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Effect of ultraviolet irradiation on beef carcass yield

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The results of testing the use of ultraviolet radiation (UVR) spectrum C (wavelength 253.7 nm) to reduce the contamination of chilled beef in half carcasses are presented. It is determined that the efficiency of UVR with a wavelength of 253.7 nm is directly proportional to the distance between meat half-carcasses in the cooling chamber: if the distance is longer than the efficiency of processing is higher. The efficiency of UVR processing of beef half-carcasses is proved by placing them in a chill chamber and storing them at 17 cm distance one from the other. This condition and the exposure of UVR for 45 min, gives a decrease in the number of mesophilic aerobic and facultative-anaerobic microorganisms on the carcasses surface to 95.15±0.23%. An ecological regime to storage of chilled cattle in half carcasses (suspension) was developed using a UVR with a wavelength of 253.7 nm at 45 minutes exposure and 17 cm distance between the carcasses without breaking the technological process. The developed mode allows to double the shelf life of the raw meat in the chilled state. For efficient UVR processing of slaughtered animals (cattle) the half-carcasses are needed to be placed in a cooling and storage chamber (air temperature – 6.0±2.0°C, relative humidity – 85.0±0.5%) at 17 cm distance one from other. In this condition and 45 minutes exposure the number of mesophilic aerobic and facultative-anaerobic microorganisms on the surface of the experimental half-carcasses decreased from (4.45±0.17) × 10^3 CFU/cm^2 at the beginning of the experiment to (0.22±0.01) × 10^3 CFU/cm^2 after UVR treatment.
Key words: muscle tissue, half-carcasses, ultraviolet irradiation, air-recirculator, temperature, exposure, microorganisms.

Introduction

Thermal and chemical methods of inactivation of microorganisms have been traditionally used to extend the shelf life of food products. However, thermal methods are not suitable for temperature sensitive foods and may reduce their quality. Chemical methods give concern of safety and environmental friendliness as they may be toxic and allergenic. Scientists around the world are developing and testing alternative methods of preventing food spoilage (without the use of heat and chemicals) to extend their shelf life and storage" (Koutchma et al., 2009; Hardin, 2014; Gorbunova & Tunieva, 2016). Among these methods, ultraviolet radiation (UVR) has attracted a great deal of attention in recent years due to its safety, environmental friendliness, low cost and ease of use. UV light is a non-ionizing, non-chemical and non-thermal method, making it environmentally friendly, easy to use and economical. Ultraviolet light is generated by mercury lamps and consists of electromagnetic radiation in the range of 100 to 400 nm. The UV light spectrum is divided into four parts: UV-A (315-400 nm), UV-B (280-315 nm), UV-C (200-280 nm) and vacuum-UV (100-200 nm). UV-C is extremely bactericidal, causing photochemical changes in microbial DNA that eventually inactivates microorganisms (Keklik et al., 2012). Many studies have demonstrated the effectiveness of UV radiation on pathogens contained in water and in food, including bacteria, fungi, viruses and protozoa (Stefanova et al., 2010; Degala et al., 2018).

Isohanni & Lyhs (2009) provides results on the effects of UV radiation on *Campylobacter jejuni* isolated from broiler chickens. Ultraviolet exposure for 14-18 seconds at a dose of 32.4-32.9 mW/cm^2 resulted in a maximum decrease log10 of 0.7, 0.8, and 0.4 during the processing of contaminated *C. Jejuni* broiler meat, skins and carcasses.

