



OPEN O ACCESS Received: 27.7.2023 Revised: 20.8.2023 Accepted: 31.8.2023 Published: 12.9.2023

# Slovak Journal of **Food Sciences**

Potravinarstvo Slovak Journal of Food Sciences vol. 17, 2023, p. 759-776 https://doi.org/10.5219/1905 ISSN: 1337-0960 online www.potravinarstvo.com © 2023 Authors, CC BY-NC-ND 4.0

# The microscopic structure of pork neck after cooling with showering stiving and processing by culture *Lactobacillus sakei*

Volodymyr Vovkotrub, Olga Iakubchak, Leonid Horalskyi, Nataliia Vovkotrub, Larysa Shevchenko, Nataliia Shynkaruk, Tatyana Rozbytska, Yuliia Slyva, Olga Tupitska, Oksana Shtonda

#### ABSTRACT

Microstructural changes in meat that occur during refrigerated storage depend on the hygiene of slaughtering and primary processing of animal carcasses, their cooling conditions, storage period, and microbial contamination and reflect the processes of meat maturation and spoilage. To extend the shelf life of pork in half-carcasses in a chilled state, 20 heads of 6-month-old large white pigs were used, which were delivered to the meat processing enterprise for slaughter. All half carcasses were cooled in a refrigerating chamber using showering, 1 hour later they were divided into 2 groups: control (without treatment) and experimental with the final treatment with a suspension of lactic acid bacteria of the SafePro® B-2 strain (Lactobacillus sakei). It has been found that cooling of pork half-carcasses in a refrigerating chamber with stiving and final processing by a culture suspension of lactic-acid microorganisms of strain SafePro® B-2 (Lactobacillus sakei) on the 4th day of storage had a positive effect on the microscopic structure of the pork neck and was characterized by a uniform color distribution when histologic specimens of muscular tissue are colored with hematoxylin and eosin, and minor cracks in the sarcoplasm, preservation of transverse and longitudinal striation of muscular fibers in comparison with that of the unprocessed pork half-carcasses with cultures of lactic-acid microorganisms. The microscopic structure of the muscular tissue of the pork half-carcass neck after cooling with stiving and final processing by a culture of lactic-acid microorganisms of strain SafePro® B-2 for 7 days of storage had a more distinct histoarchitecture in comparison with that of the unprocessed pork half-carcasses, as well as was characterized by insignificant areas of muscular fibers with transverse cracks, suspended development period of autolysis processes, partial preservation of transverse and longitudinal striation of muscle fibers. This points to a positive effect of lactic acid bacteria of strain SafePro® B-2 (Lactobacillus sakei) on the quality of the pork meat and contributes to the extension of its shelf life under chilled vintage.

#### Keywords: pork meat, storage, lactic-acid bacteria, his structure, neck muscles

#### INTRODUCTION

The quality of fresh pork includes a significant number of properties, which are essential to the suitability of the meat for use as food or the preparation of various dishes [1], [2]. Postmortem changes, that occur when the muscles are transforming into the meat, are crucial in developing quality characteristics and the overall consumer comprehension of the fresh product [3], [27]. Biochemical processes and structural changes that occur in the muscles during the first 24 hours after slaughtering play a major role in the meat's final quality and taste characteristics and depend upon the cooling processes to which the carcasses are subjected after slaughtering. After the animal is slaughtered, glycogen is anaerobically mobilized in the muscular tissue for maintaining

homeostasis. Lactate and  $H^+$  accumulate in the muscles and cause a decrease in pH value due to postmortem glycolysis. Due to exposure to high temperatures of the muscles and low pH value, the pork must apply a faster cooling process with a recommended core temperature of the muscles of 10 °C after 12 h and 2-4 °C after 24 h. For the achievement of this effect, it is often applied spray cooling (stiving) – a system in which chilled water is supplied to the half-carcasses at the initial stage of post-slaughter cooling, which makes it possible to control the shrinkage of the half-carcasses and increases the cooling rate due to evaporative cooling [4], [5]. When postmortem metabolism is ended, structural proteins are proteolytically destructed in the muscles, improving meat tenderness and taste [6], [28].

It has been established that in the process of bleeding, approximately 50% of the total blood volume is removed from the carcass, which amounts to 3.0-3.5% of the body weight of the animal [7], [8], and the blood, which is remaining in the carcass, is a perfect environment for the bacteria growth and spread. Because the fact that chemical agents for the meat to be preserved have several harmful effects on the consumer's body, the need arose to develop and use preservatives [9].

Among these are suspensions of lactic-acid bacteria, particularly *Lactobacillus sakei*, which are capable of inhibiting the growth of related bacteria species by secreting ribosomally synthesized antimicrobial peptides called bacteriocins. Therefore, the use of lactic-acid bacteria *Lactobacillus sakei* for the final processing of the pork half-carcasses during their cooling with stiving is a highly topical issue.

The study aimed to determine changes in the structure of the pork neck muscles in the half-carcasses after cooling in a refrigerating chamber with stiving and final processing by the suspensions of lactic-acid microorganisms *Lactobacillus sakei* during the storage.

#### **Scientific Hypothesis**

The scientific hypothesis lies in the fact that the storage duration of the pork half-carcass meat in a chilled condition can be optimized if we apply the cooling in a refrigerating chamber with stiving and final processing of the half-carcass surface by the suspension of lactic-acid microorganisms, which are antagonists of putrefactive and pathogenic bacteria. Such processing will make it possible to extend the shelf life of chilled pork while preserving its quality and safety. Due to such processing, it will be possible to extend the shelf life of chilled pork meat while preserving its quality and safety.

#### MATERIAL AND METHODOLOGY

#### Samples

The pork half-carcass meat was obtained when the animals were slaughtered under LLC "Antonivsky meat processing plant" conditions, Kyiv region, Ukraine.

#### Chemicals

Formalin (analytical grade, LLC "Khimlaborreaktiv" Ukraine).

Paraffin (brand A, analytical grade, LLC "Khimlaborreaktiv" Ukraine).

