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EVALUATION OF STORAGE METHODS OF BEEF BY MICROBIOLOGICAL AND CHEMICAL INDICATORS

Mykola Kukhtyn, Volodymyr Salata, Oleksandra Berhilevych, Zoya Malimon, Anatoliy Tsvihun, Bogdan Gutyj, Yulia Horiuk

ABSTRACT
Meat and meat products are a major part of a person's ration. However, due to their high nutritional value, they are a favorable environment for the development of microorganisms and require refrigerated storage. The purpose of this work was to evaluate the storage methods for refrigerated and frozen beef by microbiological and chemical parameters and to suggest criteria for evaluating beef by the content of psychrotrophic microorganisms. It was found out that the storage of beef meat with an initial mesophilic bacterial content of about 4.88 log CFU.cm^{-2} of surface and psychrotrophic bacteria 3.79 log CFU.cm^{-2} at temperature 0 °C is only possible for 8 days, further, the microbiological indices exceed the acceptable standards. Investigation of the dynamics of microflora reproduction during the storage of beef in the frozen state at temperature -2 to -3 °C for 20 days established a decrease in 1.3 times the number of mesophilic bacteria in 10 days of storage. At the same time, the number of psychrotrophic microorganisms during this storage time was increased in 4.5 times, and 20 days in 7.9 times and amounted to 5.3 log CFU.cm^{-2} of surface area. This indicates that the storage of meat in the frozen state inhibits or completely stops the development of mesophilic microorganisms for 20 days. It was found out that storing of beef in the cooled state at a temperature of 0 ±0.5 °C for more than eight days is impractical, as its biochemical indices are worsening and signs of spoilage are appearing. At the same time, storing of beef in the frozen state at a temperature of -2 to -3 °C for 20 days does not cause such significant biochemical changes as in beef stored in the cooled state at a temperature of 0 ±0.5 °C for 16 days. Therefore, we have experimentally substantiated the quantitative indicators of the content of psychrotrophic microorganisms on the surface of beef intended for storage in a cooled or frozen state. The proposed microbiological criteria will improve the safety of beef.

Keywords: psychrotrophic microflora; microbiological criteria; beef chilled; beef frozen; volatile fatty acids of meat

INTRODUCTION
Meat and meat products are a significant part of a person's ration as they are a source of complete proteins. Due to its high nutritional value, meat is a favorable environment for the development of microorganisms **(Alonso-Hernando, Alonso-Calleja and Capita, 2013; Dave and Ghaly, 2011; Gunvig, Hansen and Borggaard, 2013; Lerma et al., 2015)**. Therefore, during its storage, short-term (cooling), long-term (freezing), or long-term (freezing), different temperature regimes are used to stop microbiological and physico-chemical processes **(Bruckner et al., 2012; Jeremiah, 1997; Pennacchia, Ercolini and Villani, 2011; Pothakos et al., 2014)**. According to the definition of the British Institute of Research in the field of food technology (UK Institute of Food Science and Technology, IFST) the storage date of the food is the time during which the product remains safe, ie, meets all the proper organoleptic, chemical, physical, microbiological properties, as well as food and nutritional requirements.

The beef and half carcasses of the beef are kept cool at 0 to -1 °C at 85% relative humidity for 16 days. Freezing involves storing of beef meat at -2 to -3 °C at 90% relative air humidity for 20 days, and freezing at -12, -18, -20, -25 °C at 95% relative humidity for 8 months, 12 months, 14 months. and 18 months, respectively standard DSTU 6030:2008 **(DSTU, 2009)**.

In the **Commission Regulation (EC) No. 2073/2005** and DSTU 6030:2008 **(DSTU, 2009)** of beef and veal in carcasses, half-carcasses and quarters specify the parameters and terms of refrigerated storage of beef and veal, microbiological standards for the safety of meat in excess of which indicate the need to improve the hygiene of slaughtering and review of measures to control the technological process. However, even within the standard temperatures of refrigerated storage of meat, there is different intensity of reproduction for certain groups of microflora **(Bruckner et al., 2012; Casaburi et al., 2014; Cerveny, Meyer and Hall, 2009; Zhang et al., 2019)**.

Therefore, even if the microbiological parameters meet the standard requirements before cooling, freezing, or chilling the meat, they may well exceed these standards at the end of the storage period.

Normally, after animals slaughtering, micro-organisms should be detected only on the surface of the carcasses, this is due to exogenous contamination and meets the sanitary and technological requirements **(Bruckner et al., 2012; Kameník, 2013; Salata et al., 2017; Serraino et al., 2012)**. According to **Commission Regulation (EC) No. 2073/2005**, the number of colonies of aerobic mesophilic micro-organisms on cattle carcasses before cooling should be from log 3.5 CFU.cm^{-2} to log 5.0 CFU.cm^{-2} area, and the content of bacteria of *Enterobacteriaceae* ranges from log 1.5 CFU.cm^{-2} to log 2.5 CFU.cm^{-2}.

Scientific publications and regulatory documents pay more attention to the contamination of beef carcasses, mainly mesophilic aerobic facultative anaerobic microorganisms and bacteria of the genus *Enterobacteriaceae*, which are indicators of compliance with sanitation during the slaughter of animals **(Cantalejo, Zouaghi and Pérez-Arnedo, 2016; Commission Regulation (EC) No. 2073/2005; Leroy et al., 2009; Nyamakwere et al., 2016)**.