Yeh et al. (2018) proved the positive effect of the combined use of UV radiation and bacteriophages on bacteria of the *Salmonella* genus during the experiment on chopped beef. Sommers et al. (2017), microbiological parameters of sausages, containing sodium diacetate and potassium lactate, were studied after treatment with UV radiation at 4.0 J/cm^2. Further pasteurization of the products at exposure for 3 seconds at a steam temperature of 121°C led to complete inactivation of *Listeria innocua*. The presence of the *L. innocua* test specimens was not observed after the treated sausages were stored for 8 weeks at 8 °C. This study showed that the combination of ultraviolet light with other processing methods can be an effective and economical method of eliminating listeria in cooked sausage products. Begum et al. (2009) have demonstrated in their work that UV radiation effectively reduces the number of fungi such as *Aspergillus flavus, Aspergillus niger, Penicillium corylophilum and Eurotium rubrum.*

Some researchers (Begum et al., 2009; Sommers et al., 2017) proved that ultraviolet light is effective for inactivating a wide range of microorganisms. However, the question of the ability of UV radiation to cause decontamination of beef and pig carcasses, ensuring their long-term storage, has not been studied. In addition, all of these studies were conducted in a laboratory setting but were not tested in a production setting, which casts doubt on their effectiveness directly at the meat processing plants. Zhou et al. (2010) and Keklik et al. (2012) have shown that when light acts on the surface of a food, its energy is partly reflected and partly absorbed, and, depending on the transparency of the product, it can penetrate deep into it. It was found that the effect of light on the food product depends on both, the optical properties of the product and the properties of light, including duration, intensity, and spectrum. The maximum absorption of ultraviolet light by nucleic acid occurs at a wavelength of about 260 nm, at which UV light causes the greatest damage to microbial DNA. Miller et al. (1999) suggested that the germicidal effects of UV light appear under photochemical changes when UV light is absorbed by living cells. The absorption peak of ultraviolet light by nucleic acids, especially DNA, in the UV-C range makes them the targets of the photochemical action of UV light. UV light directly causes to photo-dimerization between neighboring pyrimidine bases and cytosine photohydration. In addition, ultraviolet light plays a role in the generation of reactive oxygen species that also react with DNA and proteins. Replication of microbial DNA is prevented by these lethal photodimers. As a consequence, cellular functions are inhibited and eventually the cell becomes inactivated. Pulsed UV light inactivates microorganisms not only by photochemical reactions but also by other mechanisms associated with the use of high intensity pulses. The microbial cell becomes inactive after UV-induced photochemical reactions, but the cell structure remains intact. However, pulsed UV light also damages cellular structures. All this suggests that the use of UV radiation is an appropriate and effective means to control pathogens that cause food spoilage.

To date, UV radiation is reasonably used in the food industry to disinfect equipment (Rodionova & Paliy, 2016; Paliy et al., 2018), packaging materials (Lonergan et al., 2019), during storage of fruits and vegetables, increasing their shelf life, storage (Begum et al., 2009). However, the absence of industrial-scale ultraviolet systems for meat and meat products limits the use of this technology in the meat industry. The question of the use of UV radiation in the food industry to refurbish of the air in refrigeration chambers, as well as the possibility of its use to decontaminate the meat carcasses surface during their storage remains unresolved.

To solve such problems, namely to continuous disinfection of air in laboratory and other rooms with the presence of people, the modern industry produces a recirculating air recirculator closed type ORUB-01- "KRONT", which was tested in our work.

Materials and methods

The purpose of the study is to develop a regime of beef half-carcasses processing in the chill state (pendulous) using the ultraviolet irradiation.

To achieve this goal, the following tasks were set:

- determine the distances between the beef half-carcasses in the cooling and storage chamber to achieve the most effective surface decontamination using UV light (with a peak value at 253.7 nm, UV-C);
- develop the regime of the meat processing with UV radiation.

The experimental part of the work was carried out during 2018-2019 on the basis of the Department of Infectology, Quality and Product Safety of the Agroindustrial Complex of Lugansk National Agrarian University (Kharkiv, Ukraine), the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center "Institute for Experimental and Clinical Veterinary Medicine" (Kharkiv, Ukraine) and meat processing enterprises of Lugansk and Kharkiv regions.

