amount found in the sheep liver and muscles. The analysis of the residual content of amoxicillin and tylosin showed the similar differences between muscle tissue, liver and kidneys [6]. It was found that liver and kidneys contain a large volume of antibiotic residues in comparison with other organs, which is logical, since liver and kidneys belong to the excretory organs. The residual amount of veterinary drugs also greatly depends on the animal species and on the duration of the rearing period. However, the antibiotics of the same group accumulate in various parts of the muscle tissue of the same animal species [6]. However, antibiotics accumulate to the greatest extent in the internal organs of the animal, not in the muscle tissue. The philic or phobic nature of antibiotics can influence their distribution in various areas of the muscles, liver and kidneys due to the different metabolic roles of these organs. For example, Yang et al. [7] when analyzing poultry found higher levels of antibiotics in chicken giblets than in chicken meat and eggs. Khattab et al [8] demonstrated that the percentage of egg whites samples positive for amoxicillin residues was higher than the percentage of egg yolks samples.

The content of antibiotics in animal tissues depends on the specific type of antibiotics administered and their clearance rate, which is influenced by the factors like the interval between the drug administration and the animal slaughter. The prevalence of antibiotic residues varies among livestock species and is formed by regional practices of antibiotic applications [9]. It is necessary to note, that broilers were found to feature the higher rates of antibiotic residues detection than the farm egg-layers and the locally-raised chickens [10]. Understanding the route of drug administration (oral, intramuscular, parenteral, intramammary) and drug concentration is crucial to interpreting antibiotic residue levels in the animal products [11]. Differences in antibiotic levels among the livestock species may also be related with the antibiotics properties and the animals' physiology or structure. For example, Huong et al. al. [12] found high levels of tetracycline residues in chicken, and sulfonamide residues in pork. The lipophilic nature of sulfonamides may result in higher retention in pork in comparison with chicken, thus indicating the influence of animal developmental processes on elimination of antibiotics.

Data on content of veterinary drugs residues in products of animal origin depending on the type of production.

The literature review showed that meat sold in the suburban shops contains more antibiotic residues than meat sold in city stores [13,14]. The study [15] found that pork from animals slaughtered in city slaughterhouses contains less veterinary drug residues than pork obtained from the local slaughterhouses. In their study, Zhang et al. al. [16] found that total antibiotics concentration in pasture-raised cattle and sheep was very low.

Although only a few cases of acute toxicity in humans caused by the antibiotic residues have been documented [17], it is essential to raise awareness of the potential risks related with the consumption of food products that contain antibiotics. The average consumer is generally unaware of the dangers of antibiotic residues contained in food products. However, the absorption of antibiotics from food products and their accumulation in the human

body may pose a threat to human health [18]. The diseases caused by exposure to toxic substances, including drug residues, are classified as the diseases of toxicological origin [19]. Antibiotic residues may serve as potential toxicological agents, the exposure to which may be potentially dangerous for humans, especially for the children. Their presence in animal products may cause pathogens resistance to the effect of antimicrobial drugs [20]. Long-term consumption of the food products with excessive residues of veterinary drugs may lead to chronic poisoning, as well as teratogenic, carcinogenic and mutagenic aftermaths. Food products contaminated with antibiotic residues are able to cause bone marrow dysfunction, disrupt intestinal flora, and cause skin allergies to sulfonamides when found in high concentrations in the food. It was found that the products of decomposition resulting from heat treatment of the antibiotics like oxytetracycline and ceftiofur provide cytotoxic effects on human lung, liver, and kidney cells [21].

Ways for reducing veterinary drugs residual amounts in the food products

The absence of antibiotics in a food product is one of the main indicators of its safety. As it was already noted, the issue of the content of veterinary drug residues in food products is of concern for the governments of most countries worldwide. In the developed countries, this issue is addressed for safety and public health reasons, but in many low-income and middle-income countries, food safety issues are addressed for reasons of economic benefit, because the unsafe food products cannot be sold on international markets [22]. During the production of food, various methods of processing raw materials are used, which provide different effects onto all groups of antibiotics. In most cases, the technological process leads to the significant reducing of antibiotics content in the finished food product, which makes it safer for consumption. Table 2 below sums up the results of studies devoted to analyzing the effect of certain technological processes on the concentration of antibiotics in the food products.

Heat treatment

The various processing steps during cheese production (pressing, salting, boiling of cheeses, whey acidification) and various types of heat treatment like pasteurization (72 °C for 15 sec and 63 °C for 30 min) resulted in a 52% to 99% reduction in enrofloxacin. In general enrofloxacin is very sensitive to high temperatures and is prone to strong decomposition. For example, frying and grilling reduced oxytetracycline content by 91–95%, whereas the reducing of enrofloxacin residues was lower, ranging from 25.6% to 33.3% within the same methods of heating [23]. Temperature, method and time of cooking play an important role in the reducing of antibiotic residues. Slow cooking of broiler carcasses led to significant reducing of tetracycline antibiotic concentrations, reaching 86–89%. This ensures that the meat becomes safe for human consumption [24]. Long cooking times at low temperatures, such as braising / simmering, are considered to be an efficient method for reducing antibiotic residues in chicken meat before its consumption. Microwave cooking for 1 minute has proven to reduce tetracycline residues in pork by up to 67% [25]. However, not only the method of heat treatment affects the "behavior" of the antibiotic during heat treatment of the raw materials; the type of animal also matters [27]. In addition, the decomposition of veterinary drug residues is also affected by the pre-heat treatment of raw materials; for example, a greater degree of antibiotic decomposition is observed during heat treatment of minced meat in comparison with heat treatment of whole raw materials [28].

Table 2. Brief review of ways of reducing antibiotics content by the food products processing.

Way of processing	Product	Achieved result	Antibiotics	Sources
Heat treatment/ Pasteurization	Dairy products	Various processing steps (pressing, salting, boiling cheeses, whey acidification) and pasteurization (72 °C for 15 sec and 63 °C for 30 min) resulted in a 52–99% reduction in the amount of the veterinary drug	Enrofloxacin	[6]
Boiling and frying	Meat and offal	Boiling reduces the amount of veterinary drug residues in muscle tissue and has greatly reduced the residual amount in the liver.	Oxytetracycline	[23]
Frying and grilling	Meat	Frying and grilling reduced oxytetracycline levels by 91–95%, while reductions in enrofloxacin residues were less expressed, ranging from 25.6% to 33.3% under the same heating conditions.	Oxytetracycline; Enrofloxacin	[23]
Long-term heat treatment	Broiler chicken meat	Braising / simmering reduces the content of tetracycline antibiotics by 86–89% in broiler carcasses.	Tetracycline	[24]
Microwave irradiation	Pork	Microwaving for 1 minute reduces tetracycline residues in pork by 67%.	Tetracycline	[25]
Freezing	Meat	Freezing at –10 °C for 9 days provided no profound effect on the concentration of antibiotic residues.	Oxytetracycline	[23]
	Meat	Chilling meat at 4 °C for 3 days resulted into a small reduction in antibiotic residues of approximately 16%, whereas freezing meat at –18 °C for six weeks had minimal effect.		[23]
Food processing	Milk	Drug content increases 5 times in cottage cheese in comparison with the original raw material, while only a small amount of drug is lost in the whey	Monesin	[26]
Food processing	Milk	Milk skimming reduced enrofloxacin content by 95%	Enrofloxacin	[6]

Notwithstanding many studies that demonstrate the benefits of heat treatment for reducing antibiotic residues in animal products, it is important to note that heat treatment is able not only to decompose antibiotics, but also to form the new compounds on their bas, or to convert them into another form. Side-products of veterinary drug decomposition are able to provide a detrimental effect on the human body, like it does oxytetracycline decomposition product — 4-epioxytetracycline, or the ceftriofur decomposition product — cephalhyde, which products provide cytotoxic effect on lung, liver and kidney cells. Veterinary drug decomposition products can also react with food components. For example, the products of ciprofloxacin decomposition, formed as a result of heat treatment, react with lactose during heat treatment of milk. Planche (2022) showed that sulfamethoxazole lost its antimicrobial activity by 45%, but six sulfamethoxazole decomposition products were found in the cooked meat [29].

In relation to that, heat treatment of raw materials is not a panacea in the issue of reducing the veterinary drugs content. For sure, in general it is wrong to use raw materials with antibiotics for its further processing, and in many countries of the world this is prohibited, as, for example, in the Russian Federation the presence of antibiotics in food raw materials is not allowed according to the TR CU 021/2011[1]. But in countries where the legislation is still not so strict in relation to the veterinary drugs content, it is important to choose the right modes of processing raw materials, as it will reduce the amount of antibiotics in the finished product, but at the same time it will not lead to the formation of other compounds that can be potentially dangerous for the human health.

Methods for detecting antibiotic residues in food products

To ensure food safety, the regulatory authorities have set maximum residues levels (MRLs) for various medicines.

In the Russian Federation, MRLs for the content of veterinary drugs in food products are defined in the TR CU 021/2011[1]. To conduct testing of products, first of all it is necessary to use the methods presented in the List enclosed to the TR CU 021/2011[2]. This List contains methods for detecting the antibiotics based on HPLC–MS/MS and ELISA [30] methods. It is also necessary to note that previously the content of veterinary drugs in meat products was regulated by the TR CU 034/2013[3], but according to the adopted Decision of the EEC No. 70[4] all groups and classes of the antibiotics previously regulated in the TR CU 034/2013[3] have been included into the TR CU 021/2011[1].

In global practice antibiotics in food products are detected by various analytical methods, like chromatography and by biosensors. These methods allow qualitative and quantitative controlling of the veterinary drugs content in almost any food matrix. It should also be noted that other methods, such as electrophoresis [31], Raman spectroscopy [32], and voltammetric methods [33] are also applicable for detecting the antibiotics in meat tissues, but their use for this purpose is not as widespread. Biosensors are used as a screening methodology for detecting the antibiotics animal products [34]. Biosensors are mainly used to detect the presence of antibiotics in milk and honey, but nevertheless, some published articles devoted to using the biosensors have shown the possibility of their using for the antibiotic's detection in meat.

Figure 2 shows a diagram of the analytical methods, used in analyzing the presence of veterinary drug residues in the food products, and also lists their advantages and disadvantages.

Iimmunoassay methods

One of the types of enzyme immunoassay used to detecrmine the content of veterinary drug residues in food products is the indirect enzyme-linked immunosorbent assay (ic-ELISA). The difference between the direct and indirect ELISA method is that in direct ELISA an enzyme-conjugated primary detection antibody is added that binds to the antigen coating the well, while in indirect ELISA, after immobilization of the antigen, a primary antibody is added that binds to the antigen, followed by the addition of an enzyme-conjugated secondary antibody. Direct ELISA is a faster diagnostic method, but it cannot amplify signals, which inability results to poor sensitivity. The amplification step in indirect ELISA is able to increase sensitivity, so ic-ELISA can be more sensitive than direct ELISA. In [35], ic-ELISA was used to detect the presence of residues of quinoxaline-based antimicrobials. Quinoxaline is a semi-finished product widely used for the production of pharmaceuticals, it posesses anti-inflammatory, antimalarial, and antibacterial action. When using quinoxaline-based preparations, a large amount of its deoxymetabolites with toxic properties may remain in the raw material. As a result of the work, the limits of detection (LOD), limits of quantification (LOQ), and recovery rates of quinoxalines determined with the help of ic-ELISA for analyzing pork, pork liver, pork kidneys, chicken meat, and chicken liver

[1] TR CU 021/2011. "Technical Regulations of the Customs Union "On food safety" (as amended as of July 14, 2021)" Retrieved from https://docs.cntd.ru/document/902320560#8Q20M0. Accessed December 4, 2024 (In Russian)

[2] EFES (2019). List of international and regional (interstate) standards, and in case of their absence — the national (state) standards containing rules and methods of analyzing (testing) and measuring, including rules for sampling, necessary for the application and implementation of the requirements of the technical regulations of the Customs Union and the implementation of technical regulation objects conformity assessment. Approved by the Decision of the Board of the Eurasian Economic Commission of December 24, 2019 N 236. Retrieved from https://www.gostinfo.ru/trts/List/45 Accessed on December 4, 2024 (In Russian)

[3] TR CU034/2013 Technical Regulations of the Customs Union "On the safety of meat and meat products" Retrieved from http://docs.cntd.ru/document/499050564. Accessed on December 4, 2024 (In Russian)

[4] EAEU (2023). "On Amending Certain Decisions of the Customs Union Commission and the Council of the Eurasian Economic Commission" Decision No. 70 of 23.06.2023. Retrieved from https://docs.eaeunion.org/documents/418/7522/ Accessed on December 4, 2024 (In Russian)

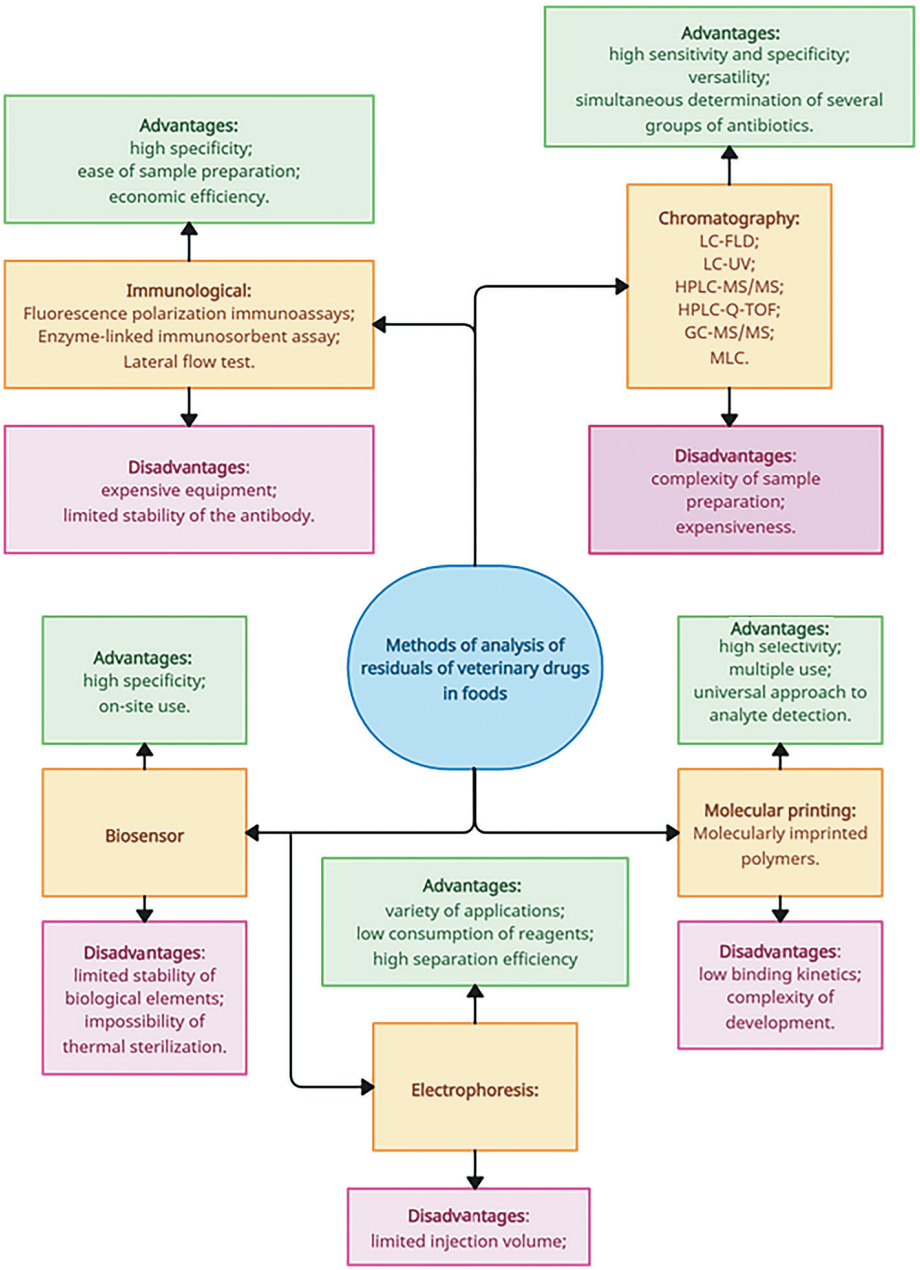

Figure 2. The most widely used methods for analyzing the content of veterinary drug residues in food (created with app.creately.com)

were 0.48–0.58 μg/kg, 0.61–0.90 μg/kg, and 73.7–107.8%, respectively. These results show that the ic-ELISA-based technique can effectively detect quinoxaline residues. The simplicity of this analytical method reduces the time required for the sample pre-treatment, improves efficiency, and complies with the requirements for quinoxaline residue analysis. This is the difference between the immunochemical methods and instrumental methods of detection like HPLC or LC–MS/MS, which require expensive equipment, complex technology and long process of testing.

Another immunoassay for detecting the presence of veterinary drugs residues in food products is fluorescence polarization immunoassay (FPIA). FPIA has gained wide application in laboratory practice due to its high sensitivity, good reliability and fast implementation [36]. The basic principle of FPIA for the small molecules is the interaction between a tracer (a chemical conjugate of an antigen with a fluorophore) and the competing antigens. As the concentration of analytes in the reaction solution grows up, the analytes occupy the antibody binding sites, thus preventing the binding of the tracer to the antibody, which leads to the formation of free tracer molecules. When the reaction mixture is irradiated with plane-polarized light, the presence of such free molecules is demonstrated as fluorescence depolarization. In view of this, the FPIA method is promising for detecting various low-molecular compounds, including veterinary drugs in food products. For example, this method was used to determine erythromycin in dairy products [37]. Extraction of erythromycin from milk for FPIA is quite simple and consists in protein precipitation with organic solvents. The LOD of erythromycin was 14.08 μg/L, and the detection rate was 96.08–107.77%.

Another type of immunoassay, the immunochromatographic assay (ICA) (or lateral flow test), due to its execution simplicity and portability of the necessary equipment, has also found wide application in the management of

veterinary drugs residues in the areas like biomedicine, agriculture, and the food industry [38].

Moreover, test systems based on the ICA method using gold nanoparticles for the ultra-sensitive detection of antibiotic residues are now being actively developed.

Chromatographic methods

Probably the chromatographic methods are the most frequently used methods for determining the content of veterinary drugs residues in food products. Despite the active growth in popularity of the other methods (ELISA, biosensors, etc.) due to their simplicity and high speed of their use, chromatographic methods still remain the arbitration technique in tasks of determining the content of antibiotics in products, i. e. in the cases when the antibiotics were already detected by some other methods, the obtained results get confirmation from chromatographic methods.

Today, there are methods for determining antibiotics content in food products via chromatography for almost all groups of antimicrobial drugs (quinolones, sulfonamides, nitrofurans and their metabolites, penicillins, amphenicols, etc.) in all types of food products of animal origin. In the Russian Federation the majority of these methods have passed the state standardization procedure.

From among all currently existing chromatographic methods, the most preferable one for testing food products for presence of veterinary drugs residues is the method HPLC–MS/MS. This method is used because of its high sensitivity, while the multiple reaction monitoring (MRM) technology eliminates false positive or false negative results. The HPLC–MS/MS equipment itself is quite expensive, but is not a rarity anymore. Instrumental settings for determining antibiotics are not very complicated and often allow determining several groups of antibiotics in one test run, moreover the tests do not require some rare and/or specific reagents. The biggest disadvantage of determining antibiotics with the help of HPLC–MS/MS is, perhaps, the process of the sample preparation. In majority of cases, it is necessary to use solid-phase extraction to extract the necessary analytes from the sample, which action significantly increases the total time of one analysis and increases its final cost [39].

Biosensors

The concept of biosensors is the combination of a biological component with a physicochemical detector. Biosensors are user-friendly and economically advantageous means of detecting the antibiotics residues due to their low cost [40]. Enzymes, antibodies, nucleic acids or whole cells that can react with the analyte of interest can be used as a biological component (or bioreceptor) in biosensors, and the physicochemical detector, in its turn, "reads" this reaction and converts it into a measured signal [41]. Today, the biosensor techniques are actively developing with involvement of more complex devices based on sensing principles, like piezoelectric biosensors, optical biosensors, molecularly imprinted polymer biosensors, fluorescent biosensors or electrochemical biosensors due to their high speed, sensitivity and selectivity [42].

Today one of the most promising biosensor methods is the analysis based on using the biochips. To detect veterinary drugs in this way, a set of micro matrices arranged on a solid substrate is used, which allows for multiple tests to be conducted simultaneously. Analysis using biochips is focused on the precise recognition of analyte binding to biological receptors on an ordered substrate, which allows for quantitative or semi-quantitative recording of the content of the corresponding analyte. Analysis based on the use of biochips allows simultaneous monitoring of significant number of several tens of analytes [43].

The paper [44] provides a review of widely used biosensors for testing food products for antibiotics residues. It reviews various types of sensors that involve enzymes, antibodies and nanobodies, aptamers, DNAzymes, molecularly imprinted polymers (MIPs) and cells as selective antibiotic-binding reagents. Various groups of nanostructures including carbon-based nanomaterials, metallic nanomaterials, quantum dots, luminescent upconversion nanoparticles (UCNPs), and magnetic nanoparticles integrated into biosensor detection platforms are discussed, as well as different detection methods including optical methods (colorimetry, fluorescence, chemiluminescence, and surface plasmon resonance). The authors review the pluses and minuses of each type of biosensors. Fluorescent biosensors, for example, can serve an excellent option for quantitative and semi-quantitative detection due to their high sensitivity and reproducibility. Using nanomaterials such as UCNPs, sensitivity at the pg/mL level has been achieved. The minus is that to read the result a fluorometer is needed. Another option is colorimetric biosensors with fairly high sensitivity. The sensitivity of colorimetric biosensors can be further increased by signals amplifying and by using DNAzymes, but the duration of analysis can be significantly increased. In general, most authors who are involved in the use of biosensors for analyzing food products for residual antibiotic content agree that it is necessary to carry out work to improve their performance. Improvement of the electrode material, metal nanoparticles, metal oxides, and carbon nanostructures for creating electrochemical biosensors will significantly increase sensitivity and reduce analysis time. In general, the use of nanomaterials improves the characteristics of almost all types of biosensors. Nanomaterials are able to improve optical and magnetic properties in optical biosensors, thus providing higher sensitivity and accuracy of detection [44]. Due to their high stability in various reaction environments, long shelf life, ease of synthesis, low production costs, and *in vitro* development, MIPs are also the promising tools for their use in biosensors. Yue et al. [45] reviewed the latest developments and using the aptamer-based sensors, oligonucleotide receptor molecules, for detection of

aminoglycoside antibiotics. They selected nine methods of signals detection used in aptamer biosensors for detection of aminoglycosides: optical fluorescence, colorimetric, chemiluminescence, surface-enhanced Raman scattering, electrochemical impedance, voltammetric, potentiometric, electrochemiluminescent and photoelectrochemical. The authors came to the conclusion that each aptasensor has its own distinctive properties and that the choice of the instrument for detection of aminoglycosides depends on certain conditions and purposes. It is important to note that aptomer-based sensors have been accepted for their application for detection of other veterinary drugs and pesticides as well [46,47].

Microbiological methods

Microbiological methods are usually used as the initial screening tools for the qualitative or semi-quantitative detection of veterinary drug residues. Microbiological methods are based on the microbial growth inhibition and the use of receptor molecules that bind antibiotics [48–50]. Microbial inhibition test detects drug residues based on their ability to inhibit the growth of microorganisms. For example, the strain of *Escherichia coli* was used to screen the fluoroquinolones and quinolones residues in the animal products [48,49].

Discussion

The main part of the modern food production is based on the processing of the ingredients purchased from various suppliers. It is necessary to inspect and control pretty long list of safety parameters in the raw materials delivered for the production, including the content of veterinary drugs residues in raw materials of animal origin. But not all manufacturers, especially the small-scale ones, have the opportunity and the necessary funds to transport raw materials for testing to some third-party laboratories, and even more so to maintain their own laboratory for the purposes of incoming and production control. In this regard, control methods that can be used "on site" will be promising and attractive, providing the opportunity to at least qualitatively determine the presence of standardized substances in raw materials.

However, there are only a few methods for the simultaneous extraction and detection of antibiotics residues of various classes in animal products due to their unique physicochemical properties. One of the potential reasons that put obstacles for the integrated detection is the difficulty of simultaneous extraction of several antibiotics. Although antibiotics have different structures, still they feature certain common physicochemical properties, like polarity and solubility [51]. Therefore, the development of a unified pretreatment using the common properties of these antibiotics is a promising direction to overcome this limitation. Low acceptable levels of antibiotics in animal products require high-throughput methods of detection with high sensitivity. Over the past few decades, many analytical methods have been developed for antibiotics detection, including instrumental analysis methods, microbiological methods, immunoassays, etc. [32]. Highly sensitive instrumental analysis methods, like HPLC–MS/MS (which is the basis for almost all methods included in the List of methods in the TR CU 021/2011[5]), are not suitable for continuous monitoring of the samples within production due to the high cost of equipment, the complexity of sample preparation and the need for professional personnel. Therefore, the development of highly effective and sensitive methods for monitoring the antibiotics residues of various classes in the products of animal origin is very important.

Over the recent time the developing of rapid methods based on immunochromatography for the purpose of detecting veterinary drugs in food products has gained particular popularity. However, in the same List of methods in the TR CU 021/2011[5], there are practically no express analysis methods. Partially, the methods based on the ELISA method can be attributed to the express determination of antibiotics. The List contains about 30 such methods. Basically, they use the test systems RIDASCREEN and MaxSignal. Nowadays their use is a big challenge due to western sanctions imposed onto the Russian Federation due to the absence of official representations or headquarters, the withdrawal of the manufacturers from the Russian market and the extension of delivery terms and, accordingly, prices increase. Only one method included in the List is related to the express testing, but its scope of application applies only to milk — MVI.MN 5930-2018[6] and is applied till the relevant interstate standard is included into the list of standards.

Conclusion

Summing up the conducted review, it can be concluded that at present the field of control of residual quantities of veterinary drugs in food products is very well developed. Almost all groups of antibiotics used today in the animal husbandry are supported with a methodological basis for their detecting in raw materials and finished food products. Moreover, almost any group of antibiotics can be determined using various methods. The further development of this field is necessary for the development and improvement of already existing methods and techniques, for example, in the development of a unique method for the samples preparation, with the help of which it will be possible to extract absolutely all known groups of antibiotics for HPLC, thus increasing the sensitivity of immunoanalytical methods or reducing the time of analysis with the help of biosensors.

[5] TR CU 021/2011. "Technical Regulations of the Customs Union On food safety (as amended as of July 14, 2021)" Retrieved from https://docs.cntd.ru/document/902320560#8Q20M0. Accessed on December 4, 2024 (In Russian)

[6] MVI.MN 5930–2018 "Methodology for measuring the content of lincomycin in dairy products using test systems manufactured by Beijing Kwinbon Biotechnology Co., Ltd, China". Certificate of Attestation No. 1086/2018 dated 03.01.2018 (In Russian)

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ANTIOXIDANT EFFECT OF NIGELLA OIL ON HEATED CAMEL AND RABBIT MEAT

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Keywords: *heat stress, oxidative stress, meat, rabbit, camel, Morocco*

Abstract
The exposure of meat to heating (cooking) or cold storage could alter its chemical composition. In this work, the oxidizing effect of heating on rabbit and camel meat, and the protective effect of nigella oil (NO) were evaluated and compared. Samples of rabbit and camel meat were treated with increasing doses of NO, and then were heated until reaching an internal temperature of 80 °C. Water loss, thiobarbituric acid reactive substances (TBARS) and carbonyls levels, as well as activities of catalase (CAT), glutathione peroxidase (GSHPx) and superoxide dismutase (SOD), were analyzed at different meat storage times. The results showed that heating of camel and rabbit meat induced a significant increase in the water loss, and TBARS and carbonyls levels, associated with a significant decrease in the activities of CAT, GSHPx and SOD. In both species, TBARS and carbonyls in the meat samples treated with NO before heating were significantly ($P < 0.05$) lower than those in the untreated samples, and decreased more and more when the NO dose increased. In the samples treated with NO, all enzymatic activities were significantly ($P < 0.05$) higher than those observed in the untreated samples, and increased more and more with increasing NO dose. The findings reveal a potential antioxidant power of NO preserving the organoleptic composition of heated camel and rabbit meat.

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Introduction

Meats are an excellent source of nutritional and bioactive compounds for the consumer [1]. While health benefits are the main factor influencing consumer demand for all meat products available on the market, dromedary [2,3] and rabbit [4] meat have excellent nutritional and dietary features, characterized by high protein and essential amino acid content, low fat and cholesterol content, and high B vitamin content. It is clear that meat processing by cooking and cold storage sometimes leads to a deterioration of its nutritional quality. Therefore, scientists are increasingly interested in the evaluation of nutritional losses caused by meat product processing methods. It is mainly lipids and proteins that are affected by both cold storage [5] and heat treatment [6,7], which could compromise health properties due to newly formed oxidation compounds. In combination with oxygen, free radicals generated during oxidative stress (OS) generally trigger oxidative chain reactions, which mainly target lipids, pigments, proteins, and vitamins of meat and meat products, altering their organoleptic composition and quality parameters such as color, flavor, tenderness, and water-holding capacity [8]. In addition, the antioxidant stability of meat is impaired due to the reduced activity of endogenous enzymes such as catalase (CAT), glutathione peroxidase (GSHPx) and superoxide dismutase (SOD), which play an important role in the elimination of reactive oxygen species, causing lipid and protein degradation. Most of the processes affecting the activity of these enzymes and organoleptic composition occur through heat and/or low-temperature storage of camel [9,10] and rabbit meat [4]. Therefore, researchers are moving towards the use of natural antioxidants to prevent meat spoilage due to OS during heat treatment and cold storage [11,12]. These antioxidants are able to minimize lipid oxidation [13] and meat rancidity, thus preserving its organoleptic and nutritional quality, increasing its acceptability, and reducing the development of unpleasant odors in meat products [14,15]. Natural antioxidants of plant origin, such as fruits, vegetables, herbs, spices and seeds, scavenge free radicals and inhibit oxidative reactions, including lipid and protein oxidation in meat and meat products, which allows the preservation of their quality, extension of their shelf life, improvement of their stability and maintenance of their organoleptic characteristics [16,17]. As an aromatic plant with multiple biological activities, one could cite the example of nigella (*Nigella sativa* L.) (NS) or black cumin, which is an annual herbaceous plant belonging to the *Ranunculaceae* family. The seeds of this plant have

extraordinary and promising curative properties allowing a wide spectrum of medicinal applications, such as antioxidant, immunostimulant and antitumor [14,18]. NS seeds contain phytochemicals such as thymoquinone, α-pinene, carvacrol, p-cymene, β-pinene, α-thujene and longifolene. These compounds have biological properties that make them exploitable by researchers for their application in food and drug manufacturing [18]. The objective of our investigation was to evaluate and compare the oxidizing effect of heating on meat of rabbits and camels, and the protective effect of nigella seeds oil against this thermal stress, by analysis of water loss upon heating, thiobarbituric acid reactive substances (TBARS) and carbonyl contents, and enzymatic activities of CAT, GSHPx and SOD.

Objects and methods

Study site and animals

The study was carried out on five healthy male camels (*Camelus dromedarius*) from the municipal slaughterhouse of Casablanca, Morocco (2 to 3 years, 170 to 260 kg) and five male *New Zealand* rabbit (1.5 to 2 kg) carcasses purchased from a market located in the same city. Casablanca is located in the west of Morocco (North Africa, latitude 33° 34′ 42″ N, longitude 7° 36′ 24″ W). The animals lived in similar conditions, were exposed to the same conditions before slaughter and were fed, respectively, barley concentrate and dry hay straw. They were deprived of water and food, transported and carefully unloaded upon arrival. They were calmly handled and then slaughtered using the halal method without any stunning. The slaughter and then all handling of the carcasses were carried out according to common traditional manual practice. No ethical approval was obtained because this study only involved non-invasive procedures in the form of meat samples collection.

Muscle sampling and treatment

Skinning, evisceration and then cutting of the camel and rabbit carcasses were carried out four hours after slaughter. A portion of the long dorsal muscle (longissimus or *Longissimus dorsi*) on the right side of the carcass of each animal was collected in a sterile plastic bag, using a sharp knife. The muscles were transported for 10 to 15 minutes in a cooler at a temperature of 4 ± 1 °C, from the slaughterhouse to the Physiopathology and Molecular Genetics laboratory in Ben M'Sik Faculty of Sciences, Casablanca (Morocco), and all external fat and connective tissue were removed. The meat portion of each animal was ground two times at a temperature of 4 °C through a meat grinder plate with 6 mm holes. Each minced portion of about 200 g was divided into five patties (P) (numbered P_1 to P_5) of about 30 ± 5 g in weight and 6 ± 1 cm in diameter. The patties were treated as follows (Table 1):

- P_1 (control 1) and P_2 (control 2) were untreated with NO.
- P_3, P_4 and P_5 were treated with 0.5 mL NO.100 g^{-1}; 1 mL NO.100 g^{-1} and 2 mL NO.100 g^{-1} of meat, respectively.

These patties were separately mixed with NO by hand protected by a latex glove.

All the patties were immediately cooled and stored at a temperature of 4 °C until 72 hours after slaughter. At the end of this time, P_2, P_3, P_4 and P_5 were heated to reach a temperature of 80 °C and then were immediately cooled on ice. All patties were stored separately in plastic bags in a freezer at a temperature of –80 °C until the analysis of water loss, TBARS, carbonyls, CAT, GSHPx and SOD (Table 1).

Meat samples heating

In the absence of ingredients, the meat patties were packed separately in polyethylene bags and then completely immersed in a water bath preheated to 100 °C for 10 min, until reaching a temperature of 80 °C. The temperatures were monitored using a Digital Kitchen Cooking Thermometer (model Alla France, Reference 91000AF003) with the food-grade stainless steel percing probe and a measurable temperature range from –50 to +300 °C (accuracy: ±1 °C).

Table 1. Experimental Design

Patties	NO (mL.100 g^{-1})	Internal temperature	Parameters analyzed
P_1 (control 1)	0 (Untreated)	Unheated	Water loss, TBARS, carbonyls, CAT, GSHPx and SOD
P_2 (control 2)	0 (Untreated)	80 °C	
P_3	0.5		
P_4	1		
P_5	2		

TBARS: thiobarbituric acid reactive substances, CAT: catalase, GSHPx: glutathione peroxidase, SO: superoxide dismutase.

Determination of water loss during heating

The water loss during heating (heating loss: HL) was calculated as the percentage of the weight of the heated samples compared to the weight of the unheated ones, using the method of Honikel [19]. After heating, each sample was removed and dried with a paper towel. HL was calculated as the difference in the sample weight before and after heating, and expressed as a percentage of the initial sample weight:

$$HL\ (\%) = [(\text{initial sample weight} - \text{final weight}) / \text{initial weight}] \times 100. \quad (1)$$

Determination of values of thiobarbituric acid reactive substances

Lipid oxidation of raw and heated minced meat was estimated by the thiobarbituric acid reactive substances (TBARS) assay using the method described by Botsoglou et al. [20] with slight modifications. Briefly, 3 g of meat samples were homogenized at 37,000 x g for 1 min, with 20 mL of ultrapure water. After adding 5 mL of 25% trichloroacetic acid, the homogenate was centrifuged (SIGMA centrifuge, model 2–16 K, Germany) at a temperature of 4 °C for 15 min at 10,000 x g, and then the supernatant was filtered. In a test tube, 3.5 mL of the extract solution was added to 1.5 mL of 0.6% aqueous 2-thiobarbituric acid, and

the solution was kept in a water bath at a temperature of 70 °C for 30 min, then cooled in tap water for 10 min. The absorbance was measured at 532 nm (spectrophotometer JENWAY6320D visible range, JENWAY, GB) to calculate the TBARS value, and the results were expressed as nmoles of malondialdehyde/mg of proteins.