It is believed that microbiological changes in meat occur due to the reproduction of psychrotrophic microflora when stored beef in a cooled and frozen state. **(Ercolini et al., 2009; Nieminen et al., 2011; Pothakos, Samapundo and Devlieghere, 2012; Pothakos et al., 2014; Stellato et al., 2017)**.

However, in recent years there has been a tendency to increase consumption and use as raw material for the frosted meat food industry compared to frozen **(Kukhtyn et al., 2020; Wei et al., 2019; Zhang et al., 2019)**. In this regard, technologies are being advanced that aim to extend the term of storage of frosted meat without altering organoleptic, physico-chemical and microbiological parameters **(Hilgarth, Behr and Vogel, 2018; Adam, Flint and Brightwell, 2010; Kukhtyn et al., 2019; Moschonas et al., 2011; Robertson et al., 2013)**. However, the term of storage of any product cannot be implemented in the production technology without a comprehensive scientific justification for all parameters that influence safety.

Therefore, a properly selected storage regime should ensure the maximum term of storage of the food product without disturbing its organoleptic, physico-chemical, and microbiological characteristics. With this in mind, it is relevant to study the content of psychrotrophic microflora during refrigeration storage of beef.

The purpose of this work was to evaluate the storage methods for refrigerated and frozen beef by microbiological and chemical parameters and to suggest criteria for evaluating beef by the content of psychrotrophic microorganisms.

Scientific hypothesis

It is possible to use the psychrotrophic group of microflora to evaluate the hygiene of the technological process of cattle slaughtering and beef processing and the suitability of meat for storage in a cooled and frozen state.

MATERIAL AND METHODOLOGY

A sampling of beef and carcass washes was carried out at meat processing enterprises of Lviv and Ternopil region, preparation for the investigation was performed according to ISO 6887-1:2017 and ISO 6887-2:2017 **(ISO, 2017a; ISO, 2017b)**. One part of the beef (half-carcass) was stored in a refrigerated state at 0 ±0.5 °C for 16 days and the second in the frozen state at 2 – 3 °C for 20 days. At the beginning of the experiment (cooled beef) and in 8, 16 days of storage in the cooled state and 10 and 20 days of storage in the frozen state, samples were taken and microbiological and biochemical parameters were determined.

Microbiological investigations were carried out in the laboratory of the Stepan Gzhytskyj Lviv National University of Veterinary Medicine and Biotechnologies. To determine the number of psychrotrophic microorganisms was sown 1 cm^3 of flushing or its ten-fold dilutions in Petri dishes, poured 15 cm^3 of molten and cooled to 45 ±5 °C MPA, incubation of crops was carried out at a temperature of +7.0 ±0.5 ºC for 10 days, and to determine aerobic mesophilic microorganisms, the crops were incubated at 30 ±1 °C for 72 ±1 h. The identification of pure cultures was performed according to the morphological, tinctorial, cultural, and biochemical properties, which are described in the Bergey bacteria determinant **(Vos et al., 2011)**. Tests were also used for the biochemical identification of non-fermenting microorganisms "Neferm test-24" (Rliva-lachema, Czech Republic).

The amount of volatile fatty acids was determined by a method based on the isolation of volatile fatty acids, which are accumulated in the meat during storage and determination by the titration amount of the distillate obtained with a solution of caustic potassium (or caustic soda). Herewith meat was considered fresh in terms of volatile fatty acids 4.0 mg KOH; – doubtful freshness – from 4.1 to 9.0 mg KOH; stale more than 9.1 mg KOH. The content of lipid peroxidation products in beef meat was determined by conventional methods, so the concentration of TBK-active products in tissue homogenates was determined by the method of Korobeinikova, 1989. To precipitate proteins to 1 mL of tissue homogenate was added 4.5 mL of 20% phosphoric acid and the samples were centrifuged. The supernatant was drained and 1.0 mL of 0.8 th thiobarbituric acid (TBK) solution was added to the precipitate and was kept for 1 h in a water bath at 100 °C. The tubes were then cooled and centrifuged. In the obtained centrifuge, the absorbance was measured at 535 and 580 nm against a control sample that contained bidistilled water instead of the homogenate. Double absorption measurement eliminates the absorption of colored complexes of thiobarbituric acid by substances of non-lipid nature. The content of TBK-active products was calculated by regression equation:

$$C = 0.21 + 26.5\Delta D$$

where C is the concentration of TBK-active products; ΔD – indicator D535 – D580 in the centrifuge. Diene conjugates (DCs) in the meat were determined spectrophotometrically.

Statistical analysis

Statistical processing of the results was carried out using methods of variation statistics using the program Statistica

9.0 (StatSoft Inc., USA). Non-parametric methods of research were used (Wilcoxon-Mann-Whitney test). The arithmetic mean (x) and the standard error of the mean (SE) were determined. The difference between the comparable values was considered to be significant for $p < 0.05$.

RESULTS AND DISCUSSION

The results of research on the dynamics of the microflora of beef meat cooled during storage are shown in Figure 1.