The subjects of the study were beef half-carcasses in the cooled state (pendulous). The experiment used 5080 beef carcasses with the traditional course of autolysis (pH = 5.6-6.0). For each experimental group we selected 20 meat carcasses obtained from one slaughter group, which according to microbiological indicators meet the requirements of DSTU 6030: 2008 "Meat. Beef and veal in carcasses, half carcasses and quarters". Meat temperature was measured in the thickness of the thigh and scapular muscles at 6 cm depth. Beef half-carcasses were treated with ultraviolet (UV) radiation in a chill and storage chamber (total area 32 m^2). To carry out the research it has been used a portable vertical irradiator-recirculator of the closed type ORUB-01-"KRONT" (trade name "DESAR-8") (CJSC "KRONT-M", Russia). Ultraviolet radiation source have been ozone lamps (TUV-30W - three units) from "Philips" (Netherlands). According to the bactericidal lamp manual instruction, the Philips TUV 30W produces shortwave ultraviolet radiation with a peak wavelength of 253.7 nm (UV-C) having a bactericidal effect. The average life of the test lamps meets the requirements of the regulatory documentation and does not exceed 1000 hours. The bulb flasks have a special coating that, by delaying radiation shorter than 200 nm, prevents the formation of ozone in the air environment and at the same time increases the life of the lamps by up to 9000 hours. The absence of direct UV rays and ozone makes the DESAR-8 recirculator absolutely safe to use in the presence of humans.

Before the meat raw material was placed in the chill and storage chamber, it was thoroughly cleaned and disinfected in accordance with the guidelines of the Disinfection of technological equipment and production facilities of the meat processing enterprises. Detergents were used with Anti-Germ SR S 25 detergent (Vetsynthesis LLC, Ukraine) (2.0% - 30 min) and CD-256 disinfectant (IPAX Cleanogel, Inc., USA) (0.3% - 1 year). After disinfection, the chamber air was sanitized using a portable vertical air recirculator of closed type ORUB-01-"KRONT" for 30 min in order to achieve sterility of the air, which was confirmed by microbiological studies. SPD 08.04 passageway (INOX TIME, Ukraine) was placed In front of the entering the camera, in order to avoid secondary contamination of raw material during contact with working personnel. Only the members of the control panel approved by the company order had access to the raw material. We also used a non-destructive method of selection of swab sampling from the surface with a sterile swab in accordance with the requirements of DSTU ISO 17604:2014 "Microbiology of food and animal feed. Sampling of carcasses of animals for microbiological analysis" Samples were randomly sampled from different sections of the meat carcass (lower pelvis, outer part of the subclavian and the inner part of the fatty tissue) from 10% of the carcasses stored in the experimental chamber. Swab sampling

from the tested objects were sampled from an area of 100 cm^2 using a 10×10 cm metal stencil, which limited the required area. Before each overlay on the surface of the object under study, a the stencil frame was flamed over an alcohol.

To have swab sampling there were made the swabs on wire rods, placed in a cotton-gauze bung. They were used to close the tube, which was previously filled with distilled water in a volume of 2.0 cm^2. The tubes were then sterilized in an autoclave at 0.5 atmospheres pressure for 30 min. From each 100 cm^2 area, limited by a stencil frame, swabs on wire rods dipped in distilled water, wiped the test surface and put it back into the test tube. We poured 8.0 cm^3 of sterile distilled water into aseptic conditions into the swabs after swabs were selected for the production dilution of these washes from the sites. A well-wrung swab was sent for disinfection. This washout in tubes was considered the initial (initial) dilution.