Ethanol (brand A, analytical grade, LLC "Khimlaborreaktiv" Ukraine).

Hematoxylin and eosin (ready-made solutions produced by "Lieca", Germany).

#### Animals, Plants and Biological Materials

For research, 20 heads of young fattening pigs of the large white breed aged 6 months were used, which were slaughtered from a private farm in the Kyiv region, Ukraine. To form groups in the experiment, pork half carcasses weighing 43-44 kg were selected.

Natural lactic acid culture (sourdoughs) SafePro® B-2 (LLC "Hr. Hansen Ukraine").

#### Instruments

Microtome MS-2 (Tochmedprilad, Ukraine).

Microscope Biolam-Lomo (approx. 10, vol. 8; approx. 10, vol. 40) (Medyka, Ukraine).

Microscope Micros MC-50 (approx. 10, vol. 8; approx .10, vol. 40) (Micros, Austria).

Microphotography of the histologic specimens was carried out with the use of a video camera CAM V200 (InterMed, Ukraine)

Mounted in microscope Micros MC-50 (Micros, Austria).

#### Laboratory Methods

For histological studies, pieces of pork neck muscles (length, width, thickness - 5-15 mm) were selected, which were immediately fixed in a 10% aqueous solution of neutral formalin for 24 hours, after which they were washed with water and dehydrated using ethyl alcohol of increasing strength: 50°, 70°, 80°, 90°, 96° and absolute alcohols. To seal the samples, they were poured into paraffin. To study the morphology of cells and tissues, sections were made, which were deparaffinized, stained with hematoxylin and eosin, and subjected to microscopy [10].

#### **Description of the Experiment**

**Sample preparation:** Pork half-carcasses subjected to carcass ablution with stiving and processing by starters of culture SafePro® B-2 in a dose of 107/cm<sup>2</sup>.

**Number of samples analyzed:** The total number of samples was 52 samples: 26 samples each from half carcasses of the control and experimental groups of pork.

Number of repeated analyses: All measurements were performed 3 times.

**Number of experiment replication:** The number of replicates of each experiment to determine one value was 5 times.

**Design of the experiment:** The control and experimental samples of 10 pork half-carcasses each were formed to experiment (Table 1).

**Table 1** Experiment scheme for cooling effect determination of pork half-carcasses with stiving in combination with processing by starters of lactic-acid bacteria.

Sample	Experiment conditions	Collection of samples for histological examination
20 half carcasses	Wet toilet half carcasses of pork by showering with water at a temperature of 2°C	1 hour after showering
Control 10 half carcasses	Storage in the refrigerator	4 days of storage 7 days of storage
Experimental 10 half carcasses	With exceptional treatment and starter culture of strain SafePro <sup>®</sup> B-2 ( <i>Lactobacillus sakei</i> ) at a dose of $10^{7}$ /cm <sup>2</sup> and storage in the refrigerator	4 days of storage 7 days of storage

All pork half-carcasses were stored in the refrigerating chamber at a temperature of  $3 \pm 1$  °C until the appearance of the meat deterioration signs carrying out histological studies according to the method [10].

#### **Statistical Analysis**

The STATISTICA Microsoft Excel editor combined with XLSTAT processed experimental data using mathematical statistics methods. The accuracy of the obtained experimental data was determined using the Student's t-test with a confidence coefficient  $\leq 0.05$  with many parallel definitions of at least 5 (confidence probability p = 0.95).

#### **RESULTS AND DISCUSSION**

Changes in pH value and increases in solute concentration (i.e.,  $Ca^{2+}$ ), when the meat is stored and aged, can affect protease activity, and physical destruction of the muscle tissue can alter the localization of proteases (i.e., the release of lysosomal cathepsins into cytosol) into myofibrillar cells and disrupt their integrity [11], [12].

One of pork carcasses' most microbially contaminated areas is the neck [13], [23].

Therefore, for the analysis of the histostructural changes that occur in the muscles, when the half-carcasses are cooled and stored, it is the most indicative part of the carcass.

According to the study results, the muscle tissue of the pork neck muscles (MTPNM) in 1 hour after cooling in the refrigerating chamber with stiving was formed by the muscle fibres (MFs) and intermuscular layers, which are formed by the presence of loose connective tissue. Adipose tissue, blood, and lymphatic vessels are found in the intermuscular connective tissue (Figure 1).

MFs had a sharp outline, their sarcoplasm had a uniform color, and under sarcolemma, there was a dark blue ovate-oblong nucleus. MFs of the pork neck had different thicknesses (small, average, and large), but the average-thick fibres were more often detected (Figure 2). When coloring the histologic specimens with hematoxylin and eosin, MFs with large diameters were less colored than average and small ones. It may be because, in MFs of average and small diameter, which are colored more intensively, myofibrils are more densely arranged than those in the fibers of large diameter, which is especially noticeable in transversal sections of the muscle tissue. The layers of the loose connective tissue (endomysium), which is located around skeletal MFs, were weakly expressed (Figure 2). In some places, MFs acquired a wavy appearance.



**Figure 1** Microscopic structure of MTPNM in 1 hour after stiving: a – muscle fibres; b – endomysium; c – nuclei of muscle fibres; d – fat cells in the premise. Hematoxylin and eosin. X 120.



**Figure 2** Microscopic structure of MTPNM in 1 hour after stiving: a - small-thick muscle fibre; b - average-thick muscle fibre; c - large-thick muscle fibre; d - nuclei of muscle fibres; e - endomysium. Hematoxylin and eosin. X 120.

At high magnification of the microscope (approx. 10; approx. 40) the transverse striations, which are formed due to the presence of actin and myosin proteins, are visible in MFs of the neck (Figure 3). The nuclei of the muscle fibres were located on the fibre periphery, right next to their sarcoplasm, they were oval and located throughout MV. When coloring the histologic specimens with hematoxylin and eosin, the nuclei were basophilic ally colored in a bluish-purple color. Their nuclear chromatin was equally spaced along the entire perimeter of the karyoplasm, and the contours of the sarcolemma were preserved (Figure 3).