As can be seen from Figure 1, that in compliance with all veterinary and sanitary requirements for the procurement of beef meat in meat processing plants, the microbiological indices of the meat meet the established standards of the Regulation EU No 2073/2005 (permissible content of mesophilic micro-organisms up to 5 log CFU.cm^{-2} of carcass surface). In 8 days of storage at 0 °C, the total number of mesophilic microorganisms on the surface of half-carcasses was increased into 16.6 times ($p < 0.05$), and in 16 days – into 3 350 times ($p < 0.05$) and exceeded the allowed level following the regulations by 1.3 times and 258 times, respectively. It can also be noted that the content of psychrotrophic microorganisms was increased into 350 times ($p < 0.05$) in 8 days of meat storage and 52 thousand times ($p < 0.05$) in 16 days. If you compare the content of psychrotrophic microorganisms with the number of mesophilic during the process of meat storage, you can find the following. Psychrotrophic microorganisms on the surface of the chilled meat are 12.4 times smaller than mesophilic, but due to the faster rates of reproduction at this temperature, their number in the eighth day of storage was already 1.7 times ($p < 0.05$) higher. Psychotrophic microorganisms of chilled meat during the storage process have been the main dominant microflora, and this indicates its major role in the occurrence of microbiological defects in meat.

In Figure 2 results are given due to the microbiological investigations on the dynamics of microflora during the storage of beef in the frozen state at temperatures of -2 to -3 °C for 20 days.

As can be seen from Figure 2, that the content of mesophilic microorganisms beef meets the established requirements for 20 days of storage at temperatures of -2 to -3 °C. A decrease of 1.3 times ($p < 0.05$) in the number of mesophilic bacteria were detected after 10 days of storage, and after 20 days of their content remained practically unchanged. This does not indicate that the storage of meat in the frozen state inhibits or completely stops the development of mesophilic microorganisms for 20 days. At the same time, psychrotrophs, which can withstand low ambient temperatures, under these conditions increased their number on the surface of the beef in 10 days of storage into 4.5 times ($p < 0.05$). During the next 10 days of storage, that is, for 20 days, their number was increased into 7.9 times ($p < 0.05$) and amounted to 5.3 log CFU.cm^{-2} of surface area.

To fully characterize the microbiological changes in frozen meat, we determined the generic composition of psychrotrophic microflora, which is dominant in the storage process of beef at low temperatures.

In Figure 3 the composition of the psychrotrophic microflora of chilled beef is shown.

The identification of psychrotrophic microflora revealed that most of the cooled meat were bacteria of the genus *Acinetobacter* spp. – 55.1 ±2.2% and the smallest 14.6 ±0.7% of *Pseudomonas* spp. At the same time, an increase in the number of detected bacteria in the composition of psychrotrophic microflora of chilled beef was detected after 16 days of storage. Among the already identified three genera from the beef are bacteria of the genus *Flavobacterium* spp. and *Aeromonas* spp., which were not identified in the cooled meat, their number was 1.7 ±0.2% and 1.4 ±0.1%, respectively. This indicates the development of these bacteria during the storage of the beef in a chilled state. It is also seen that bacteria of the genus *Acinetobacter* spp. represent almost half of all psychrotrophic microflora of the cooled and chilled meat after 16 days of storage – 55.1 ±2.2 and 42.4 ±1.7% respectively. Bacteria of the genus *Alcaligenes* spp. occupy a stable niche of microflora, both cold and frozen meat – from 30.3 to 26.2%. However, bacterial growth of the genus *Pseudomonas* spp. Into 1.9 times ($p < 0.05$) on the surface of chilled beef, compared to cooled was observed.

Identification of the composition of psychrotrophic microflora of frozen beef after twenty days of storage (Figure 4) revealed an increase in bacteria of the genus *Pseudomonas* spp. into 1.3 times. At the same time, bacteria of the genus *Alcaligenes* spp. were consistently high in both cold and frozen meat – 30.3 – 31.7% respectively. Half of all psychrotrophic microflora accounted for bacteria of the genus *Acinetobacter* spp. 55.1 – 48.6%.

The research of the chemical indices of refrigerated and frozen beef during storage is shown in Figure 5.

As can be seen from the data of Figure 5, that the beef, which was stored in chilled state for 8 days in the content of volatile fatty acids, was of dubious freshness, indicating the beginning of the fat hydrolysis process. After 16 days of chilled beef storing at 0 ±0.5 °C, a certain indicator indicates the deterioration of the meat. In particular, the number of volatile fatty acids was increased into 5.1 times ($p < 0.05$), indicating that the course of intensive biochemical processes of enzymatic hydrolysis of fat and beef with such indicators is characterized as not fresh.

Thus, researches indicate that storing beef in chilled state at 0 ±0.5 °C for more than eight days is impractical, as its chemical characteristics are getting worse and there are signs of spoilage.

Research of beef stored in a frozen state at the temperature -2 to -3 °C revealed that, after 10 days, the number of volatile fatty acids remained at the level characteristic of fresh meat. In 20 days of meat storage, an increase in the amount of volatile fatty acids was found to be 2.3 times ($p < 0.05$). According to these indicators, meat is characterized as doubtful freshness.