Next, a series of consecutive dilutions of 1:10, 1:100, 1:1000, 1:10000, 1:100000 and 1:1000000 were prepared according to DSTU ISO 21528-1:2014 "Microbiology of Food and Animal Feed. Preparation of test samples, initial suspension and tenfold dilutions for microbiological examination. Part 1. General rules for the preparation of initial suspension and ten-fold dilutions (ISO 6887-1:1999, IDT)". The number of mesophilic aerobic and facultative-anaerobic microorganisms (MAFAnM) on the surface of cattle carcasses was determined from all dilutions made. For this purpose, 1.0 cm^3 of each dilution was transferred into sterile Petri dishes and filled with molten and cooled meat-peptone agar (MPA) to 55°C. The incubation of the plating was performed in a thermostat at 37°C temperature during 48 hours. The number of MAFAnM per 1 cm^2 area was determined according to the conventional method. In order to detect pathogenic microorganisms in the swab sampling, the last ones were examined with the aim to detect the presence of bacteria *Echerichia* spp., *Salтonella* spp., *Listeria* spp., *Staphylococcus* spp., anaerobic pathogens. The studies were carried out in accordance with applicable regulatory documents. Indication of *Echerichia* spp. Carried out in accordance with GOST 30518-97 "Food products. Methods for detecting and determining bacterial counts of the group of *Echerichia coli* (coliform bacteria). Microorganisms *Salтonella* spp. was isolated according to DSTU ISO 6579:2006 "Microbiology of food and animal feed. Method for detection of *Salmonella* spp". Representatives of *Listeria* spp. were isolated in accordance with DSTU ISO 11290-1:2003 "Microbiology of Food and Animal Feed Horizontal Method for Detection and Calculation of Listeria monocytogenes" (Part 1. Detection method). GOST 29185-91 "Food. Methods for detecting and determining the amount of sulfit reductive clostridia were used to isolate pathogenic anaerobes. Studies for the presence of staphylococcus culture were performed in accordance with GOST 7702.2.4-93 "Poultry meat, offal and semi-finished products. Methods of detecting and determining the amount of *Staphilococcus aureus*". We conducted two parallel studies: we determined the number of microorganisms on the meat carcasses surface (by the method of swab sampling) and the total number of microorganisms per 1 gram of deep muscle tissue (destructive method) to detect the ability of microorganisms to penetrate the meat carcasses surface during their storage in deep layers of muscle tissue,. The studies were conducted every day during 12 days while the storage of raw meat material.

To determine the number of MAFAnM in 1 gram of deep muscle the tissue muscle samples (lower part of the hip, outer part of the subclavian cut) were selected with the help of sterile instruments (stencil frame (15×15 cm), metal ruler, knife, scalpel and tweezers). Before sampling the muscle tissue for the test, the surface of the beef half-carcasses at the sampling part was wiped with sterile wipes 2-3 times and then it was cut off 0.2±0.1 mm of the superficial muscle layer (using a scalpel) with 10×10×3 cm areas of sampling (where 3 cm is the thickness of the muscle). Accepted error in the muscle layer size is ± 0,2 cm. During each study, a sample of muscle tissue was chopped with sterile scissors. For further researchers it was used an average sample of muscle tissue with 15 gr weight. Each sample was separately placed in a sterile homogenizer flask to prepare the suspension. To do this, 15 cm^3 of 1% peptone water was added to the flask and homogenized samples in an electric homogenizer for 1 min. The ready suspensions were allowed to stand for 10 minutes, and then microbiological cultures were used according to the conventional method.

Results and discussion

To obtain the most effective surface decontamination using a 253.7 nm UVR, the required distance between the half-carcasses in the chill and storage chamber was studied. The number of MAFAnM on the surface of cattle carcasses at the beginning of the experiment was (4.45±0.17) × 10^3 CFU/cm^2. When treating UVR half-carcasses for 15 minutes in 14 cm distance, the number of MAFAnM are reduced by 3.84±0.48% compared to the initial contamination. After every 10 min of treatment, for the next 20 min, inactivation of microorganisms was observed by 12.9% with each treatment step. With a treatment exposure for 35 min, the total percentage reduction in microbial contamination was 28.2±0.62% compared to the initial contamination. After 45 min, the efficiency of UVR processing was 51.73±0.39%. Increasing the distance between half carcasses in 1 cm (up to 15 cm) almost did not change the number of MAFAnM on the surface of the half carcasses compared to the distance of 14 cm (Fig. 1).