**Figure 3** Microscopic structure of MTPNM in 1 hour after stiving: a – muscle fibres; b – nuclei of muscle fibres; c – transverse striation; d – endomysium. Hematoxylin and eosin. X 400.



**Figure 4** Microscopic structure of MTPNM in 1 hour after stiving: a – muscle fibres; b – transverse striation; c – longitudinal striation; d – fat cells. Hematoxylin and eosin. X 400.

About those mentioned above, the longitudinal striation of the fibres, due to the presence of myofibrils, is somewhat smoothed, but it is sufficiently contoured on the histologic specimens (Figure 4).

The results of the analysis of the histostructure of pig meat are based on the data of other researchers, which mean that the light of the world has a consistently uniform microstructure with slightly separated regular micron fibers, which is a typical form of good-sparing myase cells [21], [24].

According to the results of the histological studies of the muscle tissue, which is selected from the pork halfcarcasses on the 4<sup>th</sup> day of storage, which were subjected to cooling in the refrigerating chamber with stiving, the characteristic structure of the tissue was preserved (Figure 5).



**Figure 5** Microscopic structure of muscle tissue, which is selected from the pork half-carcasses subjected to stiving on the  $4^{th}$  day of storage (control): a – muscle fibres; b – nuclei of muscle fibres; c – areas of the uneven coloring of sarcoplasm. Hematoxylin and eosin. X 120.



**Figure 6** Microscopic structure of muscle tissue, which is selected from the pork half-carcasses subjected to stiving on the  $4^{th}$  day of storage (control): a – rupture of muscle fibres; b – areas of the uneven coloring of sarcoplasm. Hematoxylin and eosin. X 120.

However, transverse cracks and ruptures of muscle fibres were often detected, resulting in cracks and destruction of the sarcoplasm (Figure 6). In these places, the specific histoarchitecture, which is characteristic of the muscle tissue, was destroyed. When the histologic specimens were colored with hematoxylin and eosin, the sarcoplasm of the muscle fibres of the neck was unevenly colored, which indicated the characteristic signs of the beginning of the autolysis process (Figures 5, 7). The deformed muscle fibres and their weakened transverse and longitudinal striations were also detected (Figure 7). At the same time, deformed MFs had a tortuous (wavy) shape. The nuclei in some MFs were deformed, and in some areas, they were in a state of lysis [29]. The endomysium and perimysium of the neck muscles were expanded in some places, and their fibrous structures became loose. Such changes could occur with the participation of microflora, which was located both on the meat surface and in its thickness [14], [25].



**Figure 7** Microscopic structure of muscle tissue selected from the pork half-carcasses subjected to stiving on the  $4^{th}$  day of storage (control): a – muscle fibres; b – nuclei of muscle fibres; c – transverse striation. Hematoxylin and eosin. X 400.

A wide synthetic preservative, including nitrite, nitrate, and sorbate, through its low quality and strong antibacterial activity, is widely used for the growth of micro-organic products in the food industry. At the same time, the majority of consumers will see the use of synthetic chemical preservatives nebazhanim, the stench is not safe for health. It is possible to reduce the intensity of destructive changes in the environment and the continuation of the term of its accessory [43].

According to the results of the histological studies, the muscle tissue selected from the experimental pork half-carcasses had a similar architecture compared to the control (Figure 8).



**Figure 8** Microscopic structure of muscle tissue, which is selected from the experimental pork half-carcasses on the  $4^{th}$  day of storage: a – muscle fibres; b – nuclei of muscle fibres; c – fat cells; d – endomysium; e – perimysium. Hematoxylin and eosin. X 120.



**Figure 9** Microscopic structure of muscle tissue, which is selected from the experimental pork half-carcasses on the  $4^{th}$  day of storage: a – muscle fibres; b – nuclei of muscle fibres; c – transverse striation; d – longitudinal striation. Hematoxylin and eosin. X 400.

When studying the meat microstructure in the neck area, which was stored in the refrigerating chamber for 4 days, the histoarchitecture of the muscle tissue was formed by the different thicknesses of MFs and the layers of the connective tissue [32]. However, compared with the histostructure of this tissue only after stiving, when the histologic specimens are colored with hematoxylin and eosin, the MTPNM of the experimental pork was mostly uniformly colored (Figure 8). In some areas, MFs had a tortuous appearance [33], [34]. When the pork half-carcasses were stived, only minor areas with transverse cracks in the sarcoplasm of the muscle fibres were detected. The transverse and longitudinal striations of the sarcoplasm were mostly preserved (Figure 9). It is important to talk about the positive influence of lactic acid microorganisms on the quality of milk [30], [31]. At the same time, the nuclei of the muscle fibres had an oval-elongated shape and were basophilically colored. They were located on the periphery of the muscle fibres (Figure 8).

According to the histological studies, the histoarchitecture of the muscle tissue, selected from the pork halfcarcasses subjected to cooling with stiving, lost their characteristic structure. Storage of the pork half-carcass meat in the refrigerator for 7 days contributed to the more active development of autolytic processes, which involved many muscle fibres. Thus, when the histologic specimens are colored with hematoxylin and eosin, the sarcoplasm of the muscle fibres is not almost colored, which indicates the characteristic signs of the autolysis process (Figure 10). affect the sensory characteristics of the meat.



**Figure 10** Microscopic structure of muscle tissue selected from the pork half-carcasses subjected to stiving on the 7<sup>th</sup> day of storage (control): a – rupture of muscle fibres; b – autolysis of muscle fibres. Hematoxylin and eosin. X 120.



**Figure 11** Microscopic structure of muscle tissue, which is selected from the pork half-carcasses subjected to stiving on the 7<sup>th</sup> day of storage (control): a – rupture of muscle fibres; b – fragmentation of muscle fibres; c – autolysis of muscle fibres; d – nuclei of muscle fibres. Hematoxylin and eosin. X 120.

The obvious transverse cracks of the muscle fibres, their ruptures, and fragmentation, which increased significantly, were detected in many areas of the histologic specimens (Figure 11).