Therefore, storage of beef in the frozen state at a temperature of 2 to -3 °C for 20 days does not cause such significant biochemical changes as in beef stored in the cooled state at a temperature of 0 ±0.5 °C for 16 days.

The next part of our research was to determine the content of lipid peroxidation (POL) products in chilled and frozen beef. It is well known **(Vega et al., 2009)** that the content of POL in meat is increasing with prolonged storage, which negatively affects its quality – smell, taste, structure. The results of researches on the content of TBK-active products (TBKAP) and diene conjugates (DCs) in chilled beef during storage are shown in Figure 6.

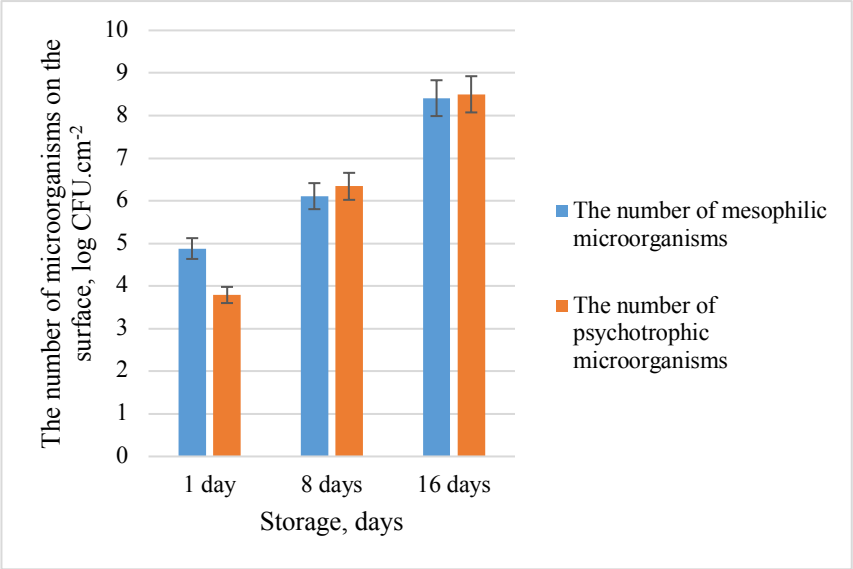

Figure 1 Changes of mesophilic and psychrotrophic microflora during storage of beef in a cooled state at a temperature of 0 ±0.5 °C.

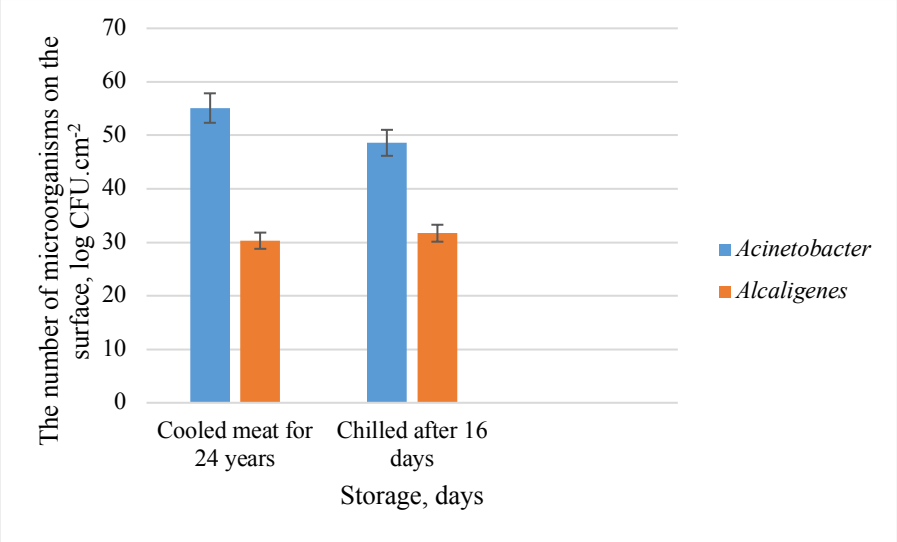

Figure 2 Changes of mesophilic and psychrotrophic microflora during storage of beef in the frozen state at temperatures -2 to -3 °C.

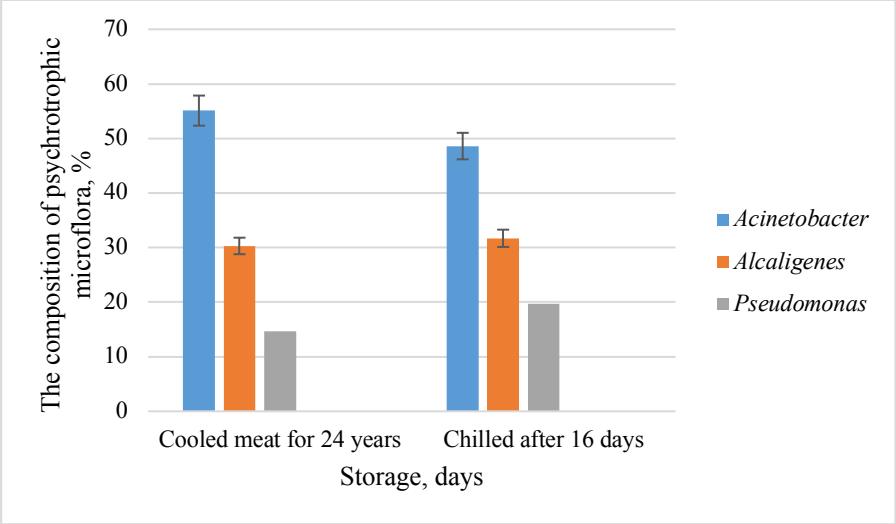

Figure 3 Generic composition of psychrotrophic microflora of beef during storage in a cooled state at a temperature of 0 ±0.5 °C.