The treatment efficiency at 15 cm distance between half carcasses was detected while exposures of 15, 25, 35 and 45 min, comparing the results with the previous experiment. We found that the efficiency of UVR processing of half-carcasses for 45 min was 56.91±0.28%, that was only by 5.18% higher, compared to 45 min of processing of half-carcasses at 14 cm distance. We detected that processing of meat half carcasses with UVR for 45 min exposure and 16 cm distance between half carcasses is more effective by 35.62±2.59% than in previous experiments (at 14 cm and 15 cm distance between carcasses). We revealed that the number of MAFAnM on the surface of the half-carcasses decreased from (3.24±0.17) × 10^3 CFU/cm^2 for 15 min exposure to (4.48±0.09) × 10^2 CFU/cm^2 for 45 min exposure. The data are almost 10 times smaller than the total amount of the original microflora on the surface of the surface before UR treatment. With the UVR processing exposure for 15, 25, 35 and 45 min at a given 16 cm distance between the carcasses, the stepwise processing efficiency is 27.22±0.9%, 40.02±0.22%, 59.92±0.24% and 89.94±0.21%, respectively. The average step-by-step exposure was 20.89±4.98% or (1.0±0.19) × 10^3 CFU/cm^2.

The efficiency of processing half-carcasses at 17 cm and 18 cm distance was almost the same. The most effective was the mode of UVR processing of half-carcasses for 45 min exposures. The processing efficiency under the given mode with the 17 cm distance between the half-carcasses was 95.15±0.23%, and at the distance of 18 cm – 95.11±0.73%. We established that it is necessary to place the carcasses in the chill and storage chamber at 17 cm distance to provide the effective UVR treatment. While saved the distance and the UVR treatment for 45 min exposure, the total number of microorganisms on the surface of the experimental surfaces was reduced from (4.45±0.17) × 10^3 CFU/cm^2 at the beginning of the experiment to (0.22±0.01) × 10^3 CFU/cm^2 after UVR treatment. Thus, the quantity of MAFAnM on the surface of carcasses decreases to 95.15±0.23% after the treatment of the surface of meat half-carcasses with UVR, at 17 cm distance between half-carcasses. The efficiency of a UVR with a wavelength of 253.7 nm was directly proportional to the

distance between meat half-carcasses in the chill chamber: the longer was the distance, the higher was the processing efficiency (Fig. 2).

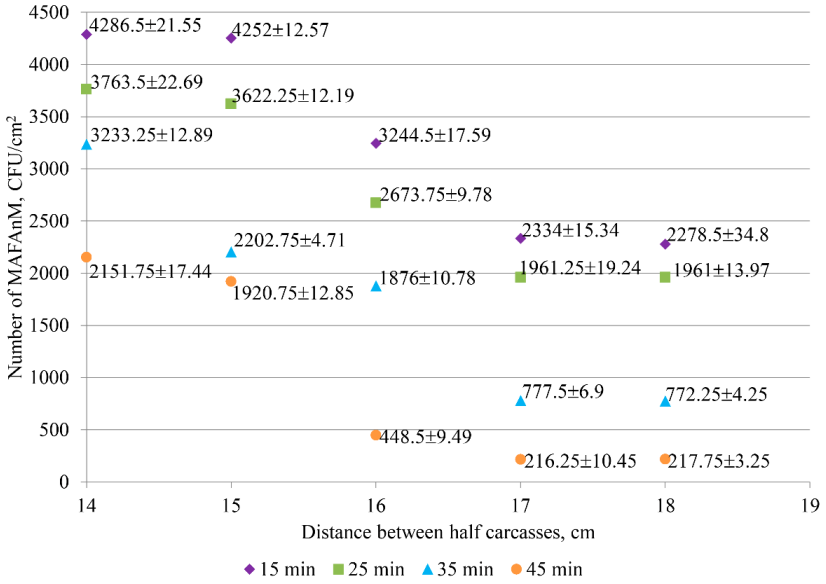

Fig. 1. The number of MAFAnM on the surface of the meat half-carcasses after treatment with UVR at intervals of 24 hours, CFU/cm^2, (M±m, n=140).