Storage of the meat for 7 days in the refrigerator, compared to the histoarchitecture of the muscle tissue for a storage duration of the meat of 4 days, contributed to the more active development of autolytic processes, which included a significant number of the muscle fibres (Figure 13).



**Figure 12** Microscopic structure of muscle tissue, which is selected from the pork half-carcasses subjected to stiving on the 7<sup>th</sup> day of storage (control): a - muscle fibres; b - nuclei of muscle fibres; c - light coloring of sarcoplasm; d - accumulation of granular protein mass. Hematoxylin and eosin. X 120.



**Figure 13** Microscopic structure of muscle tissue, which is selected from the pork half-carcasses subjected to stiving on the 7<sup>th</sup> day of storage (control): a – autolysis of muscle fibres; b – muscle fibres of tortuous shape; c – nuclei of muscle fibres. Hematoxylin and eosin. X 120.

Deformed MFs of the pork neck had a tortuous shape, and their transverse and longitudinal striations were destroyed (Figure 14). The endomysium and perimysium of the muscle tissue were expanded in some places, and their fibrous structures became loose.

The nuclei of the muscle fibres against the background of the light coloring of the sarcoplasm, due to the autolysis development processes, lost their characteristic structure and, being in a state of lysis, were detected only in the form of shadows or were outlined against the background of the destroyed sarcoplasm (Figures 10-14). Proteolysis of key myofibrillar proteins is the main reason for the ultrastructural changes in the skeletal muscle associated with meat tenderization [17], [35].

According to the results of the histological studies, the microscopic structure of the muscle tissue, which is selected from the experimental pork half-carcasses (with a storage period of 7 days) had a more distinct histoarchitecture compared to that of the unprocessed half-carcasses. This is the case with the biopreservation of *Lactobacillus sakei*. As a result, proteolytic enzymes are produced with the same substrate and are necessary for the growth of *L. sakei*. A special advantage of this microorganism is the growth rate at the time of cooling and in the presence of salt and (3-9% NaCl) in the production of bacteriocins, zocream anti listerial peptide sakacin P. *Sakei* is characterized by the creation of biological pellicle and climatic (auto-co-)aggregation, which allows you to colonize the surface **[22]**, **[38]**.



**Figure 14** Microscopic structure of muscle tissue, which is selected from the pork half-carcasses subjected to stiving on the 7<sup>th</sup> day of storage (control): a - muscle fibres of tortuous shape; b - nuclei of muscle fibres in shadow form; c - endomysium. Hematoxylin and eosin. X 120.



**Figure 15** Microscopic structure of muscle tissue, which is selected from the experimental pork half-carcasses on the 7<sup>th</sup> day of storage: a – muscle fibres; b – nuclei of muscle fibres; c – endomysium; d – pyrimidine; e – fat cells in perimysium. Hematoxylin and eosin. X 120.

Such changes in the microscopic structure of the muscle tissue caused the release of the contained muscle fibres into the intermuscular space. Granular masses visible under a light microscope were formed by hydrolytic enzymes (Figure 12). Such changes are associated with aging processes and are related to the most important factors that improve meat tenderness due to the proteolysis of structural proteins by endogenous muscle enzymes [15], [36]. In turn, protein degradation leads to the loss of structural integrity and the formation of large peptides and amino acids [16], [26].

This indicates the positive consequences of the influence of cultures of lactic acid microorganisms on the structure of the muscle tissue if the meat is stored for a long time (Figure 15). Thus, the sarcoplasm of MTPNM, which is selected from the experimental pork half-carcasses after a storage period of 7 days, when the histologic specimens were colored with hematoxylin and eosin, was mostly uniformly colored. However, the color saturation was lower than that in MFs of the muscle tissue, which is selected from the processed pork half-carcasses with cultures of lactic acid microorganisms after a storage period of 4 days (Figure 15).



**Figure 16** Microscopic structure of muscle tissue, which is selected from the experimental pork half-carcasses on the 7<sup>th</sup> day of storage: a – muscle fibres; b – nuclei of muscle fibres; c – endomysium; d – light lysed areas of sarcoplasm. Hematoxylin and eosin. X 120.

Furthermore, in certain areas of the histologic specimens, MFs were unevenly colored (light-lysed areas of the sarcoplasm were detected), which indicated the characteristic signs of the beginning of the autolysis process (Figure 16). Some of the muscle fibres had a tortuous shape (Figure 17), and their histoarchitecture was destroyed due to minor transverse cracks in their sarcoplasm (Figure 18). Such changes can probably be related to the

proteolytic activity of muscle calpains. The study results, conducted by [18], [19], indicate that calpain-1 and calpain-2 bind to myofibrils, when the meat is stored, and subsequently destroy structural proteins, including desmin.

When analyzing the histologic specimens at a high magnification of the microscope, the transverse and longitudinal striations of the sarcoplasm of individual muscle fibres were partially preserved (Figure 19).



**Figure 17** Microscopic structure of muscle tissue selected from the experimental pork half-carcasses on the 7th day of storage: a - muscle fibres of tortuous shape; b - nuclei of muscle fibres. Hematoxylin and eosin. X 120.



**Figure 18** Microscopic structure of muscle tissue selected from the experimental pork half-carcasses on the 7th day of storage: a - muscle fibres of tortuous shape; b - nuclei of muscle fibres. Hematoxylin and eosin. X 120.



**Figure 19** Microscopic structure of muscle tissue, which is selected from the experimental pork half-carcasses on the 7<sup>th</sup> day of storage: a – muscle fibres; b – nuclei of muscle fibres; c – perimysium; d – transverse striation. Hematoxylin and eosin. X 400.

The nuclei of the muscle fibres had an oval-elongated shape and were basophilically colored. At the same time, the nuclei karyoplasm was not clearly outlined by the nuclear envelope, indicating the initial autolysis processes of the muscle fibre nuclei. Such changes could also be caused by the enzymatic activity of the microflora in the meat, particularly various types of lactic acid microorganisms **[20]**, **[37]**.

