Increasing the efficiency of use, extending the freshness and ensuring the safety of animal raw materials, on the basis of "green chemistry" technological and ecological methods

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Abstract. The study has explored the theoretical foundations and conducted a number of experiments related to the methods and possibilities of using Electro-Chemical Activation (ECA) technology of aqueous solutions in the food industry. The purpose of the study has been to establish the degree of efficiency and identify ways to improve the technology to extend the freshness of raw materials of animal origin. To achieve this purpose, technical and technological methods have been developed in order to increase the efficiency of the use of food raw materials of animal origin using ECA solutions. Beef and poultry meat have been chosen as the study raw materials. A series of experiments has been aimed at establishing a rational treatment regime (treatment method, processing time and presumed storage) and checking the effectiveness of the ECA solutions action on a colony of Mesophilic Aerobic and Facultative Anaerobic Microorganisms, as well as the effect on the organoleptic (color, odor, consistency) and physical and chemical (freshness) meat properties during the experiment and for extended storage for 7 days with the results daily measurement. As the study result, it has been found that the ECA solution is an effective and economically profitable preparation for use with the purpose of the freshness prolonging of raw materials of animal origin. It has been shown that the main drawbacks of modern processing methods, such as discoloration, taste, smell and high cost of equipment are absent in the technology of processing with electrochemically activated solutions. Positive results have been found in microbiological, organoleptic, physicochemical analysis.
Key Words: food safety, Electro-Chemical Activation, "green chemistry", beef, poultry meat.

Introduction. Dishes made from raw materials of animal origin can meet the daily needs of any person in proteins, fats, carbohydrates, vitamins and minerals due to their chemical composition (Amosov 2002). The main importance of meat in human nutrition lies in the supply of essential amino acids, capable of synthesizing proteins (Importance of meat in human nutrition/Meat products-electronic resource 2017), which perform a number of important functions in the human body: building function, enzymatic function, and hormonal function. In addition, proteins carry transport, receptor, reserve, protective and other functions in the human body (Stepanov 1996). Due to the large content of proteins (14-24%) and moisture (71-76%) namely, which represent nutrients, creating favorable conditions for the microorganisms' activity from the microbiological point of view, the raw meat is a perishable product (Altunina & Petrova 2010).

Such interrelated factors as storage temperature, access to oxygen, the presence of endogenous enzymes, moisture light and, most importantly, microorganisms' content, have a significant influence on the meat shelf life and freshness (Kushevskaya et al 2004). In the modern scientific and technical literature, such modern methods and techniques for preserving and prolonging the freshness of raw materials of animal origin, such as cooling and freezing, packaging technologies, physicochemical methods for processing meat (ionizing radiation, treatment under pressure) are considered. These techniques have significant advantages and disadvantages. An actively developing technology related to the "green chemistry" solutions and used for extending the freshness terms, the quality improving and ensuring the various products safety consists in the use of the aqueous solutions Electro-Chemical Activation (ECA). The advantages of this method are environmental friendliness, safety, economy, efficiency and universality (Nikiforova et al 2015; Cloete 2005; Fabrizio & Cutter 2005; Huang et al 2008; Suvorov et al 2017).

Beef, which, according to research (Tikhmeeva 2014), is the most popular type of meat in the world, as well as chicken meat, to which special microbiological and chlorine-free requirements are applied (Bahir 2010), have been used as raw materials in our study.

It is believed that poultry meat is one of the most polluted types of raw materials and for food intake requires more thorough processing. As for beef meat, there are also some disadvantages: cows breeding in ecologically polluted areas, feeding animals with mixed fodders containing antibiotics, which significantly reduce the immunity of the animal to resist disease-causing organisms (Tikhmeeva 2014).

Our study object has included activities aimed at extending shelf life, improving quality and improving meat safety. Our study subject have constituted electrochemically activated green chemistry products, such as "Anolyte PEROX", prepared on the basis of an aqueous solution of baking soda (by chlorine-free technology), as well as "Anolyte ANK SUPER", obtained by electrochemical action on the sodium chloride aqueous solution. The Study Relevance is due to the need to improve the food services quality and safety (Suvorov et al 2017; Eco friendly disinfecting/sanitizing agent effective against all types of microorganisms-electronic resource 2017).

Material and Method. During the experiment, organoleptic, physicochemical and microbiological indicators have been monitored in accordance with regulatory documentation.

Organoleptic indicators of meat are such parameters that determine its consumer properties and directly affect the human sense organs. These include as follows: muscle tissue color, subcutaneous fat color, odor, consistency and muscle characteristics in the section.