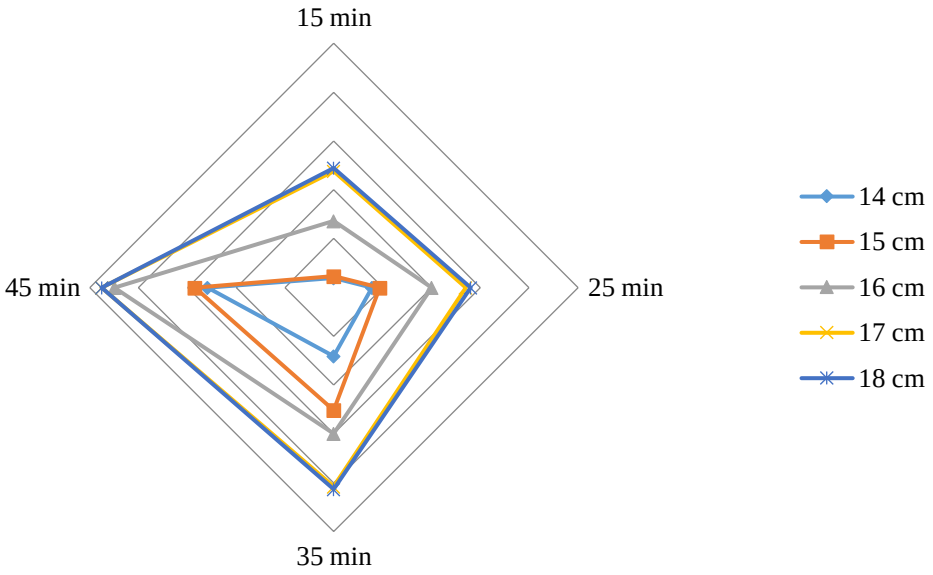

Fig. 2. Efficiency of closed-air recirculator ORUB-01"KRONT", %.

However, the processing efficiency in any experiment did not reach 100%, so viable microorganisms remain on the surface of the muscle tissue, which can multiply to penetrate deep layers of muscle tissue and cause damage to the product. This is because the effect of UV rays is cumulative: with increasing the age of microorganisms, the lethal effect of UVR became weaker, and the offspring of previously irradiated microorganisms acquires some resistance to UVR. In the next stage, we tested four modes of the UVR use in the conditions of the meat processing enterprise to test he effective sanitation regime of beef half-carcasses (pendulous) in the cooled state (Fig. 3).

The distance between the half-carcasses was 17 cm, the treatment exposure was 45 min, and the sanitation interval was once a day (every 24 h) daily during the period of control storage (up to 12 days) and relative humidity (82.0±0.5 and 85.0±0.5%). Storage temperature was from 4°C to 12°C, air flow were 1 and 2 m^3/min.

During the storage of meat half-carcasses, 24.29±3.02% of the total number of microorganisms on the surface of the halves penetrated into the deep layers muscle tissue up to the fifth day (at temperatures from 4°C to 8°C and relative humidity of 82.0±0.5%). The ability of UVR to inactivate microorganisms decreased from the sixth day of storage, the percentage of microorganisms capable to penetrate into deep layers increased to 36.01±1.69%, so from 1674.61±22.28 CFU/cm^2 to deep layers of muscle 597.05±27.02 CFU/g. We also observed the mucus formation and extraneous odor on the surface of the meat carcasses w, indicated the beginning of spoiling.