Estimation of carbonyl content

Protein oxidation was assessed by the absorbance of protein carbonyl groups at 370 nm (spectrophotometer JENWAY6320D visible range, JENWAY, GB), using the molar extinction coefficient of the hydrazone (22000 $L.M^{-1}cm^{-1}$) formed after derivatization of the carbonyl groups with 2,4-dinitrophenyl hydrazine (DNPH) [21]. The carbonyl content was expressed as nmole of incorporated DNPH/mg protein. Protein concentration was calculated by measuring the absorbance at 280 nm (UV-visible spectrophotometer, model UVILINE9400, Ref: SOC-UVILINE9400, France) and comparing it with that obtained in the case of bovine serum proteins used as standards.

Analysis of catalase activity

An enzymatic fraction was prepared from 2 g of sample homogenized in 6 ml of ice-cold phosphate buffer (100 mM, pH 7.4) using a homogenizer IKA model T18 Digital Ultra-Turrax (IKA-Werke GmbH & Co. KG, Germany) at 13,000 rpm for 1 minute. The homogenate obtained was centrifuged at 2,000 rpm for 30 minutes (SIGMA centrifuge, model 2–16 K, Germany) at a temperature of 4 °C. CAT activity was analyzed in the final supernatant. This activity was continuously monitored by the decomposition of hydrogen peroxide (H_2O_2) into H_2O and O_2, using the method of Sinha [22]. Fifty microliters of meat extract was placed in a UV cuvette with 2.9 ml of H_2O_2 solution (11 mM H_2O_2 in 50 mM phosphate buffer) and the absorbance kinetics at 240 nm were monitored at 1-s intervals for 3 minutes (UV-visible spectrophotometer, model UVILINE9400, Ref: SOC-UVILINE9400, France). The same phosphate buffer was used to prepare a blank. CAT activity was calculated using the molar extinction coefficient of H_2O_2 (39.5 $L.M^{-1}cm^{-1}$) in U/g, with U corresponding to the amount of enzyme required to decompose 1 µmol of H_2O_2 per minute, and was expressed in µmol of H_2O_2/min/mg of protein.

Analysis of superoxide dismutase activity

Total SOD activity was quantified according to the method of Paoletti et al. [23]. The procedure is based on the oxidation of NADH in the presence of superoxide anions generated by EDTA, $MnCl_2$ and β-mercaptoethanol. As SOD transforms superoxide anions into hydrogen peroxide and the oxidation of NADH is itself linked to the availability of these anions in the medium, then, as soon as SOD is added to the reaction mixture it inhibits the oxidation of the nucleotide. Therefore, at high concentrations of the enzyme, the absorbance at 340 nm (spectrophotometer JENWAY6320D visible range, JENWAY, GB) remains stable, however, in the control (not added to SOD) it decreases. Twenty microliters of meat extract was placed in a UV tank with 167 µl of TDB (100 mM triethanolamine buffer, 100 mM diethanolamine, pH 7.4), 5 µl of 100 mM EDTA/50 mM $MnCl_2$ pH 7 and 8 µl of 7.5 mM NADH. The decrease in absorbance at 340 nm was monitored in kinetic mode during each 42-second cycle for 21 minutes. One unit (1 U) of SOD activity was defined as the amount of enzyme required to inhibit the NADPH oxidation rate of the control by 50%. Enzyme activity was calculated in µmol/min/mg protein.

Analysis of glutathione peroxidase activity

GSHPx activity was determined according to the method described by Chen et al. [24]. Meat extract was obtained from 5g samples homogenized in ice-cold phosphate buffer (50 mM, pH 7) using a homogenizer IKA model T18 Digital Ultra-Turrax (IKA-Werke GmbH & Co. KG, Germany) at 13,000 rpm for 1 minute. Sample tubes were always kept in an ice-water bath during homogenization. After centrifugation at 2,800 g for 20 minutes at a temperature of 4 °C, supernatants were collected in microtubes and centrifuged again (10,000 g, 10 minutes, a temperature of 4 °C) and stored at –80 °C until analysis. Twenty microliters of meat extract was placed in a UV cuvette with 80 µl of 50 mM phosphate buffer, 500 µl of the assay medium (100 mM potassium phosphate buffer, pH 7, 1 mM EDTA, 2 mM NaN_3), 100 µl of glutathione reductase (2.4 U/ml), 100 µl of 10 mM L-glutathione, and 100 µl of NADPH (1.5 mM NADPH in 0.1% $NaHCO_3$). After 1 min, 100 µl of 1.5 mM H_2O_2 was added and the kinetics of absorbance at 340 nm (spectrophotometer JENWAY6320D visible range, JENWAY, GB) were monitored at 20-s intervals for 2 min and compared to a blank consisting of the phosphate buffer. GSHPx activity was calculated using the molar extinction coefficient of NADPH (6,220 $L.M^{-1}cm^{-1}$) and expressed as units (U)/g protein, with U corresponding to the amount of enzyme required to oxidize 1 µmol NADPH/min.

Statistical analysis

For each of the analyzed parameters of each animal, measurements were performed in duplicate on five animals and SD values were therefore calculated on ten values. The results are presented as mean ± standard deviation (SD). The effect of two factors, namely nigella oil as a natural antioxidant and storage duration (0, 1, 3 and 6 days), was analyzed by ANOVA (analysis of variance) followed by Tukey's post-hoc test on GraphPad Prism 8. A P value < 0.05 was considered statistically significant.

Results

Water loss

Camel and rabbit meat samples pretreated with NO at a dose of 2 $mL.100\ g^{-1}$ and then heated to reach an internal temperature of 80 °C showed a significant decrease ($P < 0.05$) in water loss upon heating (%), compared to their controls (untreated with NO) (26.23 ± 2.62 vs. 33.26 ± 3.32

and 22.53 ± 2.44 vs. 29.67 ± 3.21, respectively) (Figure 1). Water losses of untreated and NO-treated samples for all doses showed no significant variation between camel and rabbit meat (Figure 1).

Thiobarbituric acid reactive substances and carbonyls

After heating untreated samples of the camel and rabbit meat until reaching an internal temperature of 80 °C, the contents of TBARS (nmol malondialdehyde/mg protein) and carbonyls [nmol (2,4-dinitrophényl)-hydrazine/mg protein] as indicators of peroxidation of lipids and proteins, respectively, increased significantly ($P < 0.05$) compared to unheated ones (9.12 ± 0.65 vs 3.87 ± 0.12 and 7.62 ± 0.54 vs 3.67 ± 0.22, respectively, in the camel meat, and 6.78 ± 0.54 vs 2.35 ± 0.11 and 5.65 ± 0.46 vs 2.85 ± 0.18, respectively, in the rabbit meat) (Figure 2). In both species, heated meat samples showed significantly ($P < 0.05$) lower levels of TBARS and carbonyls when pretreated with NO compared to untreated ones. Thus, at 1 mL NO.100g^{-1}, these levels were 6.81 ± 0.55 vs 9.12 ± 0.65 and 5.55 ± 0.51 vs 7.62 ± 0.54, respectively, in the camel meat, and 4.85 ± 0.35 vs 6.78 ± 0.54 and 4.48 ± 0.37 vs 5.65 ± 0.46, respectively, in the rabbit meat (Figure 2). In addition, these contents were significantly ($P < 0.05$) lower in heated samples pretreated with 2 mL NO.100g^{-1} than those measured in heated samples pretreated with 1 mL NO.100g^{-1} (Figure 2). Furthermore, TBARS and carbonyl contents in the untreated and unheated samples, and the NO-treated and heated ones were significantly ($P < 0.05$) higher in the camel meat than those observed in the rabbit meat (Figure 2).

Activity of enzymatic antioxidants

In the camel and rabbit meat, heating of untreated meat samples to reach 80 °C decreased significantly ($P < 0.05$) the antioxidant enzymatic activities of CAT (μmoles of H_2O_2/min/mg of proteins), GSHPx (GSHPx) (U/g of proteins) and SOD (μmoles/min/mg of proteins), compared to unheated samples (3.52 ± 0.53 vs 7.48 ± 0.65, 1.81 ± 0.22 vs 2.81 ± 0.32 and 3.54 ± 0.17 vs 5.24 ± 0.23, respectively, in the camel meat, and 4.06 ± 0.57 vs 9.11 ± 0.71, 1.52 ± 0.13 vs 2.12 ± 0.31 and 3.51 ± 0.18 vs 6.26 ± 0.21, respectively, in the rabbit meat) (Figure 3). Heated samples of the camel and rabbit meat, showed significantly ($P < 0.05$) higher activities of CAT, GSHPx and SOD when pretreated with NO compared to untreated ones. Thus, at 1 mL NO.100g^{-1}, these activities were 5.65 ± 0.46 vs 3.52 ± 0.53, 2.58 ± 0.16 vs 1.81 ± 0.22 and 4.78 ± 0.18 vs 3.54 ± 0.17, respectively, in the camel meat, and 5.76 ± 0.45 vs 4.06 ± 0.57, 2.52 ± 0.14 vs 1.52 ± 0.13 and 4.76 ± 0.21 vs 3.51 ± 0.18, respectively, in the rabbit meat (Figure 3).

The results showed that the enzyme activities of CAT, GSHPx and SOD were significantly ($P < 0.05$) higher in the heated samples pretreated with 2 mL NO.100g^{-1} than those measured in the heated samples pretreated with 1 mL NO.100g^{-1} (Figure 3). In addition, these activities in the untreated and unheated samples, and the NO-treated and heated ones, were significantly ($P < 0.05$) lower in the camel meat than those observed in the rabbit meat (Figure 3).

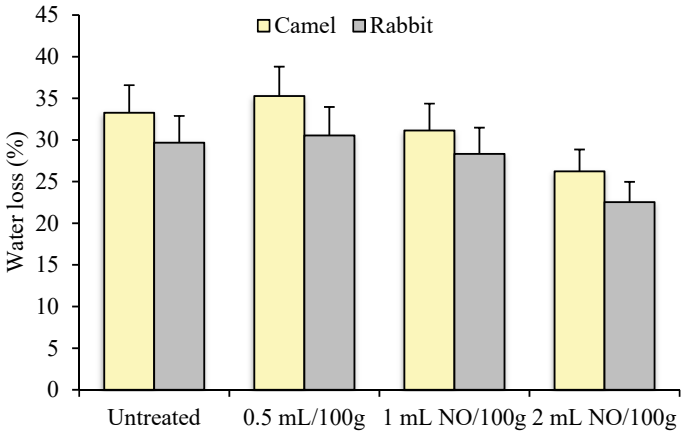

Figure 1. Values of water loss (%) in camel and rabbit meat pretreated with nigella oil (NO) at different doses, then heated until reaching an internal temperature of 80 °C. (Means ± SD, measurements were carried out in duplicate on five animals and SD values were therefore calculated on ten values)

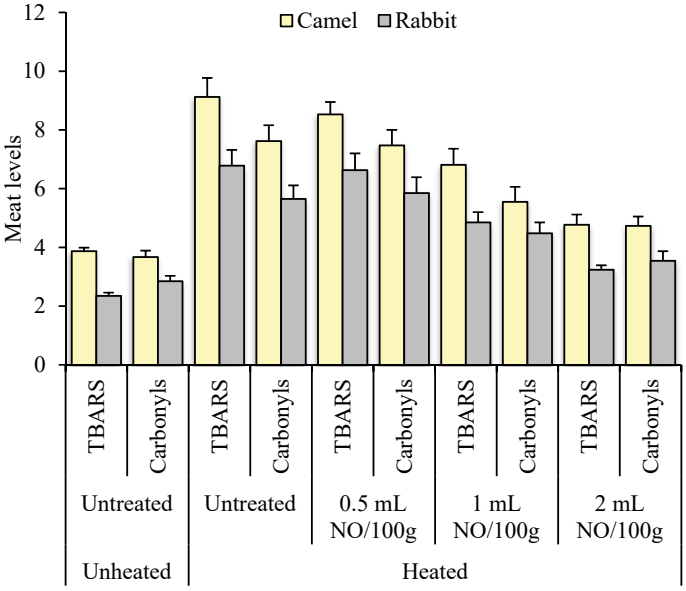

Figure 2. Values of thiobarbituric acid reactive substances (TBARS) (nmoles of malondialdehyde/mg of proteins) and carbonyls [nmol (2,4-dinitrophényl)-hydrazine/mg protein] contents in camel and rabbit meat pretreated with nigella oil (NO) at different doses, then heated until reaching an internal temperature of 80 °C. (Means ± SD, measurements were carried out in duplicate on five animals and SD values were therefore calculated on ten values)

Discussion

In the present study, heating of camel and rabbit meat induced a significant increase in the amounts of water loss, MDA and carbonyls, associated with a significant decrease in the activities of CAT, GSHPx and SOD. However, the effect of heating on oxidative stability was more pronounced in the camel meat. In both species, the treatment of meat with NO before heating, reduced significantly these levels and increased significantly these enzymatic activities in a dose dependent manner.

Water losses of camel and rabbit meat observed during heating up to 80 °C are consistent with those reported by Purslow et al. [25] for beef. These losses appear to be mainly due to thermal denaturation of some of the myosin and actin molecules at 70–80 °C, and may be accompanied by

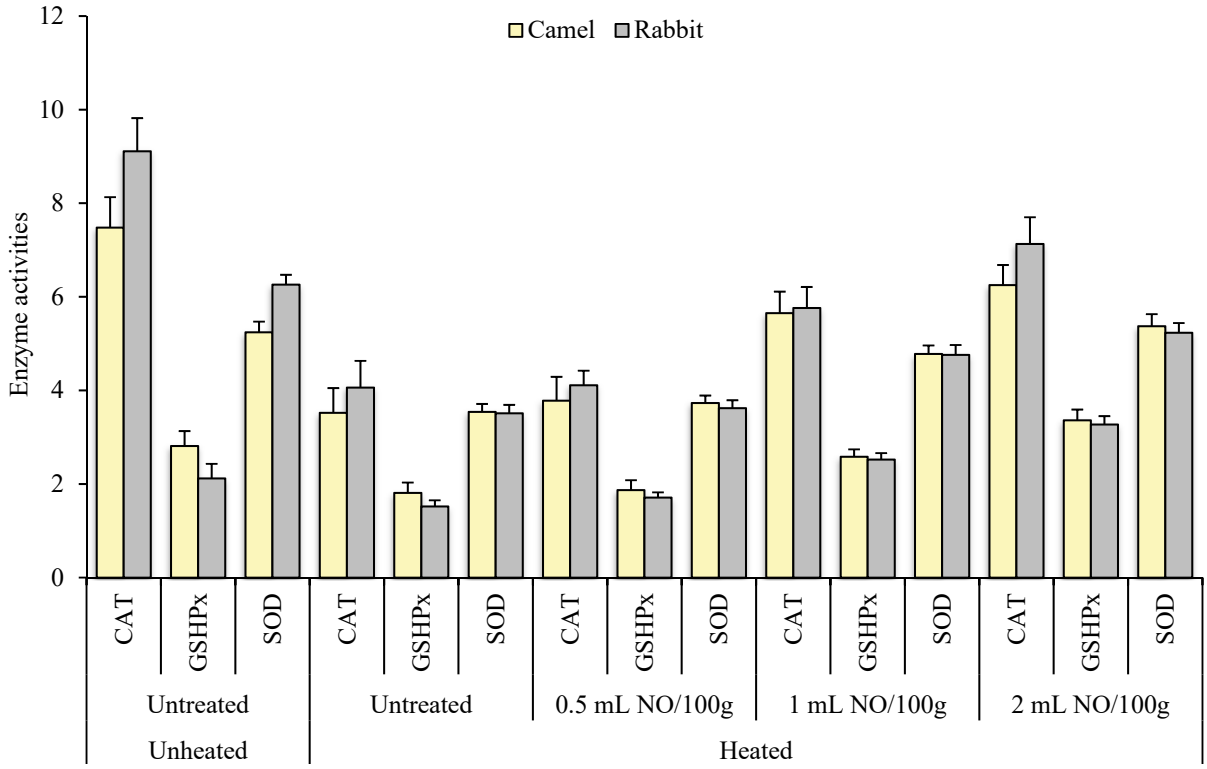

Figure 3. Activity of catalase (CAT) (μmoles of H_2O_2/min/mg of proteins), glutathione peroxidase (GSHPx) (U/g of proteins) and superoxide dismutase (SOD) (μmoles/min/mg of proteins) in camel and rabbit meat pretreated with nigella oil (NO) at different doses, then heated until reaching an internal temperature of 80 °C. (Means ± SD, measurements were performed in duplicate on five animals and SD values were therefore calculated on ten values) (1 GSHPx unit is the amount of enzyme to oxidize 1 μmol NADPH/min)

leakage of lipids, peptides and other nutrients, resulting in a decrease in meat quality [25]. Furthermore, water losses are largely dependent on the temperature and duration of heat exposure, and are dominated by longitudinal shrinkage for long sarcomeres and transverse shrinkage for short sarcomeres [25]. Haghighi et al. [26] studied the impact of protein denaturation and muscle tissue contraction during heating resulting in rapid loss of juice and moisture from meat, and the authors found that values of these parameters were closely related to temperature and not to cooking time.

In the present work, exposure of meat to heat resulted in OS revealed by high TBARS and carbonyls contents similar to what was reported for beef [27], camel [6,28,29], rabbit [30] and other species [31]. These effects could be promoted by the production of free radicals and pro-oxidant compounds, and the destruction of endogenous enzymatic antioxidants present in the muscle, thus accelerating the exposure of the lipid [28,30] and protein [27] fractions to oxidative attack and disruption of cell membranes. In their study, Shen et al. [27] examined the effect of increasing the cooking temperature up to 72 °C on the quality and carbonylation of beef proteins. These authors observed that cooking losses and protein carbonyls significantly increased with temperature [27]. The significant increase in TBARS and carbonyl contents in camel and rabbit meat during heating noted in the present work has also been reported by other studies in camel [6,28,29] and rabbit [30]. In fact, Tabite et al. [6] found that camel meat cooked at 200 °C to reach an internal temperature of 72 °C for 30 minutes, cooled to ambient temperature, then stored for 10 days at 4 °C, showed significant lipid-protein oxidation compared to raw meat. Additionally, Bahwan et al. [28] have evaluated the effects of heat treatment of camel meat samples until reaching an internal temperature of 70 °C to 75 °C, and have measured high levels of TBARS (4.5 mg/kg). These oxidations are a consequence of reactive oxygen species and generation of other oxidizing agents, which induced rapid peroxide formation and subsequently affected the structure, function and biological activity of lipids and proteins. However, these undesired modifications in physicochemical characteristics of meat by thermal stress, vary across heating methods (boiling, grilling, microwave, and frying), so, the microwave heating showed the highest levels of meat oxidation in camel [28,29] and rabbit [30].

The low oxidative stability of dromedary meat compared to rabbit meat observed in this study could be explained by the high levels of polyunsaturated fatty acids, myoglobin and other heme pro-oxidants in camel meat [3,9,32,33]. In fact, free ionic iron released from heme pigments and ferritin are considered major catalysts of lipid oxidation in cooked meat [34,35]. Furthermore, chicken or fish minced meat patties cooked to reach an internal temperature of 75 °C showed lipid oxidation closely related to their content of unsaturated fatty acids and different iron species [36]. Oxidation of oxymyoglobin to metmyoglobin generates intermediates that can accelerate the oxidation of oxymyoglobin and/or unsaturated fatty acids. This process occurs specifically after the formation of a superoxide

anion, which further generates hydrogen peroxide. The latter can react with metmyoglobin to form an activated metmyoglobin complex, ferryl myoglobin, and thus increase lipid oxidation [37,38].

In camel and rabbit meat samples having been treated with NO, then heated, the TBARS and carbonyl contents were lower, and the activities of CAT, SOD and GSHPx were higher compared to untreated and heated samples. The effect of NO was dose-dependent suggesting that this oil might promote a significant protection of the camel and rabbit meat against thermal stress. In lamb, dietary supplementation with Nigella sativa seeds (1%) for 3 months increased resistance to lipid peroxidation in meat [39], the *longissimus dorsi* muscle and the semitendinosus muscle [40]. In addition, black cumin extract showed excellent antioxidant potential on cooked beef patties when stored in refrigerator for 15 days, by increasing the stability of lipid quality which was revealed by significant low peroxide value and malondialdehyde contents [41]. Furthermore, Asghar et al. [42] found that the addition of N. sativa seeds to the diet (1 to 4%) increased the shelf life of Japanese quail meat, by reducing lipid peroxidation. In one study, Morshdy et al. [43] evaluated the antioxidant effects of NO at different concentrations (0.1%, 0.25% and 0.5%) on the physicochemical characteristics of rabbit meat stored in the refrigerator for 12 days, and the authors found that NO was able to reduce lipid peroxidation. In addition, dietary supplementation with N. sativa seeds in the presence of heat stress caused a reduction in TBARS levels and an increase in CAT, SOD and GSHPx activities in serum and liver in rabbits [44] and broiler chicken [45] suggesting a reduction in OS. Other studies reported that NO treatment induced an improvement in antioxidant potential by progressively decreasing TBARS values in cooked beef patties [41] and minced pork meat [46] during refrigerated storage for several days. Additionally, the work of Muzolf-Panek et al. [47] revealed that N. sativa seeds were able to protect ground chicken meat against SO during cold storage for 12 days. Zwolan et al. [48] showed that the ethanolic extract of *N. sativa* seeds, added to chicken meatballs at a rate of 1.2 g/100 g, reduced lipid oxidation during refrigerated storage of 14 days.

The most important free radicals generated by heat stress are oxygen derivatives, especially superoxide anion and hydroxyl radical [49]. In the present study, the improvement in the stability of lipids and proteins in camel and rabbit meat during heating in the presence of NO, could be explained by a richness of this oil in different bioactive and antioxidant compounds. According to Alberts et al. [18], black seed contains many phytochemical antioxidants such as thymoquinone (TQ), α-pinene, carvacrol, p-cymene, β-pinene, α-thujene, longifolene, tocopherols, ascorbic acid, flavonoids, thymol, tannins, magnesium and zinc, which can directly scavenge free radicals, inhibit the production of pro-oxidant nitric oxide, and modulate the activity of endogenous antioxidant systems. Indeed, TQ stimulates the activity of antioxidant enzymes such as CAT, GSHPx, and SOD, increases the expression of GSH levels, and inhibits the formation of TBARS [18]. According to another study, it was reported that polyphenol compounds showed a protective effect against OS induced by cooking and cold storage of meat in camel [10,12], sheep and goat [16].

Although studies have reported that ante-mortem dietary supplementation with black seed has improved the antioxidant potential of meat from many livestock species [39,40,42,45], a similar effect as observed in the present in vitro study following the incorporation of NO into minced meat remains to be confirmed in dromedary.

Conclusion

The study highlights, on the one hand, an impact of heat-induced oxidative stress on dromedary and rabbit meat revealed by an increase in water losses and TBARS and carbonyl contents, associated with a decrease in the enzymatic activities of CAT, GSHPx and SOD. On the other hand, the same study shows the protective role of NO against this OS by a reduction in the oxidation of lipids and proteins and by an increase in the activities of antioxidant enzymes according to a dose-dependent effect. The results of the study make it possible to promote nigella as an aromatic plant capable of playing an important antioxidant role to preserve the organoleptic composition of dromedary meat during heating. They also make it possible to consider the use of the seeds of this plant as a food supplement before the slaughter of an animal in order to minimize post-mortem OS in meat.

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BIOTRANSFORMATION OF HORSE MEAT PROTEINS BY PROBIOTIC MICROORGANISMS TO REDUCE ALLERGENICITY

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Keywords: *horse meat, microbial consortium, degree of hydrolysis, molecular weight distribution, microstructure, technological parameters, level of sensitization*

Abstract

Proteins may cause food allergies, the prevalence of which is growing worldwide. The intestinal microbiota takes an active part in stimulating and maintaining the functions of the body's immune system by producing humoral factors: enzymes, cytokines, mediators involved in the development of the immune response. The study of microbial consortium effect, which has high enzymatic activity, on reducing the reactivity of the body's immune response is of interest. The aim of the work was to study the biotransformation of horse meat proteins based on the use of probiotic consortium to obtain hypoallergenic meat raw materials. The object of the research was horse meat cooled for 24 hours at a temperature of 2 to 4 °C. The control: untreated meat samples; test 1: horse meat treated with a starter culture of one strain of Lactobacillus paracasei k-406; test 2: horse meat treated with a combined starter culture of four strains of Lactobacillus (Lactobacillus curvatus LCR-111–1, Lactobacillus plantarum 8RAZ, Lactobacillus fermentum 44/1 and Lactobacillus paracasei k-406). The microbial consortium was selected taking into account its biological compatibility and biotechnological potential, in particular, proteolytic activity. The degree of hydrolysis, technological parameters, fiber microstructure and the level of sensitization were determined. High proteolytic activity of the microbial consortium used for horse meat treatment was noted compared to the sample treated with a single-strain culture. Thus, the degree of hydrolysis after three days increased 4 times compared to the original raw material, which contributed to an increase in the hydrophilicity of the meat system by 11.8% and a decrease in the shear strength by 13.1% compared to the control (unfermented horse meat). Probiotic consortium caused a proteolytic modification of horse meat proteins, resulting in a decrease in the level of antigenic epitopes, which contributed to a decrease in the sensitizing activity of meat raw materials. This indicates the prospects for using such raw materials in the production of hypoallergenic meat products.

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Introduction

The dynamic growth of patients with food allergies all over the world indicates an urgent need to develop products that provide adequate nutrition and quality of life for people with a specific reaction to allergens. The relevance of this problem is confirmed by the Technical Regulations of the Customs Union TR CU 022/2011[1]. In Article 4 "Requirements for the labeling of food products", the manufacturer is obliged to indicate in the composition of food products the ingredients, biologically active additives, the use of which may cause allergic reactions or is contraindicated in certain types of diseases. The most common food allergens include nuts (almonds, cashews, peanuts), milk and dairy products, fish, shellfish, eggs and others. There are a number of proteins that may cause a strong allergic reaction in some people. The most well-known include casein from cow's milk, egg albumin, tropomyosin and parvalbumin from fish. Clinical manifestations of allergic reactions in humans may be expressed as gastrointestinal disorders (vomiting, diarrhea, upset stomach), rash, itching in different parts of the body, allergic rhinitis (runny nose) and even asthma attacks (anaphylaxis) [1].

Serum albumin and gamma globulin are the most prevalent and studied proteins and represent the two main potential allergens of animal meat. Serum albumin is one of the most common blood proteins; its concentration in plasma is 35 to 55 mg/ml. These are proteins with a globular spatial conformation formed by several domains [2,3,4]. Allergenic proteins contain specific segments known as epitopes. They are recognized by the immune system and trigger an allergic reaction. Epitopes may be linear or conformational. Linear (peptide) epitopes are sequences of amino acid residues in the allergenic protein that are recognized by IgE antibodies. They may be relatively short (5 to 20 amino acids) and may be present in different parts of the

[1] TR CU022/2011 Technical Regulations of the Customs Union "Food products regarding their labeling". (as amended as of April 22, 2024)" Retrieved from https://docs.cntd.ru/document/902320347. Accessed February 20, 2025 (In Russian)

allergenic protein. Conformational epitopes are formed by the three-dimensional structure of the protein, which is formed after the spatial folding of individual amino acid sequences. They may be recognized by IgE antibodies only in their three-dimensional conformation. These epitopes are usually more specific than linear ones. Epitopes on the surface of allergenic proteins are quite diverse, depending on the type of allergen, its chemical nature and spatial structure. In addition, epitopes may change depending on the storage and processing conditions of the allergen. This may affect the ability of the immune system to recognize and respond to them [5,6,7].

Food allergies most often affect children, starting from an early age. According to experts, this process is associated with problems in the formation of intestinal microflora under the influence of various factors. Due to the active growth of the urbanization process, the number of cases of milk allergy is also growing, among which there are cases of beef allergy, which is apparently associated with the antigenic affinity of bovine proteins [5]. In the absence of specific methods of therapy, one of the approaches to the prevention and treatment of such patients is the exclusion of the allergen-containing products from the diet [8,9]. Since the growing body of children requires satisfying all physiological needs, especially with regard to proteins of animal origin, deep hydrolysates of proteins from cow's milk or other dairy animal's milk are used for artificial feeding [10,11].

To date, the food industry does not produce hypoallergenic products for mass consumption. Various types of technological processing of food raw materials such as thermal treatment, hydrostatic pressure, microwave, ultrasound, chemical modification and others are not so effective. The principle of hypoallergenicity is based on the destruction of the conformational structure of the protein, on the surface of which there are IgE-antigenic epitopes capable of interacting with antibodies [12,13]. The most promising method used by many authors is enzymatic hydrolysis of proteins, resulting in short-chain peptides and amino acids formation, that lose their conformational active center of interaction with Ig E. According to a number of authors, with a molecular weight below 3.5 to 10 kDa, allergenicity becomes minimal [14–16]. However, during enzymatic hydrolysis, potential epitopes that are inside protein globules or those that are newly formed during interaction with components of food raw materials may appear [17–19].

The COVID-19 pandemic has drawn the attention of the entire population to the problems of immunoprophylaxis and products that help boost immunity. The role of probiotic consortia in the prevention of a number of diseases was discussed back in 2015 at the FEMS (Federation of European Microbiological Societies) congress [20]. Healthy intestinal microflora is one of the most important factors in human well-being. In this regard, the production of probiotic food products based on the most significant representatives of the human gastrointestinal microflora remains relevant [21].

Intestinal microflora is an integral part of the body and is involved in various life support processes. The protective function is manifested in maintaining internal homeostasis due to the release of bacteriocins and the destruction of bacterial toxins [22,23]. Microbiota takes part in digestion processes, synthesizing a number of enzymes involved in the breakdown of polysaccharides, proteins, fatty acids and other compounds involved in the regulation of metabolism. Microflora plays a major role in the synthesis of essential micronutrients: vitamins, amino acids (arginine, glutamine) [24]. As for the immunomodulatory activity of intestinal microbiota, it takes part in the formation and development of the intestinal lymphoid system, promotes the synthesis of sIgA, stimulates the synthesis of cytokines and immunomediators [25]. Thus, intestinal microflora plays an important role in the formation of sensitization to food allergens, since the maturation and development of the body's immune system occurs to a significant extent under the influence of various microorganisms inhabiting the intestine [26].

As for the mechanism of allergic diseases, according to a number of authors, the immunoregulatory effect of the intestinal microbiota is associated with its influence on the production and activity of regulatory T-cells. Intestinal biocenosis disorders are associated with an increase in the immune response from Th2 cells [27]. Th2 cells produce IL-4, IL-5 and IL-13, which contribute to the development of allergic inflammation. The effect of the intestinal microbiota shifts this response towards the development of Th1 cells, which contributes to immune tolerance and maintains the balance of Th1/Th2 cells [27]. Intestinal microbiota is also one of the environmental factors that contribute to the maturation of T-cells [28]. Finally, the intestinal microflora plays a significant role in the development and maintenance of the intestinal barrier function, the damage of which is believed to lead to allergic sensitization of the host organism [29].

Thus, the intestinal microbiota takes an active part in stimulating and maintaining the functions of the immune system by producing humoral factors: enzymes, cytokines, mediators involved in the development of the immune response. Given the above, it was interesting to search for and select a probiotic consortium with high proteolytic activity, capable of reducing the level of residual antigenicity of meat proteins and under the action of which hydrolysis products, metabolites, cytokines and other biologically active substances are formed that could be used to reduce the reactivity of the immune system. The aim of the work was to study the processes of biotransformation of horse meat proteins using a probiotic consortium to obtain hypoallergenic meat raw materials.

Material and methods

Experimental setup

Experiments were conducted in the laboratories of the department "Animal Products Technology. Commodity Science", Biotechnology Center of the East Siberian State

University of Technology and Management. Horse meat was chosen as the object of the study, as it is a traditional raw material in the places of their breeding and has a high nutritional value due to the increased content of iron, vitamins B and E. Expanded and productive horse breeding determines the feasibility of using horse meat for the production of mass-market products.

Meat was selected from animals aged 24 months, raised in the agricultural production cooperative "Ulekchin" (Ulekchin village, Zakamensky district, Republic of Buryatia). For the study, samples were isolated from the semitendinosus muscle of the hip horse cut cooled for 24 hours at a temperature of 2 to 4 °C. For the experiment, pieces of horse meat of 100 to 150 g were cut out; fat and connective tissue were separated. The control: untreated meat samples; test 1: horse meat treated with a starter culture of one strain of *Lactobacillus paracasei k-406*; test 2: horse meat treated with a combined starter culture of four strains of *Lactobacillus (Lactobacillus curvatus LCR-111–1, Lactobacillus plantarum 8RAZ, Lactobacillus fermentum 44/1* and *Lactobacillus paracasei k-406)*. The bacterial strains were obtained from the National Bioresource Center "All-Russian Collection of Industrial Microorganisms" of the National Research Center "Kurchatov Institute". The microbial consortium was selected taking into account its biological compatibility and biotechnological potential [30].

To conduct the experiment, the horse meat was injected with starters (2 units of activity per 100 kg) using syringe followed by massaging to evenly distribute the preparation inside the muscle tissue and aging for 2 to 3 hours at a temperature of 18 to 20 °C. Then, the sample was salted with 15% brine in an amount of 20% of the sample weight, massaged, packed in film and stored at a temperature of 2 to 4 °C for 3 days. The physicochemical properties, technological parameters, microstructure and sensitization level were studied using the methods given below.

Methods for determining physicochemical properties and technological parameters

The degree of hydrolysis was determined by the Formula 1:

$$DoH = (N_a / N_t) \times 100\%, \quad (1)$$

where N_a is the content of amino nitrogen, %; N_t is the content of total nitrogen, %.

The protein content was determined by the following formula:

$$P = N_t \times 6.25 \times 10\ (\%), \quad (2)$$

where N_a and N_t were determined by the Kjeldahl[2] method using KDN-812 semi-automatic installation (Xian Yima Optoelec CO. LTD, China).

Water-holding capacity (WHC) of meat raw materials was determined using the Grau-Hamm method modified by Volovinskaya-Kelman, based on the release of moisture by the test sample during light pressing (weight of 1 kg for 10 min), sorption of the released water by filter paper and determination of the amount of separated moisture by the size of the area of the spot on the filter paper.

To determine the shear strength, samples were cut out from boiled muscle tissue along the fibers using probe No. 5 (10 mm diameter) and the force required to destroy the sample by cutting was measured using Warner-Bratzler device (Russia).

Methods of morphology evaluation

For microstructural studies, horse meat samples (1.5×1.0×0.5) were fixed in 10% neutral formalin for 48 hours. After washing the samples from excess formalin, standard alcohol processing was performed through a battery of ethyl alcohol with increasing concentration (40 to 100°). Subsequently, after holding the biological material in a paraffin/chloroform mixture for 12 hours, processing was continued with three changes of melted paraffin every 60 minutes. Upon completion, melted paraffin was poured into molds with samples (1×1.5) with the addition of beeswax to improve the quality of microsections. MS-2 sledge microtome was used to make 6 to 7 μm thick sections. Sections were stained with hematoxylin and eosin according to Ehrlich, and with picrofuchsin according to the Van Gieson method to identify collagen fiber nuclei [31]. Measurements of muscle tissue structures and other cellular inclusions in horse meat samples were performed using Micromed 3 microscope and Toup Cam 5/1 video eyepiece (Optics & Electronics Co., Ltd., China) at a magnification of 100×.

Methods of biological research

The sensitizing activity of the samples was determined using a delayed type hypersensitivity (DTH) model on 21 white outbred mice weighing 22 to 24 g[3]. Three groups of seven animals each were formed: Group 1 — intact (without the administration of extracts); Group 2 — control (unfermented horse meat extract); Group 3 — test (horse meat extract fermented by the microbial consortium). The extracts were prepared from horse meat samples by homogenization in 0.15 M NaCl with water ratio of 1:10.

Sensitization with the antigen was carried out once at a tailset in amount of 60 μl of the extract and Freund's complete adjuvant (FCA) emulsion (ratio 1:1). The challenging dose of the antigen in amount of 40 μl was administered after 5 days into the pad of the mouse's hind paw (test paw). The other hind paw was a control (control paw). 24 hours after testing, the edema value was measured using MK-0-25 engineering micrometer. The difference in the thickness of both paws characterizes the edema value, which can be used to evaluate the intensity of the DTH reaction.