To determine the total microbial contamination, the selection of medium samples, the preparation of samples and dilutions in the prescribed manner have been carried out:

- products samples for microbiological analysis have been taken prior to the sampling for organoleptic and physical and chemical analyzes;
- products samples have been taken aseptically, excluding microbial contamination of the product from the environment;
- during sampling, tools, materials, utensils in contact with the product have been sterile;
- the average sample has included all the components in the ratio, in which they were in the product, so that its composition would correspond to the whole batch.

Prior to the inoculation, tenfold dilutions have been prepared: 1:10 (10^{-1}); 1:100 (10^{-2}); 1:1000 (10^{-3}). To prepare the dilutions, a Normal Saline Solution (NSS) has been used, which composition promotes the resuscitation of weakened and damaged microorganisms. To prepare the initial dilution (1:10) from the average sample, a sample weighted amount of 10±0.1 g has been selected, which was ground to a homogeneous mass after weighing and transferred to a 200-260 cm^3 flask, where 90 cm^3 of sterile NSS had been previously placed. To prepare the initial dilution, the sample weighted amount has been transferred to a flask with 9 cm^3 of NSS, and then stirred. Thus, the first initial dilution 1:10 (10^{-1}) has been obtained. When preparing the second dilution 1:10 (10^{-1}), 1 cm^3 has been taken from the sample first dilution with a sterile pipette and transferred to a flask with 9 cm^3 of NSS. The dilution has been implemented by air blowing (bubbling). The third and subsequent dilution has been prepared in a similar manner.

A determination of the Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms (QMAFAnM or a Total Viable Count (TVC)) refers to the estimation of the quantity of the sanitary indicator microorganisms group. The composition of QMAFAnM represents various taxonomic groups of microorganisms - bacteria, yeast, and mold fungi. Their total number indicates the sanitary-hygienic state of the product, the degree of its microbial content level.

As for the physical and chemical indicators, the most important for assessing the meat quality and freshness is the determination of the protein and fat mass fraction, as well as the freshness chemical analysis, which includes the evaluation of the broth transparency and aroma (GOST 23392-78 2009).

Two solutions types have been used in the study:

- (A) for chicken meat processing: "Anolyte PEROX" (Delfin Aqua, Russia), prepared by chlorine-free technology in the STEL-PEROX device, the initial component of which is a baking soda solution in fresh water with a concentration of 100-150 g L^{-1}, the final products - "Anolite-PEROX” and Catholyte (cathode liquor);

- (B) for beef processing: a disinfectant "Anolyte ANK SUPER" (Delfin Aqua, Russia), obtained by electrochemical action on an aqueous solution of sodium chloride, has been used.

Anolyte is a disinfectant and sterilizing agent, effective against microorganisms. "Anolyte PEROX" is non-toxic, does not have harmful effects on the environment, can be used in the presence of people in any way of use.

Catholyte (cathode liquor) is a non-toxic agent having pronounced detergent properties, used for washing and pre-sterilization cleaning (Bahir 2010; Suvorov et al 2017).

For a series of experiments with beef meat, two pieces of beef have been selected: one piece was subjected to multiple freezing and defrosting, laying for some time in the refrigerating chamber and at the beginning of the experiment already having a slight musty smell. The second piece of meat was bought in a supermarket of a premium class and considered to be fresh by its organoleptic indicators.

Both pieces of meat have been divided into eight portions of 10 grams and placed in disinfected glass containers, 2 pieces in each. As a result, 4 samples of fresh and stale meat have been obtained. Two containers of each sample have been left as control ones, the others have been completely filled with ECA solution and held for 5, 15 and 30 minutes, after which microbiological, organoleptic and physical and chemical parameters were checked.

The second series of experiments has been based on the data obtained in the previous experiment. It has been found that the best time for keeping meat when immersed in an ECA solution is 15 minutes. In this experiment, a repeated check of this result has been carried out. Moreover, it has been decided to use additional modes, such as irrigation and double processing (15-minute immersion with subsequent irrigation). During the whole experiment, the organoleptic parameters have been strictly monitored for 7 days, physical and chemical, as well as microbiological analyzes have been carried out twice (on the first and third days, since the shelf life of the half-finished products was two days at a temperature of 4±2°C at a relative humidity of 85% (Technological Regulations of the Custom Union 2013; SanPiN 2.3.2 1324-03 2002). Only fresh beef has been used for the experiment, completely identical to meat in the first experiment.