It is rather difficult to make a more detailed comparison of the research results obtained by us with the data of other authors, since most strains of lactic acid microorganisms, in particular *L. sakei*, are intended for the technology of fermented meat products (sausages, ham) [38], [44] and for raw pork, shock chilling or freezing is most often used, which ensures the reduction of microbial contamination during meat storage [39]. However, freezing of meat causes its quality to deteriorate due to protein oxidation and ice crystal formation, which occurs during freezing/thawing and frozen storage, which leads to irreversible physicochemical changes and quality deterioration [40]. Therefore, most researchers prefer to improve the methods of storing pork in a chilled state over freezing, but histological studies on this issue are very small and they are performed in laboratory conditions, and not in the conditions of meat processing plants. In addition, most studies used individual muscles or groups of muscles in which histological sections were made in a transverse perspective, which does not give a complete idea of the structural changes in muscle tissue [41]. As can be seen from the obtained data, the histostructure of the muscle tissue of the experimental pork neck was less damaged as a result of the autolysis process, which contributed to the extension of its storage period up to 7 days. The data, we obtained, are compliant with the study results of other scientists [42], which indicate that 8 days are enough for pork meat to acquire the sensory characteristics that satisfy the consumer requirements.

We have analyzed the results of the study of the fermentation of lactic acid microorganisms. The SafePro® B-2 can be used as a promising tool for the use of pork refrigeration in the refrigeration chamber with showers, but for this, it is necessary to increase the possible risks of microflora-control, which will be characteristic of the skin-specific micronutrient enterprises and will be characteristic of the skin-specific micro-processing enterprises.

#### CONCLUSION

Thus, the microscopic structure of the muscle tissue of the pork half-carcasses neck, which was subjected to cooling in the refrigerating chamber with stiving on the 4<sup>th</sup> day of storage, was characterized by a slight deformation of the muscle fibres, their weakened transverse and longitudinal striations. Transverse cracks and ruptures of the muscle fibres were often found in it. The sarcoplasm of the muscle fibres was unevenly colored, which indicated the beginning of the autolysis process. The nuclei of individual neck MFs were in a state of lysis. The microscopic structure of the muscle tissue of the neck, which is selected from the experimental pork half-carcasses on the 4<sup>th</sup> day of storage, was characterized by more positive characteristics of the microscopic structure (uniform coloring of the sarcoplasm, minor cracks in the sarcoplasm, preservation of the transverse and longitudinal striations of the muscle fibres, etc.) compared to that of unprocessed pork half-carcasses with cultures of lactic-acid microorganisms, which indicated the positive effect of lactic-acid bacteria on the meat quality and, as a consequence, the extension of its shelf life in a chilled condition. Therefore, when the results of the

histological studies are analyzed, it is worth pointing out that the microscopic structure of the muscle tissue of the pork half-carcass neck after cooling in the refrigerating chamber with stiving with an increased storage period of up to 7 days underwent a deformation of the muscle fibres, shown as the increased number of the transverse cracks and ruptures of the muscle fibres, characteristic signs of the autolysis process of the muscle tissue. The histoarchitecture of the pork half-carcass neck muscles after cooling in the refrigerating chamber with stiving and final processing of the surface with cultures of lactic-acid microorganisms of strain SafePro® B-2 was characterized by insignificant areas of the muscle fibres with transverse cracks, suspended development of the autolysis processes, partial preservation of the transverse and longitudinal striations of the muscle fibres, which indicates the positive effect of lactic-acid bacteria on the meat quality and contributes to the extension of its storage in a chilled condition up to 7 days. The proposed treatment makes it possible to extend the shelf life of chilled pork meat while maintaining its quality and safety. The development of research in the direction of using lactic acid micro-organisms for the production of refrigerated pork is necessary with the improvement of microbiological and physicochemical indicators, as well as in the form of a mixture that can be used to improve the optimal conditions for the preparation of a suitable source for consumption.