As can be seen from Figure 6, that when the meat was kept refrigerated at 0 ±0.5 °C, no significant increase in the amount of TBKAP and DC on the eighth day was observed. On the 16th day of storage, there is a probable increase (p <0.05) in the number of TBKAP and DC compared to the first day.

When storing beef in the frozen state at a temperature of -2 to -3 °C, a probable increase into 1.3 times (p <0.05), compared to the first one, was noted only by the amount of DC for 20 days (Figure 7).

Thus, the obtained data are shown in Figure 6 and Figure 7 indicate that as the temperature of the refrigeration treatment of beef decreases, its resistance to oxidation increases, in particular the growth of TBKAP and DC.

The results indicate that the initial amount of microflora, especially the content of psychrotrophs, is crucial for the choice of beef storage temperature. Therefore, we are offered to evaluate the suitability of beef for storage in a cooled and frozen state according to the following hygiene criteria of the technological process (Table 1).

If a microbiological investigation of five beef samples before cooling from one batch reveals at least one sample of psychrotrophic microorganisms over 4 log CFU.cm^{-2} of area (≥M), then such beef is used immediately, and measures are taken to improve the hygiene of the technological process.

If three beef samples are detected, the number of psychrotrophic microorganisms from 3 log CFU.cm^{-2} area to 4 log CFU.cm^{-2}, (between m and M), then keep such batch in the cooled state at a temperature of 0 ±0.5 °C for not more than 8 days, or in the frozen state at a temperature of 2 to -3 °C for up to 20 days.

If a microbiological examination of five beef samples reveals a number of psychrotrophic microorganisms less than 3 log CFU.cm^{-2} of area (m), then such a batch is stored in chilled state at 0 ±0.5 °C for up to 16 days, or in the frosted state for 2 – 3 °C up to 20 days.

Thus, the proposed criteria for evaluating beef before storage allowed to scientifically justify the optimal cooling or freezing temperature to obtain, at the end of the storage period, meat with satisfactory organoleptic, physico-chemical and microbiological parameters.

The urgency of the issue of fresh meat and increasing the storage term is the primary purpose for meat industry professionals. Meat has a limited storage term, creating difficulties for both producers and consumers for whom a defective product is potentially dangerous **(Jeremiah, 1997; Nyamakwere et al., 2016)**. The biggest factor which causes spoilage of meat during its storage is microbiological **(Bruckner et al., 2012; Casaburi et al., 2014; Cerveny, Meyer and Hall, 2009; Pothakos, Samapundo and Devlieghere, 2012; Zhang et al., 2019)**. Therefore, first of all, it is necessary to minimize contamination by microorganisms from the moment of slaughter to processing and to inhibit the development of existing microflora through the use of refrigeration **(Bruckner et al., 2012; Dave and Ghaly, 2011; Kameník, 2013; Serraino et al., 2012)**. However, even during refrigerated storage (cooling, freezing) of beef meat, it is spoiled by the reproduction and activity of psychrotrophic microflora. **(Bruckner et al. 2012; Jeremiah, 1997; Pennacchia, Ercolini and Villani, 2011; Pothakos et al., 2014)**. Existing regulatory documents control the hygiene of the technological process of beef only by the content of mesophilic aerobic microorganisms and bacteria of the *Enterobacteriaceae* genus **(Commission Regulation (EC) No. 2073/2005)**.

Our investigations have found that the number of mesophilic microorganisms on fresh (24 h) beef carcasses was 12.4 times higher than the number of psychrotrophic microorganisms. During storage of the beef in chilled state at temperature 0 °C for 8 days, the number of aerobic mesophilic microorganisms was increased on the surfaces of the half-carcass by 16.6 times, and in 16 days – into 3 350 times and exceeded the admissible level (up to 5 log CFU.cm^{-2} of the carcass surface) into 1.3 times and 258 times respectively. At the same time, the psychrotrophic microflora during this period of storage increased 350 times in 8 days and in 52 thousand times in 16 days. That is, on the 8th day, the amount of psychrotrophic microflora already in 1.7 times outweighed the content of mesophilic microflora. In research **(Bruckner et al, 2012; Ercolini et al., 2009; Hassan et al., 2015; Pothakos, Samapundo and Devlieghere, 2012; Pothakos et al., 2014)** was also found a higher content of psychrotrophic microorganisms in chilled foods compared to the number of mesophilic aerobic bacteria. Researchers consider the use of mesophilic aerobic microorganisms as a parameter for estimating the storage term of chilled foods rather dubious **(Maas van Berkel, van den Boogaard and Heijnen, 2004; Hilgarth, Behr and Vogel, 2018; Salata et al., 2017; Zhou, Xu and Liu, 2010)**.