For the experiment with chicken meat, a broiler chicken whole round has been chosen, which was considered to be fresh at the time of purchase. For testing, the average sample of poultry in an amount of 30 grams has been selected, and placed in sterilized glass containers, 1-2 pieces in each. As a result, 3 samples of fresh meat have been obtained.

To prepare for inoculation, the sample number one (10 grams) has been placed in a NSS without pretreatment; the sample number two has been pretreated with tap water, and the sample number three has been treated with the "Anolyte PEROX" solution. For this purpose, it has been placed in a container with anolyte for 30 minutes and then in the NSS. After processing, the meat has been analyzed for TVC and the freshness terms.

To carry out the experiment, the selected samples have been placed in a NSS: the sample number one - without pretreatment, the sample number two has been pretreated with tap water, and the sample number three has been treated with the "Anolyte PEROX" solution using a new technology in three stages: first it was thoroughly washed with a catholyte solution, then placed in a container with anolyte for 60 minutes, after which the solution was renewed and the meat was placed in fresh anolyte solution for another 30 minutes (Suvorov et al 2017).

Results and Discussion. The optimum temperature for the QMAFAnM growth is 35-37°C (under aerobic conditions); their growth temperature limit is within the range from 20 to 45°C. Mesophilic microorganisms live in the organisms of warm-blooded animals, and also survive in soil, water and air (MicroBio-electronic resource 2017). Thus, according to this indicator, it will be possible to judge the sanitary and hygienic conditions during the slaughter, meat transportation and storage.

The essence of the method for determining the QMAFAnM, inoculated into agarized nutrient media lies in the product inoculation or the product sample weighted amount dilution into a nutrient medium, the inoculations incubation and the count of all the visible colonies that have grown up. It has been established (SanPiN 2.3.2.560-96 2002; Technological Regulations of the Customs Union 2013) that the number of the QMAFAnM colonies should not exceed $5x10^5$ in beef and $1x10^4$ in chicken meat.

It should be necessary to pay special attention to the fact that ECA solutions have already found wide application in medicine, in military science, in the chemical and food industries. Anolytes can replace more than 99% of all disinfectants used in medical and preventive institutions and food industry enterprises, with the following advantages:

- effectiveness against all microorganisms, including spores, fungi, viruses with a short exposure time;
- absence of microorganisms habituation for any extended period and continuous application;
- possibility of using in a particularly clean production (food, pharmaceutical industry), etc.

The use of ECA solutions in the industry has already proved its effectiveness and relevance. An innovative approach to the use of solutions for extending the shelf life and the quality improving of raw materials of animal and vegetable origin has enormous potential success and is able to make a great contribution to the food industry development.

It should be noted that the use of chlorine in the water for cooling the poultry leads to accumulation on the surface and in the thickness of the meat by-products of oxidative activity of free chlorine, primarily organochlorine compounds (chlorophenols, chloramines, THM (trihalomethanes) and others) that are hazardous for human health. Therefore, solutions prepared using chlorine-free technology have been used for experiments with chicken meat (Bahir 2010; Suvorov et al 2017).

ECA solutions have antimicrobial properties against mesophilic aerobic and facultative anaerobic microorganisms, coliform bacteria, *Proteus* bacteria, *Salmonella*, *Staphylococcus aureus* (Sudakova 1999). Numerous studies confirm the fact that the ECA aqueous solution has antimicrobial, antiviral and antibacterial properties (Cloete 2005; Cloete et al 2009; Gulabivala et al 2004; Huang et al 2008; Martinez-Huitle & Brillas 2008; Thorn et al 2012).

The results of a determination of the QMAFAnM (beef) in the first series of experiments can be found in Table 1. The formation of mold and a large number of colonies have been observed in the control meat sample. After 5 and 15 minutes of treatment, a significant decrease in the TVC has been observed in dilution 10^3, and their complete elimination in some samples.

Based on the above data, it can be concluded that the treatment with ECA solutions within 5 and 15 minutes reduces the total microbial contamination by two-three times. The 30-minute treatment has been found to be ineffective, as the TVC indicators are approximately equal to the indices of the untreated samples. All meat samples comply with the standard ($5x10^5$ CFU g^{-1}) and are considered to be suitable for use.