#### REFERENCES

- Warner, R. D., Greenwood, P. L., Pethick, D. W., & Ferguson, D. M. (2010). Genetic and environmental effects on meat quality. In Meat Science (Vol. 86, Issue 1, pp. 171–183). Elsevier BV. <u>https://doi.org/10.1016/j.meatsci.2010.04.042</u>
- Joo, S. T., Kim, G. D., Hwang, Y. H., & Ryu, Y. C. (2013). Control of fresh meat quality through manipulation of muscle fibre characteristics. In Meat Science (Vol. 95, Issue 4, pp. 828–836). Elsevier BV. https://doi.org/10.1016/j.meatsci.2013.04.044
- Adhikari, K., Chambers Iv, E., Miller, R., Vázquez-Araújo, L., Bhumiratana, N., & Philip, C. (2011). Development of a lexicon for beef flavor in intact muscle. in Journal of Sensory Studies (Vol. 26, Issue 6, pp. 413–420). Wiley. <u>https://doi.org/10.1111/j.1745-459x.2011.00356.x</u>
- Savell, J. W., Mueller, S. L., & Baird, B. E. (2005). The chilling of carcasses. In Meat Science (Vol. 70, Issue 3, pp. 449–459). Elsevier BV. <u>https://doi.org/10.1016/j.meatsci.2004.06.027</u>
- De Oliveira, J. A., Polizel Neto, A., Bueno da Rosa, C. C., Fortuna, N. B., Groth, A., Dos Santos, R., Bento, F. C., & Gomes, H. F. B. (2021). Use of different cold water spray protocols on bovine carcasses during cooling and its effects on meat quality. In Scientific Electronic Archives (Vol. 14, Issue 5, pp. 12–21). Scientific Electronic Archives. <u>https://doi.org/10.36560/14520211345</u>
- 6. Khan, M. I., Jung, S., Nam, K. C., & Jo, C. (2016). Postmortem Aging of Beef with a Special Reference to the Dry Aging. In Korean Journal for Food Science of Animal Resources (Vol. 36, Issue 2, pp. 159–169). Korean Society for Food Science of Animal Resources. <u>https://doi.org/10.5851/kosfa.2016.36.2.159</u>
- Ockerman, H. W., & Basu, L. (2014). BY-PRODUCTS | Edible, for Human Consumption. In Encyclopedia of Meat Sciences (pp. 104–111). Elsevier. <u>https://doi.org/10.1016/b978-0-12-384731-7.00031-3</u>
- Woerner, D. R., Scanga, J. A., & Belk, K. E. (2014). SLAUGHTER-LINE OPERATION | Cattle. In Encyclopedia of Meat Sciences (pp. 284–289). Elsevier. <u>https://doi.org/10.1016/b978-0-12-384731-7.00157-4</u>
- Cherednichenko, O., Bal-Prylypko, L., Paska, M., & Nikolaenko, M. (2021). The expediency of creation of technology for the production of meat products of long term of storage of the combined structure. In IOP Conference Series: Earth and Environmental Science (Vol. 723, Issue 3, p. 032086). IOP Publishing. https://doi.org/10.1088/1755-1315/723/3/032086
- Zheplinska, M., Mushtruk, M., Vasyliv, V., Kuts, A., Slobodyanyuk, N., Bal-Prylypko, L., Nikolaenko, M., Kokhan, O., Reznichenko, Y., & Salavor, O. (2021). The micronutrient profile of medicinal plant extracts. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 528–535). HACCP Consulting. https://doi.org/10.5219/1553
- Huff Lonergan, E., Zhang, W., & Lonergan, S. M. (2010). Biochemistry of postmortem muscle Lessons on mechanisms of meat tenderization. In Meat Science (Vol. 86, Issue 1, pp. 184–195). Elsevier BV. <u>https://doi.org/10.1016/j.meatsci.2010.05.004</u>
- Pomponio, L., Bukh, C., & Ruiz-Carrascal, J. (2018). Proteolysis in pork loins during super chilling and regular chilling storage. In Meat Science (Vol. 141, pp. 57–65). Elsevier BV. <u>https://doi.org/10.1016/j.meatsci.2018.03.022</u>
- Bal-Prilipko, L. V., Patyka, N. V., Leonova, B. I., Starkova, E. R., & Brona, A. I. (2016). Trends, Achievements and Prospects of Biotechnology in the Food Industry. In Mikrobiolohichnyi Zhurnal (Vol. 78, Issue 3, pp. 99–111). National Academy of Sciences of Ukraine (Co. LTD Ukrinformnauka). <u>https://doi.org/10.15407/microbiolj78.03.099</u>

- Ajaykumar, V. J., & Mandal, P. K. (2020). Modern concept and detection of spoilage in meat and meat products. In Meat Quality Analysis (pp. 335–349). Elsevier. <u>https://doi.org/10.1016/b978-0-12-819233-7.00018-5</u>
- **15.** Perry, N. (2012). Dry aging beef. In International Journal of Gastronomy and Food Science (Vol. 1, Issue 1, pp. 78–80). Elsevier BV. <u>https://doi.org/10.1016/j.ijgfs.2011.11.005</u>
- 16. Stadnik, J., & Dolatowski, Z. J. (2013). Changes in selected parameters related to proteolysis during aging of dry-cured pork loins inoculated with probiotics. In Food Chemistry (Vol. 139, Issues 1–4, pp. 67–71). Elsevier BV. <u>https://doi.org/10.1016/j.foodchem.2013.01.079</u>
- Ugnivenko, A., Getya, A., Nosevych, D., Antoniuk, T., Kruk, O., Slobodyanyuk, N., Ivaniuta, A., Omelian, A., Gryshchenko, S., & Israelian, V. (2022). The study of "muscle eye" in bulls of Ukrainian black-spotted dairy-meat breed as a factor in improving the properties of meat products. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 519–529). HACCP Consulting. <u>https://doi.org/10.5219/1762</u>
- Lyu, J., & Ertbjerg, P. (2022). Sarcoplasmic and myofibril-bound calpains during storage of pork longissimus muscle: New insights on protein degradation. In Food Chemistry (Vol. 372, p. 131347). Elsevier BV. <u>https://doi.org/10.1016/j.foodchem.2021.131347</u>
- Lyu, J., Puolanne, E., & Ertbjerg, P. (2023). Relationship between the pre-rigor temperature of pork longissimus muscle, myofibril-bound calpain activity, and protein degradation. In Meat Science (Vol. 198, p. 109094). Elsevier BV. <u>https://doi.org/10.1016/j.meatsci.2022.109094</u>
- LI, C., Wu, J., Zhang, N., Zhang, S., Liu, J., Li, J., Li, H., Feng, X., Han, Y., Zhu, Z., Xu, X., & Zhou, G. (2009). Effects of boning method and postmortem aging on meat quality characteristics of pork loin. In Animal Science Journal (Vol. 80, Issue 5, pp. 591–596). Wiley. <u>https://doi.org/10.1111/j.1740-0929.2009.00677.x</u>
- **21.** Palamarchuk, I., Mushtruk, M., Vasyliv, V., & Zheplinska, M. (2019). Substantiation of regime parameters of vibrating conveyor infrared dryers. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 13, Issue 1, pp. 751–758). HACCP Consulting. <u>https://doi.org/10.5219/1184</u>
- 22. Rogoskii, I., Mushtruk, M., Titova, L., Snezhko, O., Rogach, S., Blesnyuk, O., Rosamaha, Y., Zubok, T., Yeremenko, O., & Nadtochiy, O. (2020). Engineering management of starter cultures in study of temperature of fermentation of sour-milk drink with apiproducts. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 1047–1054). HACCP Consulting. <u>https://doi.org/10.5219/1437</u>
- 23. Bal-Prylypko, L., Yancheva, M., Paska, M., Ryabovol, M., Nikolaenko, M., Israelian, V., Pylypchuk, O., Tverezovska, N., Kushnir, Y., & Nazarenko, M. (2022). The study of the intensification of technological parameters of the sausage production process. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 27–41). HACCP Consulting. <u>https://doi.org/10.5219/1712</u>
- 24. Ugnivenko, A., Kos, N., Nosevych, D., Mushtruk, M., Slobodyanyuk, N., Zasukha, Y., Otchenashko, V., Chumachenko, I., Gryshchenko, S., & Snizhko, O. (2022). The yield of adipose tissue and by-products in the course of the slaughter of inbred and outbred bulls of the Ukrainian beef breed. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 307–319). HACCP Consulting. <u>https://doi.org/10.5219/1758</u>
- 25. Ugnivenko, A., Nosevych, D., Antoniuk, T., Chumachenko, I., Ivaniuta, A., Slobodyanyuk, N., Kryzhova, Y., Rozbytska, T., Gruntovskyi, M., & Marchyshyna, Y. (2022). Manifestation of living and post-slaughter traits of productivity in inbred and outbred bull calves of Ukrainian meat cattle breed. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 356–366). HACCP Consulting. <u>https://doi.org/10.5219/1769</u>
- 26. Lahiri, D., Nag, M., Sarkar, T., Ray, R. R., Shariati, M. A., Rebezov, M., Bangar, S. P., Lorenzo, J. M., & Domínguez, R. (2022). Lactic Acid Bacteria (LAB): Autochthonous and Probiotic Microbes for Meat Preservation and Fortification. In Foods (Vol. 11, Issue 18, p. 2792). MDPI AG. <u>https://doi.org/10.3390/foods11182792</u>
- Sheiko, T., Tkachenko, S., Mushtruk, M., Vasyliv, V., Deviatko, O., Mukoid, R., Bilko, M., & Bondar, M. (2019). Studying the processing of food dye from beet juice. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 13, Issue 1, pp. 688–694). HACCP Consulting. <u>https://doi.org/10.5219/1152</u>
- 28. Spescha, C., Stephan, R., & Zweifel, C. (2006). Microbiological Contamination of Pig Carcasses at Different Stages of Slaughter in Two European Union–Approved Abattoirs. In Journal of Food Protection (Vol. 69, Issue 11, pp. 2568–2575). Elsevier BV. <u>https://doi.org/10.4315/0362-028x-69.11.2568</u>
- Bal'-Prylypko, L. V., Derevyanko, L. P., Slobodyanyuk, N. M., Starkova, E. R., & Androshchiuk, O. S. (2018). Using of the Ampullaria glauca snails' caviar for correction of the effects of the ionizing radiation exposure in small dose. In Nuclear Physics and Atomic Energy (Vol. 19, Issue 2, pp. 159–165). National Academy of Sciences of Ukraine (Co. LTD Ukrinformnauka) (Publications). https://doi.org/10.15407/jnpae2018.02.159