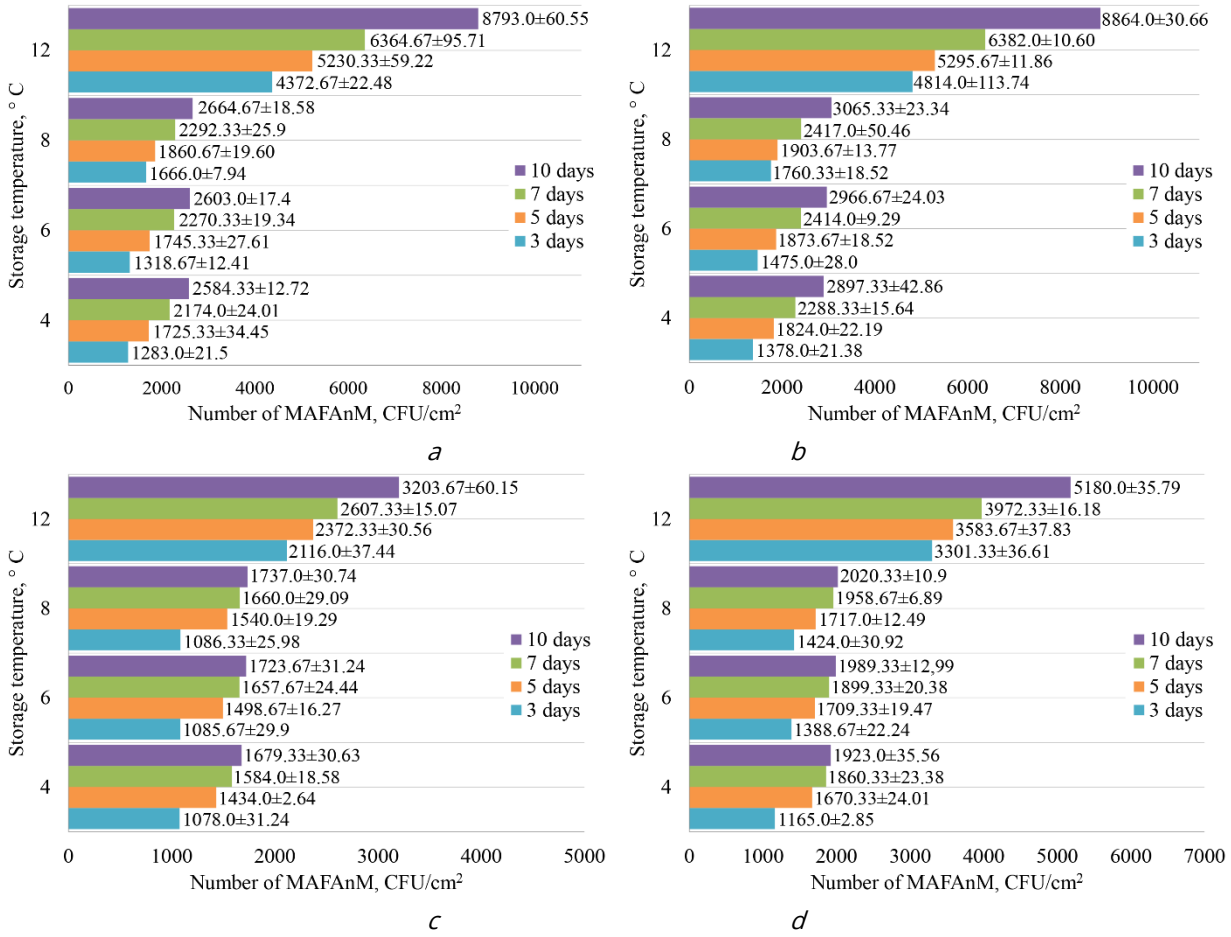

Fig. 3. The number of MAFAnM on the surface of half-carcasses during different storage regimes in 2018-2019. (M±m, n=3). *a* - air flow rate 1 m^3/min, relative humidity 82.0±0.5%; *b* - air flow rate 1 m^3/min, relative humidity 85.0±0.5%; *c* - air flow rate 2 m^3/min, relative humidity 82.0±0.5%; *d* - air flow rate 2 m^3/min, relative humidity 85.0±0.5%.

Table 1. The results of microbiological examination of beef muscle tissue samples, depending on the processing method and shelf life, CFU/g (M±m, n=3). The number of MAFAnM at the beginning of the experiment was 4.45±0.17 × 10^3 CFU/cm^2.