Table 1

Results of the experiment #1

Mode	*Fresh meat, CFU g^{-1}*		*Stale meat, CFU g^{-1}*	
Control, Initial concentration	686	$7.0x10^2$	828 + mold formation	$8.0x10^2$
Control, single dilution	857	$8.5x10^3$	740	$7.4x10^3$
Treatment with ECA solution, 5 min. Initial concentration	314	$3.1x10^2$	360	$3.6x10^2$
Treatment with ECA solution, 5 min. Three-fold dilution	5	$5.0x 10^3$	4	$4.0x10^3$
Treatment with ECA solution, 15 min. Initial concentration	330	$3.3x10^2$	331	$3.0x10^2$
Treatment with ECA solution, 15 min. Three-fold dilution	3	$3.0x10^3$	2	$2.0x10^3$
Treatment with ECA solution, 30 min. Initial concentration	626	$6.3x10^2$	760	$7.6x10^2$
Treatment with ECA solution, 30 min. Single dilution	443	$4.4x10^3$	446	$4.5x10^3$

The results of a determination of the QMAFAnM (beef) in the second series of experiments can be found in Table 2. Treatment with ECA solutions with 15-minute immersion and irrigation has allowed reducing the colonies number by several times; however, it has led to a continuous growth with a double treatment.

Table 2

Results of the experiment #2

Mode	*Day 1, CFU g^{-1}*		*Day 3, CFU g^{-1}*	
Control, Initial concentration	continuous growth	-	440	$4.4x10^2$
Control, Three-fold dilution	22	$2.2x10^4$	115	$1.2x10^5$
15-minute immersion, Initial concentration	392	$4.0x10^2$	380	$4x10^2$
15-minute immersion in Three-fold dilution	1	$1x10^3$	110	$1.0x10^5$
Irrigation, Initial concentration	900	$9x10^2$	392	$4x10^2$
Irrigation, Three-fold dilution.	3	$3x10^3$	315	$3.0x10^5$
Double treatment, Initial concentration	continuous growth	-	430	$4.3x10^2$
Double treatment, Three-fold dilution	600	$6x10^5$	178	$1.8x10^5$

On the first storage day, a 15-minute immersion and irrigation are most effective and reduce the TVC by an order of magnitude, while the number of colonies in a three-fold dilution exceeds the norm ($6x10^5$) after a double treatment. On the second day, the effect after treatment with the ECA solution is lost and the number of colonies does not decrease, in either treated or control samples, moreover, it approaches the maximum permissible limits. With regard to double treatment, the number of colonies in a three-fold dilution has decreased 3-fold compared with the first day analysis. Thus, treatment with ECA solutions shall be important only when it is applied on the first day.

Regarding the results of a determination of the QMAFAnM (chicken meat), as the result of the first series of experiments carrying out, a continuous growth of microorganisms has been observed in all samples, which indicated contamination of the meat under study and that the treatment with the "Anolyte PEROX" solution could not reduce the microbial contamination.

As a result of the second series of experiments, it has been revealed that a continuous growth of the colonies was formed in the control sample and the sample treated with tap water, which indicates the product under study contamination. In the three-fold dilution, a continuous growth has also been found in a sample treated with tap water. However, in the sample treated with "Anolyte PEROX", only 1 colony has been found in the three-fold dilution, which, according to the normative documentation (SanPiN 2.3.2.560-96 2002; Technological Regulations of the Customs Union 2013), indicates that the solution has coped with its task and destroyed pathogenic microorganisms.

As can be seen from the above, the developed technology is effective in controlling microbiological contamination of poultry meat and the commitment to improving the catering services safety has been a success (Suvorov et al 2017).

Conclusions. The ECA-solution is an effective preparation that allows to reduce the TVC by two-three times, when fresh meat processing. The treatment with the ECA solution also improved the physicochemical parameters, in particular, freshness, prolonging the meat freshness, compared to the control sample. It shall be necessary to pay special attention to the fact that the purpose of the experiment has not been to transform the stale meat into fresh meat, but to check the possibility of using the ECA solutions for processing any meat prior to storage in order to extend its shelf life and increase its microbiological safety. Summing up what has been said, the treatment with the ECA solutions is a promising direction in the food industry. The results of the performed experiments make it possible to assume the importance of using ECA solutions in the food industry in order to extend the products shelf life of, to improve the microbiological safety, which will allow consumers not to worry about the safety of their products or their dietary allowance.

Acknowledgements. The research work has been carried out within the President of the Russian Federation Grant No. MK-8362.2016.11.

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Received: 30 May 2017. Accepted: 24 June 2017. Published online: 27 June 2017.
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How to cite this article:
Suvorov O. A., Decheva E. I., Shikova I. D., Pugachev I. O., Khramtsov M. A., Voyno L. I., Ruban N. V., 2017 Increasing the efficiency of use, extending the freshness and ensuring the safety of animal raw materials, on the basis of "green chemistry" technological and ecological methods. ABAH Bioflux 9(1):37-44.