- **30.** Cherednichenko, O., & Bal-Prylypko, L. (2020). Rationale and economic feasibility of improving the technology of long-term storage of meat products. In IOP Conference Series: Earth and Environmental Science (Vol. 548, Issue 2, p. 022053). IOP Publishing. <u>https://doi.org/10.1088/1755-1315/548/2/022053</u>
- Mushtruk, M., Deviatko, O., Ulianko, S., Kanivets, N., & Mushtruk, N. (2021). An Agro-Industrial Complex Fat-Containing Wastes Synthesis Technology in Ecological Biofuel. In Lecture Notes in Mechanical Engineering (pp. 361–370). Springer International Publishing. <u>https://doi.org/10.1007/978-3-030-77823-1\_36</u>
- 32. De Oliveira, J. A., Polizel Neto, A., Bueno da Rosa, C. C., Fortuna, N. B., Groth, A., Dos Santos, R., Bento, F. C., & Gomes, H. F. B. (2021). Use of different cold water spray protocols on bovine carcasses during cooling and its effects on meat quality. In Scientific Electronic Archives (Vol. 14, Issue 5, pp. 12–21). Scientific Electronic Archives. <u>https://doi.org/10.36560/14520211345</u>
- **33.** Stadnyk, I., Bodnarchuk, O., Kopylova, K., Petrov, P., Bal-Prylypko, L., & Narizhnyy, S. (2021). Modification of the properties of milk-fat emulsions with the phase structure of "oil in water" in the dependence on the mass part of the lipoid and the stabilizing systems. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 741–748). HACCP Consulting. <a href="https://doi.org/10.5219/1389">https://doi.org/10.5219/1389</a>
- 34. Shanina, O., Minchenko, S., Gavrysh, T., Sukhenko, Y., Sukhenko, V., Vasyliv, V., Miedviedieva, N., Mushtruk, M., Stechyshyn, M., & Rozbytska, T. (2020). Substantiation of basic stages of gluten-free steamed bread production and its influence on quality of finished product. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 189–201). HACCP Consulting. <u>https://doi.org/10.5219/1200</u>
- **35.** Bhat, Z. F., Morton, J. D., Mason, S. L., & Bekhit, A. E.-D. A. (2018). Role of calpain system in meat tenderness: A review. In Food Science and Human Wellness (Vol. 7, Issue 3, pp. 196–204). Tsinghua University Press. <u>https://doi.org/10.1016/j.fshw.2018.08.002</u>
- **36.** Zheplinska, M., Mushtruk, M., Vasyliv, V., Slobodyanyuk, N., & Boyko, Y. (2021). The Main Parameters of the Physalis Convection Drying Process. In Lecture Notes in Mechanical Engineering (pp. 306–315). Springer International Publishing. <u>https://doi.org/10.1007/978-3-030-77823-1\_31</u>
- 37. Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y., & Geng, W. (2021). Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry. In Frontiers in Bioengineering and Biotechnology (Vol. 9). Frontiers Media SA. <u>https://doi.org/10.3389/fbioe.2021.612285</u>
- 38. Chaillou, S., Champomier-Vergès, MC., Cornet, M. *et al.* The complete genome sequence of the meat-borne lactic acid bacterium *Lactobacillus sakei* 23K. *Nat Biotechnol* 23, 1527–1533 (2005). <u>https://doi.org/10.1038/nbt1160</u>
- 39. Eisenbach, L., Geissler, A. J., Ehrmann, M. A., & Vogel, R. F. (2019). Comparative genomics of Lactobacillus sakei supports the development of starter strain combinations. *Microbiological Research*, 221, 1–9. <u>https://doi.org/10.1016/j.micres.2019.01.001</u>
- **40.** Hou, Q., Cheng, Y.-p., Kang, D.-c., Zhang, W.-g. and Zhou, G.-h. (2020), Quality changes of pork during frozen storage: comparison of immersion solution freezing and air blast freezing. Int J Food Sci Technol, 55: 109-118. <u>https://doi.org/10.1111/ijfs.14257</u>
- **41.** Bao, Y., Ertbjerg, P., Estévez, M., Yuan, L., & Gao, R. (2021). Freezing of meat and aquatic food: Underlying mechanisms and implications on protein oxidation. *Compr Rev Food Sci Food Saf*, 20: 5548–5569. <u>https://doi.org/10.1111/1541-4337.12841</u>
- **42.** Yu, H. H., Chin, Y. W., & Paik, H. D. (2021). Application of Natural Preservatives for Meat and Meat Products against Food-Borne Pathogens and Spoilage Bacteria: A Review. *Foods (Basel, Switzerland)*, *10*(10), 2418. <u>https://doi.org/10.3390/foods10102418</u>
- **43.** Tippala, T., Koomkrong, N., & Kayan, A. (2021). Influence of freeze-thawed cycles on pork quality. *Animal bioscience*, *34*(8), 1375–1381. <u>https://doi.org/10.5713/ajas.20.0416</u>
- **44.** Nian Lu, Ji Ma, Da-Wen Sun, Enhancing physical and chemical quality attributes of frozen meat and meat products: Mechanisms, techniques, and applications. Trends in Food Science & Technology, Volume 124, Pages 63-85, <u>https://doi.org/10.1016/j.tifs.2022.04.004</u>

#### Funds:

This research received no external funding. Acknowledgments:

#### **Conflict of Interest:**

The authors declare no conflict of interest.

#### **Ethical Statement:**

This article does not contain any studies that would require an ethical statement. **Contact Address:** 

\*Volodymyr Vovkotrub, National University of Life and Environmental Sciences of Ukraine, Department of Veterinary Hygiene, Faculty of Veterinary Medicine, Vystavkova Str. 16, 03127, Kyiv, Ukraine, E-mail: vetmed\_bcrda@ukr.net © ORCID: https://orcid.org/0009-0007-2760-4559

Olga Iakubchak, National University of Life and Environmental Sciences of Ukraine, Department of Veterinary Hygiene, Faculty of Veterinary Medicine, Vystavkova Str. 16, 03127, Kyiv, Ukraine, E-mail: yakubchak om@nubip.edu.ua © ORCID: https://orcid.org/0000-0002-9390-6578

Leonid Horalskyi, Zhytomyr Ivan Franko State University, Faculty of Natural Sciences Zhytomyr Ivan Franko State University, Department of Zoology, Biological Monitoring and Nature Protection, V. Berdychivska Str., 40, 10008, Zhytomyr, Ukraine,

E-mail: goralsky@ukr.net

© ORCID: https://orcid.org/0000-0002-4251-614X

Nataliia Vovkotrub, Bila Tserkva National Agrarian University, Faculty of Veterinary Medicine, Department of Propaedeutics and Internal Diseases of Animals and Poultry named after V.I. Levchenko, pl. Soborna, 8/1, 09117, Bila Tserkva, Ukraine,

E-mail: nataliia.vovkotrub@btsau.edu.ua

© ORCID: https://orcid.org/0000-0003-3297-454X

Larysa Shevchenko, National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Veterinary Hygiene, Heroes of Defense Str., 15, 03041, Kyiv, Ukraine, E-mail: shevchenko\_laris@ukr.net

© ORCID: http://orcid.org/0000-0001-7472-4325

Nataliia Shynkaruk, National University of Life and Environmental Sciences of Ukraine, Humanitarian and Pedagogical Faculty, Department of International Relations and Social Sciences, Heroes of Defense Str., 15, Kviv, 03040. Ukraine.

E-mail: natalisch1003@gmail.com

© ORCID: https://orcid.org/0000-0002-7724-2919

Tatyana Rozbytska, National University of Life and Environmental Sciences of Ukraine, Faculty of food technologies and quality management of products of agricultural products, Department of Standardization and Certifying of agricultural products, Vystavkova Str., 16, Kyiv, 03040, Ukraine, E-mail: roirf1991@gmail.com

© ORCID: https://orcid.org/0000-0003-0098-927X

Yuliia Slyva, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Standardization and Certification of agricultural products, Heroes of Defense Str., 15, 03040, Kyiv, Ukraine, E-mail: yuliia\_slyva@ukr.net

ORCID: <u>https://orcid.org/0000-0003-2592-6822</u>

Olga Tupitska, National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Biochemistry and Physiology of Animals named after Academician Maksym Gulyi, Kyiv, Vystavkova Str., 16, Ukraine,

E-mail: olgatup@ukr.net

ORCID: https://orcid.org/0000-0001-9067-9173

**Oksana Shtonda**, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Management of Agricultural Products, Department of Meat, Fish, and Seafoods, Vystavkova Str., 16, 03041, Ukraine,

Email: <u>oasht@ukr.net</u>

© ORCID: <u>https://orcid.org/0000-0002-7085-0934-5155</u>

Corresponding author: \*

© 2023 Authors. Published by HACCP Consulting in <u>www.potravinarstvo.com</u> the official website of the *Potravinarstvo Slovak Journal of Food Sciences*, owned and operated by the HACCP Consulting s.r.o., Slovakia, European Union <u>www.haccp.sk</u>. The publisher cooperate with the SLP London, UK, <u>www.slplondon.org</u> the scientific literature publisher. This is an Open Access article distributed under the terms of the Creative Commons Attribution License CC BY-NC-ND 4.0 <u>https://creativecommons.org/licenses/by-nc-nd/4.0/</u>, which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.