Air flow rate, m^3/min	Relative humidity, %	Air temperature, °C	Save period, days		
			3 days	7 days	12 days
1.0	82.0±0.5	4	246.67±4.91	538.0±15.37*	731.0±8.08*
		6	285.33±10,90	610.0±8.18*	862.33±26.52*
		8	296.67±9.39	664.67±4.98*	893.67±5.84*
		12	476.33±4.81	921.33±13.77*	1423.33±22.66*
	85.0±0.5	4	256.33±13.19	664.67±13.77*	887.33±26.43*
		6	280.33±4.70	664.67±13.32*	914.33±14.24*
		8	311.67±9.95	702.67±6.17*	944.33±7.05*
		12	580.0±14.18	952.0±5.68*	1384.67±12.87*
2.0	82.0±0.5	4	221.67±9.24	490.33±11.67*	711.0±7.23*
		6	238.33±14.33	563.0±11.72*	733.33±7.31*
		8	249.33±11.32	617.33±12.0*	780.67±7.96*
		12	448.67±11.32	824.33±6.49*	1242.33±13.03*
	85.0±0.5	4	238.33±17.94	525.0±7.37*	734.0±6.51*
		6	249.33±13.69	584.0±12.12*	760.67±25.78*
		8	272.67±6.12	612.67±8.99*	831.67±12.71*
		12	509.67±6.64	878.33±27.71*	1300.0±32.35*

* p≤0.05, number of microorganisms in samples were compared after 3, 7 and 12 days.

The total number of microorganisms increased after 12 days of storage from 4.45±0.17 × 10^3 CFU/cm^2 to 829.0±49.83 CFU/g (an average number of MAFAnM in 12 days at 6.0±2.0°C), that was correspond to normal limit range (1000 CFU/g). Raw meat was stored at temperatures from 4 to 8°C, relative humidity of 82.0±0.5%, air flow rate of 1 m^3/min, the distance between half-carcasses was 17 cm, and the treatment with UVR wavelength of 253.7 nm was done within 45 min every 24 hours (Table 1). While increasing the temperature up to 12°C in a given storage regime, the number of MAFAnM in 12 days is 1423.33±22.66 CFU/ g, that exceeds the established norm (1000 CFU/g) almost in 1.5 times. It should be noted that the shelf life of the control sample of raw meat material in a given mode, but without UVR treatment from 4 to 6°C is 5 days, and from 8 to 12°C is only 3

days. The amount of microorganism in 1 g of product in 10 days of storage increases by 91.65 CFU/g at relative humidity up to 85.0±0.5% (4-8°C; V air - 1 m/min). This is the optimum shelf life of the raw meat in the specified regime, however, with an increase of air temperature up to 12°C, it is 7 days (the number of MAFAnM in 1 g of meat is 952.0±5.68 CFU/g).

It should be noted that the number of MAFAnM per 1 g of meat does not change with increasing humidity up to 85.0±0.5%. The storage period of the control sample in the specified mode without the UVR processing is 5 days. The number of MAFAnM in 1 g of the control sample of meat at 4°C storage reaches 540.0±9.71 CFU/g, at 6°C – 583.33±11.05 CFU/g, and at 8°C – 669.0±18.19 CFU/g. Increasing the shelf life storage of the control samples from 4 to 8°C leads to an increase in the number of MAFAnM by two times in 1 g. Increasing the air temperature to 12°C, regardless of the set relative humidity (82.0±0.5% and 85.0±0.5%), reduces the shelf life of the experimental beef half carcasses to 7 days and in control samples - to 3 days. However, the number of MAFAnM on the surface of the control samples (without UVR treatment) at a relative humidity of 85.0±0.5% after 7 days of storage was within the limits of the accepted norm (953.67±15.6 CFU/g).

Conclusion

The processing of beef half-carcasses with UVR should be done under chill chamber and storage (air temperature – 6±2°C, relative humidity – 85.0±0.5%), 17 cm between the carcasses. In this conditions and the UV treatment exposure for 45 min, the number of MAFAnM on the surface of the experimental surfaces decreases from $4.45\pm0.17 \times 10^3$ CFU/cm^2 to $0.22\pm0.01 \times 10^3$ CFU/cm^2. The use of UVR does not break with the technological process of raw meat processing. The application of this UVR mode ensures the effective safety preservation and quality indicators of beef half-carcasses cooled during 12 days, taking into account the transportation. A veterinary health assessment of chilled meat during the storage confirmed the advantage of the UVR use.

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