



The effects of temperature regimes on the microbiological safety and biochemical parameters of frozen beef over long-term storage periods

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To guarantee the safety and high quality of meat and meat products, freezing is currently considered one of the most effective methods of preservation, commonly used in food industry. The objective of the study was to determine the activity of microbiota and the biochemical changes in frozen beef stored at different temperatures in order to substantiate optimal regimes that ensure high quality and safety of the raw material. Three regimes of storage were studied: $-12\text{ }^{\circ}\text{C}$ for 8 months, $-20\text{ }^{\circ}\text{C}$ for 14 months, and at $-25\text{ }^{\circ}\text{C}$ for 18 months. The microbiological analysis revealed that at $-12\text{ }^{\circ}\text{C}$, a large amount of psychrotrophic microflora remained vital, whereas the number of fungi and yeasts even increased by 1.86 times. Storage at $-20\text{ }^{\circ}\text{C}$ reduced the levels of Enterobacteriaceae (on average by 20 times), mesophilic, and psychrotrophic microorganisms, and also stopped the reproduction of fungal microflora. At $-25\text{ }^{\circ}\text{C}$, the results were similar to those at $-20\text{ }^{\circ}\text{C}$, indicating absence of additional advantages of this regime in terms of microbiological stability. The biochemical studies revealed that at $-12\text{ }^{\circ}\text{C}$, protein breakdown and hydrolysis of fats was more intensive, accompanied by the increases in the contents of amino-ammonia nitrogen (1.38 times) and volatile fatty acids (4.63 times), compared with fresh meat, and also an increase in pH up to 6.05 ± 0.03 . Such changes indicate the formation of signs of 'doubtful freshness'. At $-20\text{ }^{\circ}\text{C}$ and $-25\text{ }^{\circ}\text{C}$, those parameters changed insignificantly, allowing the meat to be classified as fresh. The analysis of lipid peroxidation indicated that an increase in the temperature of storage promoted a more intensive formation of TBA-active products and diene conjugates. At $-12\text{ }^{\circ}\text{C}$, their contents increased by $13.0 \pm 0.2\%$ and $27.2 \pm 0.3\%$, respectively, while no statistically significant changes were observed at $-20\text{ }^{\circ}\text{C}$ and $-25\text{ }^{\circ}\text{C}$. The yielded results indicate that an optimal regime for long (over 12 months) storage of beef is $-20\text{ }^{\circ}\text{C}$, which provided a high level of microbiological safety, slowed down the course of unsatisfactory biochemical processes, without any additional energy expenditures required for the $-25\text{ }^{\circ}\text{C}$ regime. The $-12\text{ }^{\circ}\text{C}$ regime can be used only for short-term storage given a low initial contamination and control of the development of fungal microflora.

Keywords: beef; freezing; storage temperature; microbiological safety; biochemical changes; lipid peroxidation.

Introduction

Provision of microbiological safety and preservation of nutritional value of meat over long-term storage periods are key tasks of modern meat industry (Kukhtyn et al., 2020b; Al-Dalali et al., 2022; Luo et al., 2023). In the global and domestic practice, the most effective method of preserving raw meat is considered to be freezing, which allows significantly slowing down the biochemical, physical-chemical, and microbiological processes, preserving organoleptic characteristics of the product (Lu et al., 2022; Jiang et al., 2025). At the same time, selecting optimal temperature regime of freezing and period of meat storage requires scientific validation, because these parameters are what the quality of end products and the economic effectiveness of the production depend on (da Silva Bernardo et al., 2020; Biglia et al., 2022).

According to the requirements of DSTU 6030:2008, beef in halves and quarters is allowed to be stored frozen at temperatures in the range of $-12\text{ }^{\circ}\text{C}$ to $-25\text{ }^{\circ}\text{C}$ and for a duration ranging from 8 to 18 months. At the same time, the current European regulations, in particular EU Regulation 2073/2005, establish allowable levels of microbial contamination to ensure the safety of products: The content of mesophilic aerobic microorganisms must not exceed 5 log CFU/cm^2 of the surface, whereas the number of bacteria of the family Enterobacteriaceae must not exceed 3 CFU/cm^2 of the carcass area. Adherence to these normative is crucial for preventing food poisoning and maintaining meat quality. Modern studies (Leygonie et al., 2012; Li et al., 2020) emphasize that the rate and penetration depth of freezing

have a direct effect on the product's quality: Slow rates of freezing result in the formation of large ice crystals that ruin the cellular structures, causing losses of juiciness and deterioration of the meat texture after thawing. On the other hand, excessively low temperature of storage, although providing a better stability of microbiota, requires significant energy expenditures, which necessitates the optimization of production regimes (Egelandstad et al., 2019; Kumar et al., 2020).

A number of studies (Zhang et al., 2018; Xue et al., 2021) indicate that freezing is not an absolute barrier for microorganisms: A part of psychrotrophic microflora, and also yeast fungi, can remain vital even at subzero temperatures, which explains their ability to reproduce during storage at $-12\text{ }^{\circ}\text{C}$. This is especially important for when the initial level of contamination is elevated.

From the point of view of biochemical processes, the studies confirm (Jung et al., 2025) that freezing significantly slows the proteolytic and lipolytic reactions, but does not stop them completely. Special attention is paid to peroxidation of lipids, which cause unpleasant odors and tastes, and also deteriorate the nutritional value of meat through the ruination of unsaturated fatty acids (Taha et al., 2015; Domínguez et al., 2019; Dragoev, 2024; Shui et al., 2025). Meanwhile, the temperature, rate of freezing, and storage period can have a substantial effect on the formation and accumulation of aroma compounds (Li et al., 2020).

Along with the classic methods of freezing, modern technologies are being actively explored, aimed at improving the stability of meat quality. In particular, the use of combined freezing with prior blast freezing (Zhan et al., 2018; Hou et al., 2020), the use of protective

packaging materials with modified atmosphere (Śmiecińska et al., 2018; Kandeepan & Tahseen, 2022), antimicrobial coatings, and also the introduction of natural antioxidants (extract of rosemary, green tea, tocopherols) to the processing were observed to substantially prolong the shelf life of meat without the loss of its sensory attributes (Garavito et al., 2020; Cao et al., 2023).

Therefore, modern technologies of meat freezing are oriented at its long storage without loss of its nutritional value and affecting its safety. However, selection of a particular regime of frozen storage must be based on a validated scientific research so as to ensure not only biological safety but also to prevent changes in biochemical parameters occurring at cellular level in the muscular tissue.

Therefore, the objective of the study was to determine the activity of microbiota and biochemical changes in frozen beef over the periods of storage at different temperatures to corroborate the regimes optimal for the provision of high quality and safety of the raw material.

Materials and methods

Beef samples and rinses from the carcasses were collected at a meat-processing enterprise in Ternopil Oblast, and the preparations to the studies were conducted according to ISO 6887-1:2017 and ISO 6887-2:2017. One portion of beef (half carcasses) was stored frozen at a temperature of -12 ± 0.5 °C for 12 months, the second portion was stored at -20 ± 0.5 °C for 14 months, and the third portion at -25 ± 0.5 °C for 18 months. At the beginning of the experiment (chilled beef), on day one, and at the end of the recommended shelf life of frozen beef, we collected samples and measured its microbiological and biochemical parameters.

To determine the number of psychrotrophic microorganisms, we inoculated 1 cm³ of the rinses from the halves or their tenfold dilutions in Petri dishes. The samples were covered with 15 cm³ of Mueller Hinton Agar (Farmaktyv, Kyiv, Ukraine) that had been melted and cooled down to 45 ± 5 °C. The inoculations were incubated at a temperature of $+7.0 \pm 0.5$ °C for 10 days. To determine the aerobic mesophilic microorganisms, the inoculations were incubated at a temperature of 30 ± 1 °C for 72 ± 1 h. The bacteria of the family Enterobacteriaceae were counted on an Endo medium, *Staphylococcus aureus* on a Baird-Parker agar, and *Enterococcus* on an *Enterococcus* Agar (Farmaktyv, Kyiv, Ukraine). The isolated cultures were identified according to their morphological, tinctorial, cultural, and biochemical properties using the generally accepted methods, and also using the tests for biochemical identification, namely the Staphy-test for *Staphylococcus* and the EN-COCCUS test (Pliva-lachema, Brno, Czech Republic) for *Enterococcus*.

The chemical parameters that characterize the freshness of chilled and frozen meat (as a reaction with copper sulfate, for ammonia and for peroxidase) were determined using classical methods according to Pan et al. (2025). The amounts of volatile fatty acids were determined using the method based on isolation of volatile fatty acids that accumulate in meat over the storage period and measuring their quantities by titrating of the obtained distillate using solution of caustic potash (caustic soda). According to the content of volatile fatty acids, meat was considered fresh at 4.0 mg of KOH; doubtfully fresh at KOH ranging from 4.1 to 9.0 mg of; and not fresh at a KOH of over 9.1 mg (Kukhtyn et al., 2020a).

The content of amino-ammonia nitrogen was determined using the method based on the ability of water-soluble nitrogen-containing nonprotein meat compounds (amines, ammonium salts, amino acids, etc.) to be neutralized by a base, which results in a staining in the presence of an indicator. At the same time, fresh meat was considered to contain up to 1.26 mg of amino-ammonia nitrogen, meat of doubtful freshness from 1.27 to 1.68 mg, and not fresh meat over 1.68 mg. The content of lipid peroxidation products in the beef was measured using the generally accepted methods, such as the concentration of TBA-active products in homogenates of the tissues. To settle proteins, we added 4.5 mL of 20% phosphotungstic acid to 1 mL of the tissue homogenate and centrifuged the samples. The supernatant was removed and to the sediment we added 1.0 mL of 0.8 solution of thio-barbituric acid (TBA) and kept it for 1 h on a water bath at a tempera-

ture of 100 °C. Then, the test tubes were cooled and centrifuged. In the obtained centrifugate, we measured the absorption at 535 and 580 nm against the control sample, which instead of homogenate contained double-distilled water. Two-time measurement of absorption allowed excluding the absorption of complexes stained with TBA compounds of nonlipid nature. The concentration of TBA-active products (C) was measured by adding a constant of 0.21 to the product of the coefficient 26.5 and the difference of optical densities at the wave lengths of 535 and 580 nm ($\Delta D = D_{535} - D_{580}$). Diene conjugates (DC) in meat were determined spectrophotometrically (Kukhtyn et al., 2020b).

The statistical analysis of the data was conducted using ANOVA. The results are given in the form of $x \pm SD$ (mean value \pm standard deviation). The significance of the yielded results was evaluated at a significance level of $P < 0.05$ taking into account the Bonferroni's Correction.

Results

The characteristic of the quantitative changes in the microflora of frozen beef over the 8-month storage period at -12 °C is given in Table 1. The data analysis indicates that during the 8-month storage of beef at a temperature of -12 °C, there occurred a quantitative decrease in most genera of microorganisms on its surface, except for fungi and yeasts. In particular, the number of mesophilic bacteria declined on average tenfold ($P < 0.001$), the number of psychrotrophic microflora and *Staphylococcus aureus* decreased by 1.65 and 2.34 times ($P < 0.05$) respectively, and the number of *Enterococcus* declined by 8.31 times ($P < 0.05$). The bacteria most sensitive to this temperature were those of the family Enterobacteriaceae: Their level declined by over 10 times, accounting for 1.25 log CFU/cm² of the carcass area. At the same time, this temperature of meat freezing had less effects on the death rate of fungi and yeasts, because their number over the storage period increased by 1.86 times ($P < 0.05$), which is likely explained by the ability of mold fungi to maintain their physiological activity at low temperatures.

Table 1
Microbiological parameters of frozen beef stored at -12 °C for 8 months ($x \pm SD$, $n = 6$)

Microorganisms	Day 1, log CFU/cm ²	After 8 months of storage, log CFU/cm ²
Mesophilic aerobic microorganisms	4.90 ± 3.52	$3.88 \pm 2.70^*$
Psychrotrophic	3.78 ± 2.62	$3.57 \pm 2.32^*$
Fungi and yeasts	3.11 ± 1.76	3.38 ± 2.20
Enterobacteriaceae	2.49 ± 1.23	$1.25 \pm 0.11^*$
<i>S. aureus</i>	2.07 ± 0.84	$1.70 \pm 0.19^*$
<i>E. faecalis</i>	3.17 ± 2.03	$2.25 \pm 0.05^*$

Note: * indicate a statistically significant difference between day 1 and 8 months of storage ($P < 0.05$, ANOVA with the Bonferroni's Correction).

Therefore, a temperature of -12 °C slows down the death of psychrotrophic microorganisms and promotes increase in the fungal microflora. This indicates the necessity of controlling their initial level during beef storage under such regimes.

The analysis of microbiological parameters of beef stored at a temperature of -20 °C for 14 months revealed a greater decrease in the microbial contamination on the meat surface (Table 2).

Table 2
Microbiological parameters of frozen beef stored at -20 °C for 14 months ($x \pm SD$, $n = 6$)

Microorganisms	Day 1, log CFU/cm ²	After 14 months of storage, log CFU/cm ²
Mesophilic aerobic microorganisms	4.90 ± 3.52	$3.73 \pm 2.51^*$
Psychrotrophic	3.78 ± 2.62	$3.51 \pm 2.27^*$
Fungi and yeasts	3.11 ± 1.76	$3.07 \pm 1.89^*$
Enterobacteriaceae	2.49 ± 1.23	$1.20 \pm 0.07^*$
<i>S. aureus</i>	2.07 ± 0.84	$1.61 \pm 0.36^*$
<i>E. faecalis</i>	3.17 ± 2.03	$2.20 \pm 0.92^*$

Note: see Table 1.

In particular, the most noticeable decline in the numbers of bacteria was observed for the Enterobacteriaceae, measuring on average 20 times, from 2.49 to 1.20 log CFU/cm². The number of mesophilic microorganisms on the halves' surface declined by over tenfold, from 4.9 to 3.73 log CFU/cm² ($P < 0.05$). In addition, a significant decline was noted for the psychrotrophic microflora, measuring 1.90 times ($P < 0.05$), whereas the numbers of Staphylococcus and Enterococcus decreased by 2.88 and 9.33 times, respectively. At the same time, the amounts of fungi and yeasts remained practically unaltered, indicating the absence of their reproduction at such temperature.

Therefore, frozen storage of beef at $-20\text{ }^{\circ}\text{C}$ for 14 months is a more effective method of preservation than at $-12\text{ }^{\circ}\text{C}$ for 8 months, providing a greater microbiological safety of meat for a long time.

In the technology of frozen storage of meat, an even lower temperature regime is used: $-25\text{ }^{\circ}\text{C}$. The characteristic of the changes in the microbiota in beef stored at this temperature for 18 months is given in Table 3.

Table 3
Microbiological parameters of beef
frozen at $-25\text{ }^{\circ}\text{C}$ for 18 months ($\bar{x} \pm \text{SD}$, $n = 6$)

Microorganisms	Day 1 (log CFU/cm ²)	After 18 months of storage (log CFU/cm ²)
Mesophilic aerobic microorganisms	4.90 ± 3.52	3.49 ± 2.29*
Psychrotrophic	3.78 ± 2.62	3.38 ± 2.20*
Fungi and yeasts	3.11 ± 1.76	2.92 ± 1.75*
Enterobacteriaceae	2.49 ± 1.23	1.30 ± 0.14*
<i>S. aureus</i>	2.07 ± 0.84	1.05 ± 0.04*
<i>E. faecalis</i>	3.17 ± 2.03	2.04 ± 0.85*

Note: see Table 1.

During meat storage, we found (Table 3) that the dynamics of changes in the microbiological parameters at $-25\text{ }^{\circ}\text{C}$ practically did not differ from the results obtained at $-20\text{ }^{\circ}\text{C}$ for 14 months. This indicates that for a long-term (over 12 months) storage of beef, it is suffice to maintain a temperature of $-20\text{ }^{\circ}\text{C}$. Such an approach allows reducing the expenditures of electricity necessary for freezing to $-25\text{ }^{\circ}\text{C}$. Moreover, meat frozen at $-25\text{ }^{\circ}\text{C}$ thaws slower than that stored at $-20\text{ }^{\circ}\text{C}$, which complicates the technological process even more.

Therefore, the comparative analysis of the results of microbiological studies revealed that storing beef at $-12\text{ }^{\circ}\text{C}$ for 8 months provided only a partial inhibition of the microflora: Psychrotrophic bacteria remained vital, while fungi and yeasts even grew in numbers. Freezing at $-20\text{ }^{\circ}\text{C}$ for 14 months was far more effective, decreasing the number of most groups of microorganisms (especially Enterobacteriaceae) to minimal levels, and suppressing the reproduction of fungi and yeasts. Storage at $-25\text{ }^{\circ}\text{C}$ for 18 months demonstrated almost identical results to the regime of $-20\text{ }^{\circ}\text{C}$ for 14 months, and therefore from this perspective, support of the temperature of $-20\text{ }^{\circ}\text{C}$ is optimal for long-term storage, e.g. for over a year. This allowed providing microbiological safety of the products and reducing the energy expenditures, avoiding additional freezing to $-25\text{ }^{\circ}\text{C}$. Furthermore, during the regime of storing beef at $-12\text{ }^{\circ}\text{C}$, it is necessary to provide a low level of contamination with fungal microflora, because under such conditions it can affect the microbiological safety of meat.

To characterize the changes occurring in beef subject to microbial and native enzymes over the duration of frozen storage, we used biochemical parameters. The results of the studies revealed that long-term storage of beef at different freezing temperatures affects its chemical reactions and pH. However, the most significant changes occurred only when storing meat at $-12\text{ }^{\circ}\text{C}$. In particular, under this regime of freezing, there were observed qualitative changes in meat according to reactions with copper sulfate, for peroxidase and ammonia, which characterize beef as doubtfully fresh. This indicates a slight formation of protein products and an inhibition of enzymatic activity of peroxidases. In addition, we found a slight increase in pH from 5.83 ± 0.03 to 6.05 ± 0.03 under this storage regime, which suggests an accumulation of products of protein breakdown and a change in the acid-base homeostasis, but the fluctuations were insignificant, pointing to the preservation of a relatively stable meat quality.

The assessment of the freezing regime of beef at -20 and $-25\text{ }^{\circ}\text{C}$ exhibited stable parameters with copper sulfate and for ammonia, compared with chilled meat. At the same time, according to the reaction for peroxidase, the beef was considered doubtfully fresh.

When determining amino-ammonium nitrogen and volatile fatty acids in frozen beef stored at different temperatures (Table 4), we also observed intensive changes at a higher ($-12\text{ }^{\circ}\text{C}$) temperature, compared with the lower temperatures ($-20\text{ }^{\circ}\text{C}$ and $-25\text{ }^{\circ}\text{C}$). In particular, during storage at $-12\text{ }^{\circ}\text{C}$ for 8 months, the amount of amino-ammonium nitrogen increased 1.38-fold ($P < 0.05$), compared with its content in fresh chilled meat, reaching 1.52 ± 0.03 mg. Such a level indicates the activation of processes of protein breakdown, and beef with such a parameter is classified as doubtfully fresh. The content of volatile fatty acids during the experiment increased 4.63-fold ($P < 0.05$), reaching 12.51 ± 0.23 mg of KOH. This indicates a course of fat hydrolysis, and at such a concentration beef is also considered doubtfully fresh.

Table 4
Contents of amino-ammonium nitrogen and volatile fatty acids
in frozen beef stored at different temperatures ($\bar{x} \pm \text{SD}$, $n = 6$)

Duration and storage regime	Amino-ammonium nitrogen, mg	Volatile fatty acids, mg
1 day	1.10 ± 0.02 ^a	2.76 ± 0.15 ^a
8 months at $-12\text{ }^{\circ}\text{C}$	1.52 ± 0.03 ^b	12.51 ± 0.23 ^b
14 months at $-20\text{ }^{\circ}\text{C}$	1.28 ± 0.02 ^c	6.43 ± 0.18 ^c
18 months at $-25\text{ }^{\circ}\text{C}$	1.25 ± 0.02 ^c	6.35 ± 0.19 ^c

Note: see Table 1.

Storing beef under the regimes of -20 and $-25\text{ }^{\circ}\text{C}$ for 14 and 18 months, respectively, significantly inhibited the biochemical changes in it. Because the content of amino-ammonium nitrogen insignificantly increased to 1.28 ± 0.02 and 1.25 ± 0.02 mg of nitrogen, respectively, such meat was still considered fresh. Although the content of fatty acids increased 2.37-fold ($P < 0.05$) under those regimes, compared with chilled meat, such amount is considered insufficient to cause meat imperfections.

In general, the study indicates that deep freezing (-20 and $-25\text{ }^{\circ}\text{C}$) preserves the quality of beef more effectively, slowing the development of unfavorable biochemical processes and preventing the signs of intensive breakdown of proteins.

The results of the studies of changes in the lipid-peroxidation products in beef over the periods of frozen storage at different temperatures are given in Table 5. We observed (Table 5) that an increase in the temperature of storage of frozen meat intensifies the processes of lipid peroxidation. In particular, when storing beef at $-12\text{ }^{\circ}\text{C}$, the content of TBA-active products elevated from 3.91 ± 0.09 to 4.42 ± 0.11 μmol/g. Meanwhile, at the temperatures of -20 and $-25\text{ }^{\circ}\text{C}$, this parameter remained almost unchanged, accounting for 4.03 ± 0.07 and 4.09 ± 0.08 μmol/g, respectively.

Table 5
Content of products of lipid peroxidation in frozen beef
stored at different temperatures ($\bar{x} \pm \text{SD}$, $n = 6$)

Duration and regime of storage	Content of TBA-active products, μmol/g	Content of diene conjugates, μmol/g
1 day	3.91 ± 0.09 ^a	16.45 ± 0.06 ^a
8 months at $-12\text{ }^{\circ}\text{C}$	4.42 ± 0.11 ^b	20.92 ± 0.08 ^b
14 months at $-20\text{ }^{\circ}\text{C}$	4.03 ± 0.07 ^a	18.85 ± 0.07 ^c
18 months at $-25\text{ }^{\circ}\text{C}$	4.09 ± 0.08 ^a	19.06 ± 0.07 ^c

Note: see Table 1.

A similar dynamic was recorded for the content of diene conjugates: Over the period of 8-month storage at $-12\text{ }^{\circ}\text{C}$, their amount in beef increased 1.27-fold ($P < 0.05$), whereas no significant changes occurred in the meat kept at $-20\text{ }^{\circ}\text{C}$ and $-25\text{ }^{\circ}\text{C}$. Perhaps, the increase in the level of lipid-peroxidation products at $-12\text{ }^{\circ}\text{C}$ was due to the lipolytic activity of psychrotrophic fungal microflora, which is still able to develop under such conditions. Therefore, deep freezing of beef (below $-20\text{ }^{\circ}\text{C}$) inhibits the oxidation processes more effectively,

providing a better stability of lipid fraction of meat over long-term storage periods.

Discussion

In complex, the obtained results confirm that the temperature and duration of storage of frozen beef have a decisive effect on the microbiological safety, biochemical stability, and physical-chemical characteristics of the products. The comparison of three studied regimes (at $-12\text{ }^{\circ}\text{C}$ for 8 months, $-20\text{ }^{\circ}\text{C}$ for 14 months, and $-25\text{ }^{\circ}\text{C}$ for 18 months) allowed us not only to evaluate the intensity of changes in meat but also to draw conclusions regarding their practicality under industrial conditions.

The analysis of the dynamics of the number of microorganisms confirmed the established pattern: Decrease in the storage temperature leads to slowing or complete cessation of the growth of microflora (Johansson et al., 2020; Rovira et al., 2023), but the effectiveness of this process depends on the initial level of contamination and group composition of the microbiota (Liang et al., 2021; Kukhtyn et al., 2022).

At $-12\text{ }^{\circ}\text{C}$, a large portion of psychrotrophic microflora remained vital, in particular mold fungi, the number of which over the storage period has even increased. Such a pattern is consistent with other reports (Zhang et al., 2018; Xue et al., 2021; Mohamed et al., 2023; Alves Rodrigues et al., 2025) stating that the fungal microflora is able to support the minimal metabolic activity at temperatures much lower than the water freezing point. This fact has an important practical significance in cases of hygiene negligence at initial stages of slaughter and initial processing of meat, when the risk of development of fungal cells during storage at $-12\text{ }^{\circ}\text{C}$ increases considerably.

At the same time, at -20 and $-25\text{ }^{\circ}\text{C}$, the number of Enterobacteriaceae, mesophilic aerobic microorganisms, and staphylococci decreased to the levels that do not exceed the allowable threshold normative of the EU Regulation 2073/2005. Notably, the difference in the microbiological stability between those two regimes was minimal, which aligns with the studies by Kumar et al. (2020), who reported that reducing temperature below $-20\text{ }^{\circ}\text{C}$ provides no significant additional effect in inactivating most pathogens.

Therefore, from the perspective of control of microbial contamination, the optimal temperature is $-20\text{ }^{\circ}\text{C}$, which provides a high level of safety with lower energy expenditures compared with the $-25\text{ }^{\circ}\text{C}$ regime. The results of the analysis of biochemical parameters demonstrate a certain activity of native enzymes and residual microflora even in deep-frozen meat. This manifests in a gradual accumulation of amino-ammonium nitrogen and volatile fatty acids.

At $-12\text{ }^{\circ}\text{C}$, protein breakdown and lipolysis were more intensive, as evidenced by the increases in pH to 6.05 and in the concentration of volatile fatty acids by 4.63 times, compared with fresh meat. From the technological perspective, such parameters indicate the formation of features of 'doubtful freshness', as confirmed by the reactions to copper sulfate, peroxidase, and ammonium. This correlates with the data from Jung et al. (2025) that at higher freezing temperatures, the enzymatic processes slow down insufficiently, leading to degradation of proteins and fats.

In case of using the temperatures of -20 and $-25\text{ }^{\circ}\text{C}$, the changes in amino-ammonium nitrogen and volatile fatty acids were insignificant, giving reasons for classifying meat as 'fresh'. This is explained by a rapid decline in the activity of proteases and lipases at low temperatures (Feng et al., 2020; Motaghifar et al., 2020) and the absence of development of psychrotrophic microflora (Zhang et al., 2019; Kukhtyn et al., 2020a; Yuan et al., 2024; Aydogdu, 2025).

Therefore, our study provides in-detail insights showing that the biochemical parameters of beef stored at $-12\text{ }^{\circ}\text{C}$ have significantly deteriorated at the end of the 8-month period, mostly characterizing its freshness as doubtful.

Studying the processes of lipid peroxidation revealed that an increase in the storage temperature intensifies the formation of primary and secondary oxidation products. At $-12\text{ }^{\circ}\text{C}$, there was registered an increase in the level of TBA-active products and diene conjugates, which negatively affect the taste characteristics and nutritional value

of beef by destroying polysaturated fatty acids. At -20 and $-25\text{ }^{\circ}\text{C}$, such changes were statistically insignificant, indicating an effective inhibition of the oxidation processes. Al-Dalali et al. (2021) observed an increase in the level of TBA-active products in marinated and frozen beef during storage. Furthermore, the concentrations of saturated and monounsaturated fatty acids were observed to decrease in all samples after storage, except for the control (fresh meat). At the same time, the correlation analysis revealed positive relationships between the amounts of volatile aldehydes and alcohols with fatty acids and taste perception and sensory properties. This indicates that insufficient freezing of meat can affect its biological value and taste properties. Similarly, the studies by Taha et al. (2015), Dragoev (2024), and Shui et al. (2025) revealed that freezing leads to a time-dependant increase in the accumulation of lipid-peroxidation products, malondialdehyde. At the same time, there was found cholesterol oxidation and disappearance of omega-3 fatty acids, eicosapentaenoic acid during the freezing process.

It is important to note that lipid fraction is a sensitive indicator of meat stability, because even insignificant fluctuations in the storage temperature can enhance autoxidation of fats, as confirmed by both our data and foreign studies (Domínguez et al., 2019; Li et al., 2020; Hu et al., 2025). At the same time, Ge et al. (2021) reported that the consumption of pork with oxidated products caused impaired metabolism in mice, disrupting the secretion of insulin by the pancreas and enhancing the glucose production. The authors note that protein oxidation plays a key role in the disruptions of glucose metabolism caused by poor-quality meat.

Therefore, the comparison of the studied regimes gives reasons to make the following practical generalizations.

A temperature of $-12\text{ }^{\circ}\text{C}$ can only be used for short-term storage (up to 4-6 months), given low initial contamination and thorough control of fungal microflora. A temperature of $-20\text{ }^{\circ}\text{C}$ strikes an optimal balance between microbiological safety and preservation of sensory properties on one hand and energy expenditures for storage for over 12 months on the other hand. A temperature of $-25\text{ }^{\circ}\text{C}$ does not give a significant advantage in the product quality compared with $-20\text{ }^{\circ}\text{C}$, but requires more electricity and can complicate the technological process of thawing.

The yielded results are in line with the data of modern studies (Egelandstad et al., 2019; Lu et al., 2022; Pan et al., 2025) and underline the significance of scientifically substantiated choice of freezing regime. In the future, combining optimal temperature regimes and additional methods of preserving quality (such as shock freezing, modified gas atmosphere, or the use of natural antioxidants) may allow expanding the shelf life with no loss of nutritional and biological values.

Conclusions

Temperature and duration of storage have a decisive effect on the microbiological safety, biochemical stability, and physical-chemical properties of frozen beef. Storage at $-12\text{ }^{\circ}\text{C}$ for 8 months provided only a partial inhibition of microflora: Psychrotrophic bacteria remained vital, while the number of fungi and yeasts increased 1.86-fold. At the same time, there was observed an intensive accumulation of amino-ammonium nitrogen and volatile fatty acids, an increase in pH, and an elevated level of lipid-peroxidation products, indicating the formation of features of 'doubtful freshness'.

The storage regime of $-20\text{ }^{\circ}\text{C}$ for 14 months proved to be optimal for long-term storage of beef: The number of microorganisms, including Enterobacteriaceae, decreased to minimal levels, fungi and yeasts did not reproduce, while the biochemical changes remained insignificant. According to the parameters of microbiological safety and biochemical stability, deep freezing at $-25\text{ }^{\circ}\text{C}$ for 18 months gave no significant advantage over the $-20\text{ }^{\circ}\text{C}$ storage, but required greater energy expenditures and meant slower thawing, which must be accounted for in the industrial practice.

Therefore, to prevent the risk of deterioration of beef quality while storing at $-12\text{ }^{\circ}\text{C}$, it is expedient to provide a minimal initial level of contamination and, if possible, combine freezing with other

methods of preservation, particularly shock freezing, modified atmosphere, or the use of natural antioxidants.

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