Overall, our results indicate that storage of beef meat with an initial mesophilic bacterial content of about 4.88 log CFU.cm^{-2} of surface and psychrotrophic bacteria 3.79 log CFU.cm^{-2} at temperature 0 °C is only possible for 8 days, further, the microbiological indices exceed the acceptable standards and half-corpuscles are unusable. Therefore, we believe that when storing chilled meat at temperature 0 °C, it is necessary to achieve a reduction in the initial inoculation of the carcasses by microorganisms by improving the sanitation conditions of meat provision at meat processing plants.

Investigation of the dynamics of microflora reproduction during the storage of beef in the frozen state at temperature -2 to -3 °C for 20 days established a decrease in 1.3 times the number of mesophilic bacteria in 10 days of storage. At the same time, the number of psychrotrophic microorganisms during this storage time was increased in 4.5 times, and 20 days in 7.9 times and amounted to 5.3 log CFU.cm^{-2} of surface area. This indicates that the storage of meat in the frozen state inhibits or completely stops the development of mesophilic microorganisms for 20 days. Therefore, we support the opinion of scientists **(Alonso-Hernando, Capita and Alonso-Calleja, 2013; Bruckner et al., 2012; Cerveny, Meyer and Hall, 2009; Jeremiah, 1997; Kameník, 2013; Pennacchia, Ercolini and Villani, 2011; Pothakos et al., 2014)**, which indicate that the temperature of refrigeration processing of meat has a significant influence on its storage term.

Thus, even though the meat complies with the standards by the content of mesophilic bacteria, the presence and development of psychrotrophic microorganisms in frozen meat is an integral part of the safety and quality control of beef.

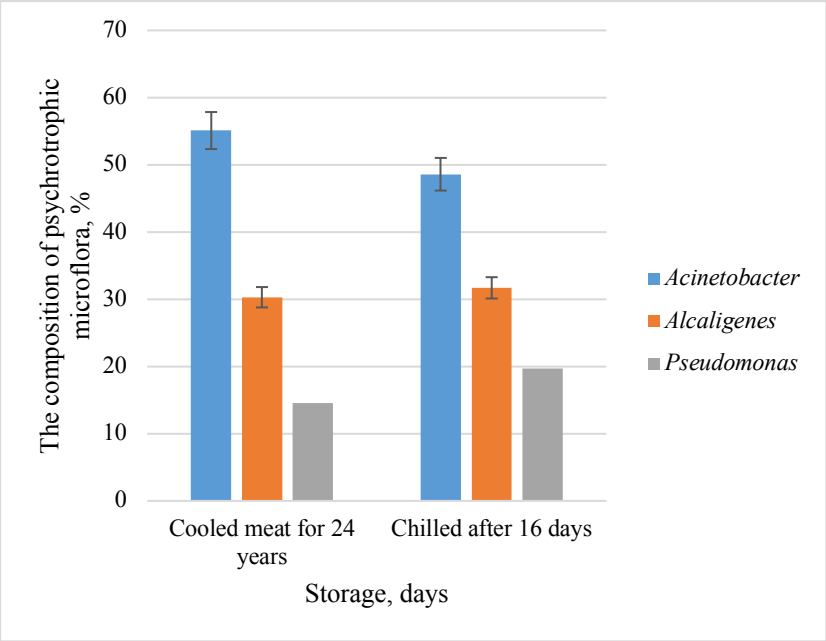

Figure 4 Generic composition of psychrotrophic microflora of beef during storage in the frozen state at temperatures -2 to -3 °C.

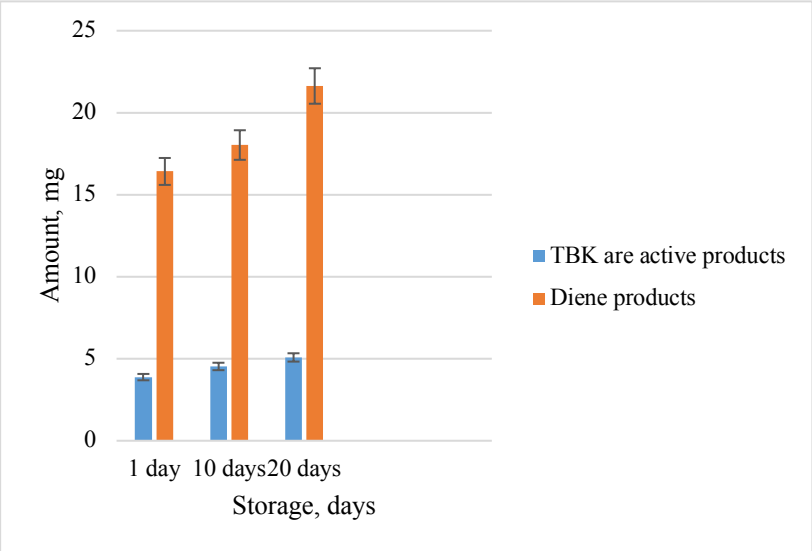

Figure 5 Changes in the content of volatile fatty acids during storage of beef in a cooled and frozen state at temperature. Note: KOH; – doubtful freshness – from 4.1 to 9.0 mg KOH; stale more than 9.1 mg KOH.

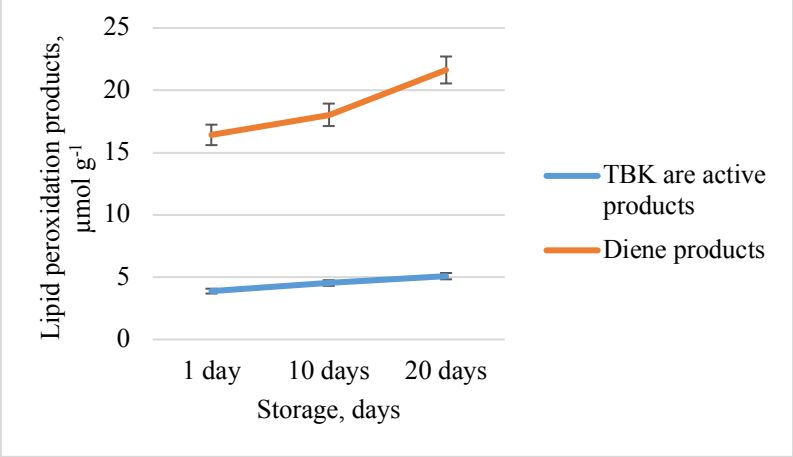

Figure 6 Changes in lipid peroxidation products during storage of the beef in chilled state at 0 ±0.5 °C.

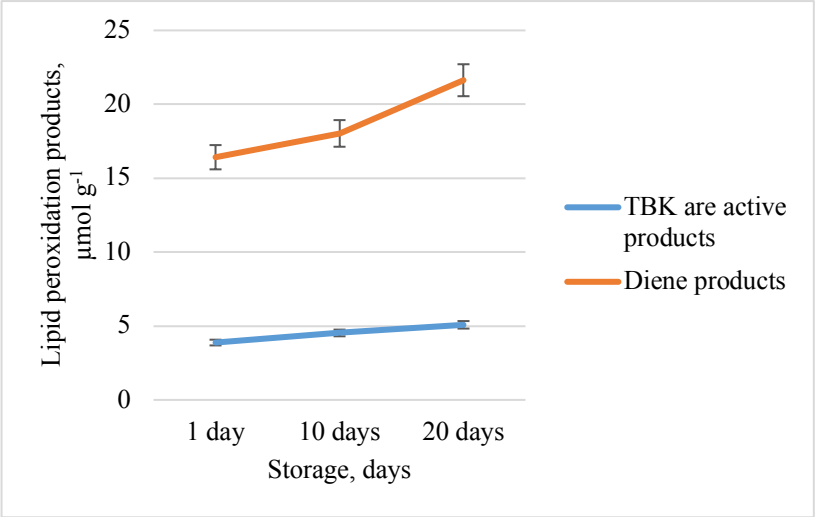

Figure 7 Changes of lipid peroxidation products during the storage of beef in the frozen state at temperatures -2 to -3 °C.

Table 1 Microbiological evaluation of chilled and frozen beef according to by the criteria of technological hygiene processes for the determination of psychrotrophic microflora.

Food category	Microorganisms	Sampling plan		Permissible limits		The stage where the metric is applied	Actions in case of poor results
		n[1]	c[2]	m[3]	M[4]		
Beef meat	Psychrotrophic	5	3	3 log CFU.cm^{-2}	4 log CFU.cm^{-2}	Before staging for refrigerated storage	improvement of slaughter hygiene and reviewing process control measures

Note: [1]n – number of samples taken from one carcass; [2]c – number of samples, parametric values, which are between *m* i *M*; [3]m – normative value of the content of microorganisms per 1 cm^2 of the carcass surface; [4]M – maximum content of microorganisms per 1 cm^2 of the surface.

An important factor that depends on the appearance of organoleptic defects in meat during its refrigerated storage is the microbial composition of the existing microflora because the production of aromatic substances and the decomposition of proteins, fats, and carbohydrates in different genera and species of microorganisms are different **(Cerveny, Meyer and Hall, 2009; Ercolini et al., 2009; Kameník, 2013; Adam, Flint and Brightwell, 2010; Pothakos et al., 2014; Stellato et al., 2017; Zhang et al., 2019)**. When identifying the psychrotrophic microflora of beef, it was found that bacteria of the genus *Acinetobacter* spp. make up almost half of all psychrotrophic microflora cooled and chilled after 16 days of storage 55.1 ±2.2 and 42.4 ±1.7% respectively. Bacteria of the genus *Alcaligenes* spp. occupy a stable niche of microflora, both cold and frozen meat – from 30.3 to 26.2%. However, bacterial growth of the genus *Pseudomonas* spp. was observed n 1.9 times on the surface of chilled beef, compared to cooling.

The identification of the composition of the psychrotrophic microflora of frozen beef after twenty days of storage revealed an increase in bacteria of the genus *Pseudomonas* spp. in 1.3 times. At the same time, the number of bacteria of the genus *Acinetobacter* spp. and *Alcaligenes* spp. in frozen meat was almost the same as in the cold. In research **(Doulgeraki et al., 2012; Ercolini et al., 2009; Stellato et al., 2017)** it is reported that the most responsible for the emergence of food defects during refrigeration storage are bacteria of the genus *Pseudomonas* spp. However, in large quantities stand out such psychrotrophic genus as *Acinetobacter* spp., *Brochothrix* spp., *Flavobacterium* spp., *Psychrobacter* spp., *Moraxella* spp., to a lesser extent *Staphylococcus* spp., and *Micrococcus* spp., lactic acid bacteria and genus Enterobacteriaceae **(Dave and Ghaly, 2011; Kameník, 2013; Pennacchia, Ercolini, and Villani, 2011)**.

Therefore, investigations indicate that bacteria of genus *Pseudomonas* spp. during the storage of beef in the cooled and frozen state show the highest intensity of development.

An assessment of the volatile fatty acid content of chilled beef found that after 8 days of storage, the meat was of dubious freshness, and after 16 days it was not fresh. In particular, the amount of volatile fatty acids was increased in 5.1 times by 16 days, indicating the course of intensive biochemical processes on enzymatic hydrolysis of fat and spoilage of meat. An investigation of frozen beef was found that after 10 days the amount of volatile fatty acids did not increase, and on the 20th day their storage their number was 2.3 times higher compared to the content in the cooled meat. With so many volatile fatty acids, the meat is considered to be of doubtful freshness.

Literary data **(Ercolini et al., 2009; Jay, Loessner and Golden, 2005; Mayr et al., 2003)** also indicate that during the storage process of meat and the development of the

microflora, there is a release of various aromatic compounds that cause organoleptic defects of meat. Therefore, we support the opinion of scientists that the amount of volatile fatty acids increases due to the microbial metabolism of psychrotrophic microflora **(Morales, Fernández-García and Nuñez, 2005; Padda et al., 2001; Popelka, Jevinová and Marcinčák, 2016)**.

CONCLUSION

Our investigations found that while storing meat in a refrigerated state, a probable increase in the amount of TBKAP and DC was detected by only 16th day compared to the first one. When storing beef in the frozen state, a probable increase in 1.3 times, compared to the first day, was noted only by the amount of DC on the 20th day. This indicates that active oxidation of polyunsaturated fatty acids in cell membrane phospholipids occurs in chilled beef meat after the eighth day of storage.

Thus, the investigations indicate that storing of beef in the cooled state at a temperature of 0 ±0.5 °C for more than eight days is impractical, as its biochemical indices are worsening and signs of spoilage are appearing. At the same time, storing of beef in the frozen state at a temperature of -2 to -3 °C for 20 days does not cause such significant biochemical changes as in beef stored in the cooled state at a temperature of 0 ±0.5 °C for 16 days. Also, to prevent the occurrence of organoleptic and biochemical beef defects during refrigeration, it is necessary to stop the development of psychrotrophic proteolytic and lipolytic microflora, which is possible by lowering the temperature.

Therefore, we have experimentally substantiated the quantitative indicators of the content of psychrotrophic microorganisms on the surface of beef intended for storage in a cooled or frozen state. The proposed microbiological criteria based on European approaches will improve the safety of beef.

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Contact address:

Mykola Kukhtyn, Ternopil Ivan Pului National Technical University, Faculty of Engineering of Machines, Structures and Technologies, Department of Food Biotechnology and Chemistry, Ruska**,** 56, 46001, Ternopil, Ukraine, Tel.: +380972392057,

E-mail: kuchtynnic@gmail.com

ORCID: http://orcid.org/0000-0002-0195-0767

Volodymyr Salata, Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyj, Faculty of Veterinary Hygiene, Ecology and Law, Department of Veterinary-Sanitary Inspection, Pekarska, 50, 79010, Lviv, Ukraine, Tel.: +380677288933,
E-mail: salatavolod@ukr.net
ORCID: http://orcid.org/0000-0002-7175-493

Oleksandra Berhilevych, Sumy State University, Medical Institutte, Department of Public Health, Sanatornaya, 31, 40018, Sumy, Ukraine, Tel. +380679038996,
E-mail: bergilevich@ukr.net
ORCID: https://orcid.org/0000-0002-3622-8942

Zoya Malimon, State Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise, Donetska, 30, Kyiv, 02000, Ukraine, Tel. +380679038996,
E-mail: z_malimon@ukr.net
ORCID: https://orcid.org/0000-0002-8616-3198

Anatoliy Tsvihun, State Agrarian and Engineering University in Podilya, Faculty of Veterinary Medicine and Technologies in Livestock, Department of Animal Feeding, Breeding and Feed Technology, Schevchenko, 13, 32301, Kamianets-Podilskyi, Ukraine, Tel.: +380673847280,
E-mail: agroargument2@ukr.net
ORCID: https://orcid.org/0000-0002-1214-1113

Bogdan Gutyj, Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyj, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Pekarska, 50, 79010, Lviv, Ukraine, Tel.: +380681362054,
E-mail: bvh@ukr.net
ORCID: http://orcid.org/0000-0002-5971-8776

*Yulia Horiuk, State Agrarian and Engineering University in Podilya, Faculty of Veterinary Medicine and Technologies in Livestock, Department of Infectious and Parasitic Diseases, Schevchenko, 13, 32301, Kamianets-Podilskyi, Ukraine, Tel.: +380976617964,
E-mail: goruky@ukr.net
ORCID: https://orcid.org/0000-0002-7162-8992

Corresponding author: *