[2] GOST 25011-2017. Meat and meat products. Protein determination methods. Retrieved from https://docs.cntd.ru/document/1200146783. Accessed February 20, 2025 (In Russian)

[3] Guidelines for conducting preclinical studies of medicinal products. Part one. Chapter 2. Moscow: Grif and K, 2012. — P. 57–58. (In Russian)

After the animals were removed from the experiment, the hind paws were cut off at the ankle joint and weighed. Local (popliteal) lymph nodes were also isolated, weighed on an analytical scale (Shinko Denshi HT224RCE, Japan), and the cellularity was counted in a Goryaev chamber. For this, each lymph node was homogenized in a glass homogenizer using 3 ml of 0.9% NaCl. Then the number of leukocytes was counted by diluting the previous sample 20 times with a 3% solution of acetic acid, colored with methylene blue.

Leukocyte count was performed in 100 large squares of the Goryaev chamber using the Formula 3:

$$X = \frac{A \times 250 \times 20}{100}, \quad (3)$$

where:

X is the number of leukocytes in 1 µl of sample;

A is the number of leukocytes in 100 large squares of the chamber.

Reaction indices (RI) were determined using the Formula 4:

$$RI = \frac{Value_{test} - Value_{control}}{Value_{control}} \times 100\%, \quad (4)$$

where:

$Value_{control}$ is the value of the control paw (or lymph node);

$Value_{test}$ is the value of the test paw (or lymph node).

Intact animals were sensitized with FCA emulsion and sterile 0.15 M NaCl solution according to the same scheme as in the groups receiving horse meat extracts.

The analysis of the sensitizing properties of the extracts was also carried out on 6 albino guinea pigs divided into 2 groups: Group 1 — control (non-fermented horse meat) and Group 2 — test (fermented horse meat). The animals were sensitized at the pads of two hind paws once with the corresponding horse meat extract mixed with FCA in amount of 0.5 ml (ratio 1:1). After 20 days, the animals were intradermally injected with a challenging dose of the corresponding extract (0.05 ml) at the shaved area of the back. To control the reactivity of the skin, 0.05 ml of sterile 0.15 M NaCl was injected intradermally at the opposite shaved area of the back. The response was assessed after 1, 24 and 48 hours by the appearance of erythema and its size (diameter)[4].

The mice and guinea pigs used in the experiment were obtained from the Buryat Republican Scientific and Industrial Veterinary Laboratory. Animal experiments were conducted in the vivarium of the Federal State Budgetary Educational Institution of Higher Education "East Siberian State University of Technology and Management".

The experiments were carried out in compliance with the principles of humanity in accordance with international moral and ethical standards and the requirements of the European Convention ETS N123[5] and Directive 2010/63/EU[6]. Animal care and maintenance were carried out in accordance with GOST 33216-2014[7] and Order of the Ministry of Health of the Russian Federation No. 199-N[8].

During the preparation for the experiment, the mice and guinea pigs underwent 10 days of quarantine and adaptation to the vivarium environment. During the experiment, the general condition of the animals, their food and water consumption were monitored daily. The experimental animals were divided into groups of 7 individuals for mice and 3 individuals for guinea pigs each in separate cages of the appropriate size, and were on the same (standard) diet with free access to feed and water. The light regime in the vivarium was provided by changing "day/night" illumination every 12 hours. The air temperature was 20 to 25 °C, the relative humidity was 60 to 70%.

Mice were removed from the experiment by cervical dislocation, and guinea pigs by chloroform vapors.

The study protocol was approved at a meeting of the ethics committee.

Statistical data processing

The obtained experimental data were processed using the calculation of mean values (M), standard error of the mean (m) and parametric test (Student's t-test) using Statistica software and Microsoft Excel. To analyze the physicochemical properties of horse meat during storage, the degree of the studied parameter change was calculated using Friedman test, which allows estimating the variability of the parameter over time. Changes were considered significant at $p \leq 0.05$.

The results of morphometric and biological studies are presented as medians (Me), upper and lower quartiles (Q1-Q3) and processed using the nonparametric Mann-Whitney test. The results were considered reliable when the significance level of differences was reached ($p \leq 0.05$).

Results and discussion

The process of meat aging includes a number of biochemical processes that may affect the technological and physicochemical properties of the raw material. The degree of hydrolysis and technological characteristics of horse meat were studied during the processing of meat raw materials with a single-strain starter (test 1) and a microbial consortium (test 2) compared to untreated meat (control). The data are presented in Table 1.

[4] Guidelines for conducting preclinical studies of medicinal products. Part one. Chapter 2. Moscow: Grif and K, 2012. — P. 58–59. (In Russian)

[5] Council of Europe — European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123). Strasbourg, 18.III.1986

[6] Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Retrieved from https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng/pdf. Accessed February 20, 2025

[7] GOST 33216–2014 Guidelines for accommodation and care of animals. Species-specific provisions for laboratory rodents and rabbits Retrieved from https://docs.cntd.ru/document/1200127506. Accessed February 20, 2025 (In Russian)

[8] Order of the Ministry of Health of the Russian Federation dated 01.04.2016 No. 199-N "On approval of the rules of good laboratory practice". Moscow — P. 9 (In Russian)

Table 1. Physicochemical changes in horse meat during processing with microorganisms

Samples under study	Storage, days			
	0	1	2	3
Degree of hydrolysis, %				
Control	5.54 ± 0.11[a]	5.74 ± 0.27[c]	6.31 ± 0.21[bc]	8.67 ± 0.34[bc]
Test 1	5.59 ± 0.12[a]	6.32 ± 0.26[b]	8.55 ± 0.25[bd]	21.75 ± 0.36[bd]
Test 2	5.63 ± 0.23[a]	7.75 ± 0.14[bd]	10.73 ± 0.32[bd]	22.87 ± 0.28[bd]
Water-holding capacity, %				
Control	70.83 ± 1.6	70.9 ± 1.8[c]	72.4 ± 2.3[c]	73.9 ± 2.7[c]
Test 1	71.24 ± 2.2[a]	76.2 ± 1.9	77.3 ± 2.1[bd]	80.9 ± 2.2[bd]
Test 2	72.17 ± 2.1[a]	78.2 ± 2.2[d]	79.9 ± 1.9[bd]	82.6 ± 1.8[bd]
Shear strength, 10^2 N/m^2				
Control	4.85 ± 0.14	4.95 ± 0.11	4.38 ± 0.12[c]	4.29 ± 0.15[c]
Test 1	4.81 ± 0.11[a]	4.55 ± 0.18	4.21 ± 0.17	3.83 ± 0.13[bd]
Test 2	4.89 ± 0.16[a]	4.43 ± 0.16	3.92 ± 0.14[bd]	3.65 ± 0.19[bd]

Note: a and b indicate that within one row, the difference in mean values is statistically significant ($p \leq 0.05$); c and d indicate that within one column, the difference in mean values is statistically significant ($p \leq 0.05$) according to Friedman test.

Analysis of the data in Table 1 showed that in the process of protein proteolysis, the degree of hydrolysis after two days increased in the control by 13.9%, in Test 1 by 53%, in Test 2 by almost 2 times compared to the initial value (Day 0). Aging for 3 days enhanced proteolytic processes, which is confirmed by an increase in the degree of hydrolysis in the control by 56.5%, in Test 1 by 3.9 times, in Test 2 by 4.1 times ($p \leq 0.05$).

A literature review showed that the treatment of protein-containing raw materials with microorganism strains promotes the development of proteolytic processes in the raw materials; it was shown that due to the effect of enzymes produced by *Aspergillus oryx season*, the degree of hydrolysis within 72 hours increased to a level of 20 to 30% of the total amount of nitrogen [32]. The authors [33] noted the proteolytic activity of propionic acid bacteria, which contributes to an increase in the amount of amine nitrogen in beef treated with microorganisms, indicating hydrolysis of meat proteins.

The hydrophilicity of the meat system plays an important role in the formation of quality characteristics of the finished meat product; a reliable increase in the degree of hydrophilicity of fermented horse meat was revealed. On Day 3 in Test 1, the increase in the value of WHC compared to the control was 10.1%, in Test 2 it was 11.8% ($p \leq 0.05$). It is possible that the synergistic effect of probiotic microorganisms in the consortium contributes to the destruction and disorientation of protein molecules and the emergence of additional hydrophilic groups. An increase in the level of WHC value was found when processing beef with propionic acid bacteria [33].

The texture of horse meat, characterized by shear strength, improved after aging with a starter culture. On Day 3 of horse meat aging, a decrease in the level of shear strength was observed in Test 1 by 8.8%, in Test 2 by 13.1% compared to the control ($p \leq 0.05$). Proteolytic processes contributed to the loosening of the muscle fiber structure (lysis), which led to a decrease in the level of horse meat rigidity.

Thus, the obtained data indicate that the microbial consortium enhances proteolytic processes in horse meat, which causes an improvement in the technological and microstructural characteristics of meat.

Proteolytic changes in muscle tissue proteins lead to the destruction of fibers, which is seen by microstructural methods. Table 2 presents the results of morphometric studies. Figures 1 to 3 show photo images with the microstructure of the studied samples.

As can be seen from Figure 7A, it was found that in the control samples of horse meat not exposed to probiotic microorganisms, the surface of the muscle fibers is uneven and transverse striation in some fibers of the control group is weakly expressed.

During the morphometric study, the thickness of the fibers in the control samples of chilled horse meat averaged 48.0 ± 5.0 μm. The fibers in the control samples are located quite densely with insignificant connective tissue layers between them. During microscopy of the control samples, the thickness of the endomysium averaged 10.5 ± 5.0 μm.

Table 2. Microstructural dimensions of the studied horse meat samples

Horse meat samples	Parameter	Diameter, μm		Thickness of connective tissue layers, μm	
		Muscle fibers	Fiber nuclei	Endomysium	Perimysium
Control	M ± m	48.0 ± 15.0[a]	4.8 ± 3.0	10.5 ± 5.0	30.5 ± 7.0
	min-max	30.5–60.5	2.5–8.5	5.0–20.0	24.5–40.0
Test 1	M ± m	80.0 ± 15.0[b]	4.0 ± 0.5	9.5 ± 2.5	25.0 ± 5.0
	min-max	65.5–95.5	3.5–4.5	7.5–12.5	22.5–35.0
Test 2	M ± m	100.0 ± 20.0[b]	5.5 ± 1.0	7.0 ± 1.5	20.5 ± 5.0
	min-max	76.5–110.0	4.5–6.5	5.5–8.5	18.0–25.0

Note: a and b indicate that within one column, the difference in mean values is statistically significant ($p \leq 0.05$) according to Student's t-test.

At the same time, the connective tissue layers between the fiber bundles (perimysium) of horse meat are more clearly visible in a longitudinal section (Figure 7A) and vary from 24.5 to 40.0 μm. As can be seen from Figure 7B, a dense row of small muscle ridges is located between the transverse fibers of horse meat.

Among the structural components in chilled horse meat, when stained according to Van Gieson, the nuclei of muscle fibers are distinguishable, which have an oval, elongated and rod-shaped form.

Histological examination of horse meat samples treated with the monoculture revealed that the sections obtained were stained pink-red and the muscle fibers were mostly folded when cut longitudinally (Figure 8A). The surface of the muscle fibers was uneven and consisted of alternating thick, swollen and loosened muscle fibers. Microscopy of sections in Test 1 showed transverse striation on most fibers in longitudinal sections (Figure 8A). In transverse sections, the muscle fibers were dense, swollen and the connective tissue layers were thinner in contrast to the control samples (Figure 8B). Among the structural elements in Test 1, the nuclei of muscle fibers attract special attention. When staining the preparations according to Van Gieson, nuclei were not detected in contrast to the control samples. Only the use of hematoxylin and eosin allowed to find that the nuclei of muscle fibers treated with the monoculture mainly had an elongated, rod-shaped form with an average thickness of 4.0 ± 0.5 μm. At the same time, the transverse striation on the surface of the fibers in Test 1 is expressed better than in the control samples, and finely looped structures are visible on some muscle fibers. In Test 1, all layers of loose connective tissue in both the endomysium and perimysium became denser and more compressed (Table 2, Figure 8B).

Microstructural analysis of horse meat samples in Test 2 treated with the microbial consortium allowed to establish the disintegration of muscle fibers into separate segments (Figure 9A). At the same time, collagen fibers significantly swelled and increased in size. The sarcolemma of the fibers was swollen and had no visible damages. As a result, collagen fibers were glued together with a decrease in the connective tissue layers between the fibers and a decrease in the width of the perimysium between the fiber bundles (Figure 9B). The results of morphometric measurements of the structural components in these samples are presented

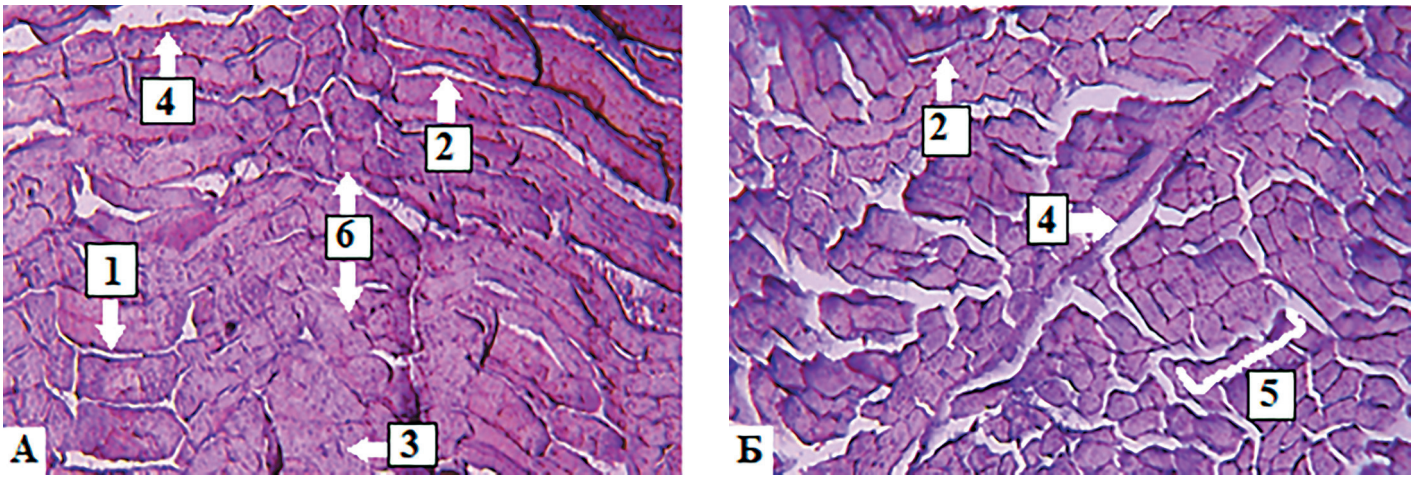

Figure 7. Micrographs of control horse meat samples: A, longitudinal section; B, transverse section: 1, muscle fibers; 2, endomysium; 3, fiber nuclei; 4, perimysium; 5, fiber bundles; 6, muscle ridges. Staining with hematoxylin and eosin (PL magnification 10×/0.25)

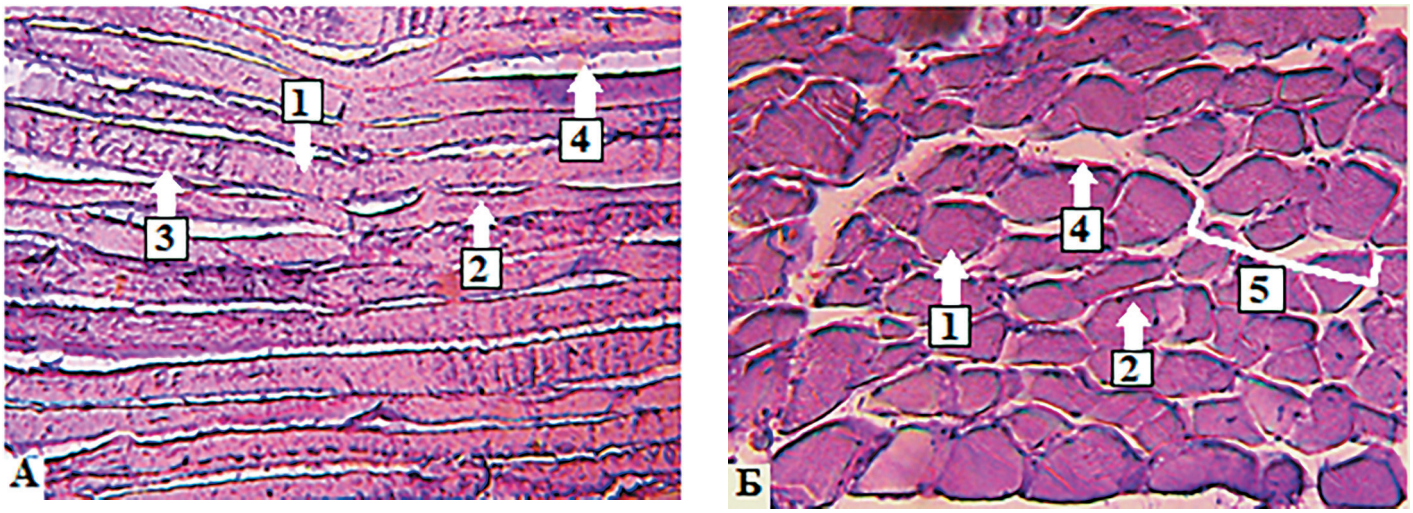

Figure 8. Micrographs of horse meat samples treated with the monoculture; A, longitudinal section; B, transverse section: 1, muscle fibers; 2, endomysium; 3, finely looped fiber structures; 4, perimysium; 5, fiber bundles. Staining with hematoxylin and eosin (PL magnification 10×/0.25)

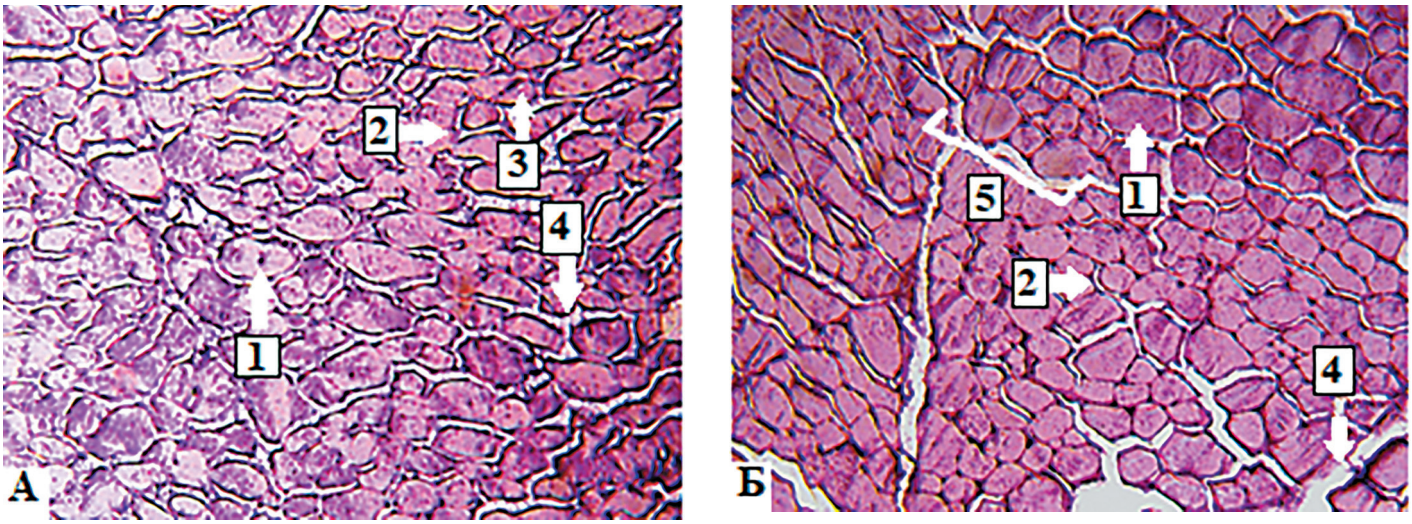

Figure 9. Histological preparations of horse meat samples treated with the microbial consortium; A, longitudinal section; B, transverse section: 1, muscle fibers; 2, endomysium; 3, thickened nuclei; 4, perimysium; 5, fiber bundles. Slight reduction of endomysium and perimysium. Staining with hematoxylin and eosin (PL magnification 10×/0.25)

in Table 2. Microscopy of sections of meat samples treated with the microbial consortium showed that most muscle fibers were completely free of transverse striation, but it was weakly visible in some fibers (Figure 9A). Treatment of horse meat with the microbial consortium affected the shape and visibility of the nuclei. Just as in Test 1, muscle cell nuclei were not detected in horse meat fibers treated with the microbial consortium using Van Gieson staining. Using another stain, slightly thickened, elongated nuclei became visible along the fibers under microscopy, in contrast to the control samples.

The conducted studies revealed that the treatment of horse meat samples with the monoculture stimulated the swelling of collagen fibers and the loosening of muscle tissue with the subsequent formation of an intense pink-red color. Microstructural analysis of horse meat samples in Test 2 treated with the microbial consortium revealed that the collagen fibers significantly swelled, increased in size, and revealed reliable differences in fiber thickness compared to the control. At the same time, the connective tissue layers between individual fibers and bundles of muscle fibers became thinner and smaller compared to those in the control samples and in Test 1 treated with the monoculture.

Based on the conducted studies, more effective hydrolysis of horse muscle tissue was revealed when using the four-strain starter. In this regard, further studies to determine the level of sensitization were carried out by comparing untreated horse meat (control) and horse meat treated with the microbial consortium (test).

Allergenicity of products may be assessed by studying the reaction of delayed type hypersensitivity (DTH) in experimental animals. Table 3 presents the parameters of the delayed type hypersensitivity reaction in white mice when administered extracts of unfermented and fermented horse meat.

When administered the extract of unfermented horse meat (control), a positive 100% reaction was noted for reaction indices 1, 2 and 4. The degree of the DTH reaction for the corresponding indices (1 to 4) was 2.2, 6.3, 7.7, and 13.4 times greater than in the intact group of animals, respectively. Administration of the extract of horse meat exposed to the consortium (test) caused a positive 100% reaction only when calculating RI-2. At the same time, in this group, the degree of the DTH reaction was lower than in the control by 1.9, 3.1, 3.1, and 2.1 times, respectively (Table 3).

Table 3. Parameters of the DTH reaction in white outbred mice when administered horse meat extracts

No.	Group name	Parameter	Reaction index			
			1 — by paw volume	2 — by paw weight	3 — by lymph node weight	4 — by lymph node cellularity
1	Intact	Me	1.49[a]	2.24[a]	5.62[a]	3.07[a]
		Q1–Q3	1.19–1.79	1.88–2.60	5.11–6.13	2.10–4.04
2	Control — extract of unfermented horse meat	reaction	+	+	+	+
		degree of reaction	100%	100%	80%	100%
		Me	3.32[b]	14.03[b]	43.21[b]	41.21[b]
		Q1–Q3	2.93–3.71	11.28–16.78	38.32–48.10	35.92–46.50
3	Test — extract of horse meat treated with the microbial consortium	reaction	+	+	+	+
		degree of reaction	60%	100%	80%	80%
		Me	1.77	4.58[c]	14.13[c]	19.92[c]
		Q1–Q3	1.33–2.21	3.76–5.40	9.80–18.46	14.06–25.78

Note: data with [b] superscript differ significantly from data with [a] superscript, data with [c] superscript differ significantly from data with [a, b] superscripts within one column ($p \leq 0.05$) according to Mann-Whitney test.

A similar pattern was established when carrying out the DTH reaction on guinea pigs (Table 4).

It was noted that after 1 hour, no pronounced reaction was observed (slight swelling and redness, the size of the erythema is difficult to determine), and after 24 and 48 hours, pink erythema was observed around the injection site. There was a reliable decrease in the severity of the DTH reaction in the test group (2) compared to the control group (1). After 24 and 48 hours, the reaction value was 1.6 and 1.5 times lower, respectively. Thus, the data obtained on two species of animals indicate a decrease in the sensitizing activity of meat raw materials exposed to the probiotic consortium. A decrease in the sensitizing properties of meat proteins is caused by the destruction of antigenic epitopes due to the proteolytic activity of the probiotic consortium.

According to the authors, one of the promising methods in the development of hypoallergenic products of animal origin is the modification of antigenic epitopes in protein molecules, which reduces their allergenicity. The degree of allergenicity reduction depends on the type of proteases used. Non-specific proteases or protease mixtures reduce protein allergenicity more effectively [6]. This approach is used by many authors to reduce the allergenicity of cow's and mare's milk, soy and egg proteins [34–36]. Considering the role of intestinal microbiota in the development of an allergic reaction of the host organism, we attempted to use the proteolytic activity of probiotic microorganisms to reduce the allergenicity of raw meat. A decrease in the sensitizing properties of horse meat due to the proteolytic activity of the microbial consortium was shown in two species of experimental animals. The role of metabolites, cytokines and other compounds produced during hydrolysis and having an immunomodulatory effect remains unclear, which requires further research.

Conclusion

As a result of horse meat treatment with the single-strain culture and the microbial consortium, data were obtained indicating high proteolytic activity of the consortium compared to the control and the sample processed with the monoculture. It was noted that the degree of hydrolysis of horse meat proteins after 3 days increased 4 times compared to the original raw material, which caused an increase in the hydrophilicity of the meat system by 11.86% and a decrease in shear strength by 13.1% compared to the control (unfermented horse meat). It is possible that the microbial consortium has a more pronounced proteolytic effect on the destruction and disorientation of protein molecules due to the synergistic effect, which contributes to the emergence of additional hydrophilic groups.

Analysis of the molecular weight distribution of proteins in the studied samples confirmed a deeper hydrolysis of horse meat proteins in the test samples compared to the control. When comparing test samples, deeper proteolytic processes were noted in the sample treated with the microbial consortium, compared to the sample treated with the monoculture. The content of proteins with a molecular weight of 65 to 170 kDa in the test sample with the monoculture decreased by 10.2 rel.%, and in the test sample with the microbial consortium this parameter decreased by 2 times. Therefore, the amounts of proteins with a molecular weight of 15 to 26 kDa increased in Test 1 by 17 rel. %, and in Test 2 by 1.88 times. The data obtained indicate a deeper destruction of protein molecules under the influence of the consortium of lactic acid microorganisms producing proteolytic enzymes.

Microstructural analysis showed that treatment of horse meat samples with the monoculture stimulated swelling of collagen fibers and loosening of muscle tissue with subsequent formation of intense pink-red color. Microstructural analysis of horse meat samples in Test 2 treated with the microbial consortium allowed to establish that collagen fibers significantly swelled, increased in size and reliable differences in fiber thickness were revealed compared to the control. At the same time, connective tissue layers between individual fibers and bundles of muscle fibers in samples treated with the microbial consortium became thinner and smaller than in the control and in samples exposed to the monoculture.

It was noted that when using unfermented horse meat extract (control), a positive 100% delayed type hypersensitivity reaction in experimental animals was observed. The administration of the fermented horse meat extract (test) caused a positive 100% reaction only when calculating the DTH reaction index by the weight of the paws. Compared with the control, a decrease in the reaction indices was noted: by paw volume by 1.9 times; by paw weight by 3.1 times; by lymph node weight by 3.1 times and by lymph node cellularity by 2.1 times. Similar results were obtained in an experimental model of delayed type hypersensitivity (skin test) in guinea pigs. Thus, the treatment of meat raw material with probiotic microorganisms caused a decrease in its sensitizing activity. Therefore, this biotechnological technique indicates the prospects for using such raw materials in the production of hypoallergenic meat products for mass consumption.

Table 4. Parameters of the DTH reaction in albino guinea pigs when administered horse meat extracts

No.	Group name	Parameter	Reaction results (d of erythema, mm) / time (hours)		
			1	24	48
1	Control — extract of unfermented horse meat	Me	No pronounced reaction	8.30[a]	6.45[a]
		Q1–Q3		7.4–9.2	5.65–7.25
2	Test — extract of horse meat treated with the microbial consortium	Me	No pronounced reaction	5.15[b]	4.41[b]
		Q1–Q3		4.25–6.05	3.61–5.21

Note: data with [b] superscript differ significantly from data with [a] superscript within one column ($p \leq 0.05$) according to Mann-Whitney test.

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IDENTIFICATION OF PRIORITY BACTERIAL GROUPS TO OPTIMIZE SANITARY PROCEDURES AT MEAT PROCESSING PLANTS

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Keywords: *microflora, meat industry, sanitation, pathogens, spoilage bacteria, ESKAPEE*

Abstract
This paper presents the study results of the microbiological composition in the industrial environment at four pork slaughter and processing plants (MPPs). The sample included plants with various production problems and different process features. The purpose of this study was to determine the priority bacterial groups typical for all studied plants, as well as to identify specific microorganisms associated with the individual characteristics of each plant. Representatives of Pseudomonas, Candida, and Escherichia genera dominated at all four plants, but each plant had its own unique characteristics. Thus, at MPP No. 1, where no preliminary decapitation was performed, a high level of industrial environment contamination with Escherichia genus microorganisms and pathogenic microorganisms, Salmonella spp. and Listeria monocytogenes, was observed. At MPP No. 2, which allows the acceptance of raw materials with defects, a significant counts of Staphylococcus genus microorganisms were detected. Pseudomonas, Carnobacterium, and Enterobacteriaceae genera were detected at MPPs No. 3 and No. 4, where systematic spoilage of finished products was revealed. Analysis results showed that individual technological stages and conditions at different plants create a unique environment that promotes the development of certain groups of microorganisms. The introduction of expanded microbiological monitoring, changes in technology, and the development of individual recommendations for each plant will reduce the risks of microbial contamination, improve product quality, and increase its safety for consumers.

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Introduction

Each plant has unique operating conditions, technological processes and ecological status, which affects the composition and dynamics of microflora. The use of standard indicators may overlook locally significant pathogens or opportunistic bacteria that may affect the product quality and consumer health in a given plant. Even meat processing plants of the same profile may differ in technological processes, features of slaughter and cutting, and face different problems (spoilage of finished products, ineffective disinfection). These aspects play a key role in the formation of a unique microflora inside the plant and, as a result, in the optimization and customization of sanitary procedures.

Microbiota in food production is often considered as a possible source of microorganisms that may affect the quality of meat products [1]. A number of studies have confirmed that microorganisms found in the product are often found on plant surfaces and equipment [2,3]. Regular use of sanitizers helps to eliminate microorganisms, however, when bacteria are found in biofilms or disinfectants are ineffective against certain groups of microorganisms, the risk of food contamination and outbreaks of infectious diseases increases [4].

According to the European Union's Zoonoses Report for 2022, *Salmonella* bacteria were identified in 951.590 meat and meat product samples, while *Campylobacter* bacteria were detected in 107.162 samples. In addition, *Listeria monocytogenes* was detected in 135.148 ready-to-eat (RTE) food samples [5].

In our country, product safety control is traditionally based on determining the presence of pathogenic microorganisms directly in the product. This approach allows identifying the potential risks of finished products before they are released to the market. However, this method does not cover the entire production process and does not take into account possible sources of contamination at different stages of production [6]. It is important to understand that product safety depends on many factors, from animal handling conditions to compliance with sanitary standards at each stage of raw material processing. To ensure comprehensive product safety, it is necessary to monitor the

entire production chain, including regular analysis of the environment, equipment, surfaces and air [7].

In addition to human pathogens, other groups of bacteria that may be present in food products and are potentially dangerous to humans or affect the quality and shelf life of the product, thereby leading to economic loss and increased costs for maintaining the sanitary status of the plant [8] should also be considered. These include spoilage bacteria and industry-specific bacterial groups, emerging microorganisms, ESKAPEE, etc.

Spoilage microorganisms. Spoilage microorganisms include bacteria, fungi, and yeasts that may cause changes in the sensory properties of food products. These microorganisms may be present even under strict sanitary conditions, so monitoring them is necessary to prevent premature deterioration of product quality. The most common bacteria that cause spoilage of chilled beef and pork during aerobic storage include *Brochothrix thermosphacta, Carnobacterium* spp., *Enterobacteriaceae* family, *Lactobacillus* spp., *Leuconostoc* spp., *Pseudomonas* spp. and *Weissella* spp. The metabolic processes of these microorganisms may cause defects such as sour taste, color changes, gas formation, mucus formation, and decreased pH [9].

Industry-specific bacterial groups. Each food industry has its own specific bacterial species. For example, in ready-to-eat food production, special attention is paid to bacteria such as *Listeria monocytogenes* causing listeriosis in humans [10]. In fish processing plants, emphasis is placed on preventing the growth of *Vibrio* spp. and *Aeromonas hydrophila*, which are capable of causing foodborne illnesses [11].

Emerging microorganisms. Emerging microorganisms are new or previously little-known pathogens that are becoming increasingly important due to changes in agricultural practices, food processing, and globalization of trade. They are of serious hazard to consumer health and require special attention from the food industry. Examples of emerging pathogens include *Escherichia coli* O157: H7 and *Campylobacter jejuni* causing serious food poisoning [12].

ESKAPE group. According to the US Centers for Disease Control (CDC), more than 2 million cases of illness and about 23 thousand deaths are associated with ESKAPE pathogens every year [13]. To attract the attention of the scientific community, CDC introduced the term "ESKAPE pathogens", which includes six types of microorganisms that are highly resistant to antibiotics and may cause hospital-acquired infections [14].

There is information about seven types of pathogens with high resistance to antibiotics, including *Escherichia coli*; in this case the abbreviation ESKAPEE is used [15]. Although these microorganisms are more often associated with medical institutions, they are also found at food plants. This is due to the uncontrolled use of antibiotics in animal husbandry and veterinary medicine, which leads to the emergence of resistant bacteria in livestock. Resistant bacteria can enter the food chain through the use of contaminated meat, milk, eggs and their processed products [16]. For example, *E. coli* and *K. pneumoniae*, commonly found in farm animals, may acquire resistance genes and transmit them to humans through the consumption of meat products [16].

Sanitary and microbiological study of microbial contamination of industrial objects at food plants. In the Russian Federation, bacteriological study of industrial objects at a food plant involves the determination of only three indicators: coliforms, *S. aureus* and total microbial count (TMC)[1].

These groups of microorganisms are considered universal indicators of food safety and production sanitation. However, such approaches have limitations. Firstly, they are usually developed on the basis of general standards and recommendations applicable to all food industry enterprises, without taking into account the specifics of a particular production. This may lead to the fact that the studied indicators do not reflect the real microbiological state of a particular plant.

Thus, a generalized approach to defining a limited group of microorganisms does not always provide accurate information on the actual conditions of microorganism circulation on a specific plant [17]. To obtain more reliable data, it is necessary to conduct individual microbiological studies that take into account the characteristics of each individual production, identify priority bacterial groups and select effective disinfectants based on these data.

The purpose of this study was to determine the priority bacterial groups typical for pork slaughter and processing plants ($n = 4$), as well as to identify specific microorganisms associated with the individual characteristics of each plant.

Objects and methods

Objects

The objects of the study were swabs ($n = 113$) collected in different production areas at pork slaughter and processing plants ($n = 4$). When collecting swabs, we used the principles of environmental monitoring and collected swabs both from surfaces in contact with food products and from remote abiotic objects [7].

Sampling of swabs was carried out in key areas of the production cycle: slaughterhouse, primary processing shop, cold rooms, and semi-finished products shop. The number of swabs collected in each area is presented in Table 1.

[1] MR4.2.0220-20. 4.2. Monitoring methods. Biological and microbiological factors. Methods for sanitary and bacteriological research of microbial contamination of industrial objects. Methodological recommendations (approved by the Chief State Sanitary Doctor of the Russian Federation on 04.12.2020) Retrieved from https://docs.cntd.ru/document/573595605 Accessed April 11, 2025

Table 1. Number of swabs collected in each area of MPP

	MPP No. 1	MPP No. 2	MPP No. 3	MPP No. 4
Slaughterhouse	$n=8$	$n=7$	$n=7$	$n=7$
Primary processing shop (boning and trimming)	$n=7$	$n=7$	$n=7$	$n=7$
Cold rooms	$n=7$	$n=7$	$n=7$	$n=7$
Semi-finished products shop	$n=7$	$n=7$	$n=7$	$n=7$

The sample included plants with various production problems and process features. MPP No. 1 slaughtered animals without prior decapitation, MPP No. 2 accepted raw materials with defects (abscesses), while MPP No. 3 and No. 4 had problems with shelf life and deterioration in the quality of finished products.

Methods

Sampling the swabs from the objects of the industrial environment

Swabs from the industrial objects at pork processing plants were collected using a sponge with a neutralizer (3M Hydra-Sponge, USA) from 100 cm^2, and in case of hard-to-reach places, using sterile cotton swabs with lethin broth (3M™ Swab-Sampler, USA). The resulting samples were incubated in Binder thermostat at 30 °C for 72 hours. After incubation, colonies with different morphology were selected from Petri dishes and identified using mass spectrometric analysis on Autof MS1000 MALDI-TOF device (Autobio, China).

An aliquot of 100 µl of liquid was taken from the bag with the sponge, which was distributed using a sterile spatula onto the surface of non-selective TSA agar (Oxoid, UK) in a Petri dish. After 72 hours of incubating the inoculations at a temperature of 24 °C, colonies were selected for species identification.

Detection of pathogenic microorganisms in the objects of the industrial environment

To detect *Listeria monocytogenes*, semi-concentrated Fraser broth was used and incubated in a thermostat (Binder, Germany) at a temperature of 30.0 ± 1.0 °C for 24 hours. To detect *Salmonella* spp., buffered peptone water was used as a diluent and incubated at a temperature of 37 ± 1.0 °C for 18 to 24 hours. To detect *Campylobacter* spp., an enrichment medium for *Campylobacter* (3M, USA) was used and incubated at a temperature of 41.5 ± 1.0 °C for 22 to 26 hours. Further studies were carried out in accordance with GOST 31659 (ISO 6579: 2002)[2], GOST 32031-2012[3] and GOST ISO 10272-1-2013[4].

Microorganism species identification by mass spectrometry

Species identification of the isolated colonies was performed on Autof MS1000 MALDI-TOF mass spectrometer (Autobio Diagnostics, China). For this, the bacterial mass of the colonies was applied to a plate and dried at room temperature. Then, 1.2 µl of formic acid was applied to each well with the dried bacterial mass for 10 min, dried, 1.2 µl of HCCA matrix (a-cyano-4-hydroxycinnamic acid, 99 %) was applied and dried again. The MALDI target was placed in the device and the equipment for microorganism identification was launched using the FlexControl software (spectra acquisition). The obtained results were analyzed using the software: if the value was below 6.0, the result was considered unreliable and was not used in further work. The result was considered reliable and taken into account at values of 6.0 to 9.0 at the genus level, and at values of 9.0 to 9.5 at the species level.

Results and discussion

Since all four studied plants specialized in pork slaughter and processing, it was reasonable to assume that they shared similar production conditions, such as the use of the same type of raw material (pork) and the same technological stages: slaughter, bleeding and subsequent cutting of carcasses. These common factors create conditions for the formation of stable microflora specific to this type of meat processing. Consequently, it could be expected that certain genera of microorganisms would be present on all four plants, playing a key role in the formation of the general microbiota of the industrial environment (Figure 1).

As a result of the microflora analysis at pork slaughter and processing plants, about 47 genera of microorganisms were found, of which 24 most common are presented in Figure 1. Genera composed less than 1 % are not shown in Figure 1.

The most common genera were *Pseudomonas* (26.3 %), *Escherichia* (8.8 %) and *Candida* (8.3 %). Bacteria of *Pseudomonas* genus were predominant and accounted for 26.30 % of the total counts of identified microorganisms. These microorganisms are considered to be one of the main causes of spoilage of meat, fruits and even beverages packaged in an aerobic environment [18]. In a study by Chinese scientists on microflora responsible for the spoilage of chilled pork, it was found that bacteria of *Pseudomonas* genus also accounted for the majority of the identified microorganisms. The average relative abundance of *Pseudomonas* in the product was 24.77 %, and the maximum abundance reached 44.43 % on the seventh day of storage [19]. This may be due to the high adaptability of pseudomonads to various conditions, including low temperatures, and the ability to effectively use available nutritional sources, which makes them dangerous for many food products [20]. There is evidence that the nature of spoilage may depend on both the species and the strain of *Pseudomonas* [21]. In a study conducted jointly by Italian

[2] GOST 31659 (ISO 6579: 2002) "Food products. Method for the detection of Salmonella spp." Retrieved from https://docs.cntd.ru/document/1200098239 Accessed April 11, 2025

[3] GOST 32031-2012 "Food products. Methods for detection of Listeria monocytogenes" Retrieved from https://docs.cntd.ru/document/1200105310 Accessed April 11, 2025

[4] GOST ISO 10272-1-2013 "Microbiology of food and animal feeding stuffs. Methods for detection and enumeration of Campylobacter spp. Part 1. Detection method" Retrieved from https://docs.cntd.ru/document/1200103500 Accessed April 11, 2025

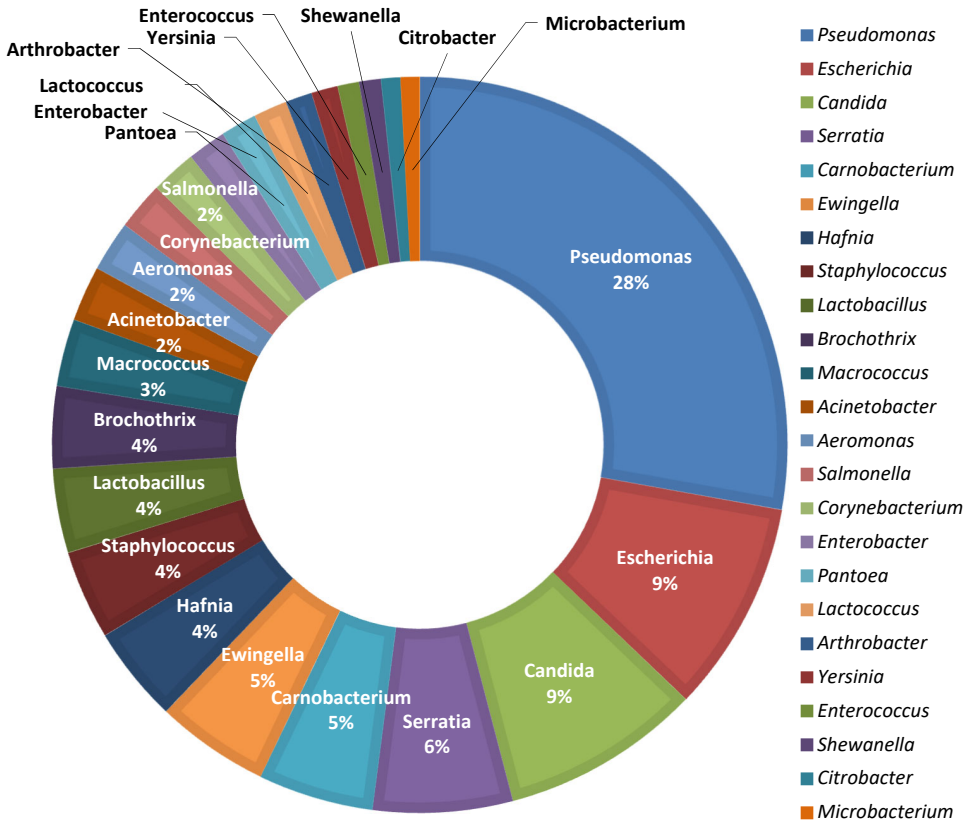

Figure 1. Predominant genera of microorganisms in the industrial environment of pork slaughter and processing plants

and American scientists, the diversity of *Pseudomonas* populations found during the processing of meat and dairy products was analyzed. It was found that the most common oligotypes were those belonging to the species *P. fragi* and *P. fluorescens* [21]. In our study at pork slaughter and processing plants, the diversity of microorganisms of this genus was represented by 40 species, of which 20.1% were *Pseudomonas brenneri*, followed by *Pseudomonas fragi* and *Pseudomonas gessardi* (9.3%), *Pseudomonas libanensis* (7.2%), *Pseudomonas tolaasi* (7.1%), and *Pseudomonas fluorescens* (5.5%). The data obtained in our study, as well as the results of other scientific works, confirm the widespread occurrence of *Pseudomonas* at meat processing plants. However, despite this, monitoring of these bacteria when taking swabs from the surfaces of equipment is not carried out. Given their high resistance to low temperatures and the ability to cause spoilage of products, regular analysis of swabs from equipment for the presence of pseudomonads would allow for their timely detection and the adoption of appropriate measures for disinfection and improvement of sanitary procedures.

The high level of *Escherichia* spp. occurrence (8.80%) indicates significant risks associated with food spoilage and potential risks to consumer health, since the vast majority of isolated strains belonged to *Escherichia coli* species. It should be remembered that pathogenic strains such as enterohemorrhagic *Escherichia coli* (EHEC) may cause severe food poisoning, accompanied by hemorrhagic colitis, which is life-threatening. The main source of *Escherichia coli* at pork processing plants is animal intestines, so these microorganisms may serve as an indicator of fecal contamination, which indicates the possible presence of other pathogenic microorganisms inhabiting animal intestines such as *Salmonella* or *Campylobacter*. It is worth noting that the distribution of pathogenic bacteria at different plants was not uniform. The largest counts of them were found on the plant, where pigs were slaughtered without prior decapitation.

The presence of yeasts of *Candida* species is also significant as these microorganisms may cause food spoilage and are of potential hazard to consumer health. *Candida* yeasts are important microbiological agents that contribute to problems in food production, especially in the context of meat processing, including pork. These microorganisms have the ability to proliferate under conditions of low pH, moderate temperatures and the presence of key nutrients such as glucose and amino acids, which determines their role as potential spoilage agents in meat products. They tend to adhere to the surfaces of equipment used for meat processing, forming biofilms that are resistant to standard sanitation and disinfection methods [22]. This phenomenon may lead to recurrent contamination of the manufactured products.

The next most common genera were *Serratia* (5.8%), *Carnobacterium* (4.8%), and *Ewingella* (4.7%). These microorganisms are also gram-negative bacteria and may be involved in food spoilage processes and also may be indicators of environmental contamination. Other genera present in smaller quantities include *Staphylococcus* (3.7%), *Lactobacillus* (3.5%), and *Brochothrix* (3.4%). *Staphylococcus* spp. are potential pathogens that may cause food poisoning, while *Lactobacillus* and *Brochothrix* may be associated with fermentation and food spoilage processes.

The presence of such microorganisms as *Carnobacterium* (4.80 %), *Lactobacillus* (3.50 %), and *Brochothrix* (3.40 %) may indicate potential problems associated with spoilage. These bacteria are known to affect the sensory properties of meat, making their presence undesirable during the processing and storage of meat products. *Carnobacterium* is a genus of Gram-positive bacteria that is often found in chilled food products, including meat. They may cause changes such as unpleasant odor and taste, as well as deterioration in the appearance of the product. These bacteria are able to actively grow even at low temperatures, which makes them especially dangerous in refrigerated storage conditions [23].

Lactobacillus is another group of bacteria that can be present in meat products. Although some lactobacilli species are used in food industry for fermentation, excessive amounts of these microorganisms may alter the sensory characteristics of meat (acidity), which in turn affects its quality and shelf life. In recent years, there has been considerable interest in the scientific community in exploring the possibilities of using lactic acid microorganisms as bioprotectants to combat spoilage bacteria such as *Pseudomonas*. Studies have confirmed that difficulties arise when attempting to transfer *in vitro* data into industrial conditions due to interactions between bacteria, antimicrobials, and food matrix structures [24].

Brochothrix is a bacterium known for its effect on the aroma and appearance of meat. It is capable of producing specific compounds that cause unpleasant odors and tastes, which reduces the quality of the final product [25].

In our country, monitoring of the sanitary and microbiological state of production is reduced to taking swabs from the hands of personnel, equipment surfaces, and inventory in contact with products. Abiotic objects not contacting with products are ignored. In addition, swabs are sampled before work or after proper surface treatment. In other words, the purpose of taking swabs is to control disinfection, and not to identify microorganisms circulating at the plant.

Given the prevalence of *Pseudomonas*, yeasts, and lactic acid microorganisms in the industrial environment, it is important to include in the monitoring program not only traditional indicators, but also other groups that are significant for the plant. In addition, to obtain an objective picture of the microbial composition, it is recommended to collect swabs not only after disinfection, but also during the work process. This will reveal the real degree of contamination and circulation of microorganisms at the plant, which will help to more effectively develop strategies for the prevention and management of microbiological safety risks [26].

After completing the analysis of the common microbiota at the four studied plants, a detailed analysis of the individual microflora at each plant was carried out, taking into account the features of technological processes and specific problems in production. This approach allowed to better understand the structure of the microbiological community at each plant and identify the features of microorganism distribution depending on the nature of production operations (Figures 2 to 5).

Figure 2 shows the microflora at MPP No. 1, where the technology of slaughtering animals without preliminary decapitation is used. This plant showed a high level of bacteria of *Escherichia* genus, which constituted the largest percentage among all identified strains. Such growth indicates a direct connection between the slaughter technology used and an increased risk of microbiological contamination of both the industrial objects and the finished product. As described earlier, a high level of *Escherichia* (a sanitary indicator microorganism) serves as an indicator of the possible presence of other microorganisms that are part of normal gastrointestinal microflora of animals, including potentially pathogenic species. This fact was confirmed in this study: it was MPP No. 1 that had the highest level of pathogenic microorganisms in the industrial objects.

Figure 3 shows the microflora at MPP No. 2, where raw materials with defects (abscesses) were accepted, which subsequently affected the results of microbiological analysis. The high percentage of staphylococci detected (14.5 %

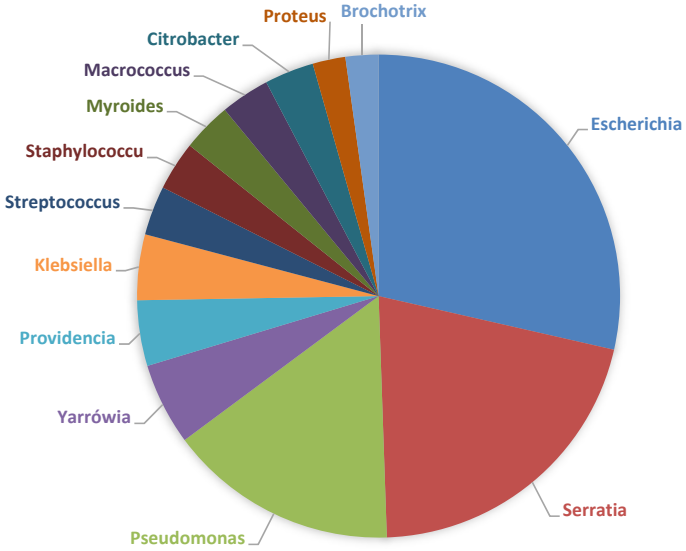

Figure 2. Microflora of the industrial environment at MPP No. 1

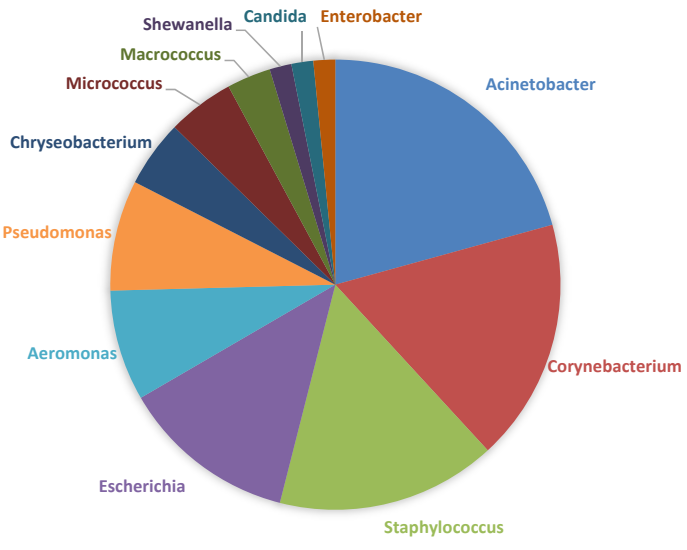

Figure 3. Microflora of the industrial environment at MPP No. 2

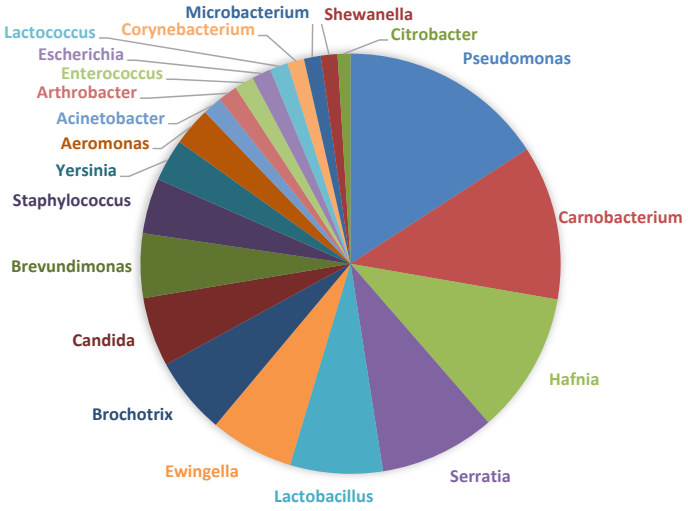

Figure 4. Microflora of the industrial environment at MPP No. 3

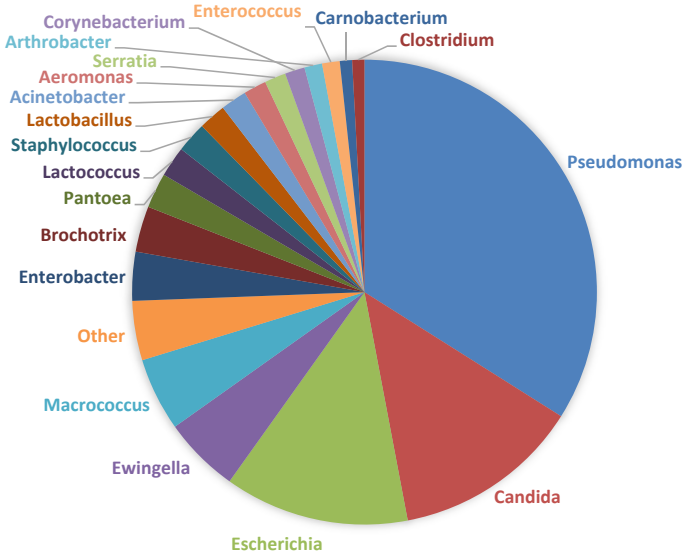

Figure 5. Microflora of the industrial environment at MPP No. 4

of all identified strains) in swabs confirmed the contamination of the industrial objects due to the use of low-quality raw materials. One of the main causes of abscess development is *Staphylococcus aureus*, which is highly virulent and can penetrate deep into the body tissues. *Staphylococcus aureus* is also a member of pathogenic group known as ESKAPE, which is characterized by multidrug resistance, making the treatment of infections in humans extremely difficult [27,28]. It is worth noting that *Staphylococcus aureus* is very dangerous not only due to its multiple antibiotic resistance, but also due to its ability to produce enterotoxins (A, B, C, D, E), which may cause severe food poisoning associated with the consumption of raw, undercooked or improperly processed products [29]. In our previous study, the microbial composition of minced meat intended for the production of dry-cured sausages was analyzed. During the study, 2 enterotoxigenic strains of *Staphylococcus aureus* were identified [30]. The detection of such strains emphasizes the importance of strict quality control of raw materials and compliance with sanitary and hygienic standards at all stages of production.

At MPP No. 2, the dominant genus of microorganisms was *Acinetobacter*. A representative of this genus, *Acinetobacter baumannii*, is also included in the ESKAPE group of pathogens. The risk of foodborne ESKAPEE infections is particularly high for hospital patients, as these microorganisms may be spread through hospital kitchens [31]. There is a report that strains of *Acinetobacter baumannii* causing enterogenous sepsis have been isolated from hospital kitchens in Portugal and Brazil [32].

At meat processing plants No. 3 and No. 4, problems related to spoilage and shelf life of finished products were observed. These difficulties negatively affected the quality of the products, and also led to financial losses and a decrease in consumer confidence (Figures 4 and 5). Bacterial groups responsible for spoilage of chilled meat and meat products are usually *Pseudomonas* spp. and *Brochothrix thermosphacta* [33]. Microbiological analysis performed at these two plants showed that the dominant group of microorganisms at plant No. 3 was *Pseudomonas* spp., followed by *Carnobacterium* spp. (12 %). At MPP No. 3, the share of *Brochothrix* genus microorganisms was 6 %, and at MPP No. 4 it was 2 %. In addition to *Pseudomonas* spp., at MPP No. 3, high levels of other *Enterobacteriaceae* family representatives were revealed: *Hafnia* genus amounted to 11 %, *Serratia* genus amounted to 9 %, and *Ewingella* genus amounted to 6.5 %. It is known that enterobacteria are considered indicator bacteria of the sanitation state of the production process, since their presence may indicate poor compliance with hygienic standards, violation of raw material processing technologies, or improper equipment disinfection [34].

Hafnia spp. are facultative anaerobes, motile due to flagella. The best-known representative of this genus is *Hafnia alvei*. These bacteria live in soil, water, food products and the intestines of animals. They rarely cause diseases in humans, but may sometimes cause urinary infections, gastrointestinal infections and even sepsis in patients with weakened immunity [35,36]. In healthy people, they most often act as saprophytes.

Serratia spp. is a genus of gram-negative bacteria that can play a significant role in the process of meat spoilage. These microorganisms are capable of producing proteolytic enzymes destroying proteins that are part of muscle tissue. Proteolysis leads to changes in the structure of meat, unpleasant odor and deterioration of the sensory properties of the product [37].

Ewingella spp. is a genus of bacteria from *Enterobacteriaceae* family, which is represented by only species, *Ewingella americana*. They are facultative anaerobes and have low motility [38]. *Ewingella americana* is found in the environment, particularly in soil and water. It rarely causes diseases in humans, although cases of bacteremia and septicemia have been reported, especially in immunocompromised individuals. Its clinical significance remains poorly understood. This genus of bacteria is rarely found in meat products, and its presence may be due to contamination from equipment, water, or other external sources [39].

Table 2. Example of individual recommendations for improving sanitation at meat processing plants

Plant	Process failure/production problem	Microbiological consequences	Problem solution
MPP No. 1	Slaughter without prior decapitation	Growth of *Escherichia* spp.	Changing slaughter technology (preliminary decapitation). Strengthening sanitation of surfaces and equipment. Introducing tests for enterohemorrhagic strains of *E. coli*.
MPP No. 2	Acceptance of raw materials with defects (abscesses)	Growth of *Staphylococcus* spp. and *Corynebacterium* spp.	Separating defective and non-defective carcass processing lines. Strengthening sanitary procedures.
MPP No. 3	Systematic spoilage of finished products	Growth of psychrotrophs (*Pseudomonas* spp., *Carnobacterium* spp.)	Using disinfectants effective against biofilms; microbiological control of water.
MPP No. 4	Systematic spoilage of finished products	Growth of *Pseudomonas* spp., *Candida* spp.	Using disinfectants effective against yeasts, as well as biofilms; microbiological control of water.

At MPP No. 4, *Ewingella* genus microorganisms were also detected, which, along with representatives of *Pseudomonas, Candida, Escherichia,* and *Macrococcus* genera, formed a stable microflora. *Pseudomonas* genus microorganisms accounted for 32 % of all identified strains. The shares of *Candida* spp., *Escherichia* spp., *Ewingella* spp., and *Macrococcus* spp. were 12.3 %, 12.1 %, 5 %, and 4.8 %, respectively. *Macrococcus* genus microorganisms are often associated with the skin of animals or personnel. Their presence may be due to insufficient processing of carcasses or violation of sanitation processes [40].

Thus, the microbiological profile of MPP No. 4 reflected both the bacteria typical for meat processing plants (*Pseudomonas* spp., *Candida* spp., *Escherichia* spp.) and the unique features associated with the presence of *Ewingella* and *Macrococcus*.

The data obtained may form the basis for developing individual recommendations for improving sanitation at each plant.

Conclusion

Meat production technology plays a key role in the formation of microbiota at a plant, since each stage of the technological process creates unique conditions that promote the growth of certain groups of microorganisms. A general trend towards the dominance of *Pseudomonas, Candida* and *Escherichia* genera was revealed at the studied pork slaughter and processing plants ($n = 4$). However, each plant had its own unique microbiological characteristics due to the specifics of technological processes and production conditions.

Thus, at MPP No. 1, where preliminary decapitation was not carried out, a high level of industrial objects contamination with *Escherichia* and pathogenic *Salmonella* spp. and *Listeria monocytogenes* was revealed, indicating contamination with gastrointestinal microorganisms. At MPP No. 2 using raw materials with abscesses, significant counts of *Staphylococcus* genus microorganisms were found. At MPP No. 3 and No. 4, where spoilage of finished products was observed, *Pseudomonas* spp., *Carnobacterium* spp. and *Brochothrix* spp. were identified.

Given the prevalence of *Pseudomonas*, yeasts (*Candida*), and representatives of *Enterobacteriaceae* family in the industrial environment of all studied plants, it is important to expand the microbiological monitoring program. In addition to traditional indicators (total microbial count, coliforms, *Staphylococcus aureus*), it is necessary to include priority groups of microorganisms for each plant, such as *Pseudomonas, Candida, Enterobacteriaceae*, as well as pathogens (*Salmonella* spp., *Listeria monocytogenes*). This will allow for more accurate assessment of microbial contamination risks and timely measures to eliminate them.

The data obtained may form the basis for developing individual recommendations for improving sanitation at each plant. For example, at MPP No. 1, it is necessary to strengthen control over cutting carcasses and preliminary decapitation in order to minimize contamination with gastrointestinal microorganisms. At MPP No. 2, it is important to pay attention to the quality of incoming raw materials and their preprocessing. At MPP No. 3 and No. 4, it is necessary to optimize the product cooling and storage system, as well as strengthen equipment disinfection measures.

Thus, the implementation of advanced microbiological monitoring and the development of individual recommendations for each plant will reduce the risks of microbiological contamination, improve product quality and increase its safety for consumers.

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THE THERAPEUTIC ROLE OF THE ALCOHOLIC EXTRACT OF *GUNDELIA TOURNEOFORTTI* L. IN *SALMONELLA* TYPHIMURIUM INFECTION AND ITS HEALTH EFFECTS IN LABORATORY RATS

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Keywords: *Gundelia tournefortii L., Salmonella Typhimurium, children, health effects*

Abstract

This study investigates the effectiveness of the alcoholic extract of Gundelia tournefortii L. in mitigating the effects of Salmonella Typhimurium infection on hematological parameters and liver and kidney function in laboratory animals. S. Typhimurium was isolated from 23 samples of food and stool collected from pediatric patients (aged 1–6 years) with diarrhea at Salah al-Din Hospital. The findings indicate that infection with S. Typhimurium led to a significant increase ($P < 0.05$) in total white blood cell (WBC) and platelet counts, reaching $28.16 \times 10^3/\mu L$ and $713 \times 10^3/\mu L$, respectively, compared to the control values of $8.50 \times 10^3/\mu L$ and $658 \times 10^3/\mu L$. Conversely, red blood cell (RBC) and hemoglobin (Hb) levels were significantly reduced ($P < 0.05$) in infected animals, measuring $5.79 \times 10^6/\mu L$ and 13.0 g/dL, respectively, compared to $4.60 \times 10^6/\mu L$ and 14.10 g/dL in the control group. Liver function tests revealed elevated levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in infected rats, with values of 164.0, 142.0, and 66.0 IU/L, respectively, compared to the control values. Renal function analysis showed a significant increase ($P < 0.05$) in urea concentration in the infected group (48.5 mg/dL) relative to the controls (41.2 mg/dL), while no significant differences were observed in creatinine levels between groups. Notably, administration of the alcoholic extract at doses of 200 and 400 μg/kg significantly mitigated the adverse effects of infection, demonstrating its potential therapeutic efficacy.

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Introduction

Salmonella spp. are recognized as significant zoonotic pathogens, posing serious threats to public health and contributing to substantial economic burdens globally, both in developing and industrialized nations, due to the high costs associated with disease treatment, prevention, and control efforts [1]. The genus encompasses over 2,500 serovars, each varying in its ecological niche and pathogenic potential, although only a limited number are responsible for the majority of human infections [2].

Members of the genus *Salmonella* are Gram-negative, facultatively anaerobic, rod-shaped, and non-spore-forming bacteria belonging to the family *Enterobacteriaceae*. Most strains are motile via peritrichous flagella and can metabolize substrates through oxidative pathways. Furthermore, many strains characteristically produce hydrogen sulfide during metabolism [3]. The genus *Salmonella* includes numerous pathogenic serovars implicated in both human and animal diseases. A notable serovar is *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) [4].

Salmonella spp. are notorious for causing gastroenteritis and systemic infections, frequently associated with symptoms such as abdominal cramps, fever, and diarrhea [5]. These pathogens have a broad host range and can contaminate a variety of foods, particularly meats from poultry and cattle, often without producing overt signs of disease in the animal reservoir [6,7].

Typhoidal serovars, such as *Salmonella enterica* serovar Typhi (*S.* Typhi) and *S.* Paratyphi, are responsible for typhoid and paratyphoid fevers, which are life-threatening

systemic infections disproportionately affecting populations in low- and middle-income countries [8]. Globally, *Salmonella* is consistently ranked among the most common causes of foodborne illnesses [9]. According to estimates from CDC, *Salmonella* is responsible for more foodborne illnesses than any other bacterial pathogen, with poultry, particularly chicken, being a major source. It is estimated that approximately 1 in every 25 packages of raw chicken sold in grocery stores contains *Salmonella* [10].

Despite its well-established status as a public and veterinary health concern, the taxonomy of *Salmonella* remains complex and often confusing for clinicians, veterinarians, and microbiologists due to its constantly evolving nomenclature [11].

The majority of the approximately 2.500 serovars are capable of causing self-limiting gastroenteritis in both humans and animals. However, certain serovars have evolved host-specific adaptations, resulting in systemic, extraintestinal infections in particular species [12]. For instance, *S.* Typhimurium and *S.* Enteritidis circulate among diverse vertebrate hosts and are leading agents of foodborne infections in humans. Non-typhoidal *Salmonella* (NTS) serovars are estimated to cause around 75 million cases of gastroenteritis annually, resulting in approximately 27.000 deaths worldwide [13]. Conversely, typhoidal serovars, such as *S.* Typhi and *S.* Paratyphi A, are strictly human-adapted and cause about 2.5 million infections annually, leading to roughly 65,000 deaths due to typhoid and paratyphoid fevers [8]. Other host-adapted serotypes include *S.* Gallinarum in poultry and *S.* Dublin in cattle, each associated with invasive systemic infections in their respective hosts [12].

Traditionally, culture-based techniques have been regarded as the gold standard for the isolation and identification of foodborne pathogens, including *Salmonella* [14]. Nevertheless, alternative methods such as immunoassays and nucleic acid-based techniques have also been employed to detect foodborne microorganisms [15], although these approaches may be hindered by limitations in sensitivity and specificity [16]. Polymerase chain reaction (PCR) is widely recognized for its rapidity and high sensitivity in detecting foodborne pathogens. However, its performance can be compromised by inhibitors commonly present in complex food matrices [17]. Consequently, PCR-based detection is often supplemented with conventional culture methods to confirm the presence of target organisms [14].

The extensive use of chemical compounds in food production and preservation has led to numerous health concerns. Therefore, humans have been searching for natural sources, including medicinal plants or their extracts. Some plants have been used in herbal medicine for therapeutic purposes in various disease conditions to reduce symptoms due to their content of effective compounds against many pathogens, in addition to their direct use in food [18]. The plant produces these compounds as by-products and they are called phytochemicals. They are biologically active components and have multiple therapeutic properties. These compounds protect the plants themselves from various parasites, and these compounds can be divided on the basis of their chemical composition into multiple compounds [19,20]. Because of their biological effectiveness due to their content of active compounds (phytochemicals), such as phenols, alkaloids, flavonoids, essential oils and resins, these plants have confirmed their ability to treat many pathological conditions and inhibit the growth of many microbial pathogens [20,21,22]. It may be any part of the plant that contains the aforementioned components such as seeds, leaves, roots, coat, and flowers [23].

The increasing prevalence of antibiotic resistance among various types of microbes, has intensified the search for effective natural alternatives. One of these plants is *Gundelia tournefortii L.* It is a valuable wild herbaceous spiny perennial plant with a height of about 60 cm, which belongs to the *Asteraceae* family and grows in semi-desert or sandy plains in Palestine, Jordan, Syria, Iran, Iraq, Azerbaijan, temperate regions in West Asia, Armenia, Turkey and other regions. Its leaves are leathery and hard with thorny serrated lobes, and their color alternates between yellow and red [24]. Its flowers can be hermaphrodite and can be yellow, white, green or red [25].

Traditionally, G. *tournefortii* has been consumed as food in Palestine, Syria, Turkey and Jordan in particular [25,26]. Due to its high economic values for culinary uses, it is used as a food ingredient in making pickles, appetizers, and soups [23]. This plant is characterized by its nutritional benefits. It has been used as food for more than 2000 years in the Babylonian civilization and continues to provide food, medicine, and income to local communities, especially the poorest, in an economically, socially and environmentally sustainable manner [27]. *G. tournefortii* is a rich source of minerals, vitamins and essential fatty acids, and the oil extracted from its flowers is also a rich source of essential fatty acids such as linoleic acid, and oleic acid [28]. Additionally, arachidic acid was also found. *G. tournefortii* contains b-sitosterol as the dominant sterol, which represents more than 51.76 % of the total sterols in the extracted oil, as well as other sterols, such as stigmasterol, 5-avenasterol, campesterol, 7-stigmastenol and 7-avenasterol [29,30,31].

As for its therapeutic or medicinal properties, this plant has been used in the treatment of many diseases, as it reduces the content of fats, cholesterol and blood sugar. Additionally, diarrhea, fever, cough, cold, stomach infections, kidney pains, intestinal disorders [25,32] and toothache [33] are treated with it. It also contributes to protect the liver [32], and acts as an antimicrobial, antiparasitic, and antioxidant due to its ability to inhibit and destroy free radicals. *G. tournefortii* also has anti-inflammatory properties and contributes to purify the blood and treat bronchitis, respiratory diseases, skin diseases, angina pectoris, cancer and stroke [34,35,36]. Its dry seeds are also effective in treating vitiligo and it is also used as a diuretic [37].

Therefore, the aim of this study was to determine the efficacy of the alcoholic extract of *G. tourneofortti L.* plant in diarrhea caused by *S.* Typhimurium in terms of its health effects on blood profile, liver and renal functions in laboratory animals.

Materials and methods

Identification of Salmonella Typhimurium

Food and stool samples were collected from autistic children under school age (1–6) years who had diarrhea while in the hospital at Salah al-Din Hospital during the period from October 1, 2022 to December 30, 2022. Twenty-three food and stool samples were collected.

Serial dilutions of the samples were prepared, the last two dilutions were grown on appropriate culture media (MacConkey agar, Nutrient agar, SS agar, Manitol salt agar and Blood agar) made by Oxoid (USA), they were cultured on the media for each species. Then, a number of diagnostic tests were performed to determine phenotypic, cultural and microscopic characteristics. Several biochemical tests, such as catalase, oxidase, urease, coagulase, IMViC, and fermentation of sugars (glucose, mannitol, sucrose, lactose, arabinose, galactose and sorbitol) were carried out for the colonies, depending on the Bergey's manual. The tests were performed as follows.

Microscopic tests

A microscopic examination of active bacterial cells at the age of 18 hours was performed to determine result of Gram staining and cell shapes.

Cultural characteristics

The cultural and morphological characteristics of the growing colonies included the shape of the colonies, their color, edges, size, and height.

Biochemical tests

Catalase test

A portion of the colonies at the age of 18–24 hours was transferred to a sterile glass slide and a drop of hydrogen peroxide reagent at a concentration of 3 % was added to it. The appearance of air bubbles is an indication of the positivity of the test and the production of the catalase enzyme from the tested bacterial species [38].

Oxidase test

The test was performed by adding many drops of oxidase reagent to a Whattman No 1 filter paper to the point of saturation. A wooden stick was then used to transfer a touch of active bacterial growth to the surface of the reagent-saturated filter paper. The appearance of the purple color within a few seconds was evidence of the species' ability to produce oxidase enzyme [38].

Urease test

The test was conducted by inoculating the colonies of bacteria on urea agar (slants) and incubating the tubes with urea agar for 24 hours at 37 °C. The color of the medium changed from yellow to pink, indicating that the test was positive, because of the change in the pH of the medium to alkaline due to the ammonia formed [39].

Coagulase test

The test was performed using the slide test method, where a drop of blood plasma was placed on a glass slide, then a part of a colony growing on a solid nutrient medium was taken by a sterile carrier and mixed with the drop. The reaction was considered positive if the plasma clotted within 20 seconds, indicating the presence of the bound coagulase enzyme [40].

Methyl red test

The MRVP (5g glucose, 5g peptone and 5g and K_2HPO_4) was prepared according to what was stated in [41], inoculated with bacteria, then incubated at 35 °C for 24 h. After that, the methyl red indicator was added. If the color changes from red to orange, the test is negative, and if the color remains red, the test is positive.

Voges–Proskauer test

This test was performed according to the method of [42], which was used to detect the ability of bacteria to produce acetone.

Citrate utilization test

The plates containing Simmons citrate media were inoculated with the colonies of bacteria under study, after which they were incubated at 37 °C for 24 h. The evidence of a positive result was the change in the color of the medium from green to blue [43].

Carbohydrate fermentation test

The isolates were inoculated into tubes containing the sugar fermentation medium, and incubated at 37 °C for 24 h. The change in the color of the medium from red to yellow color is evidence of a positive test [38].

The diagnosis was confirmed to the level of species by the Vitek 2 Compact system (64 cards) and the serovar was determined using the Kauffmann-White scheme [44,45,46].

Samples collection

Gundelia tourneofortti L. samples were collected from the central regions in Iraq and were diagnosed based on the taxonomic keys of the flora of Iraq [47].

Preparation of the alcoholic extract

The alcoholic extract was prepared by weighing 50 g of plant powder with 500 ml of ethanol alcohol (80 %) and holding overnight, after which it was filtered using layers of gauze for the purpose of getting rid of unwanted parts and then centrifugated (Compact Tabletop Centrifuge 2010, Kubota, Japan) for 15 minutes at 3000 rpm. Then, it was filtered using Whatman No. paper to obtain a clear liquid. The resulting filtrate was concentrated and dried in a rotary vacuum evaporator (IKA-Werke, Staufen, Germany). The dry plant extract was collected and kept in the refrig-

erator in opaque and tightly closed glass bottles [48], as shown in Figure 1.

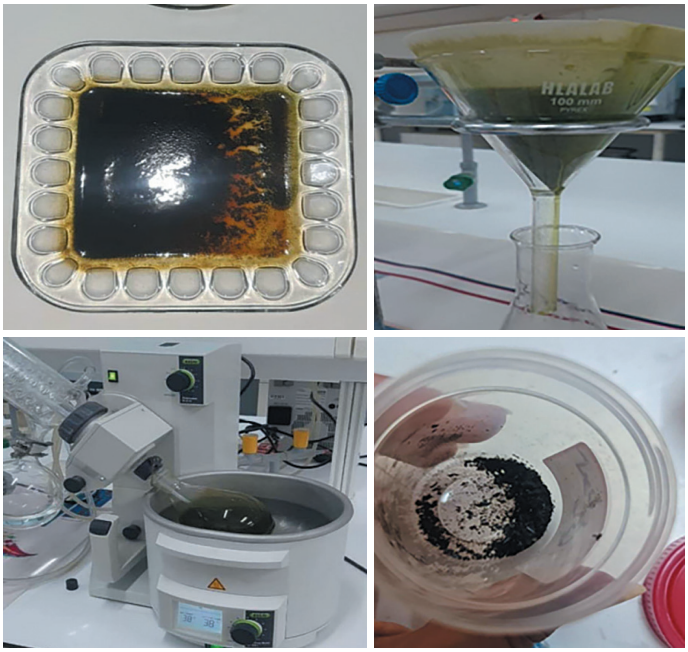

Figure 1. Preparation of the alcoholic extract of *Gundelia tourneofortti* L.

In vivo experiment

The study utilized twenty male rats, weighing 140–149 g and aged 8–10 weeks. They were housed in stainless steel cages in conditions that were suitable for them, such as 25 °C, humidity levels of 50–70 %, controlled lighting, and proper ventilation. A nutritionally adequate diet was provided, and cage cleanliness was maintained by regularly changing the sawdust, as mentioned in [49]. The experiment was designed by dividing the rats into four groups, with five replications per group, as follows: first treatment (T1), the control group second treatment (T2), rats with diarrhea caused by *Salmonella* Typhimurium; third treatment (T3), rats with diarrhea caused by *S.* Typhimurium and treated with an alcoholic extract of *G. tournefortii* at 200 µg/kg; and fourth treatment (T4), rats with diarrhea caused by *S.* Typhimurium and treated with the alcoholic extract of *G. tournefortii* at 400 µg/kg. The extract was administered orally at a dose of 2 mL. Diarrhea was induced in rats by experimental infection with *S.* Typhimurium suspension (1.5×10^8 CFU/mL) prepared using the McFarland turbidity standard for comparison and the experiment continued for 28 days. At the end of the experimental period, the animals were fasted for 20 hours, anesthetized with chloroform, and then dissected. Blood samples were collected directly from the heart for various tests, including blood profile analysis [WBC], [RBC], [PLT], [HB], and [HCT%], as well as assessment of liver enzyme activity [ALT], [AST], and [ALP]) and function of renal markers (urea and creatinine).

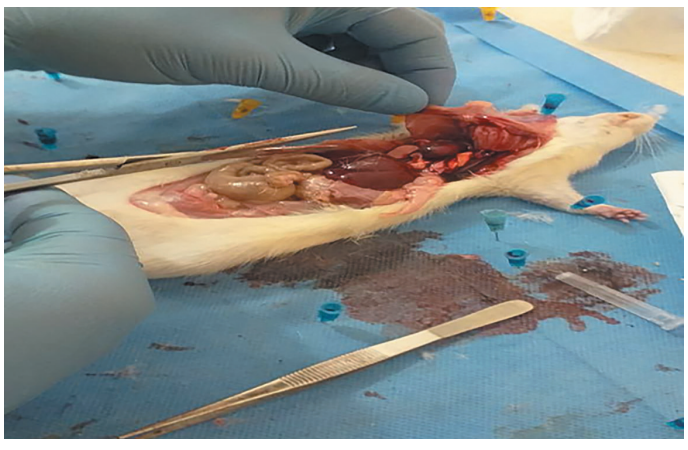

Figure 2. Anatomy of rats

Blood profile

The total counts of WBC, RBC, PLT, and HG were measured using Complete Blood Count (CBC) device.

The activity of liver enzymes

The activity of liver enzymes was estimated using analysis kits prepared by BIOLABO (France), following the method described by [32]. Aspartate aminotransferase (AST) activity was determined using these kits, while alanine aminotransferase (ALT) activity was measured based on the formation of pyruvate hydrazone through the transamination process in the presence of ALT and 2,4-dinitrophenylhydrazine, with absorbance recorded at 546 nm using the spectrophotometer (V-1100D, EMCLAB, Germany). Alkaline phosphatase (ALP) activity was also assessed using BIOLABO kits, adhering to the methodology outlined by [50].

Renal functions

Creatinine was estimated using a ready-made kit from BIOLABO (France), following the manufacturer's instructions. The absorbance was measured at 490 nm (V-1100D, EMCLAB, Germany) [32], and creatinine concentration was determined using the formula: Concentration of creatinine (20 mg/dl) = absorbance of sample / standard solution absorbance × standard solution concentration. Similarly, urea was estimated using an analysis kit from BIOLABO (France), following the supplier's instructions as described by [50].

Statistical analysis

The experimental system was analyzed using the statistical program [51] with CRD. The means were compared using Duncan's test [52] to determine the significance of differences among the factors influencing the study's traits, with a probability level of 0.05.

Results and discussion

Isolation and identification of bacteria causing diarrhea in children

Samples were collected and bacteria were cultivated using appropriate culture media, including MacConkey agar, Nutrient Agar, SS Agar, Mannitol Salt Agar, and Blood Agar. The identification of bacteria was performed by comparing the results of phenotypic, microscopic, and biochemical tests with Bergey's Manual [53] and confirmed

using the Vitek 2 compact system and the Kauffmann-White technique was used to determine the serovar. This scheme divides Salmonella enterica into six subspecies and several serotypes. A recent revision to the White-Kauffmann-Le Minor scheme was made by the World Health Organization (WHO) collaborating center for the reference and investigation of Salmonella [44,45,46]. The following bacteria contaminating meat samples were identified: *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis,* and *Salmonella* Typhimurium as shown in Table 1.

Table 1. Biochemical tests of isolated bacteria

Bacteria	Gram	Catalase	Oxidase	Urease	Coagulase	Simmons citrate	Voges Proskauer	Indole test	Methyl red	Fermentation of sugars						
										Glucose	Mannitol	Sucrose	Lactose	Arabinose	Galactose	Sorbitol
Staph. aureus	+	+	–	+	+	+	+	–	–	+	+	+	+	+	+	+
E. coli	–	–	–	–	V	–	–	–	+	–	+	–	–	+	V	V
Ent. faecalis	+	–	–	–	+	–	+	–	–	+	+	+	+	+	+	+
S. Typhimurium	–	+	–	–	–	–	–	–	–	+	V	+	+	V	V	V

(+) Positive for the test, (–) Negative for the test, (V) Differential results

Effect of the alcoholic extract of Gundelia tournefortii L. on blood profile of rats

Table 2 shows a significant increase at ($P<0.05$) in WBC count in laboratory rats exposed to experimental infection with *S.* Typhimurium without treatment, reaching 28.16 10^3/mm^3 compared to the control group (8.50). Additionally, the platelet count significantly increased to 713 in the infected and untreated group. Conversely, RBC and hemoglobin levels significantly decreased to 5.79 and 13.0, respectively, compared to the control group (6.40 and 14.10, respectively). However, when the extract was administered at concentrations of 200 and 400 µg/mg to infected rats, WBC counts significantly decreased, while RBC and hemoglobin levels increased compared to the infected group that did not receive the extract. Notably, the T4 treatment, which involved administering the extract at 400 µg/mg, demonstrated superior efficacy. Regarding red blood cell HCT%, no significant differences were observed among the treatments. These findings align with several studies [54,55,56], which concluded that a decrease in RBC counts due to bacterial infection results from impaired or lost cells, with bone marrow unable to generate or replace them. The reduced RBC count is attributed to hemolytic anemia, where RBC destruction leads to decreased hemoglobin concentration, impairing its function in transporting nutrients, ultimately affecting cellular activity and potentially leading to cell death [57,58]. The increase in WBC count is likely due to bone marrow stimulation, promoting cell production, while peripheral blood changes may result from activated helper T lymphocytes, which can elevate eosinophil levels and increase vascular permeability to facilitate cell migration to inflammation sites [54,57,59]. The reduction in leukocyte values following oral extract administration is attributed to its bioactive compounds, particularly polyphenols and glycosides, known for their anti-inflammatory properties by inhibiting cytokine activity [36]. This study is consistent with [60], who reported a significant difference in various blood indicators between the control and infected groups treated with the extract at 250 and 500 mg/kg.

Table 2. Impact of the alcoholic extract of *Gundelia tourneofortti L.* on blood profile of rats exposed to experimental infection with *S.* Typhimurium

Treatments	WBC (10^3/mm^3)	RBC (10^6/mm^3)	HB (G/dl)	HCT %	PLT (10^3/mm^3)
T1	8.50^c	6.40^b	14.10^a	44.06^a	658^b
T2	28.16^a	5.79^{ab}	13.0^c	47.80^a	713^a
T3	12.86^b	6.20^{ab}	13.7^{ab}	39.00^a	711^a
T4	11.00^b	6.33^a	13.40^b	42.26^a	652^b

At a probability threshold of 0.05, similar letters in the same column indicate that there were no significant differences between them.
T1 — control group; T2 — rats with diarrhea caused by *S.* Typhimurium; T3 — rats with diarrhea caused by *S.* Typhimurium and treated with the alcoholic extract of *G. tournefortii* at 200 µg/kg; T4 — rats with diarrhea caused by *S.* Typhimurium and treated with the alcoholic extract of *G. tournefortii* at 400 µg/kg.

Effect of the alcoholic extract of Gundelia tournefortii L. on liver activity in rats exposed to experimental infection with S. Typhimurium

Table 3 shows a significant increase in the values of liver enzymes (ALP, ALT, and AST) for the group of rats exposed to *S.* Typhimurium infection, with values of 164.0, 66.0, and 142 IU/L, respectively, compared to the control group, which had values of 104, 51.0, and 115 IU/L. However, when the rats were dosed with *Gundelia tournefortii* L. extract at concentrations of 200 and 400 µg/kg along with the infection, this treatment led to a significant decrease in the enzyme concentrations in the group treated with the extract at 400 µg/kg, with values of 159.7, 135, and 48.0 IU/L, respectively. As for the group treated with the extract at 200 µg/kg, no significant differences were recorded in the enzyme values compared to the infected group.

The elevated concentrations of liver enzymes ALT and AST in the infected rat group indicate the extent of damage occurring in the liver tissue, which causes the breakdown of the cell membrane and the release of these enzymes into the bloodstream. This results in increased enzyme concentrations in the blood serum and reflects a clear effect on the liver's various functions. The rise in AST levels could also

be attributed to metabolic alterations in the liver, followed by the production of toxins, and various pathological conditions such as acute bacterial infections and tumors, affecting organs such as the heart and muscles [61].

As for the rise in alkaline phosphatase enzyme ALP, it is due to pathological conditions, including biliary stasis, partial or complete obstruction of the bile duct, and neoplastic liver disease. On the other hand, this increase in the concentration of this enzyme may be attributed to the increase in the activity of lysosomes, which is one of the important changes before cell death [47].

The decrease in these enzyme levels in the serum of rats treated with the extract, along with the return of enzyme levels to their normal state, suggests the potential use of the alcoholic extract to support the maintenance of liver function [62]. The results of this study are consistent with those observed by Saleh et al. [63], who indicated a significant decrease in liver enzymes in rats treated with *Gundelia tournefortii* L. This plant is considered to have protective properties in supporting liver health [25,64].

Table 3. Impact of the alcoholic extract of *Gundelia tourneofortti L.* on the liver activity of rats exposed toexperimental infection with *S.* Typhimurium

Treatments	ALP	ALT	AST
	(IU/L)		
T1	104.0^{c}	51.0^{b}	115^{c}
T2	164.0^{a}	66.0^{a}	142^{a}
T3	179.0^{a}	61.0^{a}	140^{a}
T4	159.7^{b}	48.0^{b}	135^{b}

At a probability threshold of 0.05, similar letters in the same column indicate that there were no significant differences between them.
T1 — control group; T2 — rats with diarrhea caused by *S.* Typhimurium; T3 — rats with diarrhea caused by *S.* Typhimurium and treated with the alcoholic extract of *G. tournefortii* at 200 µg/kg; T4 — rats with diarrhea caused by *S.* Typhimurium and treated with the alcoholic extract of *G. tournefortii* at 400 µg/kg.

Effect of the alcoholic extract of G. tournefortii on kidney function in rats exposed to experimental infection with S. Typhimurium

Table 4 shows that in rats infected with *S.* Typhimurium, the urea level was significantly higher (48.5 mg/dL) than in the control group (41.2 mg/dL). Treatment with the *Gundelia tournefortii* L. extract at 200 and 400 µg/kg for the infected rats led to a signifiicant decrease in urea concentration, reaching 41.7 and 43.6 mg/dL, respectively. However, there were no appreciable variations in creatinine levels between the different treatments in the experiment.

The rise in urea levels is attributed to damage to renal tissues or a physiological defect in renal function, which results in a reduction in its efficiency [65]. Additionally, an increase in the concentration of free radicals in the body and oxidative stress lead to oxidation of proteins, which results in an increase in urea in the blood. The kidney removes urea from the blood, and the damage to the renal or its failure to remove urea may lead to an increase in blood urea level [66]. The results agree with [67]. Based on the results of these tests, we concluded that this plant is considered to have therapeutic and preventive properties against *Salmonella* infection. These findings align with those of Ayoubi and Baradari [7], who indicated that the plant has the bactericidal activity against *Salmonella* spp. In addition, the results are also in agreement with those of Rabizadeh and Mirian [68], who showed that natural plant extracts hold promise for preventing and treating liver diseases.

Table 4. Impact of the alcoholic extract of *G. tourneofortti* L. on the activity of kidney function of animals exposed to experimental infection with *S.* Typhimurium

Treatments	Urea	Creatinine
	mg/Di	
T1	41.2^{b}	0.3^{a}
T2	48.5^{a}	0.2^{a}
T3	41.7^{b}	0.3^{a}
T4	43.6^{ab}	0.3^{a}

At a probability threshold of 0.05, similar letters in the same column indicate that there were no significant differences between them.
T1 — control group; T2 — rats with diarrhea caused by *S.* Typhimurium; T3 — rats with diarrhea caused by *S.* Typhimurium and treated with the alcoholic extract of *G. tournefortii* at 200 µg/kg; T4 — rats with diarrhea caused by *S.* Typhimurium and treated with the alcoholic extract of *G. tournefortii* at 400 µg/kg.

Conclusion

Based on the study's findings, we concluded that infection with *S.* Typhimurium significantly reduced the total number of red blood cells and hemoglobin concentration and increased the activity of liver enzymes and the concentration of urea. Treatment of infected rats with an oral dose of the *Gundelia tourneofortti L.* alcoholic extract at 400 µg/kg had a significant effect leading to an increase in the number of RBC and haemoglobin concentration, reduction of the activity of liver enzymes and regulation of renal functions due to its content of effective compounds such as phenols, flavonoids, tannins, turbines and resins.

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EXPLORATIVE STUDY OF BLOOD UREA NITROGEN (BUN), SERUM ESTROGEN, AND CONCEPTION RATE (CR) IN SMALL-SCALE DAIRY FARMS BASED ON QUANTITY OF FEED CONCENTRATE CONSUMED BY THE CATTLE

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Keywords: *services per conception (S/C), milk yield, BUN, estrogen, conception rate*

Abstract

The purpose of this study was to measure the level of BUN in dairy cows and its influence on services per conception (S/C) rate, milk output, and the forage to concentrate (F/C) ratio. Oestrogen concentrations were also assessed based on BUN level and pregnancy rate. Three blood samples were taken for the measurement of estrogen on the day of AI (D0), seven days later (D+7), and twenty-two days later (D+22). From the entire herd, a batch of eighteen dairy cows in total were chosen at random and split to the groups based on S/C, milk output, F/C ratio, BUN, and pregnancy rate. The means of BUN based on F/C ratio showed significant difference ($p<0.05$) of the results. Each group's estrogen concentration on D0 and D+7 did not significantly differ ($p>0.05$) in regards to BUN and pregnancy rate, while the group of non-pregnant cows with high BUN featured lower value. However, in D+22, the group of non-pregnant cows with high BUN showed a lower estrogen concentration than the group of pregnant cows with low BUN ($p<0.05$), while the group of pregnant cows with low BUN had a higher BUN concentration than the group of non-pregnant cows with low BUN ($p>0.05$). These results indicated that while feed could alter BUN concentrations, non-feeding factors should also be taken into account. S/C and milk yield were found to provide no effect on BUN concentrations. According to these findings, a higher BUN concentration decreased the CR value, and concentrations of BUN ≥18 mg/dL led to lower levels of estrogen.

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Introduction

The success of a dairy farm is determined by feed management factors. Feed should contain nutrients necessary for the cow's body, in sufficient quantity and adequate quality for survival and reproduction. Nutrients needed by cows include carbohydrates, fat, protein, vitamins, water, minerals and inorganic elements [1]. Balanced and sufficient amount of feed according to the animal's needs will ensure optimal productivity and reproductive efficiency [2]. Since protein is the essential element of food primarily required to boost milk production, lactating dairy cows' milk yield can often be raised by increasing the share of protein in their diet [3]. To boost milk yield, dairy farmers as a rule increase the amount of feed given; nevertheless, it is recognized that increasing the amount of feed protein can negatively impact the reproductive functions [4].

Urea is the final byproduct of ruminant protein metabolism that enters the blood circulation [5]. High-yielding dairy cows show decreased fertility, longer days open (DO) and calving intervals (CI), increased service per conceive (S/C) and decreased conceive rate (CR) [6]. Increasing protein concentration in the ration results in increased urea nitrogen concentration in the body. The liver's process of detoxifying ammonia produces urea, a metabolite of protein included into the ration [7]. The circulatory system distributes urea, which passively diffuses across bodily fluids and contains milk urea nitrogen (MUN) and blood urea nitrogen (BUN) [8]. MUN concentration is highly correlated with BUN concentration. As a result, BUN measurement can provide farmers with crucial information regarding the health and nutritional state of the cows [9].

Ineffective intake of nitrogen for development and milk production is indicated by high BUN values [10]. Increased urea nitrogen concentrations have been shown to provide detrimental effects on dairy cow's fertility, including altered uterine fluid pH, impaired ovarian function, mineral imbalance in the uterus, lower rates of conception, and hormonal imbalance leading to reproductive disorders [11]. In addition to impaired immunity, lactating cows' secretion of K, Mg, and P, and causing hormonal imbalances, including estrogen level deviation, high blood urea prevents the removal of uterine contaminations [12]. High levels of urea nitrogen in dairy cows have detrimental effects on reproduction function because they prevent fertilization and follicle growth, decrease the ability of progesterone to attach to the ovarian receptor, and decrease the binding of luteinizing hormone to the ovarian receptor [13].

A number of risk factors, including managerial, environmental, metabolic, and nutritional problems, interact with each other to negatively impact fertility and milk yield. Reproductive function failure or infertility is a complex condition. The amount of protein in the concentrate, the resulting BUN concentration, and its potential detrimental effects on various reproductive processes are a few examples of such factors [14]. The aim of this study was to find the correlation between the level of blood urea nitrogen (BUN), level of serum estrogen and conception rate (CR) with fertility and milk yield criteria in dairy cattle.

Objects and methods

Data and sample collection

The owner of a small-scale dairy farms in Wagir, Malang, East Java, Indonesia has a total population of ± 1.000 lactating dairy cows. Among them one hundred cows were selected randomly based on the dairy cow type [Holstein-Friesian (HF)], milk yield, age, parity, health condition and measured values of body condition score BCS (1–9 points). Fifty cows were further selected based on their reproductive efficiency data [services per conception (S/C), calving interval (CI), days open (DO)] which is supported by the relevant records on cows, and the quantity of feed (forage and concentrate) consumed by them. These 50 cows were then grouped into three groups, based on S/C and milk yield criteria, (S/C 1–2, milk yield <17 L/day), (S/C 3–4, milk yield 17–21 L/day) and (S/C ≥ 5, milk yield >21 L/day). Milk yield ranges were calculated from< (means – SD); between (means – SD) and (means + SD), and ≥ (means + SD), while S/C were divided accordingly. Among the 50 cows, 18 cows complied the three groups criteria (6 cows in each group)

Blood samples were taken through the coccygeal vein (5 mL from each cow). Blood sample for estrogen measurement was taken three times, which happened on the day of AI (*D*0), seven days after AI (*D* + 7), 22 days after AI (*D* + 22), and blood sampling for BUN measurement was taken only once. Two hours after collection, blood samples were centrifuged at 2.000 rpm for 10 minutes. Serum was separated and stored (–20 °C). Pregnancy was tested 3 months after AI, through rectal palpation. After all data were obtained, regrouping was done to determine differences in the concentration of BUN and CR based on the F/C ratio, and the concentration of BUN (<18 mg/dL; ≥18 mg/dL) and rate of pregnancy on estrogen concentrations parameter.

Measurement of BUN and estrogen

BUN was measured using Berthelot method by Balai Besar Laboratorium Kesehatan Surabaya, while estrogen was measured by the lab instrument ELISA (DRG instrument GmbH, Germany) at the Laboratory of Endocrinology, Department of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga Surabaya.

Statistical analysis

Analysis was performed to identify the homogenity based on age, parity, milk yield and BCS parameters. Similarly, the homogenity of samples data of 18 cows which was further explored from among 50 cows was studied based on reproductive efficiency and composition of feed consumed. The SPSS23.0 software was used to conduct statistical analyses using One Way ANOVA and the Independent-Samples T Test ($p < 0.05$).

Results and discussion

Data collection of 100 dairy cows from a herd of approximately 1,000 lactating dairy cows in Wagir, Malang, East Java, Indonesia, showed range of ages between 3–8 years, parity 2–5, BCS 4–7 and milk yield 8–29 L/day. Then, 50 out of the 100 cows were surveyed and found to have S/C, CI and DO respectively 1–8 times, 338–697 days and 37–98 days range with a mean of 4.12 ± 0.31 times, 454.31 ± 12.9 days and 66.12 ± 2.53 days, and the range of age, parity, BCS and milk yield were respectively 3–7 years, 2–5.4–7 and 10–29 L/day, with a mean of 3.94 ± 0.36 years, 2.22 ± 0.3, 5.11 ± 0.28 and 19.17 L/day respectively. Range of forage and concentrate quantity was 20–70 kg/day and 5–16 kg/day respectively, with a mean of 35.1 ± 1.19 kg/day and 10.26 ± 0.39 kg/day. Then 18 dairy cows were selected

from among 50 cows and sorted by groups with means of milk yield, S/C, quantity of forage and concentrate equal to 19.17 ± 2.17 L/day, 3.89 ± 0.83, 34.44 ± 0.66 kg/day and 10.67 ± 1.33 kg/day respectively. Based on the results, it can be said that the number of cows per group can represent the number of dairy cow population at the study site.

S/C, milk yield and BUN

The comparison between each group showed that the mean of S/C and milk yield of dairy cows group with S/C ≥ 5 featured the highest milk yield > 21 L/day. Statistically the mean of S/C and milk yield in each group was significantly different ($p < 0.05$). The group of cows with high milk yield along with high S/C under consideration in this study had the lowest BUN concentration since there was a negative correlation between milk production and urea nitrogen content [15]. However, it was not statistically significant ($p > 0.05$) (Table 1). The concentration of urea nitrogen was not directly related to milk production and was not much affected by lactation period, but it was related to the balance of protein being fed [16,17]. Urea nitrogen concentrations can be utilized as a biomarker of how well lactating cows use nitrogen for milk production because urea nitrogen concentrations are regulated by the amount and concentration of consumed dietary crude protein [18].

Table 1. Milk yield, S/C, BUN (mg/dL), DO, S/C, CI and CR based on quantity of feed concentrate consumed by a cow

	Concentrate < 10.63 kg/d	Concentrate ≥10.63 kg/d	Grand Mean
Concentrate (kg/d)	9.50 ± 1.41[a]	11.50 ± 1.31[b]	10.63 ± 0.69
Milk yield (L/d)	13.50 ± 0.62[a]	25.17 ± 1.19[b]	19.00 ± 1.23
BUN (mg/dL)	15.61 ± 1.43[a]	15.67 ± 1.29[a]	15.64 ± 1.62
% BUN ≥ 18 mg/dL	44.44 % (4/9) [a]	22.22 % (2/9) [a]	33.33 % (6/18)
DO	61.33 ±5.87[a]	64.67 ± 4.86[a]	63.95 ±3.74
S/C	1.67 ± 0.21[a]	6.33 ± 0.21[b]	3.74 ± 0.49
CI	407.33 ± 21.32[a]	444.33 ± 34.60[b]	422.05 ± 20.04
CR	33.33 % (2/6) [a]	33.33 % (2/6) [a]	33.33 % (6/18)

Note: Different superscripts on the same row show significant differences ($p < 0.05$).

A high amount of protein feed (rumen degradable and rumen undegradable protein) with a crude protein content of > 19 % [19,20] results in a higher concentration of urea nitrogen (in urine, blood and milk) and a decrease in the efficiency of *N* utilization, which increases the excretion of nitrogen (N) [21–23]. Protein feed can be obtained from the feeding of concentrates. The highest average of F/C ratio was found in the group with low milk yield (< 17 L/day) ($p < 0.05$). This was because the quantity of concentrate is much smaller than the quantity of forage, so the protein nutrient is not enough to increase milk yield. The ratio of forages to concentrates that can increased the milk yield and raise the concentration of milk protein among the dairy cows in early lactation period was 60:40 [24].While based on the mean of concentrate feeding, the highest milk yield was recorded in the group of cows with milk yield > 21 L/day and the lowest one was recorded in the group of cows with milk yield < 17 L/day ($p < 0.05$). It indicated that concentrate supplementation is able to increase milk production [25]. Thus, the lactating cows in this group may have a balance of feed management and efficient utilization of *N* for milk production, without increasing the concentration of urea nitrogen, because a balanced feeding for lactating cows was 14–16 mg/dL in average of BUN concentration [26].

S/C can be affected by the high concentration of BUN [27], but no significant difference ($p > 0.05$) between S/C and BUN concentrations was found in this study. Yoon et al. [28] also reported that there was no effect of nitrogen urea concentration on frequency of successful artificial insemination. The high ratio of S/C (repeated attempts of conception) was not only influenced by BUN but also by fertilization failure and embryonic mortality caused by many factors, among others — the flaws of artificial insemination, environmental issues, ovulatory failure, poor genetics and uterine infection. Therefore, the conception rates in each milk yield and S/C based group also showed no significant difference ($p > 0.05$) [29]. Furthermore, regrouping was performed based on F/C ratio and BUN concentrations, each group with greater and less parameters than the grand mean of CR.

BUN, conception rate and F/C ratio

The mean of forage to concentrate (F/C) ratio and BUN (from grand mean) in this research were equal to 3.52 and 15.64 mg/dL. Then the cows were grouped into: < 3.52; ≥ 15.64 mg/dL, < 3.52; < 15.64 mg/dL, ≥ 3.52; ≥ 15.64 mg/dL and ≥ 3.52; < 15,64 mg/dL, with significant difference ($p > 0.05$) in mean result of BUN concentration. The quantities of forage and concentrates in the F/C ratio ≥ 3.52 group was lower than that of the F/C ratio < 3.52 group ($p < 0.05$), which was linear correlation to the share nutritional protein in each group.

Protein consumption, the effectiveness of *N* use for milk production, and energy balance can all affect a dairy cow's urea nitrogen content [30,31]. The absence of BUN that was ≥ 15.64 mg/dL in F/C ratio ≥ 3.52 in a group indicated that the amount of protein feed given was not high, thus unable to increase the concentration of BUN, whereas in cows that obtained F/C ratio ≥ 3.52 with BUN < 15.64 mg/dL (Table 2). BUN was averagely < 12 mg/dL, which indicated protein deficiency and it was suggested to give additional feed [8]. In cows given F/C ratio < 3.52 BUN concentration were ≥ 15,64 mg/dL. It was caused by the higher concentrate feed F/C ratio < 3.52 than F/C ratio ≥ 3.52 ($p < 0.05$). Adding the concentrate feed may increase the concentration of urea nitrogen [32]. Cows given F/C ratio < 3.52 with BUN < 15.64 mg/dL showed that dairy cows are able to efficiently utilize *N* from feed for productivity, whereas cows given ratio of F/C < 3.52 with BUN ≥ 15.64 mg/dL had a high concentration value of ≥ 18 mg/dL, which showed that there was a decrease in efficiency of utilization *N*, thus increased the excretion of *N* [18,22].

Table 2. Blood urea nitrogen (BUN) (mg/dL) and conception rate (CR) based on quantity of feed concentrate consumed and BUN (mg/dL) were more or less than the mean of whole sample

	Concentrate ≥10.63 kg/d		Concentrate < 10.63 kg/d
	BUN≥15.64	BUN < 15.64	BUN < 15.64
Concentrate (kg/d)	11.43± 0.95[a]	11.63± 0.88[a]	6.33 ± 0.88[b]
BUN(mg/dL)	19.93 ± 0.80[a]	13.38 ± 0.53[b]	11.67 ± 1.27[b]
CR (%)	14 % (1/7)	50 % (4/8)	33.3 % (1/3)

Note: Different superscriptson the same row show significant differences ($p < 0.05$).

In addition to utilizing protein from feed, ruminants can also synthesize proteins on their own with the help of microbes available in rumen. Ruminants can also utilize sources of nitrogen that are not derived from proteins (named non protein nitrogen, NPN), for the synthesis of their body proteins, in which microbial proteins have very high biological values. Thus, the amino acid supply of the body comes from feed proteins and rumen microbes. Although some proteins are resistant to rumen degradation, feed proteins and NPN that ruminants consume partially break down in the rumen, i. e. amino acids break into ammonia and branched chain fatty acids, which then provide amino acids and peptides that cattle can absorb in their intestines and use to boost its productivity [33]. Ammonia is necessary for rumen microorganisms to flourish. The rumen contains an excess of *N-NH3* if amino acids and peptides that have not been utilized for milk production are absorbed. If not utilized for microbial protein synthesis, the excess of *N-NH3* in the rumen is absorbed through the rumen wall, transformed into urea in the liver, and partially excreted in the urine [34]. As a result, cows may not always use high protein diets to their full potential; this relies on how well they process nitrogen to produce milk. If not used nitrogen raises the urea nitrogen concentration [31].

Group of cows which was given F/C ratio < 3.52 with BUN ≥ 15.64 mg/dL showed the lowest CR value. This indicated that the increase of feeding quantity that could increase BUN, in accordance with previous study where BUN concentration > 16 mg/dL resulted to the lower pregnancy rate [35], and BUN ≥ 18 mg/dL could lead to decreased fertility [9]. The group of cow given F/C ratio ≥ 3.52 with BUN < 15.64 mg/dL also had low CR values, which indicated that feeding with low quantity of forage and concentrate could also reduce CR and BUN concentrations.

BUN, estrogen and pregnancy

Pregnancy detection of 18 cows showed that 6 cows were pregnant and the other 12 cows were not pregnant. Previously reported that the decrease of conception rates in dairy cow can be influenced by the high concentration of BUN that reach ≥ 18 mg/dL [36] while half of the 12 non-pregnant cows had BUN concentrations of ≥ 18 mg/dL in accordance to the results of this study. Pregnant cows in this study had a mean concentration of BUN < 18 mg/dL, reflected they could achieve maximum fertility if the concentration of urea nitrogen ranged within 12–16 mg/dL [37]. The lack of pregnancy of cows with BUN < 18 mg/dL may be caused by other factors that contributed the repeated attempts of conception. Cows were divided into three groups according to their BUN and pregnancy status, they were grouped to low BUN and pregnant, high BUN and non-pregnant and low BUN and non-pregnant with mean concentrations of 13.92, 20.42, and 12.58 mg/dL of BUN respectively. Statistically the group of low BUN and pregnant and the group of low BUN and non-pregnant group showed no significant difference between each other ($p > 0.05$), but significantly different from the group of high BUN, non-pregnant cows ($p < 0.05$).

High BUN concentrations in dairy cows may be due to intense feeding with crude protein (CP). There are several reasons why too much dietary CP reduced reproductive performance [9,38]. Excess dietary CP has been linked to poor energy status because it can raise energy requirements, which can range from 13.3 kcal of digested energy per gram of excess *N*. Due to delayed ovulation and decreased plasma progesterone levels, poor energy status may decrease fertility. Sperm, oocytes, and embryos may be toxically affected by high BUN concentrations. It has also been noted that high BUN levels reduce the formation of prostaglandin (PGF2α), luteal phase P, Mg, and K concentrations, and pH of uterine fluid. Additionally, a high BUN may lessen the binding of leutinizing hormone (LH) to ovarian receptors. Reduced LH binding would result in lower levels of progesterone in the blood, which would lower fertility [39,40].

Estrogen concentration measured on D(0) showed that its value in the group of low BUN and non-pregnant cows < the group of high BUN and non-pregnant cows < the group of low BUN and pregnant cows; and on D(+7) it changed the following way: the group of high BUN and non-pregnant cows < the group of low BUN and pregnant cows < the group of low BUN and non-pregnant cows, but each group showed no significant difference in estrogen concentration ($p > 0.05$), whereas on D(+22), estrogen concentrations in the group of low BUN and pregnant cows was lower than the group of low BUN and non-pregnant cows and showed significant difference ($p < 0.05$), but a mean of estrogen concentration on D(+22) in group of low BUN and pregnant cows and the group of high BUN and non-pregnant cows were not significantly different ($p > 0.05$) (Figure 1).

Cows with BUN concentrations of ≥ 18 mg/dL showed false positive pregnancies because estrogen concentrations in blood were not significantly different from the pregnant cows with BUN concentrations < 18 mg/dL, they also had a lower estrogen concentrations than pregnant and not pregnant cows with BUN concentrations < 18 mg/dL. Silva et al. [41] reported that there was a negative correlation between the concentrations of urea nitrogen and estrogen, and higher protein intake (undegradable protein) is able to cause low estrogen concentrations. High feed intake can increase excessive blood flow to the gastrointestinal tract

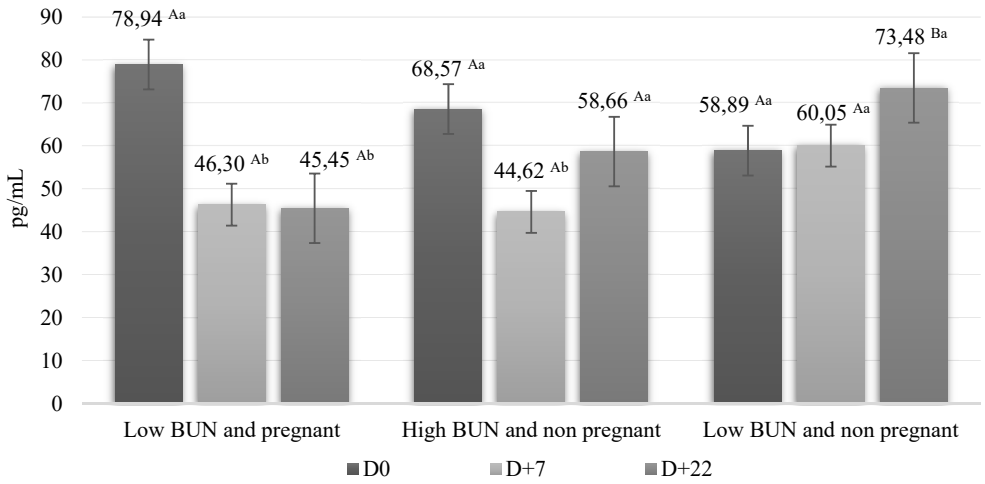

Figure 1. Serum estrogen levels (pg/mL) at day-0, day-7 and day-22 (day-0 = estrus) grouped by BUN and pregnancy status; Different superscripts ($^{A, B}$) show significant differences ($p < 0.05$) between groups; Different superscripts ($^{a, b}$) show significant differences ($p < 0.05$) between day of sampling

and to the liver. The liver has a major function for the metabolism of progesterone and estradiol 17β, increased liver blood flow causes cessation of hormone metabolism in the liver, thus lowering the concentrations of progesterone and estrogen in the blood [42]. High concentrations of urea nitrogen may also affect hormonal secretion in the ovaries, namely decreased the concentrations of luteinizing hormone (LH) that binds to ovarian receptors, insulin and insulin-like growth factor-1 (IGF-1), which all three hormones play a role in the process of steroidogenesis [43]. The IGF-1 and insulin stimulate cells proliferation and differentiation, and act synergistically with FSH in steroidogenesis by increasing the activity of P450 aromatase, and increase the secretion of estradiol. In addition, LH is also a stimulator of aromatase activity in granulosa cells, along with its being the physiological factor in the follicular ovarian of estradiol production regulator (E2) in cattle [44]. Reduced reproductive function could result from the product of *N* metabolism changing the hypophyseal pituitary-ovarian axis's activity [28].

Conclusion

The results of this study confirm that the occurrence of pregnancy can be affected by BUN concentration as the factor altering estrogen concentration. An increased concentrations of BUN ≥18 mg/dL showed false positive pregnancy, based on estrogen concentration, and caused lowered conception rates.

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EFFECT OF *CISTUS LADANIFERUS* L. ESSENTIAL OIL ON THE MICROBIOLOGICAL, PHYSICOCHEMICAL AND TEXTURAL QUALITY OF MINCED CHICKEN PATTIES DURING REFRIGERATION

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Keywords: *Cistus ladanifer essential oil, antimicrobial activity, meat preservation, minced meat, alginate encapsulation*

Abstract
This study investigated Cistus ladanifer essential oil (CLEO) as a natural preservative for minced chicken patties during 10-day storage at 4 ± 1 °C. CLEO was applied either directly or encapsulated in sodium alginate beads and compared to controls (sterile water). Encapsulated CLEO demonstrated superior efficacy, reducing total mesophilic bacteria by 2.03 log CFU/g, Enterobacteriaceae by 1.83 log CFU/g, and Pseudomonas spp. by 1.91 log CFU/g versus controls ($p < 0.05$). It also stabilized pH, maintaining values 1.6 units lower than spoilage thresholds. Color (L, a*, b*) and texture (hardness, cohesion, springiness) were significantly preserved, with encapsulated CLEO showing 25 % greater texture retention than direct application. The results highlight CLEO's dual antimicrobial-antioxidant capacity, enhanced by encapsulation and controlled release. While prior research confirmed CLEO's bioactivity, this is the first demonstration of its meat preservation potential. The findings support CLEO-alginate systems as a clean-label solution for extending poultry shelf life, aligning with industry demand for natural alternatives to synthetic additives.*

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Introduction

Minced meats are highly perishable due to their high water activity, nutrient-rich composition, and increased surface area from processing, which promote rapid microbial growth and spoilage [1]. Unlike eggs, which contain natural antimicrobial agents such as lysozymes, or citrus fruits with their naturally low pH inhibiting microbes, fresh poultry and minced meats lack inherent protective barriers, making them far more susceptible to deterioration.

Plant essential oils (EOs) have emerged as effective natural food preservatives due to their antimicrobial and antioxidant properties, derived from herbs and spices, such as oregano, thyme, and cinnamon. EOs contain bioactive compounds that inhibit bacterial growth and delay oxidative spoilage in foods. However, challenges such as strong flavor, volatility, and variable efficacy require further research on encapsulation and optimal application methods for wider industrial use [2]. The application of essential oils (EOs) in food preservation faces limitations due to their volatility and low solubility in aqueous matrices, as well as their sensitivity to environmental factors such as temperature, oxygen, and light. These factors reduce their stability and effectiveness in aqueous food systems, leading to rapid evaporation or chemical breakdown. To overcome these challenges, encapsulation techniques (e. g., nanoemulsions, liposomes, or cyclodextrins) are being explored to enhance solubility, protect bioactive compounds, and enable controlled release, thereby improving their practical use in food preservation [3]. In this perspective, encapsulation techniques, particularly those using sodium alginate, have also become a suitable approach to improve food preservation as well as the stability of bioactive molecules. Among them, encapsulation based on sodium alginate enhances stability, bioavailability, and controlled release of active molecules within food matrices. This method not only prolongs the antimicrobial and antioxidant effects of essential oils but also masks strong flavors, enabling broader application in fortified foods. By forming hydrogel beads or nanoparticles through ionotropic gelation,

alginate encapsulation ensures targeted delivery and enhanced bioavailability, making it a promising approach for advanced food preservation systems [4–6]. Moreover, sodium alginate-based encapsulation not only enhances the stability and controlled release of essential oils but also improves overall product quality by maintaining flavor, color, and nutritional integrity. By minimizing degradation and maximizing bioactive performance, alginate encapsulation offers a scalable, economical solution for sustainable food preservation [7]. Various encapsulation techniques, including spray drying, coacervation, liposome entrapment, and nanoemulsification, have been explored to overcome the limitations of essential oils in food applications. Among these, spray drying stands out as a cost-effective and scalable method, producing stable microcapsules with high encapsulation efficiency. This dual functionality makes alginate encapsulation particularly valuable for maintaining the antimicrobial and antioxidant efficacy of essential oils throughout a product's shelf life without compromising sensory qualities [8,9]. Among the most widely used microencapsulation methods are coacervation, emulsion extrusion, and supercritical fluid precipitation [10–13].

Cistus ladanifer essential oil (CLEO), extracted from the Mediterranean rockrose plant (*Cistus ladanifer*, Cistaceae family), is valued for its unique aromatic profile and bioactive properties. This drought-resistant shrub yields an oil rich in terpenes and phenolics, contributing to its reported antimicrobial, antioxidant, and anti-inflammatory effects. As a natural resource, CLEO holds potential for applications in food preservation, cosmetics, and pharmaceuticals, though further research is needed to optimize its extraction and stabilization for industrial use [14]. Renowned for its antimicrobial, anti-inflammatory, and wound-healing effects, CLEO has been employed to treat respiratory ailments, skin disorders, and digestive issues [15,16]. CLEO emerges as a promising natural alternative to synthetic preservatives due to its richness in natural bioactive compounds, such as α-pinene, camphene and borneol (monoterpenes, monoterpenols and sesquiterpenols), exhibiting strong broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, which is crucial for food safety. Additionally, its antioxidant properties help prevent lipid oxidation, extending shelf life while meeting consumer demand for clean-label ingredients [16–18]. The chemical composition of *Cistus ladanifer* essential oil shows notable regional variations, influencing its bioactive potential. In Algerian varieties, α-pinene (4.2 %), camphene (12.2 %), borneol (12.5 %), alongside 5-epi-7-epi-α-eudesmol (13.6 %), contribute to its antimicrobial efficacy [16]. In contrast, Moroccan CLEO is dominated by viridiflorol (17.64 %) and pinocarveol (11.02 %). These geographical differences highlight the need for standardized profiling to tailor CLEO for specific applications, such as food preservation or pharmaceutical/cosmetic uses [18].

Recognized by the Food and Drug Administration (FDA) [19] as a food additive and flavoring agent, CLEO has interesting applications in the food industry due to its antimicrobial and antioxidant properties, and its bioactive compounds, such as α-pinene and viridiflorol, helps preserve foods by inhibiting pathogens and preventing oxidation, while its distinct aroma enhances flavor profiles [14]. Moreover, *Cistus ladanifer* extract (CL extract) has demonstrated a strong safety profile in toxicological studies, with no adverse effects observed in rats administered doses up to 1000 mg/kg for 90 days, and this supports its potential as a safe, natural ingredient for food and pharmaceutical applications [20]. Encapsulation has been proposed as an effective solution for the stabilization of essential oils extracted from *Cistus* spp., given their high content of sesquiterpenes (48 %) and diterpenes (>18 %), while *C. ladanifer* oil is mainly composed of monoterpenes (70 %). Encapsulation methods (e. g., alginate microbeads, nanoemulsions, or cyclodextrin complexes) can protect these thermolabile compounds from degradation, mask strong flavors, and enable controlled release in food or pharmaceutical matrices [5]. Despite their potent antimicrobial and antioxidant properties, rockrose essential oils (CLEO) remain underutilized in food applications, currently limited to flavoring agents or in dietary supplements [17], rather than as natural preservatives, particularly in meat products. However, *Cistus ladanifer* leaves have been studied as a food additive to improve the quality of lamb meat, significantly reducing lipid oxidation while increasing beneficial unsaturated fatty acid content, without altering the color or organoleptic properties of meat [21].

This study aimed to explore CLEO as a natural preservative for minced chicken meat by evaluating its effect on the microbiological, physicochemical, and textural quality of minced chicken patties during 10 days of refrigerated storage at 4 °C. Particular attention is paid to encapsulation technique, which could improve the preservation potential of CLEO and extend its shelf life. By comparing encapsulated and unencapsulated CLEO with control treatments, this approach refers to effective methods for measuring meat spoilage, which can be classified into microbiological, sensory, and physicochemical analyses [22].

Objects and methods

Chemical composition, formulation and preparation of Cistus ladanifer essential oil (CLEO)

The *Cistus ladanifer* essential oil (CLEO) used in this study was produced during the year 2024 through steam distillation of leafy branches at a plants distillation facility in Tlemcen, Algeria. The resulting oil presents as a transparent liquid ranging in color from light yellow to pale orange, with characteristic physical properties, including a refractive index of 1.44, a density of 0.95 g/cm^3 and a flash point measured at 65 °C. These specifications confirm the oil's typical physicochemical profile for this botanical source. According to GC–MS analysis, the studied oil (CLEO) contains camphene (14.2 %), borneol (13.5 %),

α-pinene (4 %) and tricylene (3 %) as major components. Based on the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) [16,18], a dilution ratio of 1:8 (5 mL (CLEO): 40 mL (CO)) was used to emulsify *Cistus ladanifer* essential oil (CLEO) with corn oil (CO). The addition of vegetable oils, especially corn oil (CO), stabilizes the essential oil, prevents its rapid decomposition, and extends its shelf life. This combination improves encapsulation efficiency, while the beads ensure a slow, controlled release and mitigate strong flavors [23].

Encapsulation method for Cistus ladanifer essential oil (CLEO)

The encapsulation of CLEO into sodium alginate was performed using the extrusion dripping method adapted from [24] and [25] with slight modification. A food grade sodium alginate (Cape Crystal Brands, USA) was dissolved in deionized water, stirred at 40 °C for 2 hours, and refrigerated for 12 hours to remove air bubbles. CLEO solution diluted in corn oil was blended to a 0.5 % (w/v) sodium alginate solution, homogenized and stirred continuously using an ULTRA-TURRAX®. The encapsulation process involved dripping the CLEO-alginate mixture through a 21-gauge syringe into a 1.5 % (w/v) calcium chloride solution from a 2 cm height. After 20 minutes of gelation, the formed beads were thoroughly washed, filtered, and air-stabilized for 15 minutes to ensure structural integrity. For comparative analysis, control beads were prepared following identical procedures but with sterile distilled water replacing CLEO in the alginate mixture.

Bead characterization

The physical characteristics of the alginate beads were quantitatively analyzed using ImageJ software based on 2D image analysis captured with a Canon G7X camera following established protocols according to [24] and [25]. The mean diameter of both CLEO-encapsulated and water-containing (W) beads measured 2.36 ± 0.15 mm. Density analysis revealed that 1 gram of alginate beads contained 156 ± 4 beads, whereas one gram of beads encapsulating sterile distilled water contained 140 ± 2 beads. These measurements demonstrate the formulation-dependent variations in bead packing density while maintaining consistent spherical morphology.

Preparing chicken minced meat patties

Pectoral fillets (m. pectoralis) were obtained from 52-day-old Cobb500 broiler chickens collected from the Batna slaughterhouse (Algeria) using industrial processing methods. The fillets were skinned, minced, and transported to the LSA laboratory in a portable refrigerator at 4 ± 1°C. The minced meat was mixed with 0.8 % (w/w) NaCl and divided into three 1 kg experimental units. Each unit was formulated as described in Table 1 with concentrations based on prior studies by [26] and [27]. Standardized patties (approximately 40 g, 6 cm in diameter, 1 cm in thickness) were prepared, coded (Table 1), and aerobically packaged in polyethylene film for refrigerated storage (4 ± 1) °C. Analyses included microbiological parameters, pH values, color attributes (CIE Lab) and texture properties evaluations at 0, 2, 4, 8 and 10-day intervals.

Table1. Experimental units and treatments for chicken minced meat patties

Treatment		Beads per patty	CLEO + CO	Sterile water concentration	NaCl concentration (w/w)
Encap-CLEO	Minced meat	305 ± 6	5 % (v/w)	—	0.8 %
Direct-CLEO	Minced meat	—	5 % (v/w)	—	0.8 %
Encap-W*	Minced meat	280 ± 4	—	5 % (v/w)	0.8 %
Direct-W*	Minced meat	—	—	5 % (v/w)	0.8 %

*** W is water-containing.**

Microbiological assessment

The antimicrobial efficacy of CLEO oil, applied either directly or in encapsulated form, was evaluated by monitoring the growth of the microbial population during refrigerated storage (4 ± 1) °C at 0, 2, 4, 8, and 10-days intervals. For analysis, 10 g meat samples were aseptically placed in a stomacher filter bag containing 90 ml of sterile buffered peptone water. After homogenizing the mixture for 2 minutes, serial dilutions were prepared. For monitoring of total mesophilic bacteria (TMB), *Enterobacteriaceae* and *Pseudomonas*), appropriate dilutions were inoculated into Petri dishes containing plate count agar (PCA, MERCK, Germany) and 0.1 % cycloheximide solution, violet red bile glucose agar (VRBGA, MERCK, Germany), *Pseudomonas* agar, supplemented with *Pseudomonas* CFC selective agar supplement (SR0103) CFC (Merck, Germany). The plates were incubated respectively, under aerobic conditions at 30 °C / 72 h for TMB, at 37 °C / 24 h for *Enterobacteriaceae*, and finally at 25 °C / 48 h for *Pseudomonas* spp. Bacterial colonies were counted from three replicates, and results were expressed as log CFU/g (colony forming units) of minced chicken meat ± standard deviation (SD).

Physicochemical analysis: pH, color parameters, and texture

The pH of the samples was measured in triplicate using a calibrated SENSION+PH31 GLP pH meter (Hach Company, USA), with results expressed as mean ± standard deviation. Color evolution was monitored throughout the 10-day refrigerated storage period using a Konica Minolta CR-10 Plus colorimeter (Konica Minolta Sensing Europe B. V., Bremen, Germany) with an Ø8 mm aperture.

The surface color of the minced meat patties was quantified and described in terms of lightness (L*), redness (a*), and yellowness (b*) in the CIE Lab* color space. The color was measured during 10 days in cold storage and presented as the mean ± SD of three random readings on each sample.

Texture attributes of the samples were analyzed on days 0, 2, 4, 8, and 10 using a texture analyzer (EZ-LX Shimadzu, Kyoto, Japan) based on Witte et al. [28]. Texture profile

analysis (TPA) measured hardness (maximum force to rupture), springiness (ability to return to shape), and cohesiveness (ratio of compression area). Standardized sample molds were utilized to standardize (6 cm diameter, 1 cm height, 40 g weight). The tests were conducted at 30 % compression depth, 0.01 N preload, 50 N maximum force, and 50 mm/min speed.

Statistical analysis

Statistical analysis of all experimental data was carried out using R Studio statistical software (version 4.3.1), with data expressed as mean values (±SD). To evaluate significant differences between treatment groups, one-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple range test for post-hoc comparisons. Statistically significant differences between means were established at $p < 0.05$, with this threshold used to identify meaningful variations in measured parameters across different experimental conditions and storage periods.

Results

This study investigated the impact of different treatment methods on the microbiological and physicochemical stability of minced chicken patties during 10-day refrigerated storage (4 ± 1) °C. Four distinct treatments were compared: direct water addition (Direct-W), encapsulated water (Encaps-W), direct CLEO oil application (Direct-CLEO), and encapsulated CLEO (Encaps-CLEO). The experimental design allowed for comprehensive evaluation of how these treatments influenced microbial population dynamics (total mesophilic bacteria, and *Pseudomonas* spp.) alongside critical quality parameters, including pH evolution, color stability (L*a*b* values), and textural properties (hardness, cohesion, springiness).

Microbiological assessment

Figure 1 illustrates the growth of total mesophilic bacteria (TMB) in minced chicken patties stored at (4 ± 1) °C over 10 days. Initial TMB counts were comparable across different treatments (Direct-W, Encaps-W, Direct-CLEO, Encaps-CLEO; $p > 0.05$). By day 2, a uniform increase (2.3 log CFU/g) occurred in all groups, suggesting limited early antimicrobial action from CLEO. A critical divergence emerged on day 4, when control samples (Direct-W, Encaps-W) exceeded the spoilage threshold (7 log CFU/g), showing an increase of approximately 8.5 log CFU/g, while CLEO-treated samples showed significantly ($p < 0.05$) lower TMB levels. This antimicrobial effect became most pronounced by day 10, where controls peaked near 9 log CFU/g, whereas Direct-CLEO maintained lower counts (8 log CFU/g), demonstrating an inhibition of microbial proliferation, which is due to its antibacterial effect. Notably, Encaps-CLEO demonstrated superior inhibition throughout the study, validating both CLEO's antibacterial properties and the role of encapsulation in enhancing its sustained efficacy.

Figure 2 shows changes in the number of *Enterobacteriaceae* (log CFU/g) in chicken patties stored at 4 °C for 10 days, according to the four treatments (Direct-W, Encaps-W, Direct-CLEO, Encaps-CLEO). Initially, no significant difference was observed between the treatments ($p > 0.05$). According to [29], these indicators of fecal contamination show the initial microbiological quality of the product and can accelerate meat deterioration. On day 2, bacterial counts increased in all samples, reaching average values of approximately 6 log CFU/g. This may be explained by the absence of CLEO's bactericidal effect at this phase. This adaptation phase is common in minced meat [30,31], when bacteria adapt to inhibitory conditions [22]. From the 4th day, the values recorded for Direct-W and Encaps-W increased significantly (by 7.8 log CFU/g for day 4 and by approximately 8.2 log CFU/g for day 8) while the Direct-CLEO and Encaps-CLEO samples had significantly lower values ($p < 0.05$) compared to the controls. This can be explained by the inhibitory effect of CLEO on the bacteria. At day 10, bacterial counts in Direct-CLEO (6.13 ± 0.05 log CFU/g) and Encaps-CLEO (6.24 ± 0.06 log CFU/g)

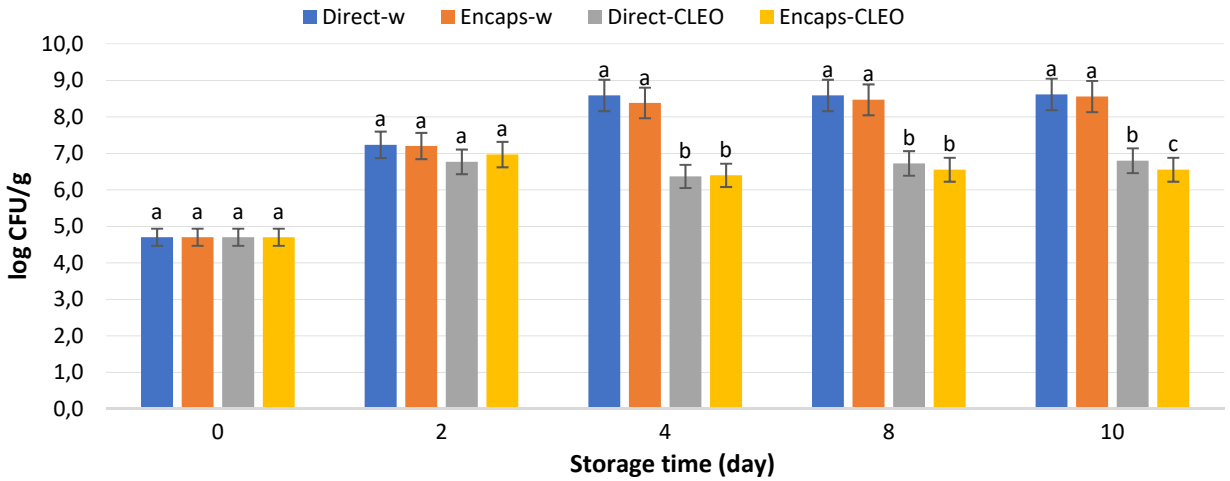

Figure 1. Evolution of total mesophilic bacteria (TMB) in chicken patties during 10 days of storage at (4 ± 1) °C according to treatments incorporating *Cistus landanifer* essential oil (CLEO) or sterile distilled water (W). The letters a, b, and c represent the results of a statistical analysis (typically ANOVA followed by a post-hoc Duncan's test). They indicate whether the means of the groups are significantly different from one another at a given storage time point

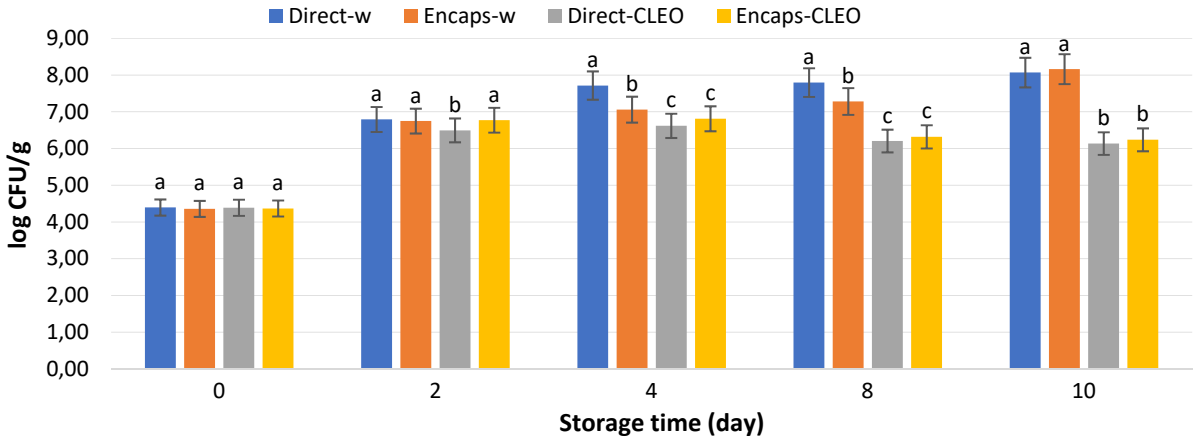

Figure 2. Evolution of *Enterobacteriaceae* in chicken patties during 10 days of storage at (4 ± 1) °C according to treatments incorporating *Cistus landanifer* essential oil (CLEO) or sterile distilled water (W). The letters a, b, and c represent the results of a statistical analysis (typically ANOVA followed by a post-hoc Duncan's test). They indicate whether the means of the groups are significantly different from one another at a given storage time point

were lower than those in controls (Direct-W: 8.07 ± 0.21 log CFU/g, Encaps-W: 8.16 ± 0.18 log CFU/g), with reductions of 1.83 and 1.92 log CFU/g, respectively. These results show that encapsulation improved the antimicrobial efficacy of CLEO, suggesting better stability and bioavailability during storage. This 24–25 % inhibition efficiency underscores how encapsulation optimizes CLEO's bioactive stability and gradual release, extending its antibacterial action against these critical Gram-negative spoilage organisms throughout storage.

Pseudomonas spp. are recognized as primary spoilage organisms in poultry stored aerobically under refrigeration (0–4 °C) [32]. These psychrotrophic bacteria thrive in cold environments, contributing to protein and lipid degradation, which leads to off-odors and off-flavors [33].

As shown in Figure 3, CLEO effectively inhibited the growth of Pseudomonas spp. in refrigerated chicken patties, with encapsulated CLEO demonstrating superior efficacy. Although all treatments started with identical initial counts (3.20 ± 0.03 log CFU/g), by day 2 the samples treated with CLEO already showed a slight antimicrobial effect, but the differences were not statistically significant (all "a"). The antimicrobial action of CLEO became evident as early as day 4, when the treated samples (Direct-CLEO and Encaps-CLEO) showed a significant reduction ($p < 0.05$) compared to the controls (Direct-W, Encaps-W reaching 4.62 log CFU/g). At day 10, the controls exceeded the spoilage thresholds (7.13 ± 0.04–7.16 ± 0.05 log CFU/g), while the Direct-CLEO and Encaps-CLEO samples maintained significant reductions compared to the Direct-W control (– 1.98 ± 0.03 and — 1.91 ± 0.53 log CFU/g; $p < 0.05$, Duncan's test) or nearly 98.7 % bacterial reduction. The addition of CLEO, whether direct or encapsulated, significantly reduced the growth of mesophilic bacteria during storage, the antimicrobial efficacy is clear from the 4th day and is maintained until the 10th day, which potentially extends the shelf life of the products.

This persistent inhibition reflects the membrane-disrupting effects of bioactive terpenes (α-pinene, camphene, borneol, with encapsulation enhancing stability and

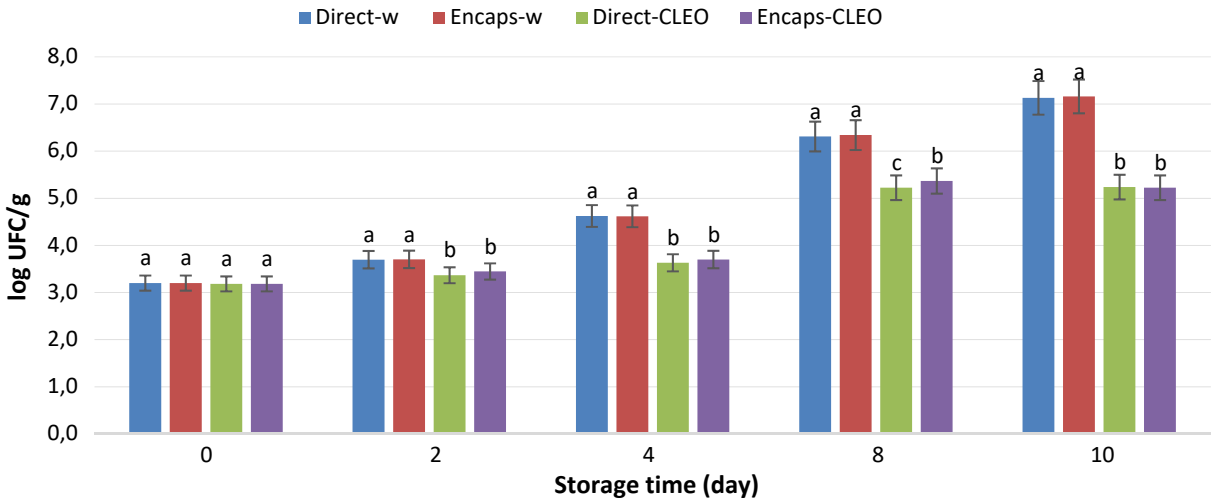

Figure 3. Evolution of *Pseudomonas* spp. in chicken patties during 10 days of storage at (4 ± 1) °C according to treatments incorporating *Cistus landanifer* essential oil (CLEO) or sterile distilled water (W). The letters a, b, and c represent the results of a statistical analysis (typically ANOVA followed by a post-hoc Duncan's test). They indicate whether the means of the groups are significantly different from one another at a given storage time point

controlled release for prolonged efficacy. The results confirm CLEO's potential to extend poultry shelf life by specifically targeting dominant spoilage pseudomonads [18].

Physicochemical analysis: pH, color parameters, and texture

pH determination

Table 2 demonstrates that CLEO treatments effectively stabilized pH in minced chicken patties treated during refrigerated storage at (4 ± 1) °C, with encapsulated CLEO (Encaps-CLEO) showing superior performance. While control samples (Direct-W, Encaps-W) exhibited a significant pH increase ($p < 0.05$) from day 4 onward, reaching alkaline levels by day 10 due to microbial metabolite accumulation, CLEO-treated groups maintained significantly lower pH values ($p < 0.05$). This stabilization was particularly pronounced in Encaps-CLEO samples, which showed the smallest pH fluctuation (ΔpH < 0.3), attributable to the alginate beads-controlled release of antimicrobial compounds that suppressed spoilage bacteria and their alkaline byproducts.

Table2. pH values of chicken patties during the refrigerated storage (at (4 ± 1) °C

Time (days)	Direct-W	Encaps-W	Direct-CLEO	Encaps-CLEO
0	5,60 ± 0,03^{b}	5,67 ± 0,03^{a}	5,67 ± 0,03^{a}	5,70 ± 0,02^{a}
2	5,75 ± 0,05^{a}	5,76 ± 0,05^{a}	5,40 ± 0,05^{c}	5,67 ± 0,02^{b}
4	6,48 ± 0,03^{a}	`6,56 ± 0,06^{a}	5,11 ± 0,05^{b}	5,16 ± 0,05^{b}
8	6,82 ± 0,03^{a}	6,78 ± 0,04^{a}	5,33 ± 0,04^{b}	5,27 ± 0,03^{b}
10	7,13 ± 0,03^{a}	7,06 ± 0,04^{b}	5,46 ± 0,02^{c}	5,51 ± 0,05^{c}

Data are presented as mean ± standard deviation. Statistical significance was assessed using Duncan's multiple range test at a significance level of $p \le 0.05$. The letters a, b, and c represent the results of a statistical analysis (typically ANOVA followed by a post-hoc Duncan's test). They indicate whether the means of the groups are significantly different from one another at a given storage time point.

At the beginning of storage (day 0), the pH difference was not significant ($p > 0.05$) between treatments, confirming uniform starting conditions. By day 4, the pH increased significantly in the Direct-W samples (6.48 ± 0.03) and Encaps-W (6.56 ± 0.06). This is due to the presence of by-products released by the germs and the absence of a preservative. The Direct-CLEO and Encaps-CLEO samples recorded pH values of 5.11 ± 0.05 and 5.16 ± 0.05, respectively, which were significantly lower compared to the Direct-W and Encaps-W samples. This can be explained by the microbial inhibition exerted by CLEO. During day 8, the pH values of the Direct-W and Encaps-W control samples increased significantly ($p < 0.05$) recording values of 6.82 ± 0.03 and 6.78 ± 0.04, respectively, while the CLEO-treated samples remained stable with values of 5.33 ± 0.04 for Direct-CLEO and 5.27 ± 0.03 for Encaps-CLE. At the end of storage by day 10, pH values reached 7.13 ± 0.03 for Direct-W and 7.06 ± 0.04 for Encaps-W, while Direct-CLEO and Encaps-CLEO samples maintained significantly ($p < 0.05$) lower pH values of 5.46 ± 0.02 and 5.51 ± 0.05, respectively. These results show that Encaps-CLEO demonstrated better pH stability (ΔpH < 0.35 vs. ΔpH > 0.6 in controls) than direct application, suggesting a controlled release of antimicrobial compounds (α-pinene, borneol), thereby prolonging activity and maintaining meat freshness.

Evaluation of color parameters

Figure 4 represents the color values lightness (L*), and redness (a*), along with increased yellowness (b*) observed in minced chicken patties treated with *Cistus ladanifer* essential oil (CLEO) and sterile distilled water (W) directly or encapsulated in sodium alginate, during 10 days of storage at 4 ± 1 °C. These color parameters are indicators of meat quality [34], as high L* values improve visual appeal, while a decrease in a* values may indicate oxidation or spoilage [31].

As shown in Figure 4a, the initial L* values were highest in Direct-CLEO samples (55.95 ± 0.07), followed by Direct-W (55.85 ± 0.05), Encaps-W (55.62 ± 0.4) and Encaps-CLEO (55.69 ± 0.07). The slight improvement in brightness with CLEO treatments is probably due to the presence of natural pigments in the essential oil. From day 4 onwards, the brightness (L*) of Direct-W and Direct-W samples significantly decreased ($p < 0.05$), recording mean values of 50.33 ± 0.08 and 51.24 ± 0.23, respectively. During the intermediate storage on days 4 and 8, Direct-CLEO and Encaps-CLEO were considerably brighter compared to Direct-W and Encaps-W, which was indicated by darkening of Direct-W and Encaps-W samples, most likely due to lipid oxidation or microbial spoilage. At the end of storage (day 10), Direct-W and Encaps-W had the lowest L* values with a mean value of 50.33 ± 0.08 showing a great discoloration. However, the samples Direct-CLEO with a mean value of 56.08 ± 0.03 and Encaps-CLEO with 55.95 ± 0.06 retained and even slightly improved their L* values at the end of storage.

The results presented in Figure 4b show the redness (a*) values of chicken patties over 10 days at (4 ± 1) °C under the four treatments. Initially, all groups had similar a* values (4.96–5.03, $p>0.05$). However, the progression of redness values varied considerably between treatments. Day 4 and day 8 also recorded the Direct-W and Encaps-W samples to exhibit a significant reduction ($p < 0.05$) in a* values by about 2 units of the mean a* values, thus making the minced meat patties pale and less red in color, perhaps due to oxidation. On day 8, Direct-CLEO and Encaps-CLEO samples exhibited significantly higher values, ranging from 3.74 ± 0.41 to 3.95 ± 0.07, respectively. On the 10th day, a* values decreased considerably in Direct-W and Encaps-W to 2.06 ± 0.04 and 2.10 ± 0.27, respectively. This can be attributed to microbial alteration and associated biochemical changes in pH, which generally result in a color shift towards green [26,35], as well as accelerated oxidation of oxymyoglobin to metmyoglobin. Direct-CLEO and Encaps-CLEO treatments retained significantly ($p < 0.05$) higher a* values of 3.55 ± 0.02 and 3.75 ± 0.49, respectively, probably due to the controlled release of the active compounds [31].

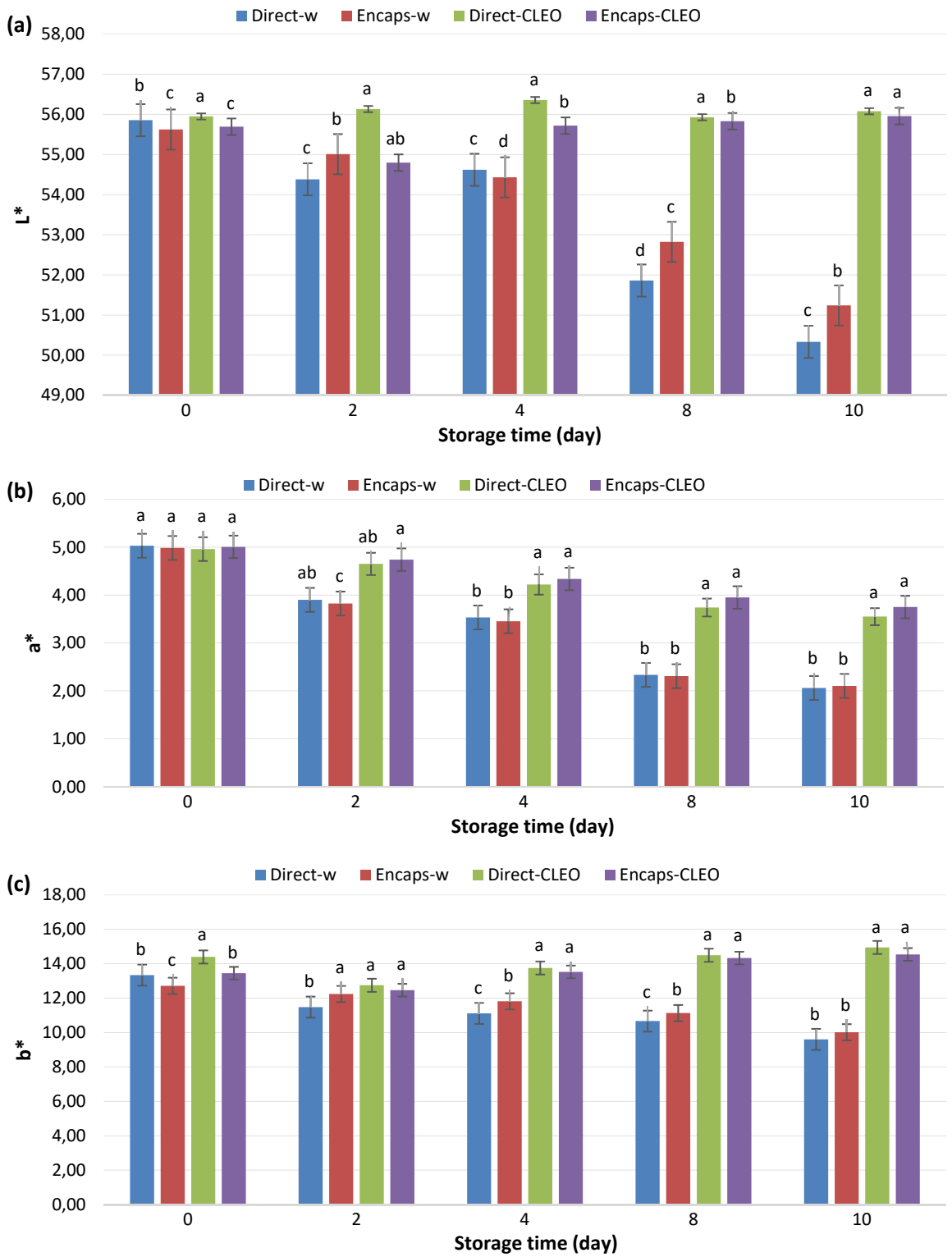

Figure 4. Color values L* lightness (a), a* redness (b), and b* yellowness (c) of chicken patties during 10 days of storage at 4 ± 1 °C according to treatments incorporating *Cistus landanifer* essential oil (CLEO) or sterile distilled water (W). Vertical bars indicate the standard deviation of the mean. The letters a, b, and c represent the results of a statistical analysis (typically ANOVA followed by a post-hoc Duncan's test). They indicate whether the means of the groups are significantly different from one another at a given storage time point

As can be seen in Figure 4c, the b* (yellowing) values decreased significantly during the 10 days of storage at 4 °C. The b* value in Direct-W decreased from 13.34 ± 0.51 to 9.6 ± 0.35 a reduction by 3.74 units), while that of Encaps-W decreased from 12.72 ± 0.17 to 10.02 ± 0.27 (a reduction by 2.7 units), indicating oxidative degradation of the pigment. The value of Direct-CLEO increased from 14.39 ± 0.16 to 14.95 ± 0.16 (an increase by 0.56 units). In addition to pH-related color changes, changes in b* values in the muscle may be associated with diet-induced postmortem glycogen changes. Unlike controls, which showed a significant decrease (p>0.05) due to oxidative degradation, CLEO-treated samples not only retained but also slightly improved their yellow color.

Texture analysis

According to the data on the texture evolution of minced meat patties over a 10-day storage period at 4 °C, presented in Figure 5, the textural properties (hardness, cohesion, and springiness) were significantly influenced ($p < 0.05$) by CLEO treatment, both in direct (Direct-CLEO) and encapsulated (Encaps-CLEO) forms, depending on the storage duration.

Figure 5a shows that the Encaps-W samples, which recorded a mean value of 16.56 ± 0.07 N, and Encaps-CLEO with a value of 15.98 ± 0.36 N initially presented significantly ($p < 0.05$) higher hardness values compared to the Direct-W treatments with a value of 11.89 N and Direct-CLEO with a value of 10.61 N. This is probably due to the firm and elastic structure of the alginate beads. After day 4, Direct-W and Encaps-W samples showed a significant ($p < 0.05$) reduction in hardness, with a 33 % decrease for Direct-W (from 11.89 ± 0.18 N to 7.95 ± 0.07 N) and a 41 % change for Encaps-W (from 16.56 ± 0.07 N to 9.82 ± 0.04 N), which is likely due to protein degradation and moisture loss due to

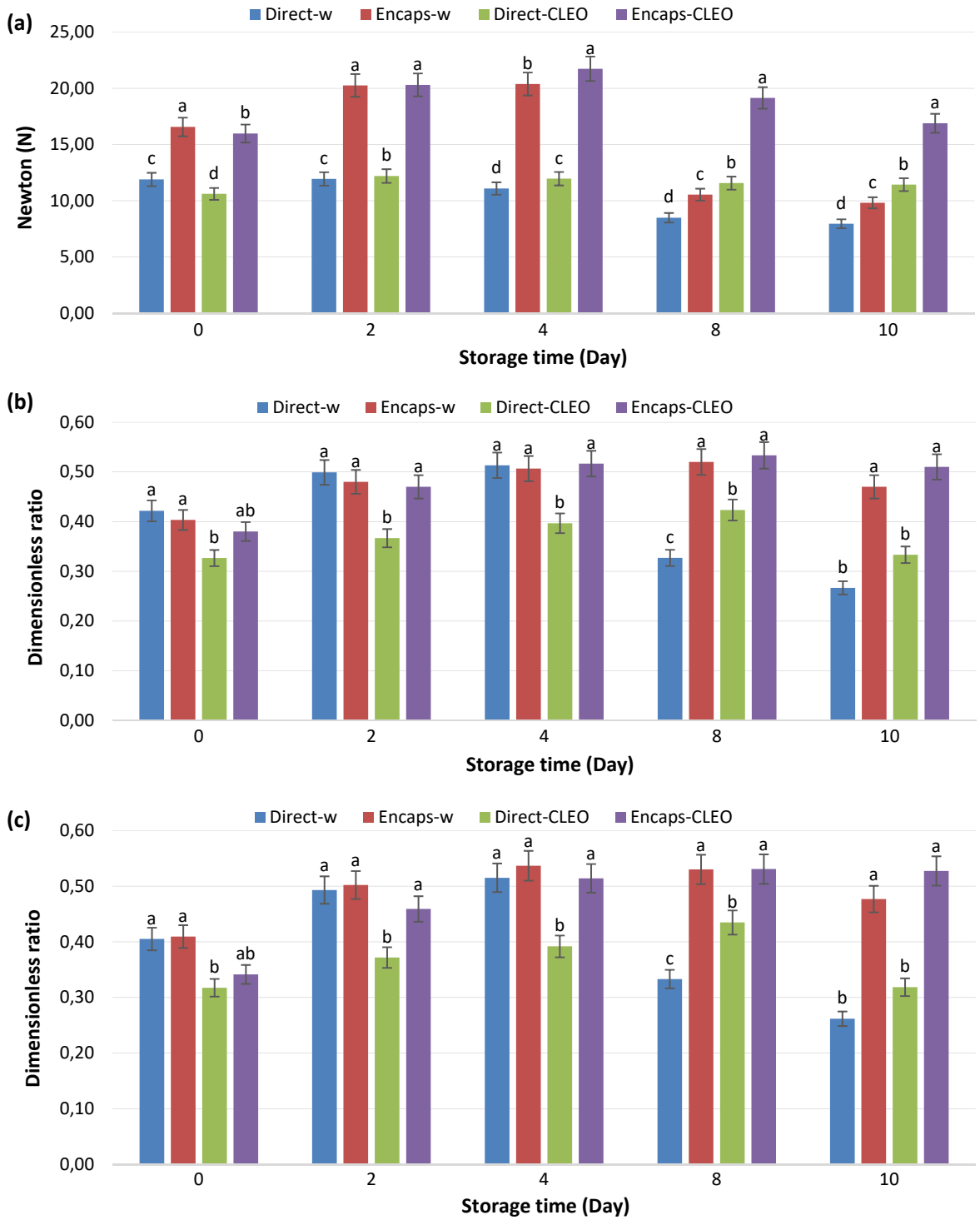

Figure 5. Textural properties (a) hardness, (b) springiness and (c) cohesiveness of chicken patties during 10 days of storage at (4 ± 1) °C according to treatments incorporating *Cistus landanifer* essential oil (CLEO) or sterile distilled water (W). Vertical bars indicate the standard deviation of the mean. The letters a, b, and c represent the results of a statistical analysis (typically ANOVA followed by a post-hoc Duncan's test). They indicate whether the means of the groups are significantly different from one another at a given storage time point

microbial activity. On day 4, the hardness of Direct-CLEO increased from 10.61 ± 0.15 N to 11.96 ± 0.07 N and then stabilized at 11.43 ± 0.06 N on day 10, which can be explained by CLEO's bioactive compounds interacting with muscle proteins, forming cross-links that improve firmness and delay proteolysis. Encaps-CLEO recorded the highest hardness value throughout storage, increasing from 15.98 ± 0.36 N to 21.74 ± 0.52 N on day 4 and stabilizing at 16.89 ± 0.21 N on day 10. This increase suggests a controlled release of CLEO's bioactive compounds, improving protein stability and water retention.

Figures 5b and 5c show that springiness and cohesion followed similar trends over the 10 days of the experiment. Indeed, samples treated with CLEO addition, either directly or by encapsulation, maintained better structural integrity than controls. Springiness decreased significantly ($p < 0.05$) in Direct-W samples, from 0.42 ± 0.04 at baseline to 0.27 ± 0.01 at the end of storage, due to protein degradation, while in the Encaps-W samples it decreased to an average value of 0.47 ± 0.05 on day 10, maintaining higher elasticity. CLEO treatments helped preserve springiness. In Encaps-CLEO, it increased from 0.38 ± 0.04 to 0.51 ± 0.05, suggesting that encapsulation delays proteolysis and improves water retention. Similarly, cohesion remained stable in Encaps-CLEO, increasing from 0.34 ± 0.07 to 0.53 ± 0.03, while it decreased in Direct-W by 37 % (from 0.41 ± 0.00 to 0.26 ± 0.02). These results confirm that CLEO, especially in encapsulated form, improves textural stability during refrigerated storage. The encapsulation technique prolongs the antioxidant and antimicrobial activity of CLEO, thus reducing biochemical degradation and preserving patties texture.

Exploration of complex relationships

The PCA biplot (Figure 6A) and correlation matrix (Figure 6B) illustrate the relationships between microbiological (*TMB, Enterobacteriaceae, Pseudomonas* spp.), physicochemical (pH, color attributes), and textural (springiness, cohesiveness, hardness) properties during storage.

Multivariate analysis of quality parameters

Principal Component Analysis (PCA) (Figure 6A) demonstrates the relationships between quality parameters, with Dimension 1 (57.43 %) separating microbiological parameters (TMB, *Enterobacteriaceae*, *Pseudomonas* spp.), pH, and color attributes (L*, a*, b*), while Dimension 2 (25.91 %) distinguishes textural properties (cohesion, springiness, hardness). The arrangement in the diagram shows that microbial proliferation and pH are negatively correlated with the color parameters lightness (L*) and redness (a*) but are positively correlated with yellowness (b*), demonstrating a link between oxidative degradation, pH increase, and yellowing. Finally, textural attributes (hardness, cohesion, and springiness) are distinct from microbiological and physicochemical properties, implying that they are influenced by independent factors, likely related to protein denaturation or water retention.

Correlation matrix

The correlation matrix (Figure 6B) reveals significant relationships between quality parameters, with pH demonstrating strong positive correlation with microbial growth (*Pseudomonas* spp. (0.70), TMB (0.71) and *Enterobacteriaceae* (0.59)), which shows that microbial growth influences pH variations during storage. On the other hand, the color parameter b* is strongly correlated with *Pseudomonas* spp. (0.94) and moderately correlated with *Enterobacteriaceae* (0.60) and TMB (0.62). This indicates that the proliferation of *Pseudomonas* spp. contributes significantly to the yellow coloration. It is also noted that the parameter L* shows a negative correlation with *Pseudomonas* spp. (–0.77) and *Enterobacteriaceae* (–0.68), which indicates that microbial growth causes meat darkening. Hardness shows a weak

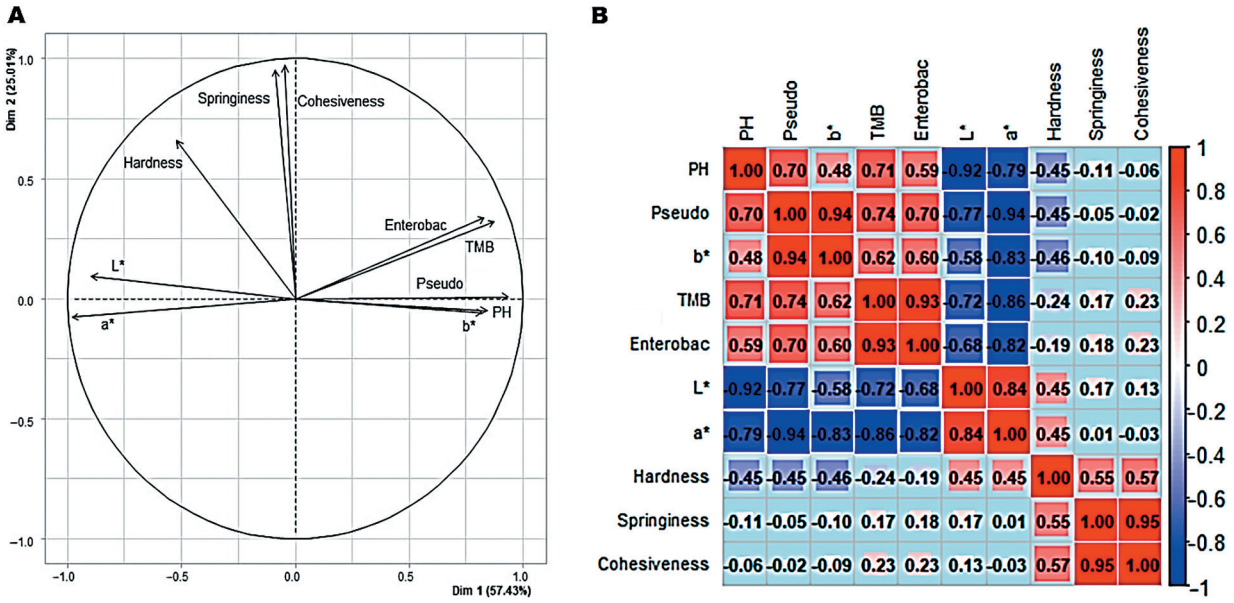

Figure 6. Graphical Representation of PCA (Figure 6A) and Correlation Matrix (Figure 6B) for the studied variables: microbiological parameters (*TMB, Enterobacteriaceae* = Enterobac, *Pseudomonas* spp.= Pseudo), physicochemical attributes (pH, color attributes a*, b*, L*), and texture properties (hardness, springiness, cohesiveness) over the storage period

negative correlation with *Pseudomonas* spp. (–0.45) and b* parameter (–0.46), indicating that its growth activates oxidative processes and contributes to softening. Elasticity shows a weak correlation with *Enterobacteriaceae* (0.18), suggesting a minor impact on elasticity. These patterns collectively demonstrate that *Pseudomonas* spp. serves as the primary driver of both color changes (yellowing/darkening) and textural degradation in stored meat products.

Discussion

The chemical composition of the essential oil used in this study is close to that reported by [16], who identified significant levels of borneol (12.5 %), camphene (12.2 %) along with a lower proportion of α-pinene (4.2 %). This compositional pattern indicates that the essential oil derived from this particular geographic region contains relatively reduced levels of monoterpene hydrocarbons, potentially influencing its physicochemical properties and biological activity.

The results revealed that samples treated with CLEO and in particular Encaps-CLEO, which contained CLEO encapsulated in alginate beads, were more stable in terms of microbial control, pH, color and texture than the Direct-W controls based on sterile distilled water. The results showed that the populations of total mesophilic bacteria (TMB), *Enterobacteriaceae* and *Pseudomonas* spp. in both Direct-W and Encaps-W samples increased significantly at the end of the storage period, while treatment with Encaps-CLEO successfully inhibited microbial growth, validating its antimicrobial efficacy. This decrease could be due to the mode of action of essential oils on bacterial cells, as already reported by [36]. In addition, and as shown in the results, the antibacterial effect of cistus essential oil in Encaps-CLEO samples became particularly effective after the fourth day of storage. Shabkhiz et al. [3] reported that the release of bioactive compounds from encapsulated *Thymus daenensis* essential oil (Td-EO) in alginate beads occurs in two distinct phases, which are short-release and long-release. They also found that encapsulation in β-cyclodextrin, combined with the formation of an alginate hydrogel, effectively slows down the release of bioactive compounds, thus improving their stability and prolonged activity.

Cistus ladanifer essential oils exhibit significant antimicrobial activity tested against both Gram-positive and Gram-negative bacteria [17]. This antimicrobial effect of CLEO is due to the presence of bicyclic organic compounds belonging to the terpene family. In most articles, the chemical profile of cistus essential oils revealed that monoterpene hydrocarbons are represented mainly by high percentages of α-pinene, with values ranging from 19.46 to 47.1 % [17]. The Algerian essential oil contains minor levels of monoterpene hydrocarbons of 0.7 to 5.2 %, but major levels of 5-epi-7-epi-α-eudesmol (13.6 %) and borneol (12.5 %) [16]. In this study, camphene (14.5 %), a monoterpene ($C_{10}H_{16}$), and borneol (13.5 %), which is a monoterpenol ($C_{10}H_{18}O$), are both antimicrobials and antioxidants, possessing broad-spectrum antibacterial properties through a membrane-rupturing mechanism [37]. The antioxidant activity of *Cistus ladanifer* essential oil stems partly from its chemical structure, which allows it to interact with reactive oxygen species [37]. Essential oils cause bacterial membrane rupture, leading to leakage of intracellular components, loss of bacterial viability and ATP depletion, disrupting energy functions and lowering intracellular pH (pHin), thus influencing metabolic reactions [38]. Parafati et al. [26] and Mehaya et al. [39] also reported the same results showing that encapsulation increases the antimicrobial activity of bioactive compounds due to stability, controlled release, and prolonged activity against meat spoilage bacteria. The inhibition of *Pseudomonas* spp. in Encaps-CLEO samples is particularly interesting because these bacteria are considered dominant in the spoilage flora of poultry in cold storage and are responsible for the emission of unpleasant odors and discoloration [40].

The pH of the samples Direct-W and Encaps-W showed a significant increase ($p < 0.05$), which can be explained by bacterial metabolism that produces alkaline compounds such as protein metabolites and essential amines [41]. The Direct-CLEO and Encaps-CLEO samples demonstrated a non-significant pH variation. This stability is due to the antimicrobial effects of CLEO in addition to the prolonged release of the latter by the alginate beads, which decrease bacterial growth and the production of by-products during storage. Similar results were reported by Lu et al. [40] and Yu et al. [42], where thyme and eucalyptus oil microcapsules delayed the increase in pH of refrigerated meat by inhibiting bacterial enzymatic activity.

Samples treated with sterile distilled water alone showed a decrease in color throughout the storage period, while a strong correlation existed between color parameters (L*, a*, b*) and the presence of CLEO upon both encapsulated and direct addition into minced meat patties. However, the Encaps-CLEO intervention preserved a* values better and showed better b* scores than Direct-CLEO. This is attributed to the controlled release of bioactive compounds present in CLEO, such as α-pinene, camphene, and borneol, which participate in the capture of free radicals, slowing down the oxidation of lipids and pigments and degradation of myoglobin [43]. Previous studies corroborate these findings, demonstrating that oregano essential oil maintained a* values in chicken meat [44], encapsulated beetroot extract improved color stability [27], and iron additives improved a* values but accelerated oxidation [45].

The results showed that the texture parameters (hardness, cohesion, and springiness) of the control samples were significantly reduced, causing meat softening. The most pronounced decline was recorded after day 4 of storage, coinciding with an increase in microbial growth and proteolysis. Texture deterioration during storage is linked to protein degradation, microbial growth, and lipid oxidation. At the same time, Encaps-CLEO also maintained

texture better than Direct-CLEO, indicating a synergistic effect of encapsulation on protecting structural integrity.

Multivariate analysis revealed significant relationships between microbial growth and quality parameters. *Pseudomonas* spp. proliferation exhibited a strong positive correlation with pH and yellowness (b), while inversely correlating with lightness (L) and redness (a*) parameters. However, the statistical analysis did not reveal a direct correlation with the texture parameters, which predicts a direct impact of yellowing, loss of redness, and blackening associated with an alkaline pH of a product, and an indirect impact of texture loss during storage of the minced chicken patties. Furthermore, according to the experimental results of the studied parameters and the statistical interpretation of the correlations, the addition of *Cistus ladanifer* essential oil (CLEO), either directly or encapsulated in alginate beads, controlled bacterial growth even of *Pseudomonas* spp., which are Gram-negative species, and delayed the modification of the physicochemical and texture parameters of the product, which validates its antimicrobial and consequently, antioxidant activity.

Conclusion

This study demonstrates that encapsulated *Cistus ladanifer* essential oil (Encaps-CLEO) significantly enhances the shelf life and quality of minced chicken patties by effectively controlling microbial growth, stabilizing pH, preserving color, and maintaining texture attributes of refrigerated samples during 10 days of storage. The antimicrobial efficacy of CLEO, particularly against spoilage-related bacteria such as *Pseudomonas* spp., is attributed to its bioactive compounds (camphene, borneol, and α-pinene), which disrupt bacterial membranes and inhibit metabolic activity. Encapsulation in alginate beads further optimizes CLEO's performance by ensuring controlled release, prolonged activity, and improved stability, thereby overcoming limitations associated with direct application.

The preservation of color (especially redness and lightness) and texture in Encaps-CLEO-treated samples underscores its antioxidant potential, preventing oxidative degradation of lipids and proteins. Notably, the delayed pH increase in treated samples confirms reduced bacterial metabolism, further validating CLEO's role in inhibiting spoilage. Statistical analysis (PCA) reinforced the strong correlation between microbial inhibition and maintained physicochemical quality, with encapsulated CLEO outperforming both direct CLEO and control treatments (Direct-W and Encaps-W).

These findings highlight CLEO, particularly in encapsulated form, as a promising natural alternative to synthetic preservatives in meat products. Its dual antimicrobial and antioxidant effects, combined with the technological advantages of encapsulation, offer a sustainable solution for improving food safety and extending shelf life while meeting consumer demand for clean-label ingredients. Future research should focus on scaling up encapsulation techniques and exploring synergies with other natural preservatives for broader industrial applications.

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EVALUATING THE INFRARED TECHNIQUE AS A NOVEL DRYING METHOD OF THE TURKISH PASTIRMA

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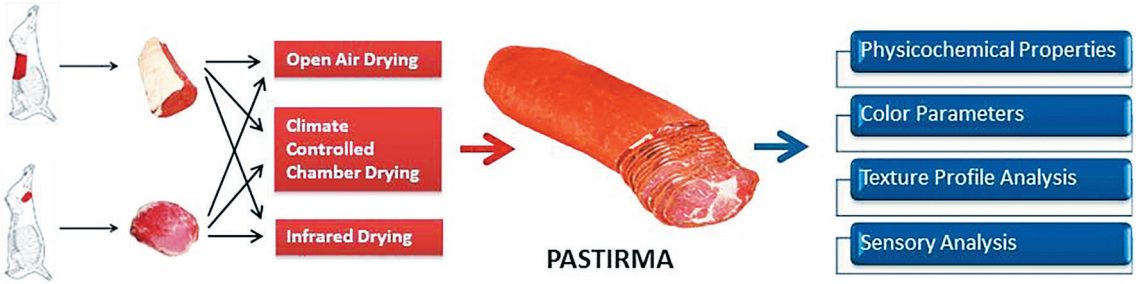
Keywords: *dry-cured meat, meat drying, microbial quality, pastirma, physicochemical quality, traditional meat product*

Abstract

Pastirma, a traditional dry-cured meat product from Turkey, is manufactured after a long drying process. In the study, we aimed to expedite the primary drying process, which is the lengthiest phase in pastirma production, by implementing the innovative infrared drying method, while preserving the sensory attributes of traditionally dried pastirma. Various physicochemical, microbiological and sensory characteristics of pastirma samples from two different muscles obtained by three various drying methods: traditional open-air (OA), climate-controlled chamber (CC) and infrared (IR) drying, were studied. The findings showed that IR drying, which reduced the drying time from 7 days to 2 days, produced the best quality in terms of sensory properties. Also, the lowest TBA values (0.07–0.13 mg/kg) were observed in IR dried samples. IR and CC drying solved the problem of uneven drying process of pastirma which was often encountered during the OA drying process. With the use of IR, the drying process of pastirma was rapid and the product quality was equivalent or even better than that of the other methods used in this research. This innovative approach of using IR drying can be adopted by the pastirma industry to facilitate the production of traditional products.

Graphical abstract



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Introduction

Pastirma, a traditional dry-cured meat product consumed for centuries in Turkey, epitomizes the intersecting historical and cultural heritage of the Middle East, Central Asia, the Mediterranean, the Balkans, and Europe as a significant gastronomic artifact; its name derives from the Turkish word 'bastırma,' meaning 'pressing' [1]. According to the Turkish Food Codex standards, pastirma is a meat product that has been cured and dried, but not thermally processed. It is made from various cuts of beef, such as loin, rib, and round, to achieve a tender texture desired by consumers [2].

The production process of pastirma involves several steps. It begins with curing the meat using a combination of salt, nitrite, sucrose, and glucose as curing agents. After curing, the meat is rinsed and pre-dried, and then it undergoes pressing, primary drying, and finally coating with a cemen paste. The cemen paste is prepared by mixing garlic, paprika and fenugreek seed powder (*Trigonella foenum-graecum*) with water. This paste is applied to the dried meat and plays a crucial role in enhancing the characteristic aroma of pastirma. Additionally, the cemen coating helps preserve the dried meat due to the antimicrobial properties provided by its ingredients [3]. For example, it acts as a barrier against oxygen and prevents mold growth on the surface. Traditionally, the production of pastirma takes place under natural weather conditions (open-air) and ambient temperatures [4]. This method allows the meat to dry and cure naturally, resulting in the desired flavor and texture.

In pastirma water activity (a_w) governs both microbial safety and structural integrity. Inadequate drying during ripening accelerates spoilage microorganism growth, causing degradation prior to product stabilization. Achieving target a_w levels (e. g., 0.84–0.92 as reported by Akköse et al. [5] and İnat et al. [6]) is thus vital to inhibit pathogens, extend shelf life, and permit safe raw consumption while preserving traditional quality [7]. According to the Turkish Food Codex, pastirma should possess a maximum moisture content of 50 %, pH of 6, salt up to 10 % (dry basis), and the coated cemen should not exceed 10 % by weight [2]. Furthermore, the pastirma must be free from rancidity and *Salmonella* contamination, and the presence of coagulase-positive *Staphylococci* and sulfite-reducing bacteria should not exceed 10^4 CFU/g [8].

The drying process reduces free water in pastirma to a very low level, resulting in a stable product with an extended shelf life of about 1 year at room temperature [9]. Low a_w inhibits physicochemical changes, biochemical reactions, and microbiological growth [10]. However, uneven drying can cause cracks in the cemen-coated surface, making these open areas susceptible to mold growth and maggot infestation from hatched fly eggs [11,12]. As pastirma is traditionally air-dried in the shade for about a month, climatic conditions such as temperature, air currents and humidity affect its quality [9]. The final drying phase is extensive, lasts for 5–12 days and poses several disadvantages [13]. The laborious processing of pastirma slows down its availability on the market and exposes it to microbial contamination during production [14]. Open-air drying, with its susceptibility to variable weather conditions, is challenging to regulate. For instance, rapid drying due to excessive air circulation, can lead to case hardening, leaving the meat core too moist [15]. Therefore, slower drying in climate-controlled chambers may resolve this issue, resulting in a more uniformly dried product.

Water removal, one of the oldest preservation techniques, is commonly used for meat and other food products. Recently, alternative drying methods using convective or radiant heat transfer have emerged. Infrared (IR) radiation, used in the food industry for heating, drying, roasting, cooking, thawing, surface pasteurization and packaging sanitization, is energy efficient. In IR drying, the food absorbs electromagnetic energy directly, unlike conventional drying where heat is transferred by convection. Fast, uniform heating and easily controllable process parameters make IR drying preferable to conventional methods. In addition, IR radiation is safe for heating food and is not harmful to human health or the environment [16].

The lengthy drying process has created logistical challenges for modern food production facilities, prompting the search for innovative techniques to speed up this stage while maintaining the traditional sensory characteristics that consumers associate with pastirma. The duration of the initial drying phase in pastirma production depends on climatic conditions and often exceeds five days in an open-air environment. However, the duration of the drying phase could be reduced by the use of IR energy or climate-controlled chambers. Neither infrared nor climate-controlled chamber drying has been investigated as part of the pastirma production process before. This innovative approach to meat drying could benefit the pastirma industry. Therefore, this research aimed to evaluate the effects of OA, CC, and IR drying techniques on some physicochemical and sensory characteristics of pastirma made from two types of beef muscles, loin and round. In addition, this research represents a pioneering effort to harness the potential of IR drying techniques to produce pastirma.

Materials and methods

Selection of meat for pastirma

The beef used in the study was sourced from a commercial meat processing company (Saray Meat Processing Co., Kayseri, Turkey). For the production of pastirma, two types of muscle were used, loin (*longissimus thoracis et lumborum*) and round (vastus group), 24 hours after slaughter. The meat was from 2-year-old steers of the Charolais breed. The steps of the pastirma production process, starting with meat selection and preparation, are detailed in Figure 1. The loin and round sections, meticulously removed from the carcass from an animal slaughtered the day before, were divided into three equal parts, each weighing approximately 1 kg. Thus, six pieces of pastirma grade meat of the same thickness and length were obtained from two sides. The meats were then trimmed to remove excess fat, tendons, and nerves. Each piece of meat was prepared to have the same length and thickness [17]. The temperature of the environment in which the samples were prepared was around 12 °C. An identification tag was attached to each of the pastirma-grade meat samples after the preliminary preparations were completed.

Preparation of curing salts and cemen paste

In the preparation of pastirma, a selection of curing agents was incorporated, including medium grain salt, sodium nitrite, and sucrose. These agents serve dual purposes in food preservation: inhibiting the growth of pathogenic microorganisms, thus enhancing food safety, and contributing to the flavor enhancement of the product.

Furthermore, an integral component of the pastirma preparation process involves the use of a spicy coating referred to as "cemen paste". The formulation of this paste involves the integration of fenugreek seed powder (50 % of the mixture), garlic (35 %), and paprika powder (15 %). These components were procured from local markets located in Kayseri, Turkey. The process of paste formation was carried out according to the methodology proposed by Gökalp et al. [9]. Specifically, an aqueous mixture was prepared by adding 1.2 L of tap water to 1 kg of the dry ingredient mixture. The result of this procedure is a homogeneous paste, which is essential for the production of pastirma. The use of this paste not only enriches the sensory appeal of the final product, but also improves its preservation properties.

Pretreatment of meat slabs for pastirma production

Pastirma samples were prepared at Saray Meat Processing Co. (Kayseri, Turkey) in accordance with the procedure described by Gökalp et al. [9] and Oz and Kaya [18]. The loin and round muscles removed from the carcass were divided into three equal parts weighing approximately 1 kg each. The excess fat, nerves and tendons were removed from six slabs of meat, each 25 cm long, 10 cm thick and 6 cm wide. To facilitate salt absorption, the slabs were stabbed twice on both sides with a knife, not penetrating more than 2/3 of the thickness of the slab. The meat slabs were cured in a stainless-steel tank containing 65 g NaCl, 80 mg $NaNO_3$ and 2 g of sucrose per kg of meat. The slabs were rubbed manually with the curing mixture and left in the tank for 1 day. The following day, the meat slabs were rinsed twice with cold fresh water to remove excess salt and hung on a metal trolley for pre-drying. All meat slab samples were dried under the same conditions for 1 day. Pre-drying was performed in the climate-controlled chamber (Kerres Anlagensysteme GmbH, Backnang, Germany) at 20 ± 2 °C and 75 % relative humidity at 1.5 ± 0.5 m s^{-1} air velocity. A period of 2 days of pre-drying was found to be effective in removing residual water that was absorbed during the rinsing process. Moisture was further removed using a custom-made press (Yıldızer Ltd., Istanbul, Turkey) manufactured for pastirma production, which applied a pressure of 1.0 kg/cm^2 for a period of 1 day. Pastirma samples were prepared in two batches to replicate the production.

Primary drying

Three different drying treatments were tested to perform primary drying, which is the longest drying stage in pastirma production. Since, it is common practice to carry out primary drying of pastirma in open air, the processing parameters for other drying methods were determined to produce a pastirma sample similar to open-air dried pastirma in terms of sensory properties. These parameters, which include drying temperature, RH, air velocity and drying time were determined as a result of preliminary studies. The similarity of controlled chamber and infrared dried samples to open-air dried samples was evaluated by traditional pastirma masters.

The meat slabs hung on a trolley were dried in the open air (OA) in the shade to avoid direct sunlight. The final drying process took 7 days and was carried out at a temperature of 37 ± 3 °C and a relative humidity of 55 ± 5 % with the air velocity of 1.5 ± 0.5 m s^{-1}. CC drying was carried out in a chamber at a temperature of 38 ± 3 °C and a relative humidity of 75 % with the air velocity of 1.5 ± 0.2 m s^{-1} for 5 days. The dimensions of the climate-controlled chamber (Kerres Anlagensysteme GmbH, Backnang, Germany) were 9 × 4 × 3 m^3. Infrared (IR) drying was performed in a custom-built drying chamber. Drying took 2 days at 38 ± 3 °C and 75 % relative humidity with 1.5 ± 0.2 m s^{-1} air velocity. An IR lamp (250-Watt, wavelength of 1100 nm) was installed in a stainless-steel cabinet (40 × 50 × 34 cm^3) to form the IR drying system. A temperature data logger (Model 174T, Testo, Lenzkirch, Germany) was used to monitor the temperature and a fan was installed to circulate the air inside the cabinet. The cabinet contained a horizontal bar on which slabs of meat were hung for drying. Ambient relative humidity (RH) and airflow velocity were measured periodically every 15 min using an anemometer (Model 410–2, Testo Co., Lenzkirch, Germany).

Application of cemen paste and final drying

To produce a traditional pastirma, dried and cured meat slabs were coated with cemen paste. The dried slabs of meat were left in the paste for 1 day and then uniformly coated with the paste. The thickness of the paste should not exceed 3–5 mm. The application of the cemen paste was followed by a final drying step in which the samples were dried at 20 ± 2 °C and 60 ± 5 % relative humidity with 1.5 ± 0.5 m s^{-1} air velocity for 1 day. This step was performed in a climate-controlled chamber (Kerres Anlagensysteme GmbH, Backnang, Germany) for all samples in one session.

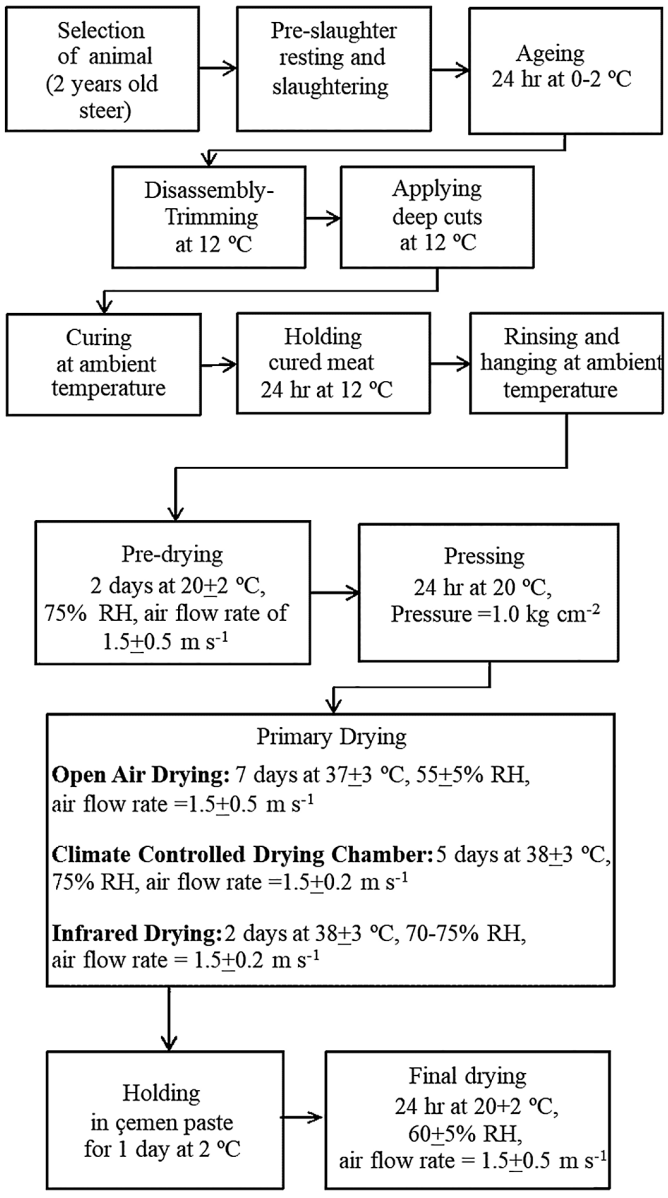

Figure 1. Flow chart of pastirma production

Physicochemical, microbiological and sensory analysis of fresh meat and pastirma

The fresh meat and pastirma samples were stored at 4 °C until analysis. Physicochemical analyses of fresh meat and pastirma included pH (Hanna HI 2211, Hanna Instruments Ltd., Bedfordshire, UK), water activity (a_w) (Aqua Lab 3TE, Meter Group Inc., Pullman, WA, USA), moisture (MA30, Sartorious AG, Göttingen, Germany), fat (B-811, Büchi Labortechnik AG, Flawil, Switzerland) and protein contents (FP, LECO Co., St. Joseph, MI, USA). Color (CIE L* a* b* color space values) was measured with a Minolta colorimeter (CR-300, Konica Minolta Sensing Inc., Osaka, Japan) with a 50 mm aperture size, D65 illuminant and 10° standard observer [19]. Residual nitrite content was determined by the AOAC method [20]. For the TBA test, the method described by Ulu [21] was used. Salt was measured by the Mohr method [22]. A texture analyzer was used to perform the TPA analysis (Microstable TA. XT Plus, Stable Micro Systems Ltd., Surrey, UK). This included hardness, adhesiveness and springiness of pastirma. The samples of pastirma were prepared by cutting 3.5 × 3.5 × 1 cm pieces. A cylindrical P25 probe of 25 mm diameter was used. Analysis was performed by compressing pastirma samples to 35 % strain at a rate of 1 mm/s. The trigger force was set to 0.01 N with a total test time of 10 s. Test samples were taken from the central part of the pastirma slabs. Fresh meat was analyzed for *Escherichia coli* O157: H7 using the ISO 16654 [23] standard method and for *Staphylococcus aureus* by the ISO 6888-1 [24] standard method. Samples of pastirma were analyzed for the presence of *Salmonella* [25], coagulase-positive *Staphylococci* [24], and sulfite-reducing bacteria [26].

The sensory analysis of pastirma was carried out by 10 expert panelists. They scored the pastirma samples according to taste, color, appearance and texture. The panelists were selected from among the pastirma masters working at Saray Meat Processing Co., Kayseri, who routinely evaluate pastirma products as a part of their job. The panelists were informed of the objectives of the study and signed an informed consent form before tasting. Furthermore, the factory management also approved the study. Pastirma samples were cut into 1.5–2 mm thick slices. The samples were labeled with 3-digit random numbers. The age of the panelists ranged from 20 to 55 years. The panelists all had a great deal of experience with pastirma. They were provided water between the samples to clear their palate. Analyses used a 5-point scale for descriptive testing, where 1 is reject, 2 is acceptable, 3 is good, 4 is very good and 5 is excellent. The test was conducted on both production batches but it was not replicated. The panelists tested a total of 12 samples in four sessions.

Statistical analysis of data

Pastirma production was replicated twice and, analysis was replicated three times. Minitab software was used for General Linear Model (GLM), as 3 different drying methods were tested on 2 different muscle types (Minitab version 16, Minitab Inc., PA, USA). One-way ANOVA with post hoc Tukey test ($P < 0.05$) was used for separation of means according to the results obtained from the GLM. The results of the sensory analysis were interpreted using Mood's median test, as the analysis was not replicated.

Results and discussion

Physicochemical and microbiological properties of fresh meat and pastirma

The pH of fresh loin and round ranged from 5.80 to 5.90. The moisture content was between 75 and 77 %. The a_w of fresh meat was between 0.965–0.998. The protein contents varied between 19.2–21.3 %. The fat content of the meat samples analyzed in this study was 5.6 % for loin and 6.3 % for round cuts. The fat content of the muscles from different parts of the carcass is often not similar [27].

Some physicochemical properties of pastirma are provided in Table 1. pH, a fundamental parameter in meat quality assessment, has an impact on the functional attributes, palatability, and meat product shelf life. The pH of dried meat products is significantly influenced by the drying process [28]. The pH of the pastirma samples

Table 1. Physicochemical properties of pastirma

Physicochemical properties	OA	CC	IR
pH			
L	5.73 ± 0.00^{bA}	5.78 ± 0.01^{aA}	5.78 ± 0.00^{aA}
R	5.68 ± 0.01^{bB}	5.72 ± 0.00^{aB}	5.74 ± 0.01^{aB}
Moisture (%)			
L	35.69 ± 0.63^{bA}	42.61 ± 0.39^{aA}	41.17 ± 0.37^{aA}
R	37.57 ± 1.82^{bA}	42.61 ± 0.27^{aA}	39.00 ± 0.32^{aA}
Water activity (aw)			
L	0.842 ± 0.003^{bA}	0.852 ± 0.002^{aA}	0.867 ± 0.003^{aA}
R	0.846 ± 0.001^{bA}	0.871 ± 0.001^{aA}	0.867 ± 0.002^{aA}
Salt (%)			
L	9.08 ± 0.15^{aA}	8.42 ± 0.12^{aA}	7.82 ± 0.10^{bA}
R	8.67 ± 0.04^{aA}	8.95 ± 0.11^{aA}	8.25 ± 0.09^{bA}
Fat (%)			
L	5.38 ± 0.39^{aB}	4.21 ± 0.37^{aB}	4.14 ± 0.20^{aB}
R	7.06 ± 0.22^{aA}	5.54 ± 0.39^{aA}	8.07 ± 0.56^{aA}
Protein (%)			
L	36.88 ± 0.06^{cA}	37.08 ± 0.09^{bA}	38.70 ± 0.02^{aA}
R	36.50 ± 0.07^{cA}	37.15 ± 0.06^{bA}	38.70 ± 0.02^{aA}
Nitrite (mg/kg)			
L	18.76 ± 0.13^{aA}	17.64 ± 0.12^{bA}	17.67 ± 0.23^{bA}
R	18.75 ± 0.10^{aA}	17.54 ± 0.13^{bA}	17.34 ± 0.06^{bA}
TBA (mg malondialdehyde/kg)			
L	0.29 ± 0.00^{bA}	0.31 ± 0.01^{aA}	0.07 ± 0.01^{cA}
R	0.16 ± 0.01^{bA}	0.32 ± 0.01^{aA}	0.13 ± 0.01^{cA}

Data are means and standard deviation of six replicates. All data were calculated on a dry weight basis. $^{a, b, c}$ Means on the same line that do not have a common superscript are different ($P < 0.05$). $^{A, B, C}$ Means within the same column that do not have a common superscript are different ($P < 0.05$). OA — Open-air drying, CC — Climate-controlled chamber drying, IR — Infrared drying. Muscle type: L — loin, R — round.

ranged from 5.68 to 5.78. Open-air (OA) dried samples had a significantly lower but comparable pH to the other samples ($P < 0.05$). The influence of drying techniques on pH dynamics in pastirma production is highlighted by this observation. The pH values of the pastirma samples in this study were in accordance with the Turkish Food Codex [2]. In other studies, the pH was found to be 5.69 to 5.92 by Karabıyıklı et al. [3] and 5.86 by Kaban [12]. In a study on the effect of salt types and drying methods, the pH was reported to be 5.72 and 5.75 for samples cured with NaCl and dried naturally or under controlled conditions, respectively [29]. Their results were similar to those obtained in the present study.

Moisture is a critical determinant of product characteristics, including texture, shelf life and sensory attributes, making it a key parameter in meat preservation and quality assessment [30]. The moisture content of the pastirma samples dried with the OA method was significantly lower than that of the pastirma dried with the other drying methods for both types of muscle ($P < 0.05$). The moisture contents of CC and IR dried samples were not significantly different. The drying process in CC and IR chambers was completed when the samples matched the sensory properties of OA dried pastirma samples. When visually inspected, the central part of the OA dried samples was wetter than the parts close to the surface in this study. The inner part of traditional pastirma is often wetter than the outer part. A pastirma product with an almost raw meat appearance in the center, although not considered to be of premium quality, is traditionally acceptable to consumers. In this study, IR and CC dried samples were observed to have a more uniform moisture distribution throughout the cross section of the sample. When infrared radiation is used to heat or dry a substance, the radiation falls on and penetrates the surface of the material and is then converted into thermal energy [31]. Therefore, infrared drying may have the potential to improve the uniformity of drying with a more homogeneous moisture distribution through the cross section of the pastirma. It was observed that IR dried samples had a more homogeneous moisture distribution when compared visually with OA dried pastirma samples. The drying time was significantly reduced from 7 days to 2 days by using IR drying instead of OA drying. The energy losses in IR drying are lower compared to conventional drying methods as the energy is absorbed directly by the food. This not only improves energy efficiency but also increases the heating rate. In addition, IR heating can promote better product quality due to a more uniform temperature distribution.

Muscle types are inherently different in their composition, which includes variables such as intramuscular fat content, connective tissue distribution and water-binding capacity [32]. Muscles with different fat content may have different moisture retention capabilities, which will affect the overall moisture content of the pastirma product. Pastirma moisture content was not significantly affected by using different muscle types. Dried OA loin pastirma had the lowest moisture content (35.69 %). Both types of muscle dried with the CC method had the highest moisture content (42.61 %). The moisture contents observed in this study were in accordance with the Turkish Food Codex [2]. The moisture contents of pastirma were determined as 47.56–48.23 % by Çakıcı et al. [33] and 45.84 % by Uğuz et al. [34].

Water activity profoundly impacts microbial stability, as microorganisms require available water for growth and metabolic activity. In addition, moisture content, which is closely linked to a_w, directly affects the texture, tenderness and mouthfeel of the product [32]. A previous study reported the effect of drying temperature and time on meat product moisture and a_w [35]. The a_w of pastirma samples in this study was between 0.842–0.871. In another study, the a_w of pastirma samples produced under controlled or natural air-drying conditions were determined as 0.91–0.92; moisture contents were 51.94–53.43 %, respectively [29]. The a_w for OA dried samples was considerably lower (0.842 to 0.846 for loins and rounds, respectively) than for other samples ($P < 0.05$). This observation may be associated with the circumstance that samples dried with the OA method exhibited the least moisture content. The lower water activity levels observed in our samples suggest the potential for all drying methods to contribute to microbial stability and extended shelf life. Furthermore, the results emphasize the importance of carefully managing moisture content to achieve the desired water activity range in pastirma production. The results also indicate that, within the parameters of this study, the choice of meat type did not significantly affect the change in water activity of pastirma.

The most important function of salt in pastirma production is its role in microbial control and preservation. The application of salt directly affects the water activity (a_w) of the meat, reducing it to a level that inhibits the growth and proliferation of spoilage microorganisms and pathogenic bacteria [36]. Salt also plays a crucial role in defining the texture, tenderness and sensory attributes of pastirma. It acts as a water-binding agent, promoting moisture retention within the meat matrix. This contributes to the characteristic chewiness and juiciness of the product, enhancing its palatability and sensory appeal. In our study, the salt content of the samples was determined between 7.82–9.08 %. These values were in accordance with the Turkish Food Codex [2]. The lowest levels of salt were found in IR dried loin (7.82 %) and round (8.25 %) samples ($P < 0.05$). The observed differences in salt content may be due to minor inconsistencies in the rinsing of pastirma slabs after the curing process to remove excess salt. Variability in rinsing practices could result in residual salt levels contributing to the observed differences in salt content between samples. In addition, the drying and pressing steps inherent in the pastirma production process exert a concentration effect on salt. As the moisture content decreases due

to drying and pressing, the salt concentration in the meat matrix naturally increases. According to Hastaoğlu and Vural [29], the drying method of pastirma did not have a significant effect on salt content. In this study, OA and CC chamber dried samples had similar salt content, while IR dried sample had slightly lower salt content (Table 1). The salt content of pastirma was determined as 6.32–7.92 % by Çakıcı et al. [33], 7.83 % by Abdallah et al. [17], and 5.96 % by Uğuz et al. [34].

Fat acts as a moisture retention agent in pastirma, helping to reduce moisture loss during the curing and drying processes. This moisture retention is crucial for maintaining the juiciness of the product and preventing undesirable drying [37]. Furthermore, the lipid content in pastirma acts as a natural barrier to microbial growth, enhancing its preservation attributes and shelf life. Fat also plays a role in controlling lipid oxidation, a critical consideration in pastirma production. The presence of fat in meat products helps mitigate the effects of lipid oxidation, ensuring the product maintains its intended flavor and sensory characteristics [38]. Fatness varied between 4.14–8.07 % depending on the muscle type but not on the drying process. The elevated fat content observed in pastirma samples derived from round could be attributed to the inherent higher fat content in fresh round compared to the fresh loin utilized in the production of pastirma. Fat content of pastirma was not substantially changed by the choice of drying method. This result suggests that the fat content remains relatively stable and consistent regardless of the drying method used. In some other studies, the fat content of pastirma samples was determined between 8.80–5.05 % by Çakıcı et al. [33], and 4.65–4.71 % by Hastaoğlu and Vural [29].

The relationship between protein content and the drying process in pastirma production is a multifaceted interplay that encompasses texture development, flavor creation, preservation and nutritional aspects. The dynamic changes in protein structure and composition during drying are crucial in shaping the sensory attributes and overall quality of the pastirma. The protein levels of the samples ranged from 36.50 to 38.70 % were affected by the type of drying but not by the type of muscle. The protein content was highest in the IR dried pastirma samples, while the OA dried samples had the lowest protein content. However, the differences between the samples were negligible. As a result, IR drying contributed to the textural and structural properties by creating a denser protein network in the product with its higher protein content. Uğuz et al. [34] reported the protein content of pastirma as 27.06 % in their study where they tested the effect of different salts on the quality of pastirma. Our results are similar to those of Uğuz et al. [34], which is considerably higher than the result.

The nitrite concentration of pastirma samples was between 17.34–18.76 mg/kg. It was influenced by the drying method ($P < 0.05$). Samples dried by the OA method had higher nitrite levels than those dried by other methods. In another study, the nitrite concentration of pastirma was reported as 9.45–14.79 mg/kg [33].

The study reveals notable differences in the levels of lipid oxidation, as measured by the TBA values, among pastirma samples prepared using different drying techniques. TBA values represent the concentration of malondialdehyde, a secondary product of lipid oxidation, and therefore serve as a measure of the oxidative rancidity in food products. The drying process plays a critical role in modulating the extent of lipid oxidation, with certain methods potentially promoting higher TBA levels due to prolonged exposure to oxidative conditions. In the samples examined, TBA values ranged between 0.07–0.32 mg malondialdehyde/kg, indicating varied degrees of lipid oxidation. The CC chamber dried samples exhibited the highest TBA values. In contrast, IR dried samples showed the lowest TBA values, with 0.07 mg/kg for loin and 0.13 mg/kg for round cuts. These differences suggest that the drying method can significantly influence the rate of lipid oxidation in pastirma. The lower TBA values observed in IR dried samples can be attributed to their likely limited exposure to oxygen due to the shorter primary drying phase. The rapid and uniform drying facilitated by the IR method may have minimized the time that lipids in the pastirma were exposed to oxygen, thus limiting the potential for oxidation reactions to occur. Oxidative rancidity is primarily driven by the reaction of lipids with oxygen, forming peroxides and ultimately secondary oxidation products such as malondialdehyde. Therefore, methods that limit oxygen exposure can effectively reduce lipid oxidation. To put the TBA values into context, it is generally accepted that TBA values of 0.5 mg/kg may indicate the onset of noticeable oxidation, while values above 1 mg/kg may indicate an unacceptable level of oxidation in meat products [27]. Notably, Abdallah et al. [17] reported a TBA value of 0.81 mg/kg for uncoated pastirma, demonstrating the potential benefits of novel preservation methods such as chitosan coating in reducing lipid oxidation. The drying method has a significant effect on the degree of lipid oxidation in pastirma, as evidenced by the differences in TBA values among samples prepared by different techniques. Among the methods studied, IR drying appears to be particularly effective in minimizing lipid oxidation, probably due to its ability to reduce oxygen exposure. These results provide valuable insights for pastirma manufacturers wishing to optimize the oxidative stability of their products, thereby enhancing their shelf-life and sensory qualities.

Microbiological characteristics are key determinants of the safety and shelf life of pastirma. The drying process, especially when combined with salting, helps to create an inhospitable environment for undesirable microorganisms, thereby extending the shelf life of the product [39]. In the study, *E. coli* O157: H7 and *S. aureus* were absent in the fresh meat samples. Catalase-positive cocci and lactic acid bacteria predominated in the pastirma microflora. *Salmonella* was not detected in any of the sample during micro-

biological analysis of the pastirma. There were less than 10 CFU/g of both coagulase-positive *Staphylococci* and sulfite reducing bacteria. According to the Turkish Food Codex, *Salmonella* should not be present in pastirma while low levels of *Staphylococci* and sulfite-reducing aerobic bacteria are allowed [8]. *Salmonella* was also not detected in another study by Karabıyıklı et al. [3]. They observed that the coagulase-positive *Staphylococci* and sulfite-reducing bacteria in the pastirma ranged from 4.37 to 7.55 log CFU/g and from 1.00 to 4.18 log CFU/g, respectively.

Color parameters of pastirma samples

The color parameter of pastirma represents a fundamental aspect of its sensory and aesthetic properties, contributing to consumer acceptance and quality perception. Color is a sensory attribute that profoundly influences the consumer's perception of the quality and palatability of pastirma. The bright red color, traditionally associated with well-cured and well-preserved meat products, is emblematic of the visual appeal of pastirma. Consumers often equate a rich, even color with freshness and superior quality, making it an important determinant of product acceptance. The choice of drying method in the production of pastirma can have a noticeable effect on its color properties. Traditional open-air drying, characterized by prolonged exposure to environmental conditions, can lead to color variations in pastirma due to factors such as oxidative reactions and microbial activity. Controlled drying techniques, including climate-controlled chambers or infrared (IR) drying, are designed to speed up the drying process and can result in a more consistent and visually appealing color. No significant effect of muscle type on the color parameter of pastirma samples was found in this study (Table 2). However, the drying method played a critical role in determining these parameters, including lightness (L*), redness (a*), and yellowness (b*). In terms of lightness (L*), open-air (OA) dried samples were the darkest, displaying values between 25.02–27.48 for loin and round cuts, respectively. Conversely, the samples dried in the CC chamber had the lightest color ($P < 0.05$). Infrared (IR) dried samples, with L* values of 30.15 and 30.94 for loin and round respectively, were intermediate between the OA and CC dried samples in terms of lightness. An intriguing correlation was observed between moisture content and color intensity among the pastirma samples. Notably, the OA dried samples, which exhibited the darkest color, also possessed the lowest moisture content. This connection raises the possibility that reduced moisture content may contribute to increased color intensity in pastirma. On the other hand, the CC dried pastirma samples exhibited the lightest color of all the samples tested. This observation is consistent with the effect of controlled drying conditions, where reduced exposure to environmental factors may contribute to a lighter color profile. While IR dried samples resulted in a color profile falling between the extremes of OA and CC drying, lending a balanced visual appearance to the pastirma. Prior studies have demonstrated a range of L* values for pastirma, with values of 40.47–42.64 by Çakıcı et al. [33], 37.88 by Abdallah et al. [17], and 42.94–44.75 by Hastaoğlu and Vural [29]. These variations in L* values between the present study and previous research may be attributed to differences in the moisture content of pastirma samples.

Table 2. Color properties of pastirma

Color parameters		OA	CC	IR
L*				
	L	25.02 ± 0.15^{c}	32.88 ± 0.17^{a}	30.15 ± 0.48^{b}
	R	27.48 ± 0.29^{c}	34.26 ± 0.68^{a}	30.94 ± 0.42^{b}
a*				
	L	9.47 ± 0.23^{c}	14.01 ± 0.46^{a}	11.99 ± 0.17^{b}
	R	10.40 ± 0.28^{c}	15.45 ± 0.21^{a}	13.30 ± 0.12^{b}
b*				
	L	4.49 ± 0.17^{a}	1.73 ± 0.20^{c}	2.51 ± 0.06^{b}
	R	3.58 ± 0.16^{a}	1.37 ± 0.36^{c}	2.27 ± 0.18^{b}

Data are means and standard deviation of six replicates. $^{a, b, c}$ Means on the same line that do not have a common superscript are different ($P < 0.05$). OA: Open-air drying, CC: Climate-controlled chamber drying, IR: Infrared drying. Muscle type: L — loin, R — round. L* — Lightness, a* — Redness, b* — Yellowness.

In the context of pastirma, the analysis of the a* values revealed notable differences associated with the choice of drying method. As for the redness (a*) of pastirma, this study found it to range between 9.47–15.45. The CC dried samples exhibited the highest a* values, indicating increased redness, while the OA dried samples had the lowest a* value, indicating decreased redness. This result suggests that controlled drying conditions that limit environmental exposure may contribute to the development of a richer red color profile. A brighter red color is often associated with premium quality fresh pastirma, whereas a darker hue might suggest an older, potentially inferior quality product [40]. The a* values of the IR dried samples were close to those of the CC chamber dried samples. Pastirma a* values reported in prior research show a wide range, with values of 30.22–27.45 by Çakıcı et al. [33], 16.13 by Abdallah et al. [17], and 12.72–14.79 by Hastaoğlu and Vural [29].

Yellowness (b*) of pastirma was highest for OA dried samples. In contrast, CC chamber dried samples showed b* values ranging between 1.37–4.49, indicating a lower degree of yellowness compared to the other drying methods A darker red color with pronounced yellowness, as seen in OA dried pastirma, may be less desirable given the traditional preference for a lighter, pinkish-red color. The b* values reported in previous studies vary, with values of 16.50–17.53 by Çakıcı et al. [33], 4.98 by Abdallah et al. [17], and 42.94–44.75 by Hastaoğlu and Vural [29].

In summary, while muscle type does not significantly affect the color parameters of pastirma, the drying method used does. IR dried samples were intermediate between OA and CC dried samples for all color parameters.

The results of this study underline the importance of the drying method in shaping the color parameters and, consequently, the perceived quality of pastirma. These findings provide valuable insights for pastirma manufacturers seeking to optimize the color characteristics and overall quality of their products.

Texture profile analysis of pastirma samples

Texture is a fundamental attribute that significantly influences the palatability and overall sensory experience of food products [41]. One of the important factors in the consumer's perception of dried meat product quality is the texture of the dried meat. Compared to alternative drying methods like air and oven drying, sun-dried meat exhibits comparatively elevated textural and flavor ratings. Nevertheless, high temperatures can lead to the denaturation of essential proteins that play a role in shaping the texture and quality of the dried meat product [28]. In this study, there was no major effect on textural parameters of muscle types (Table 3). Hardness is defined as the peak force that occurs during the first compression cycle. The hardness of the pastirma samples ranged between 16.33–33.21 N. OA dried samples had the lowest hardness ($P < 0.05$) while CC and IR dried samples were similar. Despite OA dried samples had lower moisture content than IR dried samples, they displayed lower hardness values. This observation might be attributed to the fact that the texture analyses were conducted around the central part of the pastirma samples, which had a higher moisture content compared to the outer parts in the OA dried samples. In contrast, the moisture was evenly distributed throughout the cross section of the IR and CC dried samples, contributing to their relatively higher hardness values.

The results of this study show that the muscle type does not significantly affect the textural parameters of pastirma, as shown in Table 3. However, the drying method used have an effect on the textural properties of the final product, specifically on hardness, adhesiveness, and springiness. Hardness, defined as the peak force occurring during the first compression cycle, varied between 16.33–33.21 N among the pastirma samples. Surprisingly, despite having lower moisture content, open-air (OA) dried samples had the lowest hardness values ($P < 0.05$). A plausible explanation for this is the moisture distribution within the pastirma samples. The texture analyses were performed around the central part of the pastirma samples, which in the case of the OA dried samples, retained a higher moisture content than the outer parts. In contrast, both the climate-controlled (CC) chamber and the infrared (IR) dried samples exhibited a uniform moisture distribution throughout their cross section, resulting in comparable hardness values.

Table 3. Texture profile characteristics of pastirma

Texture parameters	OA	CC	IR
Hardness (N)			
L	16.33 ± 0.61^{b}	32.21 ± 0.70^{a}	25.53 ± 1.71^{a}
R	18.46 ± 0.44^{b}	28.49 ± 1.39^{a}	27.91 ± 0.35^{a}
Adhesiveness (J)			
L	82.58 ± 1.86^{a}	65.90 ± 0.73^{b}	64.10 ± 0.38^{b}
R	84.84 ± 1.32^{a}	67.39 ± 1.21^{b}	64.79 ± 0.09^{b}
Springiness (mm)			
L	1.52 ± 0.03^{b}	1.68 ± 0.01^{ab}	1.70 ± 0.01^{a}
R	1.64 ± 0.01^{b}	1.61 ± 0.04^{ab}	1.68 ± 0.01^{a}

Data are means and standard deviation of six replicates. $^{a, b, c}$ Means on the same line that do not have a common superscript are different ($P < 0.05$). OA: Open-air drying, CC: Climate-controlled chamber drying, IR: Infrared drying. Muscle type: L — loin, R — round.

Adhesiveness, recorded as the area of the negative force curve, was highest for OA dried pastirma. Adhesiveness, as a textural characteristic, delineates the effort required to overcome the cohesive forces between the surface of food and the contacting materials. The highest adhesiveness observed for OA dried pastirma may be linked to the higher moisture content in the center of these samples, which may promote greater interaction and adhesion. As with hardness, CC chamber and IR dried samples showed similar adhesiveness, possibly due to their uniform moisture distribution. Springiness was found to be significantly higher in the IR dried pastirma samples. These samples displayed values between 1.68–1.70 mm, while the OA dried samples demonstrated lower springiness values between 1.52–1.64 mm ($P < 0.05$). Springiness is desirable in sliced pastirma, as consumers typically prefer elastic slices to inelastic ones. The uniform moisture distribution in IR dried samples may contribute to their enhanced springiness. Drying conditions, including time, temperature, and humidity, are variables that control the textural quality of the final product [4]. IR drying conditions may provide a more favorable balance of these variables to maintain the springiness of the product. Pastirma dried in the CC chamber showed a springiness comparable to both IR and OA dried samples.

Our study reveals the pivotal role of drying methods in determining the texture properties of pastirma. Drying method influenced the hardness, adhesion and springiness of the final product, while muscle type had no discernible effect on texture. The results highlight the importance of carefully controlling drying conditions, including time, temperature, and humidity, to ensure the textural quality of the final product that is in line with consumer preferences. Further research could explore the underlying mechanisms of these drying methods on moisture distribution and their consequent effects on pastirma texture.

Sensory parameters of pastirma samples

Sensory characteristics are an integral part of pastirma quality and consumer satisfaction. These properties are significantly influenced by the drying process. A drying mechanism should be chosen with a temperature that does not affect meat sensory quality and with sufficient drying time [42]. In the exploration of factors influencing the sensory parameters of pastirma, a type of cured and dried meat product, our findings revealed that the muscle type had no significant effect (Table 4). Instead, the drying method was found to be a more decisive fac-

tor in determining these parameters, including the color, odor, texture, and flavor of the product. Our study showed that the highest sensory scores for all parameters were obtained from samples dried using the infrared technique ($P < 0.05$). This radiant heating method allows the heat to be transferred directly from the IR source to the product in the form of electromagnetic waves. When absorbed, this energy is converted into heat, facilitating a more uniform and faster drying process when compared to conventional drying methods. IR drying is characterized by its specificity in targeting water molecules for evaporation, as well as its effective and rapid heat transfer [31]. Together, these attributes enable rapid and uniform removal of the moisture from the product. This uniformity, in turn, prevents localized overheating, thereby reducing the risk of scorching and associated adverse changes. Such changes can include unwanted color changes, structural deformation or onset of oxidative problems that can negatively impact product quality. It was observed that the sensory scores given by the panelists to the pastirma samples ranged from 2.00 to 3.50. A score of 2 corresponded to an acceptable product, while a score of 3 indicated a good product. In general, the sensory scores were low, probably reflecting the high-quality standards of the trained panelists.

Table 4. Sensory properties of pastirma

Sensory parameters	OA	CC	IR
Taste			
L	2.20 ± 0.20^{b}	2.20 ± 0.29^{b}	3.10 ± 0.23^{a}
R	2.00 ± 0.21^{b}	2.00 ± 0.20^{b}	3.30 ± 0.37^{a}
Color			
L	2.20 ± 0.36^{b}	2.60 ± 0.33^{b}	3.40 ± 0.3^{a}
R	2.60 ± 0.34^{b}	2.80 ± 0.20^{b}	3.40 ± 0.34^{a}
Appearance			
L	2.30 ± 0.26^{b}	2.20 ± 0.20^{b}	2.90 ± 0.31^{a}
R	2.10 ± 0.18^{b}	2.40 ± 0.13^{b}	2.80 ± 0.29^{a}
Texture			
L	2.10 ± 0.23^{c}	2.40 ± 0.16^{b}	2.70 ± 0.37^{a}
R	2.00 ± 0.15^{c}	2.20 ± 0.25^{b}	3.50 ± 0.37^{a}

Data are means and standard deviation of scores of ten panelists. [a, b, c] Means on the same line that do not have a common superscript are different ($P < 0.05$). OA: Open-air drying, CC: Climate-controlled chamber drying, IR: Infrared drying. Muscle type: L — loin, R — round.

It can be inferred that the IR dried samples that showed superior sensory scores were more consistent in texture, color and overall quality. This consistency in the sensory qualities is consistent with the results of another research. Cherono et al. [43] found that IR dried biltong had lower hardness scores compared to air-dried samples. Similarly, Kate and Sutar [44] reported superior texture in IR dried ginger rhizome samples as revealed by Scanning Electron Microscopy (SEM) images.

Moreover, the superior sensory evaluation of IR dried pastirma may also be due to the preservation of heat-sensitive flavor and aroma compounds. Traditional drying methods can result in the loss or alteration of these compounds, whereas the rapid and uniform drying characteristic of IR is likely to aid their retention. This could result in a product that is more appealing and acceptable to the trained panelists, as evidenced by the higher sensory scores.

In summary, our results highlight the central role of IR drying in improving the sensory parameters of pastirma. Its drying efficiency, rapidity and uniformity help to maintain the product quality in terms of color, texture and flavor while avoiding the negative effects of overheating and oxidation [45]. Future research directions could include extending the application of IR drying to other food products to further exploit the benefits of this technology in the food industry.

Conclusions

It can be concluded that a standard compliant pastirma product can be produced by using the three drying methods investigated in this study. This research clearly showed that the application of IR drying is capable of reducing the primary drying time from 7 to 2 days in pastirma processing while maintaining product quality. Indeed, for all sensory characteristics, including taste, color, appearance and texture, IR dried samples were found to be superior to other samples. The TBA values of the IR dried samples were the lowest, indicating less lipid oxidation and therefore a more acceptable flavor. Uneven drying can occur as a result of OA drying, resulting in a product with excessive central moisture and a raw appearance. It was shown that IR and CC chamber drying can help to avoid this problem and deliver a better product. The CC chamber dried pastirma samples had the highest L^* and a^* values, followed by the IR dried samples. The OA dried samples had the darkest color, hence poor color quality. To further shorten the drying process of pastirma without negatively affecting the product quality, it could be suggested that the IR drying method could be enhanced.

Within the scope of this study, IR and CC drying methods were tested together. Both methods have different advantages. While the drying time was reduced with IR drying, color parameters were better with CC drying. By using both drying methods together, it is possible to produce pastirma in a shorter time and with better quality parameters. It is acknowledged that the shortcoming of this study was that these two processes were not tried simultaneously.

The scientific significance of this study lies in its successful application of IR drying to substantially reduce the drying period of pastirma, from 7 days (as conventionally observed) to a mere 2 days. Such a remarkable reduction in processing time holds substantial implications for the food industry, particularly within the pastirma manufacturing sector. This work represents a notable innovation in food science, mainly by using IR drying technique to produce pastirma. Traditionally, the pastirma drying process has been time-consuming, necessitating prolonged open-air exposure. This research provides an approach that significantly reduces drying time while preserving the sensory

qualities cherished by consumers. Furthermore, IR drying mitigates the issue of inconsistent drying encountered during the open-air drying process, ensuring uniform product quality. Importantly, the study highlights that the utilization of IR drying not only expedites the pastirma production process but also enhances the final product's quality.

In conclusion, this study has explored the experimental implementation of IR drying technology in the manufacturing process of pastirma, an esteemed cured meat product with cultural significance. The findings demonstrate that IR drying can substantially reduce the drying period while simultaneously improving sensory characteristics and product quality. This approach holds considerable promise for the pastirma industry, offering a viable means to streamline production processes and meet consumer demands for high-quality, traditionally inspired food products. As such, this research represents a remarkable contribution to food science and technology, exemplifying how modern methods can harmonize with the production of traditional foods.

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

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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.

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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 2024[1]. 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 avilability 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 proteinofs 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±1°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 troponin 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 ± 2 °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 assessments 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 % O_2) to an oxygen-free combination of N_2 and CO_2 — 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 O_2 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 % O_2 + 20 % CO_2) 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 highoxygen 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 % O_2 + 20 % CO_2) 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 aggregates 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 % O_2) 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 (N_2 and CO/CO_2) 